# 28th ESACT MEETING

European Society for Animal Cell Technology

23-26 JUNE 2024 · EDINBURGH

# BOOK OF ABSTRACTS

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# Invited Speaker and Oral Communications Abstracts

# Sunday 23 June 2024

# Impact of biological therapeutics: perspective of the cancer clinician and the cancer patient.

**Professor Charlie Gourley**[1]
[1]The University Of Edinburgh, Edinburgh, United Kingdom

Keynote Lecture: Professor Charlie Gourley, Pentland Auditorium, June 23, 2024, 6:30 PM - 7:15 PM

Since the turn of the century the field of cancer therapeutics has reaped the fruits of the labour of the discovery scientists who went before. This has had an enormous impact on cancer care and on patient outcome.

Although not limiting discussion to agents derived from animal cell culture, this talk will describe how a combination of improved understanding of tumour biology along with an acceleration of targeting strategies has revolutionised outcome in many cancers. While challenges clearly remain, the acceleration of technological development provide hope for the future. Cellular therapies, gene therapies, antibody therapies and antibody-drug conjugates will all be crucial in meeting these challenges.

As well as highlighting some key developments this talk will aim to convey an idea of what having access to these novel therapies means to patients and their families.

# Micromapping: A Chemical Approach to Biological Insights and Therapeutic Intervention

**Professor Sir David MacMillan**[1]
[1]Princeton University, Princeton, USA

Keynote Lecture: Professor Sir David MacMillan, Pentland Auditorium, June 24, 2024, 8:45 AM - 9:30 AM

This lecture will describe µMap, a new photoredox-based approach to microenvironment mapping that provides a powerful means to probe biological pathways at the subcellular level. µMap uses a light-driven energy transfer mechanism to activate warheads proximal to localized iridium catalysis, which, in turn, label neighboring biomolecules in a complex intra- or extracellular environment. We will discuss the development of this new technology and its application in a number of biological contexts, including intrasynaptic labeling, target identification, mapping of the interactome of checkpoint inhibitors and CAR-T cells, and mapping of chromatin state changes.

# Invited Speaker and Oral Communications Abstracts

# Monday 24 June 2024

# Confined bioprinting in inflatable bioreactor: toward the sterile bioproduction of tissues and organs

Dr Alexandre Dufour[1], Mr Aravind Anadan[2], Dr Emma Petiot[1], Dr Isabelle Gay[3], Dr Magali Barbaroux[3], **Dr Christophe Marquette**[1]
[1]3d.FAB - Université Lyon1, Villeurbanne, France, [2]SEGULA Technologies, Lyon, France, [3]Sartorius Stedim FMT, Aubagne, France

Innovate or Die – New Technologies, Pentland Auditorium, June 24, 2024, 9:30 AM - 10:00 AM

**Relevance to Theme 5:** The present work describes an innovative component for the bioproduction of living tissue through bioprinting.

**Impact/Novelty:** The proposed technology is the missing link between the gold standard bioproduction in confined enclosures and the non-sterile state-of-the-art 3D bioprinting of living tissues.
**Introduction:** Tissue production strategies strongly rely on 3D bioprinting which sterility remains a forefront concern. The only proposed solution to day is to bioprint in a biosafety cabinet where tissues are exposed to non-confined environment during transfer and culture medium exchanges. We developed a leading-edge bioproduction tool, the Flexible Unique Generator Unit (FUGU) system, enabling 3D bioprinting and culture in a confined and sterile environment.

**Methods/Approach:** The concept is based on an inflatable and compliant bioreactor composed of a silicone membrane fixed to a building platform, itself hosting fluid management. This concept enables the full compliance during bioprinting, the modification of the internal culture volume, the handling of culture liquid phase, the direct observation and the gas exchange through the transparent silicone membrane.
**Results:** In the FUGU system, 3 features are essential: (i) being confined (ii) enabling 3D bioprinting (iii) allowing fluid transfer and management. The selection of the membrane materials was performed through solid computational simulation. Silicone elastomers were selected for their tuneable mechanical properties. Then, confinement was evaluated with sterility assays through bacteria contamination and culture. Sterility, even in the presence of a direct bioprinting needle contamination, was obtained. Finally, culturing 12 $cm^3$ bioprinted living tissue within the FUGU system enabled us to qualify the system as a Confined Biofabrication Tool.

**Conclusion:** FUGU is a hybrid bioproduction tool composed of solid and flexible parts. The flexible part can be inflated under 200 mbar sterile air pressure, allowing the full range of motion of a bioprinting head. The FUGU system will now be challenged through multiple biofabrication applications, from organ printing to tumour model and bioproduction.

# Morphological profiling of senescent cells for label-free quality control of mesenchymal stem cells

**Mr Kenjiro Tanaka**[1], Mr. Yuto Okumura[1], Prof. Kei Kanie[2], Prof. Ryuji Kato[1,3]
[1]Graduate School of Pharmaceutical Sciences, Nagoya University, Nagoya/Chikusa-ku/Furo-cho, Japan, [2]Department of Biotechnology and Chemistry, Kindai University, Higashihiroshima/Takanoumenobe, Japan, [3]Institute of Nano-Life-Systems, Institutes of Innovation for Future Society, Nagoya University, Nagoya/Chikusa-ku/Furo-cho, Japan

Innovate or Die – New Technologies, Pentland Auditorium, June 24, 2024, 9:30 AM - 10:00 AM

**Relevance to Theme Selected:**
Our technology, which identifies various senescence types in MSCs through morphological cell quality evaluation method, represents a significant innovation in bioprocess control and stem cell therapy understanding.

**Impact/Novelty:**
Our research introduces a novel, non-invasive method using image analysis and machine learning to assess MSC quality, enhancing precision in stem cell therapies and bioprocess control.

**Introduction:**
Mesenchymal stem cells (MSCs) are prized in regenerative medicine for their immunosuppressive abilities and capacity to differentiate into various mesenchymal tissues. A major challenge, however, is maintaining their stable expansion culture. MSCs rapidly lose their proliferative ability and functionality during continuous culture, often due to stress-induced cellular senescence. Our team has been working on a non-destructive, early prediction method for assessing MSC quality. This method combines image-based analysis with machine learning. While we have previously shown cell quality decline related to over-passaging using morphological profiles, it was uncertain if we could predict different types of senescence.

**Methods/Approach:**
This study aimed to identify distinct senescence types in MSCs using our morphological cell quality evaluation method. Considering the significant variability within MSC populations, we focused on detecting stressed MSCs at a single-cell level to enhance our method's sensitivity. We exposed MSCs to various senescence-inducing conditions, including over-passaging and senescence-inducing drugs. Their morphological changes were recorded using an automated imaging system throughout the culture period. These time-lapse images were analysed to extract morphological features, which were then used for clustering and machine learning.

**Results:**
We discovered two key findings: (1) senescent cells display different morphologies depending on the type of senescence they undergo, and (2) our morphological analysis can identify and profile senescence-related sub-populations within MSCs.

**Conclusion:**
Our research shows that despite the presence of various senescence-related phenotypes, our label-free, morphology-based evaluation technique can effectively assess the condition of MSCs using only their microscopic images. This technology is promising for detailed cell quality assessments in MSC-based therapies and cell manufacturing.

# From Moonshot to One-Pot: A New Paradigm for Multispecifics Manufacturing

**Dr Guogang Dong**, Dr. Dawn Eriksen-Stapleton, Dr. Arch Creasy, Mr. Mike King, Ms. Kimberly Nguyen
[1]Pfizer, Andover, United States

Innovate or Die – New Technologies, Pentland Auditorium, June 24, 2024, 9:30 AM - 10:00 AM

**Relevance to Theme Selected:**
*Theme 5. Innovate or die – technology innovation driving the understanding and control of bioprocesses*
This work describes the development of a novel technology called one-pot capture redox (OPCR) for the manufacture of multispecifics

**Impact/Novelty:**
OPCR incorporates co-culture of two cell lines followed by a redox reaction executed during protein A purification step to significantly simplify multispecific manufacturing process and reduce timeline.

**Introduction:**
Interest in multispecific antibodies as the next-gen therapeutics has grown dramatically over the past decade. This is driven by their unique capability of binding to two or more antigens or epitopes simultaneously, thereby delivering enhanced or new therapeutic effects. The manufacturing process of these multispecific molecules can be complex. While some are expressed from a single cell line, many utilize two separate cell lines, each producing a half molecule or parental homodimer mAb. Traditionally, two mAbs are produced in separate production bioreactors and partially purified through affinity chromatography. Heterodimerization is achieved by combining the mAbs in a defined ratio via a redox reaction in a mixing vessel. This significantly increases the number of unit operations and complexity compared to a typical mAb manufacture process.

**Methods/Approach:**
Here we describe a method to co-culture the two cell lines that express the parental homodimers. The co-culture harvest fluid is then loaded on a protein A chromatography column for purification and to complete a redox reaction that forms the heterodimer. Process parameters that influence the titer ratio of the parental mAbs at harvest are investigated and optimized.

**Results:**
We will show the successful development of co-culture process combined with the column-based redox reaction for several multispecific molecules of different IgG types, with robust control of the heterodimer in the drug substance. Insights on levers to control the homodimer ratio at harvest of the co-culture process will be shared. Additionally, we will present data for successful demonstration of the process at the manufacturing scale.

**Conclusion:**
The combined technology of the co-culture and capture redox addresses many of the shortcomings of the conventional multispecific process. It eliminates multiple unit operations, reduces manufacturing time, and enables the production of multispecifics using processes typical for standard mAbs.

# The Sleeping Beauty transposon system: A molecular parasite tamed for genome engineering

**Prof. Zoltan Ivics**[1]
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Zoltán Ivics[1,2,3], Csaba Miskey[1], Lacramioara Botezatu[1], Nicolas Sandoval-Villegas[1], Matthias Ochmann[1], Guillermo Guenechea Amurrio[4], Juan Bueren[4], Tobias Bexte[5], Evelyn Ullrich[5]
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Cells as Factories and Therapies: Session 1, Pentland Auditorium, June 24, 2024, 11:00 AM - 12:30 PM

Transposons are mobile genetic elements evolved to execute highly efficient integration of their genes into the genomes of their host cells. We established robust preclinical protocols for transposition-mediated stable gene transfer into human T and NK cells by *Sleeping Beauty* (SB) vectors supplied as minicircles (MCs) and synthetic mRNA as a source of the transposase. The SB protocol is i) as efficient as lentiviral gene transfer with respect to overall stable gene transfer, sustained transgene expression and biological activity of genetically engineered cells, ii) associated with a far safer genomic integration profile than any viral system currently in clinical use and iii) fully non-viral, thereby allowing GMP vector production at significantly reduced costs on a per patient basis. We have recently adapted the MC/mRNA gene delivery protocol to hematopoietic cells, and have validated the protocol in a mouse model of Fanconi Anemia by demonstrating efficient engraftment and a repopulation advantage of gene-corrected hematopoietic stem and progenitor cells. Finally, we identified and characterized a novel hyperactive variant of the SB transposase, and established regulated assembly of catalytically active transposase through intein tagging and splicing. These modifications will enhance the efficiency and safety of gene delivery in human clinical applications.

# Deciphering key adenoviral elements in the production of recombinant AAV vectors

**Dr. Sofia Fernandes**[1,2], Julia Guerra[1,2], Prof. Ana Sofia Coroadinha[1,2]
[1]iBET, Instituto de Biologia Experimental e Tecnologica, Oeiras, Portugal, [2]ITQB, Instituto de Tecnologia Quimica e Biologica, Oeiras, Portugal

Cells as Factories and Therapies: Session 1, Pentland Auditorium, June 24, 2024, 11:00 AM - 12:30 PM

**Relevance to Theme Selected:** This study explores crucial adenoviral factors influencing AAV production, advancing strategies for safer, scalable, and high titer rAAV vectors in gene therapy manufacturing.

**Impact/Novelty:** Revealing the intricate interplay between AAV and adenoviral components propels the design of advanced production systems, crucial for achieving consistently high titer rAAV vectors, thus revolutionizing gene therapy manufacturing.

**Introduction:** The success and approval of gene therapies using recombinant adeno-associated virus (rAAV) have increased their demand. Traditional production methods face challenges in scalability and biosafety. The development of stable AAV producer cell lines without replication-competent virus has emerged as a promising alternative, offering enhanced biosafety and potential for scalable, consistent, and cost-effective vector production. However, current strategies have not yet achieved titers comparable to those obtained in transient transfection- based production. We believe that this discrepancy indicates an incomplete understanding of of AAV-helper virus interactions.

**Methods/Approach**: This study aimed to identify essential Adenoviral genes for AAV production. Utilizing a pHelper plasmid with full AdV genes (E2A, E4, and VA RNAs), eight constructs with varied gene combinations were generated. Their impact on serotypes 5, 8, and 9 productivity was evaluated through transient transfection, in suspension conditions and compared relative to control (pHelper). The resulting rAAV vectors were collected from the intracellular and extracellular fractions. Each cellular fraction was separately quantified by ELISA (TP) and qPCR (VG).

**Results:** Results revealed that E2A-DBP, E4orf6, and VA RNA alone were insufficient for successful rAAV production, resulting in significant decreases for rAAV8 and rAAV9. Restoration of production levels required specific promoter and region elements, such as L4P promoter, L4-22/33k, and E4orf3 regions. Notably, rAAV5 was less affected.

**Conclusion:** The knowledge gained from this study will ultimately facilitate the development of improved strategies for large-scale production of high titer and high-quality rAAV vectors, thereby advancing the field of gene therapy.

# Trigger-induced drug release from particle-loaded macrophages

**Mr Omkar Desai**[1], Dr. Mario Köster[1], Mr. Sushobhan Sarker[1], Dr. Albert Poortinga[2], Dr. Nina Ehlert[3], Prof. Dr. Dagmar Wirth[1]
[1]Helmholtz Center For Infection Research, Braunschweig, Germany, [2]Polymer Technology, Eindhoven University of Technology, Eindhoven, the Netherlands, [3]Institute of Inorganic Chemistry, Leibniz University of Hannover, Hannover, Germany

Cells as Factories and Therapies: Session 1, Pentland Auditorium, June 24, 2024, 11:00 AM - 12:30 PM

**Relevance to Theme Selected:**
The study evaluates a novel, cell-based delivery system with triggered drug release through external physical stimuli.

**Impact/Novelty:**
The study presents a macrophage-based drug delivery system designed for local release in response to physical cues.

**Introduction:**
While systemic drug delivery is widely employed, even for treating local diseases, it has shortcomings such as low therapeutic effectiveness and high toxicity in off-target tissues. To overcome these limitations, targeted drug delivery is an attractive, however largely unrealized concept. This study explores macrophages as carriers for drug-loaded particles and demonstrates that ultrasound and an alternating magnetic field (AMF) enable local, trigger-induced release of drugs from macrophages, both in vitro and in mice.

**Methods/Approach:**
Gas-filled antibubbles and mesoporous nanoparticles (MSNs) were loaded with drugs (4-OH-Tamoxifen, cytokines, or antibiotics). Macrophages were loaded with antibubbles only, or co-loaded with MSNs and superparamagnetic, AMF-responsive particles (SPIONs). Ultrasound and AMF were evaluated for release from respective loaded macrophages.

**Results:**
Macrophages can be efficiently (co-)loaded with various particles. While MSN/SPION-loaded macrophages showed slow spontaneous cargo release, exposure to AMF amplified the release due to heat-induced apoptosis. Antibubble-loaded macrophages demonstrated only residual spontaneous release, while exposure to ultrasound effectively disrupted antibubbles and cell membranes, releasing their entire cargo. Notably, trigger-induced cell death was restricted to loaded macrophages, whereas unloaded cells remained unaffected. Finally, we evaluated drug release from antibubble-loaded macrophages in mice. Non-invasive ultrasound triggered the release of Tamoxifen from loaded macrophages in mice, while unexposed mice did not show the release of the drug.

**Conclusions:**
The study demonstrates that external stimuli trigger the release of bioactive cargo from macrophages loaded with cargo-filled microparticles. Ultrasound emerges as an attractive non-invasive trigger for drug release from antibubbles. Together, the study supports microparticle-loaded macrophages as a potent drug delivery system with time- and space-controlled release.

# Complementary Gene Editing Technologies Enhance Rapid Generation of Stable Cell Lines Producing Enveloped Viral-Like Particles

**Dr. Corey Brizzee**[1], Dr. Tyler Kinder[1], Jasminka Bozic[2], Dr. Catalina Soare[2], Dr. Anne-Catherine Fluckiger[2]
[1]Demeetra Agbio, Lexington, United States, [2]VBI Vaccines, Ottawa, Canada

Cells as Factories and Therapies: Session 1, Pentland Auditorium, June 24, 2024, 11:00 AM - 12:30 PM

**Relevance to Theme Selected:** The work presented highlights the production of hard-to-produce enveloped viral-like particles (eVLPs). Enhanced cell factory platforms are tailor-made to express protein components of eVLPs.

**Impact/Novelty:** Combining piggyBac transposase and Cas-CLOVER targeted nuclease technologies enable rapid stable cell line development expressing therapeutic candidates for development of novel coronavirus vaccines as new variants surface.

**Introduction:** Viral-like particles are proven to be powerful therapeutics towards infectious diseases by harnessing the power of the human immune system. Currently, new variants of SARS-CoV-2 continue to surface causing decreased efficacies of vaccinations and the requirement for rapid production of new therapeutic biologics.

**Methods/Approach:** Two complementary gene editing technologies, piggyBac transposase and Cas-CLOVER targeted nuclease, were utilized to create enhanced cell bioprocessing platforms to produce eVLPs. Firstly, the piggyBac transposase/transposon system was utilized to map novel sites of interest to express an eVLP stable core protein, MLV-GAG. Subsequently, since Cas-CLOVER is a dimeric targeted nuclease system, the most efficient guide RNA pair was used to independently knock-in viral-like protein cargo (GAG, spike proteins, etc.). Knock-in constructs incorporated homologous fragments to the target sites flanking integral protein components of eVLPs. Following NEON transfection, positive cells were enriched and characterized for the presence of knock-in cargo and secreted protein expression.

**Results:** Via the piggyBac system, five total novel sites were identified in which one of particular interest was further utilized for the differential gene expression stage. Known genomic safe harbor sites, *rogi1* and *gsh31,* were also targeted for comparison. Three guide RNA pairs were tested per site which yielded over 35% cutting efficiencies at all sites in a single NEON electroporation. Antibiotic selection enriched the cell pools for positive gene-edited cells over 90%. Throughout the antibiotic selection, pools were validated via PCR and western blot, which showed increased target amplification and secreted protein expression, respectively. Comparison of each knock-in site revealed differential protein expression levels that can create different ratios of eVLP components.
**Conclusion:** These innovative cell platforms will be used to create product candidates for manufacturing novel vaccine therapies. Unlike current pipelines, our newly developed workflow will be able to rapidly deliver those novel product candidates in response to Pandemics.

## Control and Characterization of Thousands-Diverse Recombinant Polyclonal Antibodies

**Ms. Rena Mizrahi**[1]
[1]GigaGen, Inc. (A Grifols Company), San Carlos, USA

Product Quality – Relevance and Assessment: Session 1, Pentland Auditorium, June 24, 2024, 3:45 PM - 5:15 PM

Conventionally, hyperimmune globulin drugs manufactured from pooled immunoglobulins from vaccinated or convalescent donors have been used in treating infections where no treatment is available. This is especially important where multi-epitope neutralization is required to prevent the development of immune-evading viral mutants that can emerge upon treatment with monoclonal antibodies. Using microfluidics, flow sorting, and a targeted integration cell line, GigaGen established a platform for development and manufacturing of recombinant polyclonal antibodies (pAbs), which comprise a mixture of >1,000 individual antibodies produced en masse. The first drug of this class to enter clinical studies was GIGA-2050, for treatment of SARS-CoV-2. Development of this novel drug class leverages existing knowledge from mAb manufacturing, but has also required development of new techniques to analyze the diversity of antibody species present and to ensure lot-to-lot consistency.

# Product Quality Control through Raman Spectroscopy

**Prof. Thomas Villiger**[1], Dr. Patrick Romann[1,2], Mr. Sebastian Schneider[1], Ms. Daniela Tobler[1], Dr. Martin Jordan[3], Mr. Arnaud Perilleux[3], Dr. Jonathan Souquet[3], Dr. Jean-Marc Bielser[3]
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Product Quality – Relevance and Assessment: Session 1, Pentland Auditorium, June 24, 2024, 3:45 PM - 5:15 PM

**Relevance to Theme Selected:**
Ensuring consistent product quality has become increasingly important, particularly in continuous processes. Here, we propose a Raman based feeding strategy to ensure consistent N-linked glycosylation profiles during a perfusion process.

**Impact/Novelty:**
Novel Raman-controlled feeding approach that stabilized metabolic activity thus preventing product quality drifting in perfusion cultures. This approach might pave the way for parametric real-time testing in the long term.

**Introduction:**
Despite technological advances ensuring stable cell culture perfusion operation over prolonged time, reaching a cellular steady-state metabolism remains a challenge for certain manufacturing cell lines. Raman spectroscopy has the potential to measure different nutrients and metabolites simultaneously and in real-time, which builds the basis for providing a well-defined environment for intensified cell culture processes.

**Methods/Approach:**
A novel on-demand pyruvate feeding strategy was developed, leveraging lactate as an indicator for tricarboxylic acid (TCA) cycle saturation. Real-time lactate monitoring was achieved through in-line Raman spectroscopy, enabling accurate control at predefined target setpoints. This approach allowed stabilizing a steady-state perfusion process producing a bispecific antibody with drifting product quality attributes, caused by shifting metabolic activity in the cell culture.

**Results:**
The implemented feedback control strategy resulted in a three-fold reduction of ammonium levels and stabilized product quality profiles. Consistent profiles were achieved for all major product N-linked glycosylation forms. In addition, this approach effectively reduced the high mannose content which is often associated with process intensification, such as extended culture duration or increased cell culture densities.

**Conclusion:**
The Raman-controlled pyruvate feeding strategy represents a valuable tool or continuous manufacturing, stabilizing metabolic activity, and preventing product quality drifting in perfusion cell cultures. Finally, an outlook about further possibilities as well as limitations of product quality control through Raman spectroscopy will be provided.

# Unveiling mAbs & viral vectors´ attributes by Mass Spectrometry-based advanced bioanalytics

**Dr Sofia B. Carvalho**[1,2], Dr Ricardo Gomes[1,2], Dr Bruno Alexandre[1,2], Dr Ludivine Profit[3], Dr Sushmitha Krishnan[4], Dr Severine Clavier[5], Dr Michael Hoffman[4], Dr Kevin Brower[4], Dr Patrícia Gomes Alves[1,2]
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Product Quality – Relevance and Assessment: Session 1, Pentland Auditorium, June 24, 2024, 3:45 PM - 5:15 PM

**Relevance to Theme Selected:** MS-based bioanalytics explored here focused on mAbs and AAVs' product quality attributes (PTMs) and process-related impurities (HCPs) profiling, critical for bioprocess understanding and QC.

**Impact/Novelty:** MS approaches implemented can be applied to several biotherapeutics, allowing detailed characterization of multiple quality attributes in a single platform, including PTMs and HCP profiling.

**Introduction:** Characterizing products and bioprocesses is critical for R&D and manufacturing of biologics, e.g., mAbs or viral vectors. Evolving product complexity and regulatory demands are dictating the use of advanced bioanalytics. This study outlines an MS-based Multiple Attribute Method (MAM) strategy for post-translational modifications (PTMs) assessment of mAbs and adeno-associated viruses (AAVs) and host cell protein (HCP) profiling. Bioprocess impact on product attributes and process-related impurities was assessed.

**Methods/Approach:** We implemented a MAM workflow for mAbs' PTMs characterization using a QTOF platform. NIST reference and process samples were evaluated. HCP profiling was conducted by DIA-SWATH on the same MS platform. Heavy-labelled peptides from selected HCPs were used for quantification. Both workflows were optimized for different AAV serotypes and bioprocesses.

**Results:** MAM enabled the profiling of key PTMs like glycosylation, oxidation, and deamidation. Using DIA-SWATH we quantified targeted high-risk HCPs and other relevant ones. These results defined the best design space, maximizing product quality and purification performance of our polishing platform. We applied a MAM workflow to several AAV serotypes achieving a MS/MS coverage >95% of capsid viral proteins. PTMs identified (oxidation, deamidation, phosphorylation) were aligned with literature reports. Ongoing HCP profiling aims to improve further bioprocess understanding.

**Conclusion:** High-sensitivity and high-resolution MS-based approaches, namely MAM, are emerging as leading tools for biologics R&D and QC. These analytical solutions are being applied to characterize established biotherapeutics, e.g., mAbs, but also hold promise for more complex biologics, like AAVs, currently a major focus in gene therapy clinical trials.

# Novel analytics for cell and gene therapy manufacturing

**Dr. Stacy Lynn Springs**[1]
[1]Massachussetts Institute Of Technology, Cambridge, United States

Product Quality – Relevance and Assessment: Session 1, Pentland Auditorium, June 24, 2024, 3:45 PM - 5:15 PM

Commercial manufacturing of autologous cell and gene-modified cell therapies is logistically complex because of the requirement for significant coordination of clinical and manufacturing activities to ensure that the product and the patient are simultaneously prepared for administration. The final product must be held for 7-14 days pending a final negative result from growth-based compendial sterility tests, indicating product safety. Viral safety testing, which requires 28 days or longer, is uncommon. These timeframes are not optimal for cell therapy manufacturing because the patient's condition may deteriorate, or final product quality may be impacted during this hold time. There is an unmet need for methods capable of rapid detection of a broad spectrum of adventitious agents, including the ability to distinguish between live/dead and infectious/non-infectious agents. In addition to allowing rapid release of cell therapy final products, rapid AA detection would also allow process monitoring to achieve early detection of contamination events which would impact the ability to supply the cell therapy product in the desired timeframe.

We have developed multiple approaches to adventitious agent (AA) detection which can achieve the sensitivity of compendial sterility tests and the standard *in vitro* virus assay in much shorter timeframes. We have developed both targeted assays for detection of specific AA contaminants as well as untargeted approaches for broad spectrum detection of unknown contaminants using multiple platform technologies. We have developed a digital CRISPR-LAMP assay for targeted detection of known potential contaminants which provide rapid, sensitive, and quantitative measurement of live/infectious AAs. We have highly sensitive sterility and viral safety assays using next-generation sequencing using the Oxford Nanopore long-read sequencing platform by optimizing the sample preparation and bioinformatics pipelines. We have used a metabolomic approach to identify an enzyme present in bacteria but absent in mammalian cells which measures the ratio of nicotinic acid to nicotinamide as an indicator of bacterial contamination in cell culture and is being translated to both an at-line microfluidic mass spectrometry platform and a spectrophotometric platform which enables continuous, in-line cell culture monitoring. Finally, we have demonstrated that measurements of extracellular viral microRNAs serve as indicators and identifiers of live viral contamination events in cell culture spent medium. This talk will provide a review of these methods that we have developed.

# Using high-throughput sequencing to identify IgG sequence variants and the molecular mechanism for their occurrence

**Dr Claire Harris**[1]
[1]Astrazeneca, Cambridge, United Kingdom

Product Quality – Relevance and Assessment: Session 1, Pentland Auditorium, June 24, 2024, 3:45 PM - 5:15 PM

**Relevance to Theme Selected:**
Protein characterisation using methods such as mass spectrometry is critical in ensuring the correct product quality for protein biotherapeutics. RNASeq data can be used to guide and support this characterisation.

**Impact/Novelty:**
Variants identified in later-stage projects can lead to delays due to the requirement of further process development or re-initiating cell line development.

**Introduction:**
During characterisation of a purified IgG in early development, protein physiochemical methods identified a number of variants with smaller and larger masses than expected. The measured masses for the smaller species did not correspond to predicted fragments generated by hydrolysis of peptide bonds.

**Methods/Approach:**
High-throughput sequencing methods were used to characterise the transcriptome of the manufacturing cell line by RNASeq, and the genome by nanopore long-read sequencing. This was combined with protein physiochemical characterisation to investigate the identity of the variants.

**Results:**
Analysis of the transcriptome identified that the smaller molecular weight species were due to introns present in the heavy chain constant domain that were mis-spliced. This led to a change in the transcript sequence leading to the production of a premature stop codon and the production of a fragment-like species. De-novo assembly of the RNASeq reads identified an aberrant fusion transcript where the light chain constant domain sequence was fused to the c-terminal end of the heavy-chain, leading to the production of the variant with the larger than expected mass. Furthermore, nanopore genomic sequencing showed this was a result of how the transgenes had been integrated into the host genome in tandem with partial copies present.

**Conclusion:**
Combining high-throughput sequencing analysis with protein characterisation can help identify sequence variants in recombinant cell lines to ensure correct and consistent product quality and lead to optimisation of the transgene expression cassette to reduce their production.

## Developing and manufacturing CRISPR-engineered T cell therapies in the academic setting

**Dr. Brian Shy**[1]
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Transitioning from Development to Manufacture: Session 1, Pentland Auditorium, June 24, 2024, 6:00 PM - 7:30 PM

CRISPR engineering approaches are enabling a multitude of exciting new cell and gene therapies. The UCSF Investigational Cell Therapy Program streamlines the pre-clinical development, regulatory interactions, and GMP manufacturing necessary to rapidly advance and scale these technologies in the academic setting. I will discuss our experience developing and manufacturing a variety of experimental T cell therapies incorporating next generation genome and epigenome engineering strategies, highlighting upcoming products and opportunities to bring these treatments to more patients.

# Improvement of USP scale-down models for biopharmaceutical production processes: A case study

**Dr Timm Keil**[1], Dr Michael Löffler[1], Dr Bettina Knapp[1], Dr Jochen Schaub[1]
[1]Boehringer Ingelheim Pharma GmbH & Co. KG, Ingelheim am Rhein, Deutschland

Transitioning from Development to Manufacture: Session 1, Pentland Auditorium, June 24, 2024, 6:00 PM - 7:30 PM

**Relevance to Theme Selected:** Reliable scale-down models are crucial for successful process development and transfer to production-scale.

**Impact/Novelty:** We present an innovative approach to design a technical scale-down model that mimics the large-scale cellular microenvironment.

**Introduction:** Scale-down models (SDMs) are vital in the development and analysis of upstream bioprocesses involving mammalian cells in the biopharmaceutical domain. They must accurately represent large-scale bioreactors to enable knowledge transfer across different scales. Scale-up often leads to changes or inconsistencies in process performance and product quality. This is in particularly problematic when the SDM is intended for submission relevant process characterization studies.

**Approach:** The cause of these discrepancies is not always immediately apparent. Even with key parameters maintained during the scale-up process (like power-input and gassing), the cellular microenvironment may undergo changes. Matching the large-scale microenvironment in small scale is key for a reliable small-scale model. For instance, with constant power-input, the tip-speed varies across scales, affecting the distribution of bubble size and cell stress. The $CO_2$ regime is impacted by the hydrostatic pressure in larger scales, and the higher surface-to-volume ratio in small-scale bioreactors promotes evaporation. Different technical handling procedures between the scales might also impact scalability of the process. These parameters interact, leading to a cumulative effect which might significantly impact the cellular microenvironment. We applied technical methods to consider these scale-up related differences during scale-down model development.

**Results:** In this study, we illustrate the development of an innovative product-specific scale-down model. We examined the offsets present in the existing scale-down model, assessed potential technical modifications, and put into action appropriate technical solutions. The newly developed scale-down model is more effective at replicating the large-scale cellular microenvironment, thereby reducing offsets.

**Conclusion:** This improved model facilitates the process of scaling up and transfer, as well as it supports process characterization studies and submission.

# "Organized stress" for robust scale-up of intensified process with non-perfusion seed bioreactor

Mr Bassem Ben Yahia[1], **Mr Antoine PIEDNOIR**[1], Mr Thomas Dahomais[1], Mr Wolfgang Paul[1]
[1]UCB Pharma S.A., Braine L'alleud, Belgium

Transitioning from Development to Manufacture: Session 1, Pentland Auditorium, June 24, 2024, 6:00 PM - 7:30 PM

**Relevance to Theme Selected:**
This research work is focused on intensified processes with high seeding density inoculated from seed bioreactor in fed-batch mode and assess scale up and robustness challenges.

**Impact/Novelty:**
This is the first reported study highlighting a positive impact of cellular stress in seed bioreactors on intensified production bioreactor with the introduction of the "organized stress" concept.

**Introduction:**
Process intensification has been widely used for many years in the mammalian biomanufacturing industry to increase productivity, agility and flexibility while reducing production costs. The most commonly used intensified processes are operated using a perfusion or fed-batch seed bioreactor enabling a higher than usual seeding density in the fed-batch production bioreactor. Similarly to non-intensified processes, such intensified processes should be designed and characterized for robust process scale-up.

**Methods/Approach:**
This research work is focused on intensified processes with high seeding density inoculated from seed bioreactor in fed-batch mode using Chinese Hamster Ovary cells. The impact of the feeding strategy and specific power input (P/V) in the seed bioreactor and on the production step with two different cell lines (CL1 and CL2) producing two different monoclonal antibodies was investigated.

**Results:**
Cell culture performance in the production bioreactor has been improved due to more stressful conditions for the cells in the seed bioreactor and the impact of the production bioreactor P/V on the production performance was limited.

**Conclusion:**
The addition of the feeds and the specific power input of the N-1 seed bioreactor were identified as critical key parameters to significantly impact the cell culture performance of the production bioreactor. To the best of our knowledge, the impact of fed-batch N-1 seed bioreactor conditions on intensified production bioreactor is reported here for the first time. The "organized stress" concept has been introduced and provides a simple and robust scale-up approach to implement robust processes across multiple production sites

# Mimicking Impacts of Production Scale Heterogeneous Mixing on Cellular Performance Using the Single Multi-Compartment Bioreactor

**Mr Ralf Takors**[1]
[1]University of Stuttgart, Institute of Biochemical Engineering, Stuttgart, Germany

Transitioning from Development to Manufacture: Session 1, Pentland Auditorium, June 24, 2024, 6:00 PM - 7:30 PM

**Relevance**: To ensure successful *transitioning from development to manufacture* performance losses should be prevented at production scale. Hence impacts shall be identified early in lab scale for developing robust processes.

**Novelty**: The novel setting of Single-Multi Compartment Bioreactor (SMCB) allows to mimic industrial scale impacts in small scale, e.g. reflecting characteristic trends of by-product formation and product qualities.

**Introduction**: The successful transfer of lab achievements to production scale is the *conditio sine qua non* for implementing novel bioprocesses. Nevertheless, non-wanted performance losses regarding productivity and product quality may happen endangering product specifications and economics. Hence, it is of outmost importance to establish reliable lab-systems that well mirror large scale heterogeneities for investigating sensitive process parameters *ad initio*.

**Methods**: The SMCB mimics large scale mixing heterogeneities by installing multiple discs in lab-scale stirred bioreactors. The design of the discs follows detailed engineering rules leading to the simulation of realistic large scale conditions in 1.5 L stirred bioreactors. It will be shown that large scale information of mixing time and power input leads to the straightforward design of similar conditions in small scale.

**Results**: By investigating realistic power inputs between 20.4 - 1.5 $Wm^{-3}$ cultivations with the antibody producing Chinese Hamster ovary cell DP12 showed characteristic lactate formation in the SMCB in contrast to the homogenously mixed reference bioreactor. Trends of lactate production followed characteristic large scale observations. Furthermore, the analysis of antibody glycosylation patterns revealed changes of product quality with power input. The degree of galactosylation heavily depended on power input alone irrespective of the provoked heterogeneities.
**Conclusion**: The SMCB opens the door for the distinct evaluation of particular large-scale impacts which enables the development of individual solutions for scalable bioprocesses

## Lessons from the Pandemic - GMP manufacturing in academia

**Prof. Catherine Green**[1]
[1]University of Oxford, , United Kingdom

Transitioning from Development to Manufacture: Session 1, Pentland Auditorium, June 24, 2024, 6:00 PM - 7:30 PM

The manufacturing of material for use in even early-phase clinical trials must, in the UK and Europe, be under the applicable GMP rules and guidance. This poses challenges for academic groups wanting to initiate clinical testing of novel medicinal products, both in terms of cost and compliance. The University of Oxford has, since 1999, overcome these challenges by in-house GMP-compliant MHRA-licensed IMP manufacturing in the Clinical BioManufacturing Facility. I will provide an overview of this facility and its operations. With regard to the products that we have been manufacturing recently I will highlight particular issues that we have had to resolve for successful, low cost, small scale GMP activities. Building on the work that was necessitated by the COVID-19 pandemic I will also suggest future considerations for the evolving landscape for academic GMP manufacturing, both for vaccines and other biologics.

# Invited Speaker and Oral Communications Abstracts

# Tuesday 25 June 2024

## Towards genetic therapies for Rett syndrome

**Prof. Adrian Bird**[1], Dr Jacky Guy[1], Dr Raphael Pantier[1]
[1]University of Edinburgh, , United Kingdom

Keynote Lecture: Professor Sir Adrian Bird, Pentland Auditorium, June 25, 2024, 8:45 AM - 9:30 AM

Cytosine residues in genomic DNA can be modified post-synthetically and this affects local protein-DNA interactions. For example, the protein MeCP2 specifically binds to motifs containing methylated cytosine, potentially allowing it to interpret this “epigenetic” mark. Several clinical disorders are caused by mutations in the *MECP2* gene, including the profound neurological disorder Rett syndrome. The equivalent phenotype in animal models can be reversed, suggesting that the protein fine-tunes neuronal function and that the human disorder may also be curable. Evidence will be presented that the root cause of Rett syndrome is failure of the primary function of MeCP2, which is to restrain gene expression in a DNA methylation-dependent manner. It was recently reported that MeCP2 function depends on intrinsically disordered regions (IDRs) in MeCP2 which drive the formation of condensates via liquid-liquid phase separation (LLPS). Our findings contradict this hypothesis. Instead we establish that Rett syndrome-causing deletions in the C-terminal IDR greatly reduce MeCP2 abundance by a mechanism that does not depend on LLPS.

# Enabling the intensification of cell culture processes by using modified amino acids

Dr Tim Hofmann[1], Ms Corinna Schmidt[1], Ms Sascia Neumann[1], Ms Justyna Masiewicz[1], Ms Ronja Seibel[1], **Dr. Aline Zimmer**[1]
[1]Merck Life Science KGaA, Darmstadt, Germany

Innovate or Die -- New Technologies, Pentland Auditorium, June 25, 2024, 9:30 AM - 10:00 AM

**Relevance to Theme Selected:**
Enable bioprocess intensification for higher yields using modified amino acids for the generation of therapeutic modalities.

**Impact/Novelty:**
A modification of canonical Leucine and Isoleucine to Keto-(Iso)Leucine and N-lactoyl-(Iso)Leucine sodium salts allow to increase the overall solubility of cell culture media and feed (CCM/CCF) formulations. Today no soluble derivatives of Isoleucine/Leucine exist on the cell culture media market.

**Introduction:**
The increasing demand of biotherapeutics such as monoclonal antibodies, bispecifics, antibody-based fusion proteins and antibody fragments requires innovative solutions and the constant improvement and optimization of bioprocesses. Large quantities of biotherapeutics need to be manufactured in a cost- and time- efficient manner in order to obtain large doses of drugs for clinical trials and commercialization. Concentrated CCM formulations are one way to increase volumetric productivity in the generation of biotherapeutics by adding less feed volume or using existing bioreactor capacity more efficiently. However, state-of-the art feeds cannot be further concentrated due to limited solubility of many amino acids, in particular at physiological pH.

**Methods/Approach:**
Modified Isoleucine and Leucine were shown to be bioavailable in several Chinese hamster ovary (CHO) cell lines and were designed to be a suitable replacement for their canonical counterparts. This unlocks the possibility to generate highly concentrated CCF formulations which will in return increase the volumetric productivity per batch, fed-batch or perfusion through higher bioreactor starting volumes, decreased feed volumes and increased cell masses.

**Results & Conclusion:**
An increased volumetric yield of 51% was achieved in fed-batch mode using modified amino acid derivatives. Cell specific productivity and critical quality attributes like post translational modifications, aggregation and fragmentation were not negatively impacted, while maintaining or improving cell culture performance. Next generation cell culture formulations including bioavailable highly soluble Keto-(Iso)Leucine and N-lactoyl-(Iso)Leucine, will satisfy the pharma industrial demand and ultimately leading to better bioprocesses.

# Microfluidic Device for the Expansion of T Cells under Continuous Perfusion

**Mr Jorge Aranda-Hernandez**[1], Ms Oleksandra Derevianko[1], Mr Pierre Springuel[1], Dr Pedro Azevedo Da Silva Couto[1], Prof. Qasim Rafiq[1], Prof. Nicolas Szita[1]
[1]UCL, London, United Kingdom

Innovate or Die -- New Technologies, Pentland Auditorium, June 25, 2024, 9:30 AM - 10:00 AM

**Relevance to Theme Selected:**
A microfluidic approach for the expansion of T-cells will alter the way of bioprocessing in cell therapies.

**Impact/Novelty:**
Expansion of T cells to high concentrations in a microfluidic device is novel, and can lead to new bioprocessing modalities.

**Introduction:**
Microfluidics afford high control over the microenvironment and a high degree of flow control. Moreover, microfluidic devices are amenable for automation, allow sensor integration for *in situ* monitoring, therefore reducing experimental time and cost. By creating comparatively large culture chambers in the devices, we hypothesised that significant cell numbers can be achieved, rendering such devices suitable for process development.

**Methods/Approach:**
Microfluidic devices (< 300 µL) were used for cell expansion under perfusion for 7 days. Jurkat, primary T-cells, and CAR T cells were pre-cultured using standard methods and then transferred to the devices. Devices were continuously perfused starting from about 5 µL $min^{-1}$ to higher flow rates during cell growth. Cells counting and viability was performed with an automated cell counter (NucleoCounter NC-3000).

**Results:**
The microfluidic devices allowed suspension cell expansion for achieving high cell concentration. Jurkat cell culture reached 28 million cells/mL with >90% viability, primary T-cell culture yielded >40 million cells/mL and >90% viability. For CAR T cells, initial results showed high cell densities at high viabilities after 4 days, and also to be comparable to traditional cell culture platforms such as G-REX® and 24-well plate. Oxygen consumption was monitored *in situ* using optical sensors for the entire duration of a culture. The possibility of multiple reuses of the microfluidic devices with integrated oxygen sensors was also confirmed during the experiments.

**Conclusion:**
The microfluidic devices under continuous perfusion showed great potential for prolonged cell expansion. Furthermore, it was concluded that the microfluidic devices can be reused from one experiment to another resulting in lower production cost and a way to an eco-friendlier platform.

# Integration of stem cell-derived pancreatic aggregates into FN-silk network for *in vitro* maturation

**Ms Kelly Blust**[1], Dr. Carolina Åstrand[2], Dr. Siqin Wu[2], Prof. My Hedhammar[1]
[1]The Royal Institute Of Technology, KTH, Stockholm, Sweden, [2]Spiber Technologies , Stockholm, Sweden

Innovate or Die -- New Technologies, Pentland Auditorium, June 25, 2024, 9:30 AM - 10:00 AM

**Relevance to Theme Selected:** Incorporating pancreatic aggregates derived from pluripotent stem cells into FN-silk offers curative stem cell therapy for type 1 Diabetes.

**Impact/Novelty:** Incorporation of pancreatic aggregates derived from pluripotent stem cells FN-Silk networks may be a novel cell therapy to treat type 1 Diabetes.

**Introduction:** Diabetes type 1 is a life-threatening disease that accompanies a life-long insulin dependency and limits the quality of life. An efficient way to treat diabetes is pancreatic islet transplantation. However, pancreatic islet transplantation has significant disadvantages, e.g., massive loss of islets and donor shortage. These problems could be solved by using insulin-producing pancreatic aggregates differentiated from human pluripotent stem cells combined with a biomaterial based on a unique 3D spider silk (FN-Silk) scaffold to protect pancreatic aggregates during the transplantation. FN-Silk is recombinantly produced in *E.coli* and is functionalized with a fibronectin motif to promote cell adhesion.

**Methods/Approach:** We have analysed the viability of pancreatic aggregates incorporated in FN-Silk networks during a cultivation period of 3 weeks. Furthermore, we compared the functionality of free pancreatic aggregates to incorporated ones by measuring the expression of c-peptide (insulin) and glucagon expression. Specifically, the transcriptome was analyzed on a single-cell level to compare free and FN-silk aggregates gene expression profiles and heterogeneity.

**Results:** We observed high viability of pancreatic aggregates incorporated in FN-silk networks over 3 weeks of cultivation and enhanced pancreatic islet function by increasing insulin and glucagon expression. In particular, the maturation of beta cells in FN-silk networks seems to be improved.

**Conclusion:** FN-Silk is an excellent biomaterial to incorporate pancreatic aggregates and could be used for transplantation to diabetic patients.

## Annotation of the non-canonical translatome reveals that CHO cell microproteins are a new class of impurities in therapeutic antibody drug products

**Colin Clarke**[1]
[1]NIBRT, Ireland

From Big Data to Better Cells and Processes, Pentland Auditorium, June 25, 2024, 11:00 AM - 12:30 PM

Chinese hamster ovary (CHO) cells are used to produce almost 90% of therapeutic monoclonal antibodies (mAbs) and antibody fusion proteins (Fc fusion). The annotation of non-canonical translation events in these cellular factories remains incomplete, limiting not only our ability to study CHO cell biology but also detect host cell protein (HCP) impurities in the final antibody drug product. We utilised ribosome footprint profiling (Ribo-seq) to identify novel open reading frames (ORFs) including N-terminal extensions and thousands of short ORFs (sORFs) predicted to encode microproteins. Mass spectrometry-based HCP analysis of eight commercial antibody drug products (7 mAbs and 1 Fc fusion protein) using the extended protein sequence database has revealed the presence of microprotein impurities for the first time.

# miRNome analysis of HEK293-F cells during AAV production

**Ms Madina Burkhart**[1], Ms. Katrin Langenbach[1], Ms. Jamie-Ann Baiz[1], Ms. Sonja Lochmüller[1], Mr. Linus Weiß[1], Prof. Dr. Kerstin Otte[1]
[1]Institute for Applied Biotechnology, Biberach University of Applied Sciences, Biberach an der Riß, Germany

From Big Data to Better Cells and Processes, Pentland Auditorium, June 25, 2024, 11:00 AM - 12:30 PM

**Relevance to Theme Selected:**
Eukaryotic cells play a crucial role in generating viral gene therapeutics like recombinant adeno-associated viruses (rAAVs). Here we aim for molecular characterization via miRNome analysis of production cells to understand and optimize rAAV production.

**Impact/Novelty:**
We report the currently undescribed miRNome of HEK293-F cells during different phases of rAAV production to identify modulatory miRNAs as engineering tools.

**Introduction:**
Although recently a high demand for large-scale production of therapeutic rAAV has emerged, process development and cell engineering for efficient production are still at early stages. Recently, microRNAs (miRNAs) were developed as efficient engineering tools for biopharma production, however, miRNA expression profiles during rAAV production processes are currently undescribed. Therefore, we conducted a genome-wide miRNome analysis in HEK293-F production cells to characterize and identify specific miRNAs implicated in rAAV production.

**Methods/Approach:**
HEK293-F cells were cultivated and triple-AAV-plasmid transfection was performed. Triple-transfected, un- and mock-transfected cells were cultivated for 72 h and viability, viable cell density (VCD), viral genomic titer, transduction assays and Chip-based miRNome analysis were performed at different sampling timepoints. Data were bioinformatically analyzed and expression modulated using siRNAs or transposase-based miRNA transfection.

**Results:**
The entire rAAV production process in HEK293-F cells was characterized monitoring viability, VCD, and production of infectious particles. Genome-wide miRNA expression analysis at various timepoints enabled calculation of differential miRNA expression to allow for the identification of (i) early, mid-late and late expressing miRNAs, (ii) up- or downregulated rAAV production specific miRNAs, (iii) possible miRNA signatures between different production phases and lastly, (iv) pro-productive or adverse miRNAs to finally enable production cell engineering. Additionally, miRNA target prediction to identify single genes or entire cellular pathways linked to AAV production is currently performed as well as expression modulation of relevant miRNAs to increase rAAV titer and modulate viability or VCD.

**Conclusion:**
miRNome analysis is a powerful tool to characterize specific molecular mechanisms involved in rAAV production. These findings can be applied to engineer an improved rAAV production cell line meeting current medical demands.

# Culture media customization to the target molecule using hybrid AI systems

**Prof. Rui Oliveira**[1], Dr João Ramos[1]
[1]School of Science and Technology, Nova University Lisbon, Caparica, Portugal

From Big Data to Better Cells and Processes, Pentland Auditorium, June 25, 2024, 11:00 AM - 12:30 PM

**Relevance to Theme Selected:** Theme Data, Cells and Processes. This project combined a genome-scale model of CHO cells, historical data of 67 reactor experiments and machine learning for precise culture media design to the target molecule.

**Impact/Novelty:** This study presents the first hybrid model of a CHO cell line at the genome-scale – hybrid genome-scale modelling - based on AI and mechanistic knowledge.

**Introduction:** Deep learning is emerging in many industrial sectors in hand with big data analytics to streamline production. In the biomanufacturing sector, big data infrastructure is lagging compared to other industries. A promising approach is to combine machine leaning with mechanistic biological models in hybrid workflows that are less dependent on the quality and quantity of data. Such hybrid modelling workflows are recognised as efficient tools to develop cell and process digital twins.

**Methods/Approach:** A CHO-K1 genome-scale model based on omics was combined with machine learning with the ability to learn from historical data across different molecules. The resulting hybrid AI model was applied to design culture media feeds specific to the target molecule in a CHO-K1 platform. Flux balance analysis (FBA) is currently the standard method to compute metabolic fluxes in genome-scale networks. Several FBA extensions employing diverse objective functions and/or constraints have been published. Here we propose a hybrid semi-parametric FBA extension that combines mechanistic-level constraints (parametric) with machine learning constraints (non-parametric) in the same linear program.

**Results:** A CHO dataset with 27 measured exchange fluxes obtained from 67 reactor experiments was collected. Mechanistic constraints were deduced from a reduced CHO-K1 genome-scale network with 686 metabolites, 788 reactions and 210 degrees of freedom. Nonparametric constraints were obtained by principal component analysis of the flux dataset. The two types of constraints were integrated in the same linear program showing comparable computational cost to standard FBA. The hybrid FBA is shown to significantly improve the specific growth rate prediction under different constraints scenarios. A metabolically efficient cell growth feed targeting minimal byproducts accumulation was designed by hybrid FBA. Metabolic efficient feeds were designed to target molecules and validated in an Ambr 250 system. All *in silico* culture media feeds showed a titer improvement (up to 60%) in relation to the control (molecule unspecific) feed with no exception.

**Conclusion:** It is concluded that hybrid genome-scale modelling allows for a more precise optimisation of the metabolism of CHO cells. Integrating parametric and nonparametric constraints in the same linear program is an efficient approach to reduce the solution space and to improve the predictive power when critical mechanistic information is missing and when bid data is not available. The hybrid genome-scale approach is able to learn from experience. The resulting flux centric culture media design approach is intrinsically robust. In theory, with enough validation cycles across different molecules, the hybrid AI method could be applied to design ab initio custom feeds for every new molecule.

# In Silico Modelling for Bioprocesses: An Epigenetic and Machine Learning Approach

**Dr Karlheinz Landauer**[1,3], Dr. Asier Galarza[2], Dr. Daniel Luescher[2], Dr. Max Pryce[2]
[1]QBDC Gmbh, Neutal, Austria, [2]QBDC GmbH, Nusshof, Switzerland, [3]Kamosys GmbH, Neutal, Austria

From Big Data to Better Cells and Processes, Pentland Auditorium, June 25, 2024, 11:00 AM - 12:30 PM

**From Big Data to Better Cells and Processes**
The vast amount of data generated during process development and manufacturing is further amplified by epigenetic characterization, adding 20,000 data points per analysis. Extracting actionable insights from this wealth of information necessitates modern data analysis tools like machine learning and artificial intelligence. Our work addresses this challenge by presenting a solution.

**Impact/Novelty**
Epigenetic characterization of manufacturing processes is in its infancy. Our study stands out as the first of its kind, analysing distinct CpG sites across bioprocess development.

**Introduction:**
The quest for digital twins in bioprocess engineering has been ongoing since years. Our study introduces a novel methodology employing machine learning to analyse genome CpG sites using specialized microarrays. We focus on a CHO-K1 cell line and utilize a chemically defined medium strategy in a fed-batch mode.

**Methods/Approach:**
Our approach involves methylation profiling of Chinese Hamster Ovary (CHO) responsive CpG sites using microarray technology. Integration of this epigenetic analysis with machine learning enables construction of sensitive in-silico networks to predict critical quality attributes, process parameters, and media components.

**Results:**
We identified 240 pivotal expression regulation sites crucial for characterizing 10 process quality attributes. A CpG index comprising only 5 sites accurately estimated cell culture density, stage, or metabolic profile at sampling points ($R^2 > 0.98$). This index demonstrated precise forward and backward predictive capabilities over a 10-day window, validated across various feeding regimes.

**Conclusion:**
Utilizing an epigenetic-based digital twin for bioprocess development presents a significant advancement. This approach reduces the need for physical testing while enhancing understanding from a genome-translation perspective. The in-silico quality-by-design approach fostered by this methodology will significantly reduce time and costs associated with bioprocess development while enhancing product quality.

## Towards digital process development - Changing process operation with (hybrid) models and transfer learning

**Dr. Moritz von Stosch**[1]
[1]DataHow AG, Zurich, Switzerland

From Big Data to Better Cells and Processes, Pentland Auditorium, June 25, 2024, 11:00 AM - 12:30 PM

Advances in high-throughput experimentation and process analytic technology have resulted into a flood of data being produced during process development and operation. While data infrastructure and governance are often adopted from the tech industry to gather and manage the data, data analytics remains siloed, focused only on the process activity at hand. Hence, an opportunity arises for cross activity data analytics.

Hybrid modeling, which incorporates fundamental knowledge with machine learning methods, allows for knowledge to be transferred across the lifecycle of the biological product , thereby bridging early, mid, and late-stage process development. Knowledge transfer across different biological products can be accomplished with machine learning methods, such as transfer learning.

This contribution demonstrates the application of hybrid modeling and transfer learning for the development of mammalian cell culture processes. The performance of the methods will be benchmarked against standard statistical approaches and the opportunities that open up through these technologies will be presented. Lastly, a knowledge-centric modus operandi will be proposed, that relies on digital technologies to foster the capture, sharing and hence democratization of knowledge.

## Precision gene delivery through protein engineering

**Prof. Andreas Pluckthun**[1]
[1]University of Zurich, Zurich, Switzerland

Cells as Factories and Therapies: Session 2, Pentland Auditorium, June 25, 2024, 4:00 PM - 5:30 PM

To enable in situ production of therapeutic proteins, we have combined several protein engineering technologies to devise a new platform, termed SHielded, REtargeted ADenovirus (SHREAD). It is based on virus-like particles that are devoid of any viral genes, but contain 36 kb of DNA that can encode multiple genes and complex regulatory regions. To target particular cells and organs, an adapter strategy has been devised, based on the DARPin platform, to selectively target any surface receptor of interest. To hide the particles from the immune system and to minimize liver targeting, a shield was developed based on a trimerized single-chain Fv fragment, covering the facets of the icosahedron.

Applications have included expressing therapeutic anti-tumor antibodies or bispecific T-cell engagers in situ, either by targeting tumor cells in vivo or tumor-associated fibroblasts, with very encouraging in-vivo effects. Several therapeutic cytokines were expressed simultaneously, to modulate the tumor microenvironment. We have also infected and reprogrammed T-cells in vivo, for producing CAR-T cells in vivo. By targeting dendritic cells in lymph nodes and co-expressing cytokines there, we could achieve highly efficient tumor vaccination. We believe that this versatile technology holds great promise to change the paradigm of precision delivery of therapeutic proteins.

# The serendipitous discovery of a novel and highly efficient transposase to advance mammalian cell factories

**Dr Moritz Schmidt**[1], Dr Simon Fischer[1], Daniel Heinzelmann[1], Dr Patrick Schulz[1], Dr Benjamin Renner[1], Franziska Reuss[1], Dr Nikolas Zeh[1], Dr Robin Nilson[1]
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Cells as Factories and Therapies: Session 2, Pentland Auditorium, June 25, 2024, 4:00 PM - 5:30 PM

**Relevance to Theme Selected:**
Our work unveils a novel transposase system that significantly enhances the efficiency, stability, speed and control of cell factories, thereby expanding its unique value and applicability in animal cell technology.

**Impact/Novelty:**
We discovered a unique, highly active transposase system in an exotic insect species, distinct from known transposases, and optimized it for several biopharmaceutical applications.

**Introduction:**
In recent years, transposase/transposon systems have revolutionized biopharmaceutical development by enhancing cell line performance, accelerating timelines and improving cell line stability. However, the limited number of available hyperactive transposase systems represents a major challenge. To address this, we embarked on a mission to discover novel functional transposases.

**Methods/Approach:**
We conducted an expansive metagenomic screening across diverse species, identifying approximately 390 potential transposase and ITR sequences. A subset was tested in CHO cells for genomic transposition and recombinant protein expression. Intriguingly, one transposase system proved active and was further optimized through protein and transposon engineering.

**Results:**
Following the discovery of the novel transposase, systematic optimization significantly enhanced its activity in mammalian cells. This optimized transposase expedited the generation of high-performing recombinant antibody-producing CHO cell lines, as well as bioassay cell lines expressing membrane proteins, and facilitated the generation of stable AAV-producing HEK293 cells. Transposon optimization provided precise control over the transcription rates of a multi-domain novel therapeutic protein with high fidelity, thereby ensuring the correct assembly of products.

**Conclusion:**
Our research has led to the identification of a novel transposase, previously unknown and functional across a variety of species including CHO and HEK293. This transposase has broad applicability, from enhancing recombinant protein expression to facilitating stable AAV production. This significant finding paves the way for more efficient and economical biologics production, ultimately contributing to the development of safer and more effective therapeutics.

# Model-driven Synthetic Biology Approaches to Engineer Improved Stable Cell Lines for Lentivirus Manufacturing

**Dr Brianna Jayanthi**[1], Dr. Jeremy Gam[1], Mr. Omar El-Ghouch[1], Dr. Michelle Chang[1], Ms. Paulena Lieske[1], Mr. Adam Carcella[1], Ms. Manali Rajenkumar Desai[1], Dr. Jeffrey McMahan[1], Mr. Luis Gonzalez[1], Dr. Martín Carcamo[1]
[1]Asimov, Inc., Boston, United States

Cells as Factories and Therapies: Session 2, Pentland Auditorium, June 25, 2024, 4:00 PM - 5:30 PM

**Relevance to Theme Selected:**
The stable LV Edge System utilizes model-guided sequence and cell culture optimization to drive high titer and scalable lentiviral vector production.

**Impact/Novelty:**
Packaging and producer systems have been developed to provide robust, high-titer, and scalable solutions to eliminate the need for large scale GMP-grade plasmids in lentiviral vector manufacturing.

**Introduction:**
The success of CAR cell therapies has generated an exponential increase in demand for GMP lentiviral vector production. Lentiviral vectors are the preferred method for efficient, stable integration of CAR genes into immune cell genomes. To address the inherent shortfalls of current transient transfection-based lentiviral vector manufacture, we have developed inducible stable lentiviral vector packaging and producer cell line systems.

**Methods/Approach:**
The LV Edge System integrates synthetic biology, computational models, novel clone-screening approaches, and robust processes to provide solutions for lentiviral vector manufacturing. Our clonal, suspension HEK293T-based, packaging and producer cell lines are genetically engineered for inducible production of VSV-G pseudotyped lentiviral vectors. The packaging cell line requires transfection of a single plasmid encoding the transgene and has been validated across multiple scales. Our fully-stable producer cell line development process takes 4 months from transfection to research cell bank release and requires less than 1 µg of R&D grade DNA for transfection, eliminating the need for GMP-grade plasmids. Our monoclonal-verified cell lines provide for ease of scale-up and reduce process complexity that leads to consistent manufacture.

**Results:**
We achieve high-E8 TU/mL titers across multiple CAR transgenes from a 5-day, model-driven bioreactor process. High stable expression of lentiviral and transgene components is achieved by engineered plasmids for tight inducible-regulation of gene expression, which are then integrated using a proprietary hyperactive transposase. Tightly-regulated, high-copy integration allows for robust bioreactor growth pre-induction and high specific productivity during production. Extended cell culture studies have demonstrated long-term stability of both packaging and producer lines.

**Conclusion:**
The LV Edge System enables scalable and high-titer production of lentiviral vectors while eliminating the need for GMP plasmids. These systems shift the paradigm beyond the current four-plasmid transient transfection system, with the ultimate goal of delivering lower-cost cell and gene therapies to patients.

# Expression in CHO cells of a bacterial biosynthetic pathway prevents proteolysis of recombinant proteins

**Prof. Octavio Ramírez**[1], Violeta Guadarrama-Pérez[1], César Aguilar[2], Alberto Porras-Sanjuanico[1], Enrique Merino[1], Francisco Barona-Gómez[2], Laura Palomares[1]
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Cells as Factories and Therapies: Session 2, Pentland Auditorium, June 25, 2024, 4:00 PM - 5:30 PM

**Relevance to Theme Selected:**
A novel strategy for engineering the cell factory to solve the important problem of proteolysis will be presented. An improvement in product quality and yield was obtained.

**Impact/Novelty:**
This work goes beyond solving a problem for recombinant protein production. We prove that biosynthetic pathways unique to bacteria are functional in CHO cells, expanding the possibility of cell engineering.

**Introduction:**
A significant problem during recombinant protein production is proteolysis. One of the most common preventive strategies is the addition of protease inhibitors, which has drawbacks, such as their short half-life and high cost, and their limited prevention of extracellular proteolysis. Actinomycetes produce the most used inhibitors, which are non-ribosomal small aldehydic peptides.

**Methods/Approach:**
We expressed the livipeptin biosynthetic pathway, encoded by the *lvp* genes; in CHO cells. It is an unprecedented biosynthetic route involving a condensation-minus non-ribosomal peptide synthetase (NRPSs), and a tRNA-utilizing enzyme (tRUE) that directs the synthesis of a protease inhibitor peptide, livipeptin.

**Results:**
The expression of the *lvp* genes resulted in the production of livipeptine with cysteine protease inhibitory activity, implying that mammalian tRNAs were recruited by the lvp system. CHO cells transiently expressing the biosynthetic pathway produced livipeptin without affecting cell growth or viability. Expression of the *lvp* system in CHO cells producing two model proteins, secreted alkaline phosphatase (hSeAP) and a monoclonal antibody, resulted in higher specific productivity with reduced proteolysis.

**Conclusion:**
We show for the first time that the expression of a bacterial biosynthetic pathway is functional in CHO cells, resulting in the efficient, low-cost synthesis of a protease inhibitor without adverse effects on CHO cells. This expands the field of metabolic engineering of mammalian cells by expressing the overwhelming diversity of actinomycetes biosynthetic pathways and opens a new option for proteolysis inhibition in bioprocess engineering.

**References:**
Guadarrama-Pérez et al. Expression in CHO cells of a bacterial biosynthetic pathway producing a small non-ribosomal peptide aldehyde prevents proteolysis of recombinant proteins. Submitted.
Cruz-Morales et al. (2013). *Genome Biology and Evolution 5*: 1165–1175.

# A large-scale deletion screen defines the essentiality of the "dark matter" of the CHO genome

**Mr Federico De Marco**[1], Antonino Napoleone[1], Ivy Rose Sebastian[1], Alexander Molin[1], Dr. Krishna Motheramgari[1], Dr. Nina Bydlinski[1], Dr. Mohamed Hussein[1], Prof. Nicole Borth[2]
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Cells as Factories and Therapies: Session 2, Pentland Auditorium, June 25, 2024, 4:00 PM - 5:30 PM

**Relevance to: 3. Data, cells, and processes**
We present data on a truly genome wide deletion screen that demonstrates the importance of the non-coding genome on cell behaviour. The screen covers the entire genome, irrespective of annotation.

**Impact/Novelty:**
We developed a genome wide CRISPR screen in CHO cells that sheds light on the crucial role of unannotated and potentially non-coding genomic regions in controlling cellular phenotypes.

**Introduction:**
The biopharmaceutical sector relies on CHO cells as a tool to study biological processes, and as the preferred host for production of biotherapeutics. In parallel, improvements in CHO cell genome assembly have provided knowledge for designing advanced genetic engineering strategies. While most such efforts so far have focused on coding genes, with some interest in transcribed, but non-coding RNAs (e.g. microRNAs and lncRNAs), we still lack genome wide systematic studies that precisely investigate the remaining 90% of the genome. This "dark matter" includes regulatory elements as well as poorly annotated genomic regions. Here, CRISPR screens are the most powerful resource to bridge the gap between genotype and phenotype for these regions in production cell lines.

**Approach:**
In this study, we used a genome-wide CRISPR-Cas9 screening platform with 112,272 paired guide RNAs targeting 14,034 genomic regions for full deletion of the targeted sequence. Using this platform, we conducted a negative screen that actively selects dying cells to identify regions essential for cell survival. Using paired gRNAs overcomes the intrinsic limitations of traditional frameshift which will likely have little or unknown effects on the non-coding genome.

**Results:**
This work revealed, with high confidence, a number of regions essential for CHO survival, where many currently lack any annotation. For these regions we present annotation or lack thereof, transcription if any as well as annotated chromatin states. Selected regions, in particular those that were blank by all the above, were individually deleted for confirmation.

**Conclusion:**
Our study sheds a novel light on a substantial part of the mammalian genome which is traditionally difficult to investigate and therefore neglected. Our results, in the context of the high value of CHO expression systems, represent an important milestone that is likely to enable new approaches towards a streamlined cell line development platform that also integrates synthetic biology design principles.

# A Novel Cell Engineering Platform for High-Yield AAV Production and Improved Manufacturability

**Dr Kathy Ngo**[1], Professor Lawrence Chasin[2], Mr. Larry Forman[1]
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Cells as Factories and Therapies: Session 2, Pentland Auditorium, June 25, 2024, 4:00 PM - 5:30 PM

**Relevance to Theme Selected:** Our cell engineering platform for improved adeno-associated virus (AAV) manufacturing addresses the critical challenges in gene therapy manufacturing and presents an innovative modality for improving cells as factories for therapeutics.

**Impact/Novelty:** Our revolutionary platform, with improved AAV production and CQAs, can significantly bolster the efficiency and cost-effectiveness of gene therapy manufacturing, and can accelerate current development timelines.

**Introduction:** AAV has recently emerged as a novel therapeutic modality in gene therapy. Challenges such as poor production yield and product quality persist in the virus manufacturing space, which we have addressed using our cell engineering technology.

**Methods/Approach:** We used a directed-evolution approach based on repeated cell fusions to shuffle the genome and amplify chromosomes of HEK-293 host cells. Engineered clones enriched for mitochondria phenotypes were isolated, then used as transient-transfection hosts, and for creating stable producer cell lines. For generation of stable producer cell lines, we developed a novel inducible system that maximizes the capabilities of the inherent viral production machinery.

**Results:** Engineered HEK-293 clones grown in suspension culture exhibited up to 12-fold productivity improvement via triple transient transfection for AAV1, AAV2, AAV5, and AAV9 serotypes with capsid titers as high as $10^{17}$ viral particles/L (vp/L)—at least 10-fold higher than current industrial processes. Selection for certain mitochondria phenotypes resulted in a 2-fold improvement in full-to-empty ratio—up to 55% full in crude supernatants. Finally, our engineered stable producer cell lines achieved capsid titers of $10^{16}$ vp/L.

**Conclusion:** We demonstrated a multi-modal cell-engineering platform that have significantly improved yield and manufacturability in both transient transfection and stable producer cell line methods. We further propose a model regarding the role of mitochondria for enhancing capsid percent-full. Taken together, our disruptive platform technologies provide solutions for current gene therapy manufacturing challenges.

# Cord blood natural killer cells: a promising source of cytotoxic extracellular vesicles for cancer immunotherapy

**Miss Isabel Doutor**[1,2], Cristiana Ulpiano[1,2], Prof. Cláudia Lobato da Silva[1,2], Prof. Ana Azevedo[1,2], Dr. Ana Fernandes-Platzgummer[1,2]
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Cells as Factories and Therapies: Session 2, Pentland Auditorium, June 25, 2024, 4:00 PM - 5:30 PM

**Relevance to Theme Selected:**
Optimizing the manufacturing process for well-characterized umbilical cord blood-derived natural killer cells and their extracellular vesicles, with a focus on enhancing the efficacy and safety of cell-based therapies.

**Impact/Novelty:**
Production of highly cytotoxic extracellular vesicles from readily accessible umbilical cord blood-derived natural killer cells for immunotherapy.

**Introduction:**
Umbilical cord blood-derived natural killer (UCB-NK) cell-based immunotherapy holds great promise in treating diverse malignancies, including blood cancers, owing to the allogeneic nature of UCB, its low immunogenicity, and easy accessibility. While NK-derived extracellular vesicles (EVs) provide a cell-free alternative, the ongoing challenge persists in obtaining a substantial quantity of functional UCB-NK cells and NK-EVs.

**Methods/Approach:**
Enriched CD56+ cells from UCB samples were expanded in various culture media supplemented with interleukin-2, including X-VIVO 15, NK MACS (NKM) with 5% human serum (hS), CTS NK-Xpander (NKX) with 5% hS, StemSpan SFEM II, PRIME-XV NK Cell CDM, and GMP SCGM. UCB-NK cells were characterized by flow cytometry and their activation status was assessed through degranulation and cytotoxicity assays against the K562 cell line. Following expansion, a conditioning phase was conducted with NKX without hS or NKX supplemented with exosome-depleted human platelet lysate (EVdhPL). EVs were isolated by anion-exchange chromatography, characterized according to MISEV2018 guidelines and included in the cytotoxicity assay.

**Results:**
NKM and NKX were the most favorable culture media for expanding NK cells and could maintain a $CD3^{-}CD56^{+}$ NK cell percentage above 90% throughout the expansion period. NK cell activation state improved, as indicated by increased degranulation and cytotoxicity. Cells exhibited a higher particle production when EVdhPL was used, and their viability was maintained during the 96 hours of conditioning. Isolated NK-EVs exhibited the expected morphology and were shown to be cytotoxic.

**Conclusion:**
The data obtained is critical for determining the optimal conditions for producing well-characterized NK-EVs, paving the way for the development of robust manufacturing processes.

# Invited Speaker and Oral Communications Abstracts

# Wednesday 26 June 2024

# An AI- and Laser-based Biomanufacturing Platform to Scale Production of Personalized Regenerative Cell Therapies

**Dr Marinna Madrid**[1]
[1]Cellino, Cambridge, United States

Keynote Lecture: Dr Marinna Madrid, Pentland Auditorium, June 26, 2024, 8:45 AM - 9:30 AM

Autologous induced pluripotent stem cells (iPSCs) enable replacement cells/ tissues to be manufactured from a person's own blood, enabling treatment of chronic degenerative diseases without immunosuppression or donor matching. However, current manufacturing requires manual labor, qualitative human decision-making, and open systems- resulting in high variability, low scalability, and high cost ($800k/dose per Huang et al., 2019). Here we present an AI (artificial intelligence)-driven optical biomanufacturing platform for consistent, scalable production of autologous iPSC-derived therapies. Cells are imaged, AI-driven algorithms analyze images to quantify cell/ colony characteristics, and laser-generated bubbles selectively remove cells as needed.

Here we present several cell culture processes that have been replaced by AI-driven optical bioprocesses, including: estimation of well confluence, clonalization of a stem cell population, and passage-free culture of proliferating cells. We demonstrate that the cells resulting from the optical bioprocesses maintain important characteristics.

The optical bioprocesses shown here are compatible with closed biomanufacturing, which is necessary to enable parallelization while minimizing the risk of cross-contamination. We present prototypes of fluidic cassettes for closed cell culture, including demonstrations of key processes, such as: cell seeding, cell growth, and laser-based cell removal.

Together, this work enables scalable and consistent manufacturing of personalized regenerative medicines.

# Process Analytical Utility of Raman Microscopy for Cell Therapy Manufacturing

**Dr. James Piret**[1,2,3,4], H Georg Schulze[1,2], Shreyas Rangan[1,2,4], Katherine MacDonald[1,2,4,5], Diepiriye Iworima[1,4], Megan Levings[1,4,5,6], Timothy Kieffer[1,4,6,7], Michael Blades[1,8], Robin Turner[1,2,9]

[1]University of British Columbia, Vancouver, Canada, [2]Michael Smith Laboratories, , , [3]Department of Chemical and Biological Engineering, , , [4]School of Biomedical Engineering, , , [5]BC Children's Hospital Research Institute, Department of Surgery, , , [6]Department of Surgery, , , [7]Department of Cellular and Physiological Sciences, , , [8]Department of Chemistry, , , [9]Department of Electrical and Computer Engineering, ,

Product Quality – Relevance and Assessment: Session 2, Pentland Auditorium, June 26, 2024, 11:15 AM - 12:45 PM

New clinical therapies based on implanting living cells into patients have the potential to cure degenerative and deadly diseases. However, populations of living cells are far more inherently variable and complex than any molecular drug. Additionally, they cannot be purified or analyzed anywhere near as stringently as drugs. Both accelerating process development and ensuring the long-term success of cell therapies will depend largely on the development of improved methods to validate both the final cell product quality and the expected critical process parameters during manufacturing. Raman spectroscopy offers a label-free approach to distinguish cell types and physiological states by analyzing cellular macromolecular composition changes. Using Raman microscopy, we have shown that spectral markers can detect early apoptotic cell death, discriminate between stem cells and differentiated progeny, as well as between T-cell subtypes and activation states. Overall, Raman spectroscopy offers a promising approach to provide a highly informative, and even a non-destructive means to enhance our ability to monitor, validate and control the quality of cell therapy manufacturing. Importantly, given the variability of cell therapy manufacturing, this process analytical technology has the potential to provide feedback control of the harvest and other manufacturing process operations.

# Assessing Extractables and Leachables from Single-Use Systems used in Advanced Therapy Medicinal Product Production

**Dr. Roberto Menzel**[1], Dr Armin Hauk[1]
[1]Sartorius Stedim Biotech GmbH, Göttingen, Deutschland

Product Quality – Relevance and Assessment: Session 2, Pentland Auditorium, June 26, 2024, 11:15 AM - 12:45 PM

**Product Quality**
Regulatory authorities require Extractables and Leachables data to assess potential harm to process performance, product quality and patient safety, posing specific challenges for Advanced Therapy Medicinal Products (ATMP).

**Novelty**
Developing *in silico* modelling approach utilizing available extractables data and a high throughput cell painting assay for application of a novel biocompatibility test system for ATMP SUS production processes.

**Introduction**
ATMP production is increasingly reliant on single use systems, often with high surface to volume ratio but reduced possibilities facilitating a leachables clearance. While SUS extractables data can serve as a basis for assessing ATMP applications, there are significant opportunities to improve assessment tools for exposure estimation, biocompatibility testing and toxicological evaluation. Only with appropriately improved mitigation strategies it is possible to fulfil regulatory requirements on E&L in the ATMP area.

**Methods**
A mechanistic digital twin process model was developed, enabling suitable exposure estimation for process equipment-related leachables (PERLs) in an ATMP production environment. Further, we tested forty-five commonly found extractables and two SUS extracts with biocompatibility tests including a High Throughput Cell Painting Assay (HT-CPA) using a human cell line. HT-CPA is commonly employed to identify active substances, enabling unbiased determination of > 1000 cell features associated with > 500 effects on cells by morphological screening.

**Results**
The modelling exercise demonstrated PERLs exposure to cells and patients falling far below critical effective concentrations. Levels of three cytotoxic leachables including bis (2,4-di-*tert*-butylphenyl)-phosphate (bD*t*BPP) were low enough as to be unable to compromise cells.

**Conclusion**
In conclusion, in *silico* modelling, coupled with databases using prior knowledge, enable predictions, assessments, and evaluations of complex multicomponent SUS directly relevant to ATMP manufacturing.

# Identification and Characterization of CHO Host Cell Proteins that can Degrade Polysorbate in Drug Products

**Dr Inn Yuk**[1], Mr. Daniel Kovner[1], Ms. Amy Shen[1], Ms. Hong Li[1], Mr. Tobias Graf[2], Dr. Sanjay Gupta[2], Mr. Wenqiang Liu[1], Mr. Anthony Tomlinson[1]
[1]Genentech, South San Francisco, United States, [2]Roche, Penzberg, Germany

Product Quality – Relevance and Assessment: Session 2, Pentland Auditorium, June 26, 2024, 11:15 AM - 12:45 PM

**Relevance to Theme Selected: Product Quality**
We present a new approach towards furthering understanding of cell culture impact on the obligatory critical quality attribute related to visible particles in liquid drug products.

**Impact/Novelty:**
We applied a novel prospective approach towards proactively uncovering potential culprits for polysorbate degradation and succeeded in considerably expanding our current literature knowledge.

**Introduction:**
The root cause for a major product quality challenge in liquid drug products (DPs) can be traced back to its cell culture origins. The presence of trace levels of certain residual host cell proteins (HCPs) can lead to the formation of visible particles in DPs during long-term storage. These HCPs originate from production hosts during upstream processing, persist through downstream processing and are typically formulated with polysorbate into the DP. These HCPs hydrolyze polysorbate and release free fatty acids (FFAs). Over the course of DP storage, these poorly-soluble FFAs accumulate and can form visible precipitates when they exceed their solubility limit.

**Methods/Approach:**
To date, only a handful of such HCPs are known because of the difficulties in identifying these enzymes (present at trace levels in purified materials) by target-agnostic proteomic approaches. Instead of retrospective analysis, we applied a prospective approach to identify and characterize such HCPs. We selected twenty HCPs based on their known/putative hydrolytic activity, and recombinantly expressed, purified, and characterized them by orthogonal methods.

**Results:**
Through our stepwise screening process, we identified 13 polysorbate-degrading HCPs, some of which were not previously known to degrade polysorbate. By identifying and characterizing the enzymatic activities of these HCPs, we have significantly expanded our knowledge on such potentially problematic HCPs.

**Conclusion:**
Our findings thereby support targeted bioprocessing strategies for removing these problematic HCPs to reduce the risk of polysorbate degradation and associated visible particle formation in liquid DPs.

# How to address challenges in characterization and modeling of CD markers in MSCs?

**Mr Jean-françois Michiels**[1], Mr Clément Laloux[1], Mr Pierre Lebrun[1]
[1]Pharmalex Belgium, Mont-Saint-Guibert, Belgium

Product Quality – Relevance and Assessment: Session 2, Pentland Auditorium, June 26, 2024, 11:15 AM - 12:45 PM

**Relevance to Theme Selected:**
Characterization of CD markers is fundamental to the product quality.

**Impact/Novelty:**
Autologous cell therapy, involving the processing of an individual's cells or tissues outside the body and their subsequent reintroduction, poses unique challenges when analysing data.

**Introduction:**
Mesenchymal stem cells (MSCs) that are used in autologous cell therapies need to be properly characterized. A process failure is associated to a failed treatment for the patient.
MSCs should express CD105, CD73 and CD90, and lack expression of CD45, CD34, CD14, CD11b, CD79 alpha or CD19 and HLA-DR surface molecules.
The ability to analyze CD markers is crucial to the manufacturing process. Any deviation from the minimal criteria must be understood to overcome the issue.

**Methods/Approach:**
Here, we proposed a statistical model adapted to the CD markers analysis, which account for the natural bound in the data [0;1]. The model is multivariate and can include several factors that may cause the deviation observed. The goal is to identify which factors are critical and must be controlled in an appropriate way.
The statistical model is a linear mixed model with a logit link on the mean (to force the estimate to be bounded between 0 and 1). The dependent data follow either one common normal distribution or a normal distribution per group of some discrete covariates. In both cases, it is a normal distribution with untransformed mean(s).

**Results:**
Results demonstrate the usefulness of statistical modeling for characterizing and understanding the manufacturing process of autologous cell therapies. For example, it may help to identify the effects of multiple manufacturing sites and bioassays.

**Conclusion:**
Appropriate modelling is needed to characterize manufacturing process. In addition, this is in line with QbD approaches that authorities favour

## Making Analytical Characterisation Routine

**Dr. Jonathan Bones**[1], Dr Silvia Millán-Martín[1], Dr Noemí Dorival-García[1], Dr Sara Carillo[1], Mr Maikel Gaitkoski[1,2], Dr Florian Füssl[1]

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Silvia Millán-Martín[1], Noemí Dorival-García[1], Sara Carillo[1], Maikel Gaitkoski[1,2], Florian Füssl[1] and Jonathan Bones[1,2]

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Product Quality – Relevance and Assessment: Session 2, Pentland Auditorium, June 26, 2024, 11:15 AM - 12:45 PM

Living in a data rich age, we have become accustomed to having vast amounts of information readily available at our finger tips. The question must be asked as to why we continue to use relatively simple analytical methods that provide limited information for the analysis of increasing complex biopharmaceuticals? To address this challenge, we have developed and deployed different analytical strategies designed to make informative characterisation style experiments more routine. Application of the multi-attribute method (MAM) will be described, as will our approach for near real-time characterisation during upstream processing for mAb production. Not to be confused with peptide mapping, MAM is a mindset that focuses on two phases, firstly a discovery phase where product quality attributes are identified, followed in the second phase by targeted monitoring with new peak detection using liquid chromatography with high resolution mass spectrometric detection. While originally designed for deployment in the QC setting, an additional powerful application of MAM is for comparability assessment, particularly to support biosimilar development. To compliment peptide centric MAM, we developed a companion method based on high resolution intact LC-MS analysis named iMAM that provides information on additional product quality attributes. iMAM adheres to the same two phase philosophy as MAM with the advantage that minimal sample preparation is required, mitigating the risk of induction of sample preparation related artefacts. Finally, building on the concept of iMAM, the development of an integrated analytical platform for near real-time process and product characterisation of an IgG1 mAb process will be described. The described methods are capable of rapidly generating significant quantities of data and are ideally suited for embracing digitalisation of biopharmaceutical manufacture. As we look towards the development of predictive analytics and AI, these platforms will play a key role because we cannot develop data analytics without high quality analytical data.

## Mitigating risk during biologics manufacturing using discovery stage developability assays and state of the art machine learning based optimization

**Dr. Jyothsna Viswesvaraiah**[1], Dr. Ryan Peckner[1], Dr. June Shin[1], Dr. Nathan Rollins[1], Dr. Samuel Perry[1], Mr. Elliott Wittenberg[1], Mr. Ishan Sharma[1], Dr. Jordan Anderson[1], Dr. Colin Lipper[1], Mr. Stephen Lutz[1], Dr. Yi Xing[1], Dr. Daniela Cipolletta[1], Dr. Kevin Otipoby[1], Dr. Nathan Higginson-Scott[1]
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Transitioning from development to manufacture: Session 2, Pentland Auditorium, June 26, 2024, 2:00 PM - 3:30 PM

Historically, biologics discovery programs were driven purely by a molecule's potency, without considering other attributes often leading to late-stage failure. High-throughput screening and molecular property analysis during candidate selection have emerged as effective risk mitigation strategies for manufacturing. Traditional antibody optimization and development, while reliable, is iterative and cumbersome. At Seismic Therapeutic we are harnessing recent machine learning advancements to overcome these limitations in our discovery, engineering, and development pipelines.

# Safe and economic clinical manufacturing of arenavirus-based immuno-virotherapies: challenges and solutions

**Dr Binod Prasad**[1], Dr. Dethardt Muller[1], Dr Julia Zöller[1], Ms. Luisa Pickarski[1]
[1]Abalos Therapeutics GmbH, Düsseldorf, Germany

Transitioning from development to manufacture: Session 2, Pentland Auditorium, June 26, 2024, 2:00 PM - 3:30 PM

**Relevance to theme selected (04. Transitioning from Development to Manufacture)**: The presentation deals with the challenges and solutions applied from lab-scale development to clinical scale manufacturing of a novel advanced therapy medicinal product (ATMP) in the field of virus-based immunotherapies.

**Impact/novelty**: The clinical manufacturing process we developed offers solutions for coping with the most critical safety requirements related to immuno-virotherapies besides supplying 1200+ clinical doses from one 200 L scale.

**Introduction:** Since market approval of the first oncolytic viral (OV) therapy in 2015, immuno-virotherapies are advancing with increasing importance and unprecedented speed. More than 50 clinical OV studies have already been completed, far more are currently ongoing in different clinical phases, either as monotherapy or increasingly as combination therapy. We developed a non-oncolytic immuno-virotherapy against solid tumors based on a Lymphocytic Choriomeningitis Virus (LCMV) currently being at preclinical stage. Unlike with oncolytic viruses, infection with LCMV does not directly destroy tumor cells by cell lysis, it builds-up a persistent innate and adaptive immune response directed against the tumor.

**Methods/Approach:** Virus-based immunotherapeutics are live replicating biological entities produced, stabilized, and stored at titers that easily exceed viral vaccine titers by several orders of magnitude. They vary widely in type, shape, size, size distribution and particle-to-plaque ratio. Suitable up- and downstream manufacturing technologies are required able to cope with the characteristics of live virus particles. As it applies for all parenteral products, both manufacture and storage of highly concentrated virus drug substance and drug product have to comply with highest safety and stability standards required for parenteral products.

**Results:** We established a clinical manufacturing process at clinical scale in a BSL-2 facility using fully disposable technologies for upstream and downstream manufacturing. Upon virus amplification in human cells in a stirred single-use bioreactor (SUB), two major technologies are applied for multi-stage downstream purification, hollow fiber-based tangential-flow filtration for clarification and final formulation, and monolithic chromatography for virus concentration and efficient removal of process-related impurities. The virus formulation allows for long-term liquid frozen storage without appearance of visible particles or any significant increase of subvisible particles.

**Conclusion:** Challenges during process development and scale-up have been manifold and will be discussed together with technological solutions applied to enable safe and economic clinical manufacturing of this novel immuno-virotherapy

## High-Throughput Virology and Design of Experiment for Rapid Vector Production Process Optimization

Dr. Jean-Simon Diallo, Dr. Jondavid de Jong, Ms. Keara Sutherland, **Ms Andrea Vervoort**[1]
[1]Virica Biotech, Ottawa, Canada

Transitioning from development to manufacture: Session 2, Pentland Auditorium, June 26, 2024, 2:00 PM - 3:30 PM

**Relevance to Theme Selected:** Harnessing the power of high-throughput virology and DoE statistical methods to optimize gene therapy vector production rapidly and efficiently.

**Impact/Novelty:** Demonstrating the potential of high throughput assays with DoE for rapid gene therapy vector production process optimization.

**Introduction:** Biomanufacturing of cell and gene therapies (CGT) is a complex process, hampered by the scalability of manufacturing platforms. Production parameters are key drivers for vector quality, quantity and cost, making them prime process optimization targets. However, assessing all possible variables is time-intensive using traditional one-factor-at-a-time (OFAT) process optimization strategies.

**Methods/Approach:** A high-throughput, 96-well format virology platform using transduction-based quantification of luciferase reporters allows rapid quantification of viral vectors. We developed transfection-based production and quantification assays for AAV (suspension 293) and lentivirus (adherent 293T) and used DoE to optimize up to six different production parameters simultaneously. This combinatorial approach of high-throughput virology with DoE is amenable to various vector production platforms.

**Results:** AAV production in suspension HEK293s was optimized, with the methodology allowing simultaneous optimization of up to six factors, including total DNA, nucleic acid type, cell count, transfection reagents, and addition of small molecule enhancers to optimize AAV production. Additionally, LV production optimization in adherent HEK293T by full factorial combination of various plasmid ratios was performed. Importantly, the optimized conditions identified at a small scale were translatable to larger-scale formats, demonstrating scalability.

**Conclusion:** We demonstrate that our approach enables process optimization of multiple factors in time-frames not achievable through traditional OFAT methods. Furthermore, the output data can be used to optimize processes for various factors, including transduction signal, TU/mL, vg/mL or even cost/vg. The flexibility of optimizing different outputs from a single data set unlocks the potential for data-driven decisions to maximize production processes.

# End-to-end CHO Platform Across Research, PD and Manufacturing to Efficiently Identify Molecules with High Yield

**Dr Natalia Gomez**[1], Kristi Daris[1], Jian Wu[1], Noelia Blanco-Talavan[1], Fuyi Chen[1], Keshab Rijal[1], Eric Gislason[1], Marissa Mock[1], Jennitte Stevens[1]
[1]Amgen, Thousand Oaks, United States

Transitioning from development to manufacture: Session 2, Pentland Auditorium, June 26, 2024, 2:00 PM - 3:30 PM

**Relevance to "Transitioning from development to manufacture" Theme**: Successful performance of a manufacturing process starts early on by evaluating and identifying the best product candidates during molecule screening using a highly productive and manufacturing-representative cell culture process.

**Impact/Novelty:** Development of a high-yield next-gen CHO expression system for implementation from Research to Manufacturing with innovation in (a) the CHO host, (b) the vector design, and (c) cell culture process with flexibility in format, including fed-batch and perfusion.

**Introduction**
Increasingly complex Biologic pipelines predominate in Biopharmaceuticals. In particular, multi-specifics are difficult to express, emphasizing the need to re-evaluate cell line and culture platforms to increase yields. Furthermore, early identification of molecule candidates with properties conducive of highly productive commercial manufacturing processes is critical. This manufacturability assessment should be embedded during the first screening of hundreds of molecules to ultimately choose efficacious and safe medicines with lower manufacturing cost.

**Methods/Approach**
To identify molecules with superior productivity and product quality, cell culture conditions during molecule screening need to be representative of a typical manufacturing process. To achieve this objective, we first developed a next-gen CHO expression system that increased Mab and multi-specific yields versus a legacy system. Second, we implemented this system as an end-to-end platform across Research, Process Development (PD) and Manufacturing to identify molecules with desired properties from the start. Third, after screening of candidates with representative process, we calculated an estimated future manufacturing yield and corresponding impact on plant capacity according to potential product demand. These combined results ultimately informed the selection of the best molecule to advance into clinical development.

**Results**
We developed the next-gen expression system with (1) a GS-KO host, (2) a modular vector design, and (3) optimized cell culture in fed-batch and perfusion-based formats. The new platform significantly increased fed-batch titer versus the legacy system. Additionally, perfusion further increased productivity. This high yield enabled deployment in Research for high-throughput screening via miniaturized/automated production cultures. Finally, as designed, we could estimate the future manufacturing process productivity, plant capacity impact and product quality profile before PD started.

**Conclusion**
Overall, the integrated next-gen expression platform is enabling a more effective and faster advancement of a multi-modality portfolio with better productivity, and with the ability of identifying early on the best molecules to deliver cost-effective processes.

# Utilizing oxygen transfer rate monitoring to transfer cell cultures from microtiter plates to stirred-tank reactors

**Ms Anne Neuß**[1], Prof. Dr. Jørgen Magnus[1]
[1]RWTH Aachen University - AVT.BioVT, Aachen, Germany

Transitioning from development to manufacture: Session 2, Pentland Auditorium, June 26, 2024, 2:00 PM - 3:30 PM

**Relevance to Theme Selected:** Precise and non-invasive online monitoring in different scales improves the efficiency of a scale-up for promising CHO cell lines from screening to production processes.

**Impact/Novelty:** The scale-up of mammalian cell cultures from small, shaken scales to a stirred- tank reactor (STR) based on oxygen transfer rate (OTR) monitoring was shown for the first time.

**Introduction:** Real-time online monitoring of crucial process parameters is necessary for fast and efficient development of new CHO production processes. Concurrently, employing screening approaches in small, shaken scales offers time- and resource-saving possibilities. However, the small-scale screening approaches are only useful if process parameters and results are comparable with large production scales. The OTR provides extensive information about cell culture behavior and has the advantage that it can be monitored non-invasively in small scales by the Transferrate Online Monitoring (TOM) system and in stirred tank reactors by off-gas analysis.

**Methods/Approach:** Antibody-producing CHO-DP12 cells were cultivated in 96-deep-well MTPs, 250 mL shake flasks, or in a 1.5 L STR. In each scale, the OTR was monitored online. Offline samples were taken to determine further key culture parameters and antibody productivity.

**Results:** Cultivation of mammalian cells in shaken 96-well MTPs was expected to be difficult due to the delicate balance between oxygen supply and hydrodynamic force. However, by precise OTR monitoring, we first demonstrated that the comparability between MTP and shake flask cultivations is very high. Subsequently, the scale-up to a STR based on a constant volumetric power input was successfully performed, as indicated by similar OTR curves and comparable titers. Therefore, we could show that a scale-up from small, shaken cultivations to a STR is possible.

**Conclusion:** Monitoring the OTR provides insights into mammalian cell culture behavior and is a suitable parameter to evaluate scale-up experiments.

# Towards Scalable Allogeneic CAR-T Manufacturing: Perfusion Optimisation and Multi-litre Scale-up in Single-use Stirred-tank Bioreactors

**Mr Pierre Springuel**[1], Dr Tiffany Hood[1], Dr Fern Slingsby[2], Ms Nicola Bevan[3], Ms Amanda Frangleton[3], Mr Timo Schmidberger[4], Dr Winfried Geis[4], Dr Julia Hengst[4], Dr Noushin Dianat[5], Prof. Qasim Rafiq[1]
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Transitioning from development to manufacture: Session 2, Pentland Auditorium, June 26, 2024, 2:00 PM - 3:30 PM

**Relevance to Theme Selected:**
This work demonstrates Quality by Design-driven optimisation of perfusion parameters and multi-litre scale-up for industrial allogeneic CAR-T manufacturing in stirred-tank bioreactors.

**Impact/Novelty:**
Highlights the significant improvements that can be made in CAR-T yields by optimising perfusion and demonstrates for the first time the impact of automated harvesting solutions on CAR-T cell quality.

**Introduction:**
The emergence of allogeneic chimeric antigen receptor T cell (CAR-T) therapies, designed for universal patient administration, promises substantial reductions in manufacturing costs if economies of scale can be achieved. To meet this potential, scalable bioprocesses that support high cell yields are required. The aim of this work was to establish a multi-litre stirred-tank bioreactor (STR) CAR-T manufacturing workflow poised for future allogeneic production.

**Methods/Approach:**
A Design of Experiments (DOE) study was first conducted in the Ambr® 250 (250mL) to investigate the impact of perfusion parameters on CAR-T growth and quality. Optimised perfusion settings were subsequently scaled-up into the Univessel® Single-Use (SU) 2L STR and comparability across both scales was assessed. At the 2L-scale, implementation of automated harvesting using the Ksep® was also assessed.

**Results:**
Optimising perfusion conditions in serum-free media supported final CAR-T cell yields almost five times greater than the conventional fed-batch process. Harvested CAR-T cells exhibited desirable phenotypic quality attributes. Initial scale-up experiments in fed-batch mode have confirmed process scalability from 250mL – 2L and found automated cell harvesting to have no adverse impacts on CAR-T quality.

**Conclusion:**
These studies highlight the substantial improvements in CAR-T yields achievable through DOE-driven optimisation of perfusion parameters in STRs. Additionally, they highlight good scalability of the CAR-T expansion process from the 250mL - 2L scale. This work establishes a scalable multi-litre STR workflow suited for future allogeneic CAR-T manufacturing needs.

# Environmental Footprint of Biomanufacturing: strategies for sustainability

**Prof Alois Jungbauer**[1]
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Keynote Lecture: Professor Alois Jungbauer, Pentland Auditorium, June 26, 2024, 3:30 PM - 4:15 PM

The global environmental impact of the entire healthcare industry is completely ignored. The American Chemical Society Green Chemistry Institute Pharmaceutical Roundtable has reported that the global warming potential for a 2000 liter process in the US, for example, is 22.7 tons CO2eq per 1 kg drug substance. Process intensification and plant utilization are key to reducing environmental impact. A 2000 L single-use bioprocess where single-use systems to account for an estimated 769 kg per batch, compared to 226 kg of chemicals used. Bioprocesses in the pharmaceutical and biopharmaceutical industry are inherently complex and involve a number of levels of automation. This presentation will explore current issues, potential solutions and forward-looking strategies to achieve these goals. It will discuss best practices from established biologics production, how to implement sustainable production that are compatible with reduced costs, and present metrics for evaluating the environmental footprint of a bioprocess in the context of the societal impact of biopharmaceuticals. Possible solutions to improve the environmental footprint and their impact on production costs will be presented and it will be shown how a circular bioeconomy can be implemented in bioprocessing with a focus on downstream processing.

# Poster Presentations

# Cells as Factories

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# Design and validation of a semi-automated workflow for therapeutic cells expansion, harvest, and final fill.

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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
Genetically modified organisms and cells are increasingly deployed at large scales in clinical environments. Robust strategies are needed to prevent unintended contamination of therapeutics payloads in disposable systems during manufacturing.

**Impact/Novelty:**
While commercially available platforms allow for large scale production of cell therapies, they are often limited in scaling processes, are inherently inefficient, require skilled personnel, and lack flexibility.

**Introduction:**
Costly all-in-one cell processing systems are commonly used in the development and production of cultured biologics and cell and gene therapies. Large scale manufacturing of these therapies requires bioreactors and separation systems to generate enough biological material, with reproducible and aseptic workflows, often warranting precious and costly clean-room space.

**Methods/Approach:**
Employing a decentralized manufacturing strategy, cost-effective production of advanced therapeutics using modular closed-system production workflows may be achieved at a fraction of the cost of all-in-one systems.

**Results:**
This presentation describes the results of expanding bone-marrow derived mesenchymal stem cell on microcarrier beads in a modular closed-system. Our integrated system was assembled using specialized off-the-shelf bioprocessing consumables, minimal liquid-handling equipment and allowed the entire critical workflow to be performed in a single step on a bench space in a standard BSL2 laboratory. The resulting assemblies exhibit unprecedented performance to cells processing and fluid handling including storage. Our latest results obtained on a set of carefully selected particles and cells lines (e.g. mesenchymal stem cells) will be exposed. This work provides a foundation for new and safer bioprocessing solutions that are re-designed to provide lower cost and more benefit to patients.

**Conclusion:**
The study results indicate that this modular system is suitable for both suspension and adherent cell manufacture and can be easily scaled up in any R&D or clinical settings. We will also discuss how precision controlled biomanufacturing strategy impacts product quality, process efficiency and reliability.

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# Overcoming expression challenges of x-specific antibodies by balancing chain expression

**Dr Anett Ritter**[1]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
We show that we can improve the production and quality of x-specific antibodies in stable CHO cell lines by balancing chain expression using polycistronic plasmid designs.

**Impact/Novelty:**
Production of x-specific molecules with higher percentage of correctly assembled molecules decrease efforts needed for analytics and downstream purification processes to eliminate unwanted misassembled variants.

**Introduction:**
Chinese hamster ovary (CHO) cell line generation and production has been optimized to express monoclonal antibodies in high quality and quantity with reduced resource needs. Additional efforts are required to overcome expression challenges of more complex x-specific formats to drive CHO cells towards high expression of correctly assembled x-specific molecules with low amounts of misassembled variants. Expression of complex x-specific antibodies that are built out of more than two chains require co-transfection of different monocistronic plasmids. Our hypothesis is that co-transfection in addition to other factors such as different expression strength of genes can lead to unbalanced expression of chains causing preferred assembly of misassembled variants over the correct x-specific.

**Methods/Approach:**
We evaluated in head-to-head comparison mono- & polycistronic plasmid designs with the goal to determine if balanced chain expression can lead to higher expression of correctly assembled x-specifics.

**Results:**
Data confirms that we can balance mRNA levels using polycistronic plasmids and pool productivities remain similar or increase to 2-3 fold depending on the candidate in fedbatch processes. The formation of x-specifics is increased up to 90% with a decrease of misassembled variants. Our data shows that clones derived from pools generated with polycistronic plasmid designs yield in higher productivities. Selected clones produce 3.5 g/L in fedbatch process with a purity of 90% and remain stable production throughout a cultivation period of 14 weeks.

**Conclusion:**
Our data confirm that polycistronic plasmid designs enable development of x-specific molecules with decreased efforts needed for analytics and downstream purification processes to eliminate unwanted variants.

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# Lentiviral vector production in HEK293-T suspension cells with single-use bioreactors

**Dr Johanna Viiliäinen**[1], M.Sc Josefin Thelander[1], M.Sc Christine Sund-Lundström[1], Dr Greta Hulting[1], M.Sc Ann-Christin Magnusson[1]
[1]Cytiva, Uppsala, Sweden

Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Cells as factories:**
Lentivirus vectors (LVV) are extensively used in the growing cell and gene therapy field for the delivery of nucleic acids into target cells, both *ex vivo* and *in vivo*.

**Impact/Novelty:**
Generate a robust and reproducible LVV production process.

**Introduction:**
Lentivirus vectors (LVV) are extensively used in the growing cell and gene therapy field for the delivery of nucleic acids into target cells, both *ex vivo* and *in vivo*. Vector production is a major contributor to the cost of manufacturing cell and gene therapies. Improvements in vector production, such as increased vector yield per batch, are therefore paramount as they can reduce manufacturing costs and increase the availability of various therapies. We describe our scalable, robust process to produce LVV using suspension cells in single-use bioreactors.

**Methods/Approach:**
To produce LVV, we used a four-plasmid transfection system consisting of two packaging vectors, one envelope vector, and a transfer vector carrying the green fluorescent protein (GFP) reporter gene. PEI MAX (Polysciences) was used as the transfection reagent for HEK293-T cells. Three production runs were performed both in Xcellerex™ XDR-10 stirred-tank bioreactor and ReadyToProcess WAVE™ 25 rocking bioreactor. We describe the production process from cell inoculation and expansion in the single-use bioreactors to the final harvests. Our analysis of the harvested material included a transduction assay for infectious titer measured in transducing units (TU), and p24 assay for viral particles (VP) measured in VP/mL.

**Results:**
Data from our LVV production batches show that we reached our target for harvesting materials for infectious titers ($10^7$ TU/mL) and viral particles ($10^{10}$ VP/mL). The consistency between batches indicates a robust and reproducible production process.

**Conclusion:**
The study confirms that our LVV production protocol is suitable for rocking motion as well as stirred-tank bioreactors.

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# Expression of complex therapeutic proteins at very high yields with stable CHO GPEx Lightning pools

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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to the Theme selected:** **Cells as factories.**

**Impact/Novelty:**
A novel use case for an industrially relevant expression system. Complex therapeutic proteins were produced in stable CHO pools at gram scale with very high titers.

**Introduction: The GPEx® Lightning technology, protein expression in stable pools**
GPEx® Lightning is a mammalian cell line development technology with the potential to achieve high gene copy numbers for a gene of interest. The insertion is stable and directed by a recombinase to engineered expression regions and contains a glutamine synthetase (GS) gene for selection. For monoclonal antibodies, titers of up to 12 g/L at cell densities of $28x10^6$ /mL viable cells were reported in a 14-day fed-batch cell culture process.

**Methods/Approach:**
Stable transfections were conducted with Expifectamine™ and a dedicated vector system unique to the GPEx® Lightning cell line. Selection of stable pools was conducted via glutamine withdrawal. All fermentation runs were conducted in ambr® 250 Modular or Biostat® B-DCU fermenters from Sartorius.

**Results: Complex therapeutic proteins were produced at gram scale**
Complex non-antibody therapeutic proteins were produced from cell pools generated using the GPEx® Lightning cell line development system. Yields of more than 10 g/L were achieved at viable cell densities of $10\text{-}20x10^6$ /mL, conducted in a 14-day fed-batch process. The data suggest that this approach provides research functions with the capability to increase their protein output and speed up their pipeline.

**Conclusion: Are stable pools the future?**
The production of complex therapeutic proteins in stable pools might have a potential beyond research. The speed of development, high and stable expressions, and the ability to scale up the production in bioreactors has a potential to provide stable pool-derived material for early development.

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# Automated plasmid purification can reduce labour time and increase AAV9 titres in small-scale productions

**Mrs Sanne Rönning**[1], Mr Hannes Thorell[1], Dr Magdalena Malm[1], Prof Johan Rockberg[1]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
Using a system for automatic plasmid purification instead of a manual commercial plasmid purification kit, we drastically reduce hands-on time for plasmid purification while maintaining comparable or increasing AAV productivity.

**Impact/Novelty:**
Our data shows that plasmids derived from the same bacterial culture but purified on two different plasmid purification systems can have major impacts on AAV titres and working hours required.

**Introduction:**
Increasing amounts of Adeno-associated virus (AAV)-based therapies are receiving approval for the treatment of genetic diseases. However, progression is hindered by high costs associated with production of AAVs for drug candidate screening. This can partly be attributed to the amount of high-quality plasmids required for AAV production, as plasmid purification can be both labour-intensive and time-consuming.

**Methods/Approach:**
To investigate ways of reducing production costs, the automated plasmid purification system Biotage® PhyPrep (PhyPrep) was benchmarked against a commercial bench-top manual plasmid purification kit. The plasmids required for production of AAV9 were purified in GigaPrep scale on both systems, followed by evaluation of plasmid quality, preparation time and AAV9 titres in two different production systems – HEK293F and AAV-MAX production system from Thermo Fisher Scientific.

**Results:**
The PhyPrep automated plasmid purification resulted in 90% reduction of hands-on manual labour time compared to the manual kit, with 50% reduction of total process time. One of the HEK293 production systems gave AAV9 titres on comparable levels for both plasmid purification systems, while the other production system showed 20-fold increase in AAV9 titres using the PhyPrep-derived plasmids.

**Conclusion:**
These results indicate plasmid purification time can be vastly cut using the PhyPrep as well as potentially give larger AAV9 titres, depending on the plasmid purification method and the transfection system used. Additionally, buffer composition and endotoxin levels seem to be important factors when optimizing the process for increased AAV titres.

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# Interaction studies of artificial globular C1q variants with pentameric and hexameric IgM

**Ms Maria Magdalena John**[1], Ms. Monika Hunjadi[1], Ms. Vanessa Hawlin[1], Prof. Renate Kunert[1]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:**
Our research fits to the topic Cells as Factories as our future goal is to find and express an enhanced version of the C1q protein for the clinical approach.

**Impact/Novelty:**
Our research focuses on the IgM and C1q interaction and paves the way for the development of recombinant C1q, specifically variants thereof for further scientific studies.

**Introduction:**
The classical complement pathway is activated through the interaction between the globular head of C1q and the constant domain of an antigen/immunoglobulin complex. Although structural data exists for the IgM/C1q complex, the influence of immunoglobulin structure, particularly the difference between pentameric and hexameric oligomers, remains unexplored. To address this, we developed single-chain artificial globular C1q variants and expressed them alongside with pentameric and hexameric IgMs with different antigen specificities in CHO cells.
**Methods/Approach:**
Two globular variants of C1q were designed: ACB consisting of the three globular domains connected by short linkers and expressed as a single-chain protein and AD2 mirroring ACB but extended at the C-terminus by a human serum albumin domain 2, linked by a flexible glycine-serine linker.
Stable recombinant cell lines were established in CHO-K1 cells, and anti-FLAG affinity resin facilitated high-quality material purification.
Binding evaluation of C1q and single-chain variants to pentameric and hexameric IgMs involved competitive interaction and complement activation assays in ELISA format and Bio-layer interferometry using Protein L tips loaded with IgM variants.
**Results:**
Competitive complement interaction and activation assays demonstrated that the single-chain variants were able to compete with native C1q for binding to IgM in ELISA format. Bio-layer interferometry confirmed interaction, revealing small molecule binding behaviour demanding further investigation.
The C1q mimetic is used for random mutagenesis in different loops of the protein. Screening of the mutant library expressed via yeast surface display technique should identify optimal binders and critical amino acid residues on the globular head of C1q.

**Conclusion:**
Our study on IgM/C1q interaction yields promising results with engineered C1q variants and recombinant IgMs. It offers potential for identification of critical amino acid residues on C1q, advancing our understanding of this immune interaction and offering prospects for therapeutic development.

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# Sar1A overexpression improves antibody productivity of CHO cells through secretion process engineering

**Mr Yu Tsunoda**[1,2], Assoc. Noriko Yamano-Adachi[1,2,3], Prof. Takeshi Omasa[1,2,3]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
Understanding and controlling intracellular antibody transport through secretion process engineering and mechanistic analysis moves CHO cells toward becoming more robust and efficient mammalian cell factories.

**Impact/Novelty:**
This study improves antibody productivity by enhancing COPII vesicle-mediated antibody transport from the endoplasmic reticulum (ER) to the Golgi apparatus.

**Introduction:**
Chinese hamster (*Cricetulus griseus*) ovary (CHO) cells are mammalian host cells commonly used for therapeutic antibody production. We previously analyzed the secretion process in antibody-producing CHO cells and found that folded and assembled antibodies were accumulating in the ER. Thus, the transport of antibodies from the ER to the Golgi apparatus could be one of the bottlenecks in antibody production[1)]. Nevertheless, few studies have engineered the COPII vesicle-mediated antibody transport and investigated how the modification affects the CHO cell secretion process. Here, Sar1A, a protein involved in COPII formation, was overexpressed in CHO cells to improve antibody productivity and clarify its effect on the secretion process.

**Methods/Approach:**
The constructed Sar1A-overexpressed CHO cell lines were batch-cultured to examine their specific antibody production rate. The intracellular antibody accumulation and the specific localization of the intracellular antibodies were investigated by chase assay using a translation inhibitor and observed by immunofluorescence-based imaging analysis. Moreover, the effects of the engineered antibody transport on the antibody's glycosylation profile and the unfolded protein response (UPR) pathway were analyzed by LC/MS and UPR-related gene expression evaluation, respectively.

**Results:**
Sar1A overexpression significantly improved the specific antibody production rate by 48.4% through reduced intracellular accumulation, especially in the ER. It lowered the glycan galactosylation and induced a stronger UPR at the end of batch culture.

**Conclusion:**
Sar1A overexpression enhanced the antibody productivity of CHO cells by modifying their secretion process. To streamline the secretion process more, holistic secretory engineering would be necessary.

1) Kaneyoshi K. *et al*., (2019). *J. Biosci. Bioeng*., **127**, 107-113

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# Production of recombinant proteins and virus-like-particles for antibody development using baculovirus-free insect cell expression system

**Ms Seyhan Demiral**[1], Mr. Marcel Jaron[1], Mr. Jonathan Benecke[1], Ms Nina Lehmler[1], Dr. Maren Schubert[1], Prof. Dr. Michael Hust[1], Prof. Dr. Stefan Dübel[1]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
High-quality expression of recombinant proteins and virus-like-particles (VLP) is essential for vaccination and diagnostics. Our baculovirus-free insect cell system ensures efficient, high-yield and high-quality production of these for antibody development.

**Impact/Novelty:**
Despite the widespread use of baculovirus expression vector system (BEVS) for protein and VLP production, it faces significant bottlenecks. Our approach overcomes these challenges while maintaining the high yields achieved.

**Introduction:**
High-quality protein and VLPs are important for vaccines and diagnostic applications. BEVS is frequently used because of its high yields, but it faces challenges in adjusting the ratios of the required structural proteins and concurrent production of baculoviral particles and proteins leads to purification difficulties. Our solution presents a plasmid-based High Five cell expression system that overcomes these limitations.

**Methods/Approach:**
Among other applications, our system has been used to produce SARS-CoV-2 proteins and VLPs. Recombinant Spike and RBD were used for diagnostic assays. The quantity and quality of VLPs were validated and evaluated using Nanotracking analysis (NTA), ELISA, cytometry, and microscopy. Fluorescent VLPs were used to develop a high-throughput assay to screen our anti-SARS-CoV-2 antibody candidates for inhibition of the binding to ACE2-positive cells in a cytometer.

**Results:**
We demonstrate that our system achieves high yields and high quality in protein and VLP expression. The characteristics of fluorescent SARS-CoV-2 VLP were confirmed: They show a diameter of ~145 nm, ACE2 binding, and the typical "corona" aura could be observed. The transferability of this system to produce other types of VLP like Hantavirus and Influenza could be shown.

**Conclusion:**
Our plasmid-based method for expressing proteins and VLPs is efficient and advantageous compared to BEVS, allowing for the high-quality and high-quantity production of recombinant proteins and VLPs.

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# Strategic design of antibiotic selection marker drives high titer in the GOCHO™ platform

**Ms Sheffali Dash**[1], Seungjo Park[1], Michelle Sabourin[1], Peggy Lio[1]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected (Cells as factories):** Multiple approaches have been implemented in the manufacturing process to improve expression titer and stability. We explore the impact of a vector design and process change on the resulting clones for suitability in manufacturing monoclonal antibodies.

**Impact/Novelty:** In this study we explore a strategic design change of an antibiotic selection marker to obtain clones producing higher titers of antibodies. We also address the impact of this approach on expression stability and gene copy number.

**Introduction:** Changes to the expression vector has profound effects on antibody expression levels, expression stability and product quality in cell-line development. Here, we explored a combination of vector design and workflow optimization to obtain high expressing stable clones. Our approach was to lower the expression levels of the selection marker which we hypothesized could drive higher expression of the genes of interest by modulating selection pressure.

**Methods/Approach:** In our current GOCHO™ workflow, minipools selection is initiated 48 hours after transfection using the antibiotic G418. We selected minipools producing a model mAb from a plasmid with G418 selection marker modified to reduce its expression levels. The top minipools obtained from each selection were cloned using a single-cell printer. Through a series of scale-up steps, clones were ranked by titer, and the top clones were evaluated for titer, expression stability and gene copy number.

**Results:** We identified the optimal concentration of antibiotic selection for our workflow and improved the overall productivity by >2-fold, with multiple clones producing >5 g/L in a non-optimized fed-batch screen. The vast majority of the high titer clones were stable over 60 generations.

**Conclusions:** This modified selection strategy is more robust and generates stable high titer clones suitable for manufacturing.

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# Efficient Targeted Integration in CHO Using BXB Integrase

**Mr Tobias May**[1], Dr. Kristina Nehlsen[1], Dr. Anne Dittrich[1]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
A genetic engineering approach for optimization of protein manufacturing processes is presented.

**Impact/Novelty:**
Novelty of the approach is combination of high efficiency with high precision. This results in fast and versatile cell line engineering.

**Introduction:**
The fast generation of recombinant cell lines is of utmost importance for biologics production. This can be accomplished by sophisticated instrumentation or by genetic engineering approaches including e.g. Cre, Flp, Phi or Crispr/Cas.

**Methods/Approach:**
We developed a novel targeted integration approach based on the BXB integrase. First, a genomic locus is tagged with a construct harbouring the required attachment sites, which in addition, allows for selection of recombinant cells with the desired expression characteristics. Thereby tagged master cell clones are identified that display high and stable transgene expression. In the second step, the tagging construct is excised and the gene(s) of interest are integrated into tagged site, thereby conferring the positive expression characteristics from the master cell line to the desired cell line.

**Results:**
We implemented this approach in CHO cells and generated different master cell clones. These were used for initial experiments in which the exchange efficiency was determined with a fluorescent reporter protein. The integration efficiency was dependent on the amount of the plasmid and integration/exchange levels of up to 30% of transduced cells were reached, making this process highly efficient. Next, the exchange/integration process was supported by blasticidin selection. This led within 14 days to the generation of 100 Mio. recombinant cells expressing different genes of interest (e.g. PD-L1, CXCR7, Her2…) in a homogenous fashion. Simply by modifying the genetic setup of the integration cassette different cell lines were developed displaying expression levels of the transgene spanning 3 orders of magnitude. In proof of concept experiments this technology was used to establish a small library of proteins to display on the cell surface.

**Conclusion:**
The developed technology combines simplicity, speed, versatility, and reliability and thus opens the door to push cell line engineering to the next level.

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# Influenza A Defective Interfering Particles as Broad-Spectrum Antivirals

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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:**
Genetic engineering of virus-derived defective interfering particles (DIPs) and corresponding complementing animal cell lines.

**Impact/Novelty:**
Development of a new type of DIP called OP7 for use as a novel broad-spectrum antiviral for intranasal administration to improve pandemic preparedness.

**Introduction:**
DIPs of influenza A virus (IAV) are suggested as a promising new class of antivirals. DIPs are replication-deficient viral particles that depend on co-infection with infectious standard virus (STV). Here, DIPs exert an inhibitory effect on STV replication by hijacking viral and cellular resources. Typically, IAV DIPs harbour an internal deletion in their genome. We discovered a new type of IAV DIP termed “OP7”. Instead of an internal deletion, OP7 shows numerous point mutations in its genome. Further, OP7 displays a superior interfering efficacy compared to conventional DIPs (cDIPs).

**Methods/Approach:**
To avoid safety concerns for the medical use of OP7, we developed a cell culture-based production system that does not depend on co-infection with infectious STV using genetically engineered cells and reverse genetics for IAV. To test the antiviral potential, we conducted animal experiments in mice.

**Results:**
We reconstituted a mixture of DIPs consisting of cDIPs and OP7 chimera DIPs, in which both harbour a deletion in their genome. To complement the defect, a suspension MDCK cell line was established that expresses the missing viral protein. We achieved very high OP7 titres of 2.2 x $10^{11}$ DIPs/mL and a purity of OP7 chimera DIPs of 99.7% in perfusion mode. Intranasal administration of OP7 in mice was well tolerated. A rescue from an otherwise lethal IAV infection upon OP7 co-infection was observed. Further, OP7 also suppressed respiratory syncytial virus (RSV) and SARS-CoV-2 replication *in vitro* by stimulating antiviral innate immunity.

**Conclusion:**
The process is currently being adapted towards GMP production to facilitate preclinical and clinical trials.

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# Battle of the Barriers: Preventing Transgene Silencing with Barrier Elements.

**Miss Rebecca Sizer**[1], Dr. Claire Arnall[2], Dr. Emma Biggs[2], Dr. Leon Pybus[2], Prof. Nia Bryant[1], Prof. Robert White[1]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected**:
Epigenetic silencing in cells leads to recombinant protein expression loss. Insulator barriers can block epigenetic silencing, optimizing yields and improving efficacy, economy, and safety in biopharmaceutical production.

**Impact/Novelty:**
The barrier function of tRNA genes (tDNAs) has not been utilised for industrial biomanufacturing purposes. To this end, tDNA barriers are both novel and patentable if proven to be successful.

**Introduction:**
Recombinant proteins are a principal product of the biopharmaceutical industry, ~70% of which are produced by integrating transgenes into a mammalian cells genome. These systems often produce highly variable and unstable transgene expression, largely due to epigenetic silencing of the transgene over time. To combat this problem, vectors have been engineered to include genetic elements or barriers that protect against silencing, thereby improving recombinant protein production. Many endogenous barriers contain tDNAs, examples of which have been shown to block the spread of epigenetic silencing.

**Methods:**
I have assessed the ability of tDNA barriers to reduce transgene silencing using an eGFP reporter assay. Here, CHO-K1 cells were transfected with constructs containing an eGFP gene flanked by a variety of different barrier elements, and eGFP expression was monitored over 10 weeks using flow cytometry.

**Results:**
My results demonstrate that a subset of tDNAs can prevent expression instability over a period of 10 weeks to a greater extent than the current industry gold standard barrier UCOE, and that these elements can be combined to produce a fully optimised vector. If these results transfer to a more industrially relevant setting, tDNA barriers could improve biomanufacturing stability significantly.

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# Epigenetic engineering via targeted DNA demethylation can improve biologic production in CHO cells

**Miss Sienna Butterfield**[1], Miss Rebecca Sizer[1], Miss Sarah Smart[2], Dr. Fay Saunders[2], Prof. Nia Bryant[1], Prof. Robert White[1]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:** Epigenetic silencing is a major contributor to unstable therapeutic expression and suboptimal capacity of mammalian cells. Epigenetic engineering using CRISPR activating systems offers a targeted approach to combat this challenge.

**Impact/Novelty:** Use of CRISPR dCas9 to target DNA demethylation at an exogenous promoter driving a monoclonal antibody gene via the demethylase TET1 in CHO cells has never been done before.

**Introduction:** Recombinant proteins represent a substantial fraction of the biopharmaceutical market. Industrial production of these therapeutics like monoclonal antibodies (mAbs) involves integrating transgenes into mammalian genomes like Chinese Hamster Ovary (CHO) cells. However, instability of transgene expression over long-term culture poses a major challenge and necessitates costly stability studies. This silencing has been linked to changes in the CHO epigenome, including methylation of promoter DNA. Approaches to combat silencing have included use of DNA methyltransferase inhibitors, but this untargeted approach has genome-wide effects that compromise cell proliferation.

**Methods/Approach:** I have instead used a CRISPR Cas9 system to target the DNA demethylating enzyme, TET1, directly to the promoter of a mAb transgene using the nuclease inactive dCas9.

**Results:** Directly targeting the DNA demethylase TET1 significantly reduced CpG methylation exclusively at the mAb promoter. When applied in a cell line which had lost transgene expression and gained promoter methylation over long term culture, dCas9-TET1 reduced promoter methylation to match initial levels. This demethylation by TET1 was accompanied by a significant increase in mAb transcript levels.

**Conclusion:** Gain of promoter DNA methylation over long term culture is a contributor to epigenetic silencing of transgenes. Targeted DNA demethylation offers a means to reactivate expression, exemplifying how epigenetic engineering can boost CHO cell efficacy.

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# Scalable High-Density Microcarrier Suspension Culture of Human Adipose and Wharton's Jelly Mesenchymal Stem Cells

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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected**
Aligned with the theme 'Cells as Factories and Therapies,' our research presents a scalable alternative for MSC expansion crucial in effective cell therapy, eliminating the need for numerous adherent plates.

**Impact/Novelty**
Our expansion protocol allows the growth of 150-250 million hMSCs, from adipose and umbilical origin, in a single 125 mL spinner-flask, utilizing the highest reported microcarrier concentration to our knowledge

**Introduction**
Mesenchymal stem cells (MSCs), renowned for their immunomodulation, bioactive molecule secretion, and multi-differentiation potential, have significantly advanced regenerative and cell therapy. Their application extends to treating a spectrum of conditions including heart failures, bone/cartilage-related issues, diabetes, cancer, premature ovarian insufficiency, etc. Despite being a small proportion within tissues, MSCs are self-renewable and are expandable in vitro. However, conventional plate culture, involving labour-intensive procedures, extensive handling, contamination risk, and scalability issues, is inadequate for producing cells needed for cell therapy. Microcarrier culture addresses these challenges by providing an anchoring surface for cells while are homogeneously suspended in an agitated system. This culture system is scalable to various bioreactor dimensions.

**Methods/Approach**
Cells isolated from Wharton's Jelly (WJ-MSC) or adipose tissue (ASC) were expanded in an agitated suspension culture. It was seeded with 8 million cells in a spinner flask with microcarriers at 8 g/L in 50 mL αMEM-Antiox medium. Media was replaced as needed, glucose was added to prevent growth limitations, and the volume was increased from 50 to 120mL during the culture.

**Results**
Human WJ-MSCs and ASCs were successfully grown in a microcarrier suspension culture. Over the 13-day culture, 250 million cells were obtained in a single flask, resulting in a 30-fold increase in cell number.

**Conclusion**
Our developed protocol allows high-density MSC expansion. A single 125 mL spinner flask is equivalent to at least forty T175-dishes. This presents a user-friendly, contamination-resistant, and scalable system adaptable to controlled bioreactors.

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# Establishment of a scalable stable lentivirus manufacturing process in suspension perfusion mode.

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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
Enabling lentivirus manufacturing in suspension perfusion mode for viral-based gene therapies using stable producer cell lines.

**Impact/Novelty:**
Implementation of a scalable lentiviral vector perfusion process using an ultrasonic cell separation system with increased perfusion rates.

**Introduction:**
Production of lentiviral vectors for clinical gene transfer trials is currently mainly done via transient expression. This is challenging due to the high cost of GMP-grade plasmids, the labour-intensive nature of a transient process as well as the lower reproducibility and scalability.
To facilitate clinical grade lentiviral vector production, we have developed a state-of-the-art stable suspension packaging cell lines in the past. We have now significantly improved the suspension perfusion process using an ultrasonic cell separation system with optimized perfusion conditions and developed an efficient tailor-made DSP process to purify and sterile filter LVVs.

**Methods/Approach:**
We have established a HT small scale perfusion system (AMBR250) using an ultrasonic cell separation equipment. With this system we have performed a variety of suspension perfusion runs to identify the best conditions, which were confirmed at 2 L bioreactor scale and the LVV material was used to improve the LVV downstream process (purification, concentration and sterile filtration).

**Results:**
The producer cell lines are expressing high infectious titer. Conditions have been optimized to improve LVV stability, maintain high cell viability for at least 2 weeks, and increase the ratio of infectious to non-infectious particles. We have also identified more gentle culture conditions using an AMBR250 perfusion system with an ultrasonic cell retention device.

**Conclusion:**
Scalable production of LVVs for commercial and clinical use is a hurdle due to traditional transient production methods. We show in this work that we are capable to express and purify LVVs at high infectious titer over a period of 2 weeks using stable suspension producer cell lines and have significantly improved the process using state of the art HT perfusion systems.

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# Modifying Chimeric Antigen Receptor (CAR) expression in T cells using m6A-associated elements-containing UTR sequences

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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
The study evaluated the use of different sequence motifs - well-established or newly designed - to enhance the expression of Chimeric Antigen Receptor (CAR) in T cells.

**Impact/Novelty:**
Improving CAR expression should enhance the efficacy of CAR-T cells. The identified motifs that have a positive impact on CAR expression haven't been reported in mammalian cells.

**Introduction:**
$N^6$–methyladenosine (m6A) is the most abundant internal modification on eukaryotic mRNA and has been implicated in a wide range of fundamental cellular processes, and several physiological processes including immunity, memory, and cancer. Most m6A modifications on eukaryote cells are located near the stop codon, in the consensus DRACH motif (D = A, G or U, R = A or G, A = m6A, and H = A, C or U). We previously reported the importance of this motif in protein expression in Chinese Hamster Ovary cells, in this study we compared several combinations of this motif with well-established or newly discovered m6A-associated cis-elements in the 5' and 3' Untranslated Region (UTR) on CAR expression in T cells.

**Methods/Approach:**
CD19-CAR mRNAs with different 5'UTR or 3'UTR sequences were in vitro-synthesized, qualified and quantified by Tape Station and then delivered to activated Pan-T cells by electroporation. CD19-CAR protein expression was quantified by Flow Cytometry using a CD19-CAR-specific antibody and Western blot analysis.

**Results:**
We identified m6A-associated motifs that are positively associated with mRNA stability. In addition, several m6A-based combinations were found to increase CAR surface expression by up to 50%.

**Conclusion:**
The positive impact of m6A-motifs in the UTRs on the level of CAR expression in T cells is consistent with what has been found in other mammalian cells (e.g. CHO cells). The m6A-associated motif is an exciting discovery, as it has yet to be reported. On-going work is underway to understand its cellular mechanism and gain further insight into its potential significance in RNA therapies.

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# Exploring bioprocess operation modes for the expansion of human Mesenchymal Stromal Cells

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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected**
This work explores a stem cell production process, where there is need for cost-effective, scalable, and robust expansion, aligning with the theme by exploring cultivation modes for enhanced therapeutic production.

**Impact/Novelty**
The outcomes of this study have an impact on the production cost and improved process robustness, which in turn lead to reduced patient cost and increased treatment availability.

**Introduction**
Human Mesenchymal Stromal Cells (hMSCs) have a high potential in the allogenic treatment of several medical conditions. These treatments require high doses, putting a high demand on the expansion process. In order to make the treatments with high quality hMSCs available at scale, the expansion process has to be cost-effective, scalable, and robust. The current state-of-the-art production, using multi-layered tissue culture flasks, cannot meet these demands, and efforts are being made to develop automated and controlled bioreactor-based processes. Two key aspects for a scalable expansion process include an efficient feed system, and a successful migration to added surface.

**Methods/Approach**
Bone-marrow derived hMSCs were cultured on microcarriers in 100 mL stirred-tank bioreactors, where different cultivation modes (fed-batch and perfusion) were investigated and compared to repeated medium changes. Different compositions of the feed medium were explored. Fresh beads were added at confluency, and different approaches were taken to enhance the bead-to-bead migration.

**Results**
Both fed-batch and perfusion mode allowed a higher expansion factor than what could be achieved with manual medium changes. Some conditions granted bead-to-bead migration, which in turn allowed for a prolonged process.

**Conclusion**
The insights gained from the project can be used in the continued development of an efficient and safe production process to increase the availability of stem cell treatment on a global scale.

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# Design of Experiment (DoE) Enabled Scale-Up for Adeno-associated Virus (AAV) Production

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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:** Cells are used to produce AAV through triple transfection. DoE can be used to develop an understanding of this process and aid the identification of optimal conditions for scale-up.

**Impact/Novelty:** DoE enabled scale-up for AAV production would further develop our understanding around triple transfection and address the challenges around the reproducibility of process performance and scale-up.

**Introduction:** Adeno-associated virus (AAV) therapies are produced through triple transfection into a single cell line, where three plasmids are combined with a transfection reagent that undergoes interactions with DNA. While transfection of multiple plasmids gives flexibility and allows modification of transgene regulatory elements, there are complexities associated with the triple transfection resulting in challenges around reproducibility of process performance in terms of titre and product quality. Additionally, there are often difficulties encountered when scaling up.

**Methods/Approach:** This study describes a 2 stage DoE approach to optimize triple transfection using FectoVir-AAV and a HEK293F cell line. The three plasmids allowed for production of an AAV5 capsid containing the transgene encoding Green Fluorescent Protein (GFP). An initial screening design was implemented due to the large number of factors with potential to have significant effects on transfection efficiency, genome titre, capsid titre, and ratio of full to empty capsids. The factors screened included DNA amount, plasmid ratios, volume of FectoVir-AAV, complexation volume, complexation time and cell culture density. Three factors were taken forward to the second stage which implemented a central composite design. This design was chosen to accurately estimate quadratic terms in responses and expand the design space beyond levels included in the screening.

**Results:** Across the conditions screened, transfection efficiency ranged from 8.8 - 61.6%, genome titres from $1.6 \times 10^9 – 3.0 \times 10^{10}$ VG/mL and capsid titres from $4.6 \times 10^9 – 3.9 \times 10^{11}$ capsids/mL. Analysis indicated that DNA amount, complexation time and volume of FectoVir-AAV had the greatest effect. The output of the second stage of DoE was used to identify optimum conditions for triple transfection.

**Conclusion:** The optimized conditions were scaled up from 125 ml shake flasks to a 50 L Wave reactor. Successful scale up was determined by assessing genome titre, capsid titre, and ratio of full to empty capsids.

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# Novel hyperglycosylated recombinant bovine follicle-stimulating hormone (rbFSH): a potential candidate for veterinary use

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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
Protein glycoengineering and process development to produce a novel veterinary biotherapeutic with enhanced biological activity.
**Impact/Novelty:**
The aim of this work was to develop a biotechnological process to produce a novel hyperglycosylated long-lasting rbFSH variant (rbFSH-LD) as an improved substitute of commercially available porcine pituitary-derived products.
**Introduction:**
Bovine ovarian hyperstimulation is a process currently based on porcine follicle-stimulating hormone (pFSH) preparations partially purified from pituitary glands. This comprises many disadvantages, including batch-to-batch variations, possible contamination with luteinizing hormone, and an extremely short circulating half-life. For these reasons, the development of a recombinant bovine FSH (rbFSH) with improved pharmacokinetic parameters represents a powerful strategy to substitute the pituitary-derived preparations.
**Methods/Approach:**
rbFSH-LD was designed by fusing three copies of mGMOP highly O-glycosylated peptide[1] to the C-terminus. Cell lines were generated through lentiviral transgenesis of suspension CHO-K1 cells. Cells were cultured in a one-liter bioreactor for 17 days. rbFSH and rbFSH-LD proteins were purified from culture supernatants by immobilized metal affinity chromatography (IMAC). Physicochemical characterization of purified proteins was performed following SDS-PAGE, western-blot, isoelectric focusing and sialic acid quantification by HPAEC-PAD. Biopotency was evaluated in female rats using the Steelman and Pohley bioassay. Pharmacokinetic studies were conducted in female rats.
**Results:**
rbFSH and rbFSH-LD were obtained with high purity levels (>95%), with traces of free a and b subunits as main contaminants. rbFSH-LD presented a higher apparent molecular mass and a higher content of more acidic isoforms compared to rbFSH due to its greater glycosylation degree and sialic acid content. Preliminary biological assays showed that rbFSH-LD not only exhibits a 2.4-fold specific biological activity than rbFSH but it also presents enhanced pharmacokinetic parameters.
**Conclusion:**
Our strategy allows the production of a long-lasting rbFSH as an attractive alternative to the use of pituitary-derived pFSH.
**Bibliography:**
1. M. Sales et al., J. Biotech. 2021.

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# Producing Oncolytic Newcastle Disease Virus in EB66 cells

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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:**
Virotherapy using oncolytic viruses will require high-titre virus preparations produced in efficient “cell factories”. EB66 cells, potentially achieving up to 1.6E08 cells/mL, could be a manufacturing candidate.

**Impact/Novelty:**
Process development for Newcastle Disease Virus (NDV) production in GMP-compliant EB66 suspension cells. Establishment of a scale-down model using shake flasks for semi-perfusion cultures.

**Introduction:**
NDV has proven its efficacy as oncolytic virus in several (clinical) studies over the last decades. Similar to other viral vectors, production processes need to be established to meet the potential demand.
Using the suspension duck cell line EB66, perfusion processes at 1.6E08 cells/mL have been achieved for flavivirus production in our group. Here, we report the establishment and optimization of a batch process for NDV production and subsequently evaluate intensification options for semi-perfusion culture.

**Methods/Approach:**
EB66 cells (Valneva SE) were cultivated in CDM4 avian medium (Cytiva) at 37°C, 7.5 % $CO_2$ and 150 rpm in shake flasks. NDV-GFP (Valneva) was used to infect the cells with addition of TrypLE (Thermo Fisher Scientific). Titres were determined by a $TCID_{50}$ assay using adherent Vero cells. Aiming for high virus titres, we screened and optimized most important production parameters as temperature, TrypLE concentration and multiplicity of infection (MOI). Moreover, maximum viable cell concentration (VCC) in semi-perfusion culture was determined by manual media replacement (cell-specific perfusion rate of 35 pL/(cell day).

**Results:**
Optimized production parameters resulted in NDV titres up to 1E09 TCID/mL. Decreasing temperature to 33°C after infection improved virus titre by one log. During semi-perfusion, a maximum VCC of 7.2E07 cells/mL was reached.

**Conclusion:**
Our studies demonstrate EB66 cells as promising cell factories for oncolytic NDV production in batch mode and high cell density semi-perfusion culture.

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# Exploiting time-delayed IL-21 supplementation in repeated-batch cultivation for controlled B cell expansion and differentiation

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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected**: B cells could become valuable assets in bioprocess technology, particularly due to their remarkable capability for rapid proliferation and subsequent production of large quantities of protein (e.g., antibodies).

**Impact/Novelty**: An innovative methodology is proposed, involving time-delayed IL-21 supplementation in repeated-batch cultivation, while demonstrating its efficacy in enhancing B cell survival and promoting controlled PC differentiation.

**Introduction**: Managing cell proliferation and directing differentiation of primary B cells towards specific phenotypes remains a challenge, but holds undeniable potential for biotechnological applications. A critical hurdle is the precise delivery of appropriate stimuli to the B cells at the appropriate time during differentiation, ideally without the need for feeder cells to achieve robust cell expansion. soluble ligands and cytokines, such as CD40L, IL-4, and differentiation-inducing IL-21, overcomes feeder cell limitations, thereby systematically advancing on-demand cell proliferation and differentiation.

**Methods/Approach**: After isolating human B cells from adult and juvenile tonsillar tissue, we investigated the effects of cultivation with and without IL-21 in both batch and repeated-batch cultures over 11 days (with medium changes on days 4, 8, and 11).In the repeated-batch approach, cells were continuously cultured with IL-21, while in the second approach, IL-21 was added on day 4 during the first medium change. Besides cellular expansion, differentiation and gene expression were analyzed through immunophenotyping and qPCR.

**Results**: Late IL-21 supplementation delays exponential growth and enhances cellular expansion irrespective of donor age. Immunophenotyping reveals a delayed but prolonged plasma cell burst and reduced early memory cell levels, with less pronounced donor-derived variations in juvenile cells.
**Conclusion**: This study highlights the benefits of using soluble factors to eliminate challenging feeder cells, emphasizing the regulation of PC differentiation through pre-culturing cells without initial IL-21 supplementation. The proposed methodology provides a promising approach for optimizing B cell cultivation in biotechnological applications.

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# Production of Adeno-associated virus containing pro-apoptotic TRAIL is enhanced by shRNA interference.

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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
An AAV which contains a cytotoxic gene can be used as a therapeutic vector for cancer. This work aimed to improve the production efficiency of AAV containing the cytotoxic gene TRAIL.

**Impact/Novelty:**
This work increased the producing efficiency of AAV containing the pro-apoptotic gene TRAIL. The system can be easily applied for producing AAVs with other cytotoxic genes for treating different types of cancer.

**Introduction:**
Adeno-associated virus (AAV) is a popular therapeutic gene vector for treating human genetic diseases. AAV can also be used to deliver a cytotoxic gene for treating cancer. For example, TNF-related apoptosis-inducing ligand (TRAIL) can bind to DR4 and DR5 and induce cell death in tumour cells. However, to produce AAV with cytotoxic genes can be challenging since these genes are also toxic to the producer cells, leading to cell death and low production yield.

**Methods/Approach:**
In this work, a novel AAV production system was created. A viral genome containing a transcriptional fusion of gfp and TRAIL was generated and cloned into the pAAV plasmid. Subsequently, an anti-gfp shRNA cassette was placed in the backbone of pAAV-gfp-TRAIL. We hypothesized that the anti-gfp shRNA expression would down-regulate gfp-TRAIL mRNA and prevent TRAIL expression in the production (HEK293) cells.

**Results:**
Firstly, compared to the control with scrambled shRNA, the presence of the anti-gfp shRNA increased the viability of producer cells and virus yield. Secondly, in HEK293 transfected with anti-gfp shRNA, GFP expression was significantly lower than the cells transfected with scrambled shRNA. Finally, there was significantly lower DR5 expression in anti-gfp shRNA transfected cells compared to scrambled shRNA transfected cells, which is a consequence of lower TRAIL expression.

**Conclusion:**
Altogether, the results show TRAIL expression decreased the producer cells viability and AAV production yield. The anti-gfp shRNA could restore HEK293 cells viability and production yield, though not to the wildtype pAAV-gfp level (no TRAIL present). The shRNA was designed to target gfp instead of TRAIL, as a simple read-out but also meaning the plasmid could be used for generating AAV containing other cytotoxic genes.

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# Context-dependent genomic locus effects on antibody production in recombinant CHO cells generated through random integration

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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:** Given the advent of controlled CHO cell line development via site-specific integration, an understanding of relationships between integration site and its cellular effects is crucial.

**Impact/Novelty:** We demonstrated limitations of current efforts to identify and implement hot spots and presented a perspective on site-specific integration based cell line development.

**Introduction:** Site-specific integration of a protein expression cassette into a hot spot is emerging as a promising stable cell line development strategy. Although positional effects are important for therapeutic protein expression, the sequence-specific mechanisms by which hotspots work are not well understood.

**Methods/Approach:** We performed whole-genome sequencing to identify randomly inserted vectors in the genome of mAb-producing rCHO cells, and validated these locations and vector compositions. We also analyzed the epigenetic features of the integration site and experimentally validated the site and sequence. Finally, we compared rCHO cells with host cells in terms of inherent production capacity.

**Results:** The mAb-producing rCHO cell line harbored multiple copies of light chain, and the genomic context was modified by chromosome rearrangements at the 3' transgene integration site and CHO contig-contig fusions. The integration site was characterized by active histone marks and potential enhancer activities, and CRISPR/Cas9 mediated indel mutations in the region upstream of the integration site led to a reduction in $q_{mab}$ by up to 30%. Notably, the integration site and its core region did not function equivalently outside the native genomic context, showing a minimal effect in the host cell line. We also observed a superior production capacity of the recombinant cell line compared to that of the host cell line.

**Conclusion:** Developing desirable rCHO cell lines requires a balance of factors including transgene configuration, genomic locus landscape, and host cell properties.

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# Sericin Reduces Glucose Consumption and Increases Cellular Population and Amount of Biologics

**Mr. Naoki Shimizu**[1], Mr. Yuya Murakami[1], Mr. Ryo Masugi[1], Dr. Satoshi Terada[1], Dr. Jun Takahashi[2]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected**: Higher glucose concentration leads to more glucose consumption of the cells. We found that sericin suppresses glucose consumption and so improves cell culture for biologics production.

**Impact/Novelty:** Sericin accelerates cellular growth and decreases glucose consumption, achieving a rich population of cells and their abundant biologics production. Sericin is a promising supplement for various cells producing biologics.

**Introduction:** We previously reported that sericin accelerates the growth rate of various cells and increases their integral viable cells (IVC). This improvement in the cellular population resulted in higher production of their biologics. In this study, we aimed to unveil how sericin enriches the population of the cell culture without harmful influence on biologics production.

**Methods/Approach:** Hybridoma and CHO cells were seeded to the media containing various concentrations of glucose. This media was prepared using serum- and glucose-free RPMI medium with ITS-X. Supplements of *Pure Sericin*$^{TM}$ (*FUJIFILM Wako, Japan*) or BSA were utilized as positive controls.

**Results:**
1. Hybridoma
In the absence of sericin, the specific glucose consumption rate was increased with the glucose concentration in a dose-dependent manner, as well as in the presence of BSA. In contrast, sericin strictly down-regulated cellular glucose consumption and maintained it at a low level, having no relation to the glucose concentration in the media. This efficient use of glucose improved the cell population, and the increased IVC with sericin directly contributed to elevating the MoAb production.
2. CHO
In CHO cell culture, sericin had a similar effect as in hybridoma cell culture. Sericin down-regulated glucose consumption while increasing the IVC and recombinant protein production.

**Conclusion:** Sericin would be a promising supplement for cell culture to decrease cellular glucose consumption, improve higher population, and enhance biologics production of various cells.

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# Improvement of the Biological Production Supplementing with SERICIN into Hyperosmotic Culture

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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:** Our findings contribute to enhancing the biological productivity of mammalian cell cultures. Hyperosmotic media supplemented with sericin could easily be applied to various cell culture systems.

**Impact/Novelty:** Hyperosmotic pressure accelerates the specific protein production rate but reduces cellular population and so fails to increase production. Sericin maintained population at a high level and successfully enhanced production.

**Introduction:** It has been reported that hyperosmotic pressure enhances the productivity of individual cells while suppressing cell proliferation. Due to the reduced population at elevated osmolality, no enhancement in the biological titer was made. For improved production in hyperosmotic culture, the cells were cultured in the presence of sericin, an accelerator of cell proliferation.

**Methods/Approach:** A hybridoma cell line 2E3-O, MoAb producer, was seeded to a serum-free RPMI medium. To the medium, various amounts of NaCl were supplemented to increase the osmotic pressure. Betaine, osmoprotective factor, and *Pure Sericin*$^{TM}$ (*FUJIFILM Wako, Japan*) were supplemented into the media in order to regulate hyperosmotic stress and to induce proliferation of the cells, respectively.

**Results:** The addition of 0.3 g/L of NaCl, the one-third concentration of a physiological saline solution, into the culture medium, increased osmotic pressure. Under the hyperosmotic stress, the specific MoAb production rate was doubled. However, the cellular population was harmfully affected. In addition, the integral viable cells of the culture were reduced by half, indicating no improvement in the production. In the presence of sericin, hyperosmotic culture accelerated productivity without a bad influence on the cellular population, so it successfully improved MoAb production.

**Conclusion:** Results obtained here indicate that the mitogenic effect of sericin contributes to an abundant cellular population under hyperosmotic pressure. Supplementation with sericin into hyperosmolar media is a promising means to improve biological production.

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# Finding the Needle in the HEKstack - Media Screening for AAV Production in HEK293

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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:** Manufacturing processes are complex. The development of a lean culture medium will reduce complexity, lower costs, and allow robust production.

**Impact/Novelty:** In consideration of rising demand, the simplification of production processes is essential. Lean media formulations are part of the solution.

**Introduction:** Adeno-associated viruses (AAV) evolved as the leading platform for *in vivo* gene therapy. To date, transient transfection of HEK293 cells is state-of-the art. Media optimized for AAV production must primarily support viral genome replication and capsid protein expression. Defining a minimal set of components allowing for transient transfection, high growth rates, and high AAV titer will help to lower cost, reduce raw material variance and complexity as well as simplifies sourcing of raw materials.

**Methods/Approach:** Based on previous knowledge from media development, a minimal medium was produced. Left out components were supplemented and cell growth of a commercially available HEK293 cell line was monitored at shake flask scale. To further refine the initial medium, a design of experiment (DoE) was conducted using MODDE® in shake flask scale. Media variants supporting stable cell growth were tested for AAV production capacity by transient transfection with FectoVir®-AAV. Additionally, potential enhancing components were included based on AAV biology. Finally, the applicability of the developed media variants in a regulated system was tested with the Ambr15®.

**Results:** The first minimal medium variant did not allow stable growth of HEK293 cells. Also, rational supplementation was not successful in reducing the number of media components. By using a DoE approach, a group of essential vitamins was identified and 3 out of 15 media variants were chosen for subsequent AAV production. Higher titres were achieved with one variant than with the reference medium. It was used as a basis for further improvement by addition of components supporting AAV production, which led to a 5-fold increase in genomic titer compared to the reference.

**Conclusion:** The combination of a DoE approach with rational media design enabled the development of a lean HEK medium for stable cell growth and high AAV titers.

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# A CHO cell-based simultaneous display and secretion platform for accelerated bispecific antibody development

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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to the theme**
We have introduced a platform developed for the efficient development and production of bispecific antibodies (BsAbs) in CHO cell factories.

**Impact**
This platform overcomes key challenges associated with BsAb development and significantly improve the efficiency of the BsAb development process.

**Introduction**
BsAbs are a class of therapeutic molecules designed to simultaneously bind to two distinct targets. The conventional process of BsAb development starts with characterization of two sets of monoclonal antibodies (mAbs) specific to different antigens, followed by engineering BsAb variants that merge antigen-binding domains from these mAbs through subunit heterodimerization. Despite starting with pre-characterized binders, the integration into functional bsAbs proves intricate due to the complex interplay between bsAb formats and functionality. A universal design principle is lacking, necessitating technologies that enable high-throughput, in-format screening of BsAbs for functionality and manufacturability.

**Approach**
We have developed a CHO cell-based platform which allows simultaneous display and secretion of BsAb libraries. This platform comprises three key components: 1) a CHO master clone containing two single copy landing pads integrated into two genomic sites that provide stable and high-level gene expression, 2) two targeting plasmid vectors enabling the simultaneous display and secretion of two different antibodies, 3) helper vectors expressing recombinases or Cas9/sgRNA for enhancing the efficiency of targeted integration of the two targeting vectors into their corresponding land pads at controlled ratios.

**Results**
Our platform enables high-throughput screening to identify BsAbs with desirable binding affinities to their corresponding antigens. Identified CHO cells presenting promising BsAbs can be directly employed as production cell lines, streamlining material production for further developability assessment and functional studies. As a successful application of this platform, we engineered the CD3 targeting moiety in a T cell engaging BsAb, enhancing both function and manufacturability.

**Conclusion**
In conclusion, our CHO cell-based platform, enabling simultaneous display and secretion, streamlines the BsAb development process, resulting in significant time and cost savings

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# IDENTIFICATION OF TRANSGENE INTEGRATION SITES, THEIR STRUCTURE AND EPIGENETIC STATUS WITH CAS9-TARGETED NANOPORE SEQUENCING

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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
The presented topic is an important technique to aid in the characterization of recombinant cell lines or cell factories.

**Impact/Novelty:**
nCats allows to precisely identify multiple, large transgene insertions surpassing the informative value of traditional approaches. The fast turnaround time allows to identify suitable candidate cell lines in less time.

**Introduction:**
The number and sites of integration largely determine transgene expression levels in recombinant CHO cells and are therefore important characteristics for the assessment of cell lines. However, the accuracy and versatility of traditional methods for quantification are limited. In order to fully characterize transgene integrations, we have adopted a fast and accurate method to detect integration sites, their structure and epigenetic status using Cas9-targeted nanopore sequencing (nCATS) that allows targeted, directional sequencing of long, native DNA fragments and simultaneously provides information about the DNA methylation status.

**Methods/Approach:**
For nCATS applications, isolated and dephosphorylated DNA is subjected to CRISPR/Cas9 mediated cleavage with crRNAs targeting genomic loci of interest. Generation of DNA ends at the induced double strand breaks allows site-specific ligation of sequencing adapters and enriched sequencing of target sites. For proof of concept, we performed nCATS by targeting the Fut8 promoter in a CHO-K1 cell line after epigenetic modulation. Next, we identified integration sites and their structure in two recombinant CHO-K1 cell lines that were previously analyzed by conventional TLA-sequencing.

**Results:**
Sequencing of the Fut8 promoter resulted in high coverage and correct identification of the targeted region. Additionally, the DNA methylation status was accurately assessed. In the recombinant cell lines, reads spanning over exogenous and endogenous regions were used to precisely determine transgene integration sites. In addition to the integration sites reported by TLA sequencing, a third integration site was detected by nCATS. Furthermore, the conformation of the integrated sequence was disclosed identifying a plasmid concatemer of more than 21 kb, which could not be resolved previously. The method was adjusted to also work with the newest R10 nanopore flow cell chemistry.

**Conclusion:**
nCATS is a fast, versatile and affordable tool to determine integration sites in recombinant cell lines and offers the advantage to simultaneously study epigenetic modifications of the targeted sequence.

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# Biomass specific perfusion rate as a control lever for the continuous manufacturing of biosimilar antibodies

**Dr Eunice Leong**[1], Ms Wen Qin Tang[1], Mr Jake Chng[2], Ms Lyn Chiin Sim[1], Ms Zi Ying Zheng[1], Dr Wei Zhang[1], Dr Ian Walsh[1], Dr Gerben Zijlstra[3], Mr Maarten Pennings[2], Dr Say Kong Ng[1]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:**
We are reporting the use of biomass specific perfusion rate (BSPR) to reduce the process economics for continuous manufacturing. Overall, lower BSPR maximized culture media efficiency and antibody production.

**Impact/Novelty:**
BSPR could be a relevant control parameter for cells that grow in volume during the production phase. The efficient growth arrest led to reaching higher specific productivities earlier.

**Introduction:**
Continuous manufacturing enables high volumetric productivities of biologics. However, it is challenging for perfusion culture to be economically viable without high specific productivities. During the production phase, cells were observed to increase in cell diameter. This led to the hypothesis that BSPR could be a relevant control parameter for such cells. This study explored the effect of increasing perfusion rate proportionally to increasing cell volume at three BSPRs.

**Methods/Approach:**
In this study, six bioreactor vessels were set up on Sartorius Ambr250 to run the perfusion cultures in parallel. The three BSPRs in the production phase were 6.5, 8 and 12 mL/cm$^3$/day, running in duplicates. Two glucose set points were also experimented to evaluate the strength of its influence under these process conditions.

**Results:**
The three perfusion rates modelled conditions with low, medium, and high concentrations of nutrients. We observed that the BSPRs influenced the viability, cell diameter, specific productivities, lactate and ammonium concentrations.
In the presence of high concentration of nutrients, cells reproduced quickly and maintained relatively high viabilities and similar cell volumes. In contrast, in the presence of lower concentration of nutrients, cell growth slowed down, viabilities declined gradually while cell diameters increased significantly (31.5% increase compared to the diameter on the day of inoculation).
On day 14, cells in bioreactors with low BSPR had specific productivities 3 times that of cells in bioreactors with high BSPR. There were minimal differences in the charge variants and impurities in the product collected on day 8 and day 14.
Noticeable, there was lower lactate production but higher ammonium accumulation with low BSPR. At the same time, the glucose concentrations between 2.5 g/L and 6 g/L had limited influence on the cells and product quality.

**Conclusion:**
BSPR influenced cellular growth rate and, consequently, productivity. More efficient media utilization could be achieved by reaching high productivities earlier.

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# A two-dimensional model to predict influenza virus and defective interfering particle coinfection in tissue-like systems

**Dr Daniel Rüdiger**[1], Ms. Patricia Opitz[2], Dr. Sascha Young Kupke[1], Prof. Udo Reichl[1,2]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
Defective interfering particles (DIPs), which can be produced efficiently in animal cell culture**,** are promising candidates for antiviral therapy based on their efficacy against various virus species.

**Impact/Novelty:**
We combine sophisticated experimental and mathematical modeling approaches to support the advancement towards the application of DIPs for treatment of viral diseases in humans.

**Introduction:**
DIPs are mutated, replication-incompetent viruses that prey on their corresponding standard virus (STV) for viral resources to facilitate their own propagation. In recent years, the potency of DIPs derived from influenza A virus against various virus species has been demonstrated in cell culture and mouse experiments, supporting their potential application as a broad-spectrum antiviral.

**Methods/Approach:**
To improve our understanding of the mechanistic details of DIP interference, we have developed a two-dimensional model of STV and DIP coinfection in tissues focusing on the spatial dynamics of virus spread and the innate immune response. We calibrated the kinetic rates of virus spread and clearance based on infection experiments performed with interferon (IFN)-competent human lung cells. To that end, we measured coinfection dynamics, the two-dimensional spread of STVs and DIPs, virus-induced IFN production and its suppressive effect on the infection.

**Results:**
Our model simulations closely reproduce the observed patterns of STV and DIP spread in a coinfection scenario. We conclude that the prevention of STV spread does not dependent on the STV-to-DIP ratio, but rather on the cell-to-DIP ratio. Furthermore, model simulations indicate that DIPs have to be administered before or shortly after STV infection to protect cells, because induction of the IFN response is crucial to achieve a potent suppressive effect.

**Conclusion:**
We developed a two-dimensional data-driven model of STV and DIP coinfection that describes spread in tissues taking into account the virus-induced innate immune response, which supports the development of antiviral treatment strategies.

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# Adeno-associated virus production intensification by continuous bioprocess with repeated transfections in high cell-density perfusion bioreactors

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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:1. Cells as factories and therapies**
The current study focuses on transient transfection of Human Embryonic Kidney cells, HEK293, for the production of recombinant adeno-associated virus (rAAV) for gene therapy applications.

**Impact/Novelty:**
The present process intensification approach is a combination of high cell density (HCD) transfection at ≥ 20 million cells per mL (MVC/mL) and prolonged production phase of rAAV thanks to repeated transfections in perfusion stirred-tank bioreactors.

**Introduction:**
rAAV vectors are widely used for many gene therapies. The fast-growing worldwide demand for these therapies presents a major challenge for the rAAV viral vector manufacturing capacity. A prevailing manufacturing method is based on co-transfection of three plasmids pAAV-RC (viral replication and capsid genes for AAV), pHelper (adenovirus gene products required for the production of infective AAV), and pAAV (AAV inverted terminal repeats with the gene of interest) into HEK293 cells with harvest 48-72 hours post transfection (hpT).

**Methods/Approach:**
In this study, repeated triple plasmids co-transfections were performed in HCD (≥ 20 MVC/mL) perfusion cultures of suspension HEK293F-derived cells in stirred tank bioreactors at 250 mL scale for the production of GFP. The rAAV serotype 9 production process was monitored by the transfection efficiency and the quantification of the produced viral capsids (ELISA) as well as the viral genomes (qPCR).

**Results:**
The application of repeated HCD transfection in a perfusion culture ≥20 MVC/mL evidently prolonged the production phase of rAAV beyond 72 hpT with a sustained rAAV productivity per cell.

**Conclusion:**
The successful repeated HCD transfections in bioreactors demonstrated a cost-effective and efficient approach compared to existing rAAV manufacturing processes and paved the way to continuous biomanufacturing of rAAV.

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# A high-throughput screen identified microRNAs to increase AAV production in HEK293 suspension cells

**Ms Madina Burkhart**[1], Ms. Bettina Finkbeiner[2], Mr. Jona Röscheise[1], Dr. Andreas Schulze[2], Ms. Sabrina Reichl[2], Dr. Florian Sonntag[2], Dr. Markus Hörer[2], Prof. Dr. Kerstin Otte[1]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:**
Eukaryotic cells are the workhorse for the production of viral gene therapy vectors such as adeno-associated viruses (AAVs). The present work aimed to significantly increase AAV productivity of HEK293 suspension cells.

**Impact/Novelty:**
A high-throughput screen successfully identified microRNAs inducing an increased AAV yield after overexpression in HEK293 cells used in Ascends proprietary manufacturing platform.

**Introduction:**
AAV vectors have attracted significant attention for gene therapy application because of attributes such as comparably low immunogenicity and potential for long-term gene expression without vector integration. In order to bring AAV gene therapy to more common diseases and broader patient populations, AAV vector demand is significantly increasing in recent years. MicroRNAs as non-coding regulatory molecules have previously been developed as engineering tools to improve cellular productivity for protein therapeutics. Therefore, we developed a novel high-throughput screening platform to identify microRNAs that have the potential to boost AAV productivity of mammalian cells to meet therapeutic demand.

**Methods/Approach:**
We developed a screening process combining microRNA transfection, AAV production and functional cell-based read-out. Using this platform, a genome-wide microRNA mimic screen was conducted.

**Results:**
We present comprehensive data on the establishment of the complex screening platform including relevant controls. Next, the screening of 2’443 microRNAs for their impact on AAV production is presented with the identification of 160 microRNAs increasing AAV yield by means of a higher fold change compared to control. In addition, 1’044 microRNAs decreasing AAV yields were identified. Reproducibility of the observed positive effects was confirmed for 63 of the top microRNAs identified. Selected microRNAs are currently validated by a multi-parameter microbioreactor system.

**Conclusion:**
In conclusion, this high-throughput screen is the first comprehensive effort to establish microRNAs as tools for a disruptive increase of AAV production to resolve existing manufacturing bottlenecks for the application of AAV for gene therapy.

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# Establishment of a suspension-based perfusion process for the effective production of lentiviruses

**Miss Marta Arrizabalaga Cascallana**[1,2], Mr. Maximilian Klimpel[2], Dr. Stephen Goldrick[1], Dr. Holger Laux[2], Prof. Qasim Rafiq[1]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected.**
Investigation of lentivirus manufacturing in perfusion mode for viral-based gene therapies.

**Impact/Novelty.**
Evaluation and optimization of novel media and compound additions in lentivirus perfusion culture for enhanced process performance.

**Introduction**
There is an increasing demand for high-quality, high-producing lentiviral vector (LVV) processes. Process intensification has the potential of addressing these requirements, allowing for faster processing times, enhanced vector quality and reduced process costs. However, its establishment remains a challenge and is largely understudied. This project aims to optimise LVV production through the investigation of perfusion and establishing a scalable suspension platform.

**Methods/Approach**
A high-productivity, inducible producer HEK293T-based cell line for LVV production, adapted to serum-free, suspension growth will serve as the basis for our platform. Small scale studies were conducted in shake flasks and in the ambr15® system in pseudo-perfusion process by daily centrifugation for a total of 10-12 days. Infectious titre was determined by flow cytometry.

**Results**
This piece of work focuses on the identification of a favourable metabolic state and bioreactor parameter optimization. To achieve this, several experiments in small scale were undertaken. Media studies and some bioreactor parameters were investigated to identify the optimal conditions to support a high-viability and rapid cell growth, enhance virus stability and improve process performance. The second piece of work evaluated the impact of two sets of compounds with the goal of increasing process productivity and viral stability. These include Viral Sensitizers (VSEs), designed to prevent high levels of viral auto-transduction, and recombinant albumin. Finally, virus kinetic studies and process characterization with the identified optimal conditions were completed before scaling-up to the 5L-scale in perfusion.

**Conclusion**
Perfusion development for LVVs can be accomplished in small scale platforms and improve process performance with media and supplement studies.

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# Evaluation of biofabrication of 3D cellularized constructs for monoclonal antibody production

**Mr Elliott Cowles**[1], Dr Laura Chastagnier[1], Dr Julia Niemann[2], Dr Magali Barbaroux[3], Dr David Pollard[4], Dr Oscar Reif[2], Dr Christophe Marquette[1], Dr Emma Petiot[1]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:** Cells as factories _This study relies on CHO (Chinese Hamster Ovary) cells cultivated in 3D. It aims to evaluate their biosynthetic activity and potential to produce monoclonal antibodies.

**Impact/Novelty:** Envision biofabrication thanks to 3D bioprinting to enhance CHO-specific productivity.
**Introduction:** The contemporary landscape of monoclonal antibody manufacturing revolves around suspension cell culture within bioreactor setups, reaching capacities of 10,000 litres and yielding 1-10 g/L. Recent decades have seen a surge in process intensification, demanding cell densification steps and intricate feeding strategies. Growth arrest can be crucial in these strategies and significantly enhance cell-specific productivity. In the context of 3D bioprinted proliferative hydrogels, cell growth performance is commonly altered. This study focuses on assessing the impact of this altered growth environment on CHO cell line biosynthetic activity and its potential to produce monoclonal antibodies (Mabs).

**Methods/Approach:** The experimental protocol entailed seeding CHO DG44 cells encapsulated inside a proprietary biocompatible hydrogel as 3D-bioprinted slabs. Cultivation unfolded under diverse feeding strategies, utilising different media, including a serum-containing medium (DMEM/FCS), XtraCHO Stock & Adaptation Medium (SAM), and XtraCHO Production Medium (PM). Over 14 days, cultivation monitoring encompassed cell growth rates, metabolic activities, and Mabs production. A printed structure 10$cm^3$ associated with a dedicated bioreactor culture process was developed and compared to the standard CHO suspension culture process in an AmbR 250ml HT Modular bioreactor (Sartorius).

**Results:** CHO growth rates in 3D constructs were less than 0.05 $days^{-1}$, 14-fold lower than in reference suspension cultures. Such slow growth was associated with CHO cell interaction with their 3D environment. However, the performance of 3D culture production was process-and-medium dependent. Notably, constructs sequentially cultivated in SAM for amplification and then in PM for fed-batch production exhibited a notable 2.15-fold increase in cell-specific productivity compared to suspension cells.

**Conclusion:** CHO cells encapsulated in 3D structures exhibited restrained growth. While overall antibody production in 3D lagged due to reduced cell concentrations compared to traditional cultures, specific productivity increased under specific conditions. This underscores the potential for exploring novel bioprocesses that provide controlled cell proliferation and enhance productivity.

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# Optimizing defective interfering particles of influenza virus for antiviral treatment

**Ms Tanya Dogra**[1], Lars Pelz[1], Daniel Ruediger[1], Julia Boehme[2], Maike Baelkner[2,3], Olivia Kershaw[4], Yvonne Genzel[1], Dunja Bruder[2,3], Sascha Young Kupke[1], Udo Reichl[1,5]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:**
Our research focuses on using engineered MDCK cells and plasmid-based reverse genetics to produce defective interfering particles (DIPs) of influenza A virus (IAV) for antiviral therapy without infectious helper viruses.

**Impact/Novelty:**
IAV-derived DIPs are regarded as promising new antivirals. With modified reverse genetics, we generate new DIP constructs with enhanced and optimized antiviral activity.

**Introduction:**
Conventional DIPs contain internal deletions in one of their eight viral RNA (vRNA) segments, e.g. Segment 1 (S1) DIPs. Recently, our group discovered “OP7”, entailing nucleotide substitutions in S7 vRNA [1]. During co-infections, DIPs suppress standard virus (STV) production by replicating faster and inducing innate immunity.

**Methods/Approach:**
To identify new deletions in S1 vRNA, we used next-generation sequencing on samples from long-term infection cultures with DIPs and STV [2]. Using eight plasmids and PB2-expressing cells (protein missing in S1 DIPs) [3], we rescued new S1 DIP candidates. In the following study, we further improved S1 DIP constructs and added a ninth plasmid (encoding S7-OP7) in the reconstitution scheme to generate OP7 chimera DIPs [4].

**Results:**
*In vitro,* our S1 DIP candidates showed stronger antiviral efficacy than well-known DI244 [2]. Regarding OP7 chimera DIPs, suspension cell-based production resulted in 99.7% in OP7 chimera DIPs, which demonstrated strong antiviral activity in human lung cells. In mice, it led to 100% survival against a lethal STV infection without any disease symptoms.

**Conclusion:**
Newly established DIP constructs are promising antiviral candidates that strongly suppress STV replication. In future, we will generate DIPs that express surface antigens of seasonal IAV strains to facilitate their use as live-attenuated vaccines. All DIP preparations could be administered via a nasal spray.

[1] Kupke *et al.* (2019) *J Virol*
[2] Pelz *et al*. (2021) *J Virol*
[3] Bdeir *et al.* (2019) *PLoS ONE*
[4] Dogra *et al.* (2023) *Sci Rep*

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# Increased energy production in producing cells to satisfy demands for growth, productivity and maintenance energy

**Ms Jerneja Štor**[1,2], Dr. nat. techn. David Ruckerbauer[1,3], PhD Diana Széliová[3], Dr. Sarah Sacco[4], Prof. Jamey Young[4], Ao. Univ.Prof. Dr. Nicole Borth[1,2], Univ.-Prof. DI Dr. Jürgen Zanghellini[3]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
For generation of high producing cell lines it is important to understand the underlying cellular metabolism, resource distribution and energy production to unlock new targets to increase cellular productivity.

**Impact/Novelty:**
We took a new approach in maintenance energy (ME) determination. While calculations for growth phase are comparable with previous reports, ME during non-growth phase is suggested to be significantly lower.

**Introduction:**
Producing cells are expected to have higher energy production due to additional demand for protein synthesis resulting in higher fluxes through the TCA cycle, mainly responsible for NADH production.

**Methods/Approach:**
Carbon-13 metabolic flux analysis was used to establish small scale metabolic models and identify metabolic differences (i) between a mAb-producing and a non-producing *Chinese Hamster Ovary* cell line and (ii) between exponential and stationary phase of a fed-batch.

**Results:**
Higher exchange rates and intracellular fluxes were observed for the producer in exponential growth. Also, the fluxes that generate energy during glycolysis, TCA cycle and amino acid metabolism exhibit a generally higher production of energy molecules (NADH, ATP, GTP and $FADH_2$). Due to a significant decrease in metabolic activity and growth in stationary phase, we observed significantly decreased energy production, yet due to lower specific productivity, the differences between the cell lines were insignificant. Despite higher energy production, ME was similar between cell lines, however ME in stationary phase was significantly lower.

**Conclusion:**
High production phenotypes are required to generate more energy to satisfy demands for biomass, product and ME. Observed lower ME in stationary phase suggests either that the required ME here is lower compared to exponential phase or that the available energy is not sufficient to satisfy the ME demand during stationary phase, which ultimately leads to cell death.

No conflicts to declare.

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# Understanding the Mechanism of Intracellular Multispecific Antibody Aggregation in CHO Cells

**Dr Lauren Kraft**[1], Dr. Hillary Warrington[1], Thomas Kelly[1]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:** We are describing and solving emerging issues in expressing the modality of multispecific antibodies, specifically molecule design and the common product quality issue of aggregation.

**Impact/Novelty:** Aggregation of multispecific antibodies is a prevalent issue that has not been addressed. Our characterization sheds light on mechanism and potential solutions that could benefit the industry.

**Introduction:** As the pharmaceutical industry moves toward producing complex multispecific molecules, emerging product quality liabilities need to be characterized and mitigated to ensure the industry can meet the needs of patients. Protein aggregation during the expression of multispecific molecules is a chronic problem that lowers yields of active protein, adds to the complexity of purification, and raises the cost of goods. One basic question that is still not clearly resolved is whether the aggregates are formed within or outside of cells.

**Method/Approach:** In order to address the issue of biotherapeutic aggregation during cell culture by engineering of a CHO host cell, we first sought to understand when, where, and how these aggregates are forming. We developed an innovative cell biology and mass spectrometry-based workflow to separate, isolate, and analyze intracellular and secreted biotherapeutics over time.

**Results:** We developed a robust workflow, then optimized and applied it to several molecules using similar cell culture parameters to create a broad understanding of the issue of aggregation in our platform process. We monitored intracellular and secreted biotherapeutic product and related species over the course of a bioreactor run, representing a variety of cell growth and production phases. We detected aggregates in both the intracellular and supernatant samples. This suggests that the aggregates can form in the cells.
**Conclusion:** This multidisciplinary study utilizes cell biology, molecular biology, and mass spectroscopy techniques to monitor the temporal dynamics and spatial localization of aggregate formation in cells and describe an emerging issue in the pharmaceutical industry.

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# Expanding the Apollo™X Cell Line Development toolbox: Strategies for challenging modalities

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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:**
This study explores the development of process strategies to overcome complexities faced in multi-specific antibody cell line development (CLD).

**Impact/Novelty:**
Development of multi-chain vectors and high-throughput transfection approaches enabled the selection of transfectant pools with high productivity and target molecule purity.

**Introduction:**
With the biotherapeutic market gearing towards the production of novel modalities, new challenges await Chinese hamster ovary (CHO)-based CLD. Multi-specific antibodies present complications due to the requirement of efficient multi-chain expression. This poses challenges for the expression system, cell line selection strategy, analytical characterisation and downstream purification. High levels of productivity are often driven by optimised vector designs that achieve delicately balanced chain expression. Additionally, consideration of product purity earlier in the upstream process is paramount since product related impurities are common with these complex proteins.

This study investigates the holistic approach required to develop a bespoke CLD strategy for multi-specifics.

**Methods/Approach:**
Vector Development: Quad and double gene vectors with differing orientations encoding for a 4-chain bispecific antibody (BsAb) were created.
Transfections: Apollo™X CHO host underwent high-throughput transfections using the Amaxa® Nucleofector® technology. Various DNA amounts and selection pressure concentrations were tested.
Fed-batch culture: Resultant transfectant pools were tested for production and purity performance in a 14-day fed-batch process.

**Results:**
Multiple transfection conditions were trialled with either single vector transfection or co-transfection of two vectors. Optimisation of vector combinations and transfection conditions resulted in a 3-fold BsAb titre improvement in transfectant pools. Additionally, high levels of target molecule purity were obtained.

**Conclusion:**
Multi-specific antibodies are inherently complex due to their structure and physiochemical properties. Due to this, a ‘one-size-fits-all’ approach should not be used to produce this class of therapeutics. This study demonstrated that the deployment of a ‘molecular and transfection’ toolbox early in the upstream process can generate optimal transfectant pools that can form the basis of a successful CLD campaign.

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# Effect of AAV gene expression in the transcriptome of CHO cells

**Ms Konstantina Tzimou**[1], Prof Lars K. Nielsen[1], Dr Jesús Lavado-García[1]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:** Studying CHO responses upon AAV gene expression provides insights into the system, and limitations in rAAV production. Addressing such constraints could transform CHO cells into a novel rAAV production platform.

**Impact/Novelty:** CHO cells are widely used in biopharmaceutical production. However, limited studies explore CHO cells as rAAV production host. This is the first study investigating CHO responses to AAV gene expression.

**Introduction:** Among the various gene delivery systems available for gene therapy, recombinant adeno-associated virus (rAAV) vectors have gained attention due to their favourable safety profile and high efficiency in gene delivery. One major challenge in the field of rAAV-based gene therapies is the limited production yields, preventing their manufacturing at large-scale. While the well-studied HEK293 and Sf9 are the most common production platforms, due to the limitations mentioned above, there is high interest for exploring alternative production hosts, such as CHO cells.

**Methods/Approach:** AAV genes are organized in an overlapping manner within the AAV genome, maximizing its limited genetic capacity. In this work, AAV gene sequences were engineered and plasmids expressing single AAV proteins were constructed. The effect on the transcriptome caused by the expression the different AAV proteins in CHO cells was studied, by differential expression analysis using Kallisto and Sleuth.

**Results:** The results of this study demonstrate that most differentially-expressed proteins are involved in various pathways such as extracellular matrix organization, cell-to-cell signalling or RNA processing. Additionally, our data pointed towards novel candidate genes and potential targets for cell engineering.

**Conclusion:** Our findings provide an insight into the complex interplay between AAV proteins and cellular responses in CHO cells, enhancing the understanding of CHO cells as rAAV producers and shedding light on potential optimization strategies for enhancing rAAV production yields.

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# N-1 perfusion process for intensification of AAV production

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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Cells as factories**
Process intensification in the field of gene therapy and manufacturing of viral vectors.

**Impact/Novelty:**
Perfusion process of HEK293 cells to optimize adeno-associated virus (AAV) production.

**Introduction:**
The interest in genomic medicine has increased significantly over the past few years as the demand for gene therapy is high. As the field is transitioning into generic diseases rather than only rare diseases, the need for viral vectors such as AAV continues to grow. The upstream process is still one of the bottlenecks of AAV manufacturing with long and tedious cell expansion steps. To increase the efficacy of viral vector production, perfusion can be used in the N-1 step to intensify the seed train.

**Methods/Approach:**
We have developed a scale-down perfusion process at 1.5-L scale. The process was based on our ready-to-use HyClone™ peak expression medium supplemented with glucose and glutamine to support a CSPR of 80 pL/cell/day and cell concentrations between 30 and
35 MVC/mL. We scaled up the process to 50 L using Xcellerex™ automated perfusion system (APS) and used N-1 intensification to generate a high-cell density inoculum for a 2000 L production bioreactor.

**Results:**
We achieved viable cell concentrations of up to 35 MVC/mL at a CSPR value of 80 pL/c/d at both 1.5-L and 50-L scales. These results make high-density perfusion processes suitable for inoculum preparation (N-1) of HEK293 cells for production in bioreactors up to 2000 L in scale. Consistent performance with respect to cell growth, cell viability, and volumetric productivity was achieved in our process.

**Conclusion:**
We show the development of a bench-scale (1.5-L working volume) proof of concept model and an N-1 perfusion process at the 50-L scale with comparable cell growth, metabolic profiles, and volumetric productivity.

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# On-demand hydrogel microcarrier production by ink-jet bioprinting for stem **cell scalable suspension culture**

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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:**
**Bioproduction of MSCs-loaded dissolvable hydrogel microbeads with Ink-jet bioprinting for potentially increase amplification yield.**

**Impact/Novelty:**
**Novel on-demand production of dissolvable microcarriers with ink-jet bioprinting.**

**Introduction:**
Current large-scale Mesenchymal Stem/Stromal Cells (MSCs) production primarily relies on microcarriers in suspension bioreactors, facing limitations such as cell source constraints, cell-microcarrier interaction challenges, and a lack of an easily manageable, transferable, and scalable process. In our study, we used a bioink developed by our group with inkjet technology to create MSCs-laden hydrogel microcarriers. Our main hypothesis is that employing inkjet bioprinting will enable the production of micrometric hydrogel microbeads, offering both outer surface and inner structure for enhanced cell expansion, thereby facilitating greater amplification potential.

**Methods/Approach:**
A bioink formulation of fibrinogen, alginate, and gelatine was used to produce microbeads with a PICO Pulse jet valve system (Nordson), seeded with human adipose-derived MSCs into a crosslinking bath of thrombin, $Ca^{+2}$ and transglutaminase. The microbeads were characterized by measuring their size, cell density, cell viability, and stemness markers.

**Results:**
Understanding bioink rheology and MSCs enabled the customization of an in-house bioink for maintaining high cell viability during inkjet bioprinting. Despite initial cell viability challenges due to inkjet stress, our approach fosters long-term cultivation with MSCs exhibiting robust growth and sustained viability. Size and cell amplification factors align with those of commercial microcarriers, and the microbeads can be dissolved with a high cell recovery yield while retaining their capabilities. This scalable production strategy was successfully evaluated for various MSC types, with long-term conservation of stemness markers confirmed. As a scalability demonstration, cell-loaded microbeads were cultured in 25 mL Erlenmeyers.

**Conclusion:**
These results validates the proof-of-concept of an on-demand stem cell microcarrier bioproduction procedure by 3D ink-jet bioprinting.

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# Engineering and Production of a Novel Cytokine Trap for the Treatment of Canine Atopic Dermatitis

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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
This research focuses on protein engineering and process development to create a novel biotherapeutic using fusion protein technology.

**Impact/Novelty:**
This study aims to design and produce a cytokine trap with enhanced biological activity for canine atopic dermatitis treatment.

**Introduction:**
Interleukin 31 (IL-31) is pivotal in atopic dermatitis pathogenesis, inducing pruritus, disrupting the epithelial barrier, and promoting inflammation. Soluble receptors can serve as cytokine blockers, but they face limitations due to their short half-lives and reduced affinity. This is a result of the intricate binding between cytokines and receptors, which involves multiple receptors. Cytokine traps, fusion proteins comprising receptor cytokine binding domains (CBD) and IgG constant regions (Fc), overcome these limitations through dimerization and the utilization of CBDs from multiple receptors, extending half-life and enhancing affinity.

**Methods/Approach:**
Two fusion proteins (R-Fc and R-His) were designed using CBDs from canine IL-31 receptors (IL-31Rα and OSMR). For R-Fc, the Fc region of canine IgG was incorporated, while R-his was linked to a histidine tag. Cell lines were generated through lentiviral transduction of CHO-K1 cells, followed by clonal selection. Cells were adapted to suspension growth in a serum-free medium and cultured in batch mode. Purification of R-Fc and R-His was accomplished using protein A chromatography and immobilized metal affinity chromatography (IMAC), respectively. Purity and identity were confirmed through SDS-PAGE Coomassie staining and Western blot analysis.

**Results:**
R-Fc, and R-his were designed with distinctive structural features. Stable cell lines were generated, ensuring high production levels. After a single purification procedure, high purity levels were achieved for both proteins. R-his had a molecular weight of 100 kDa, while R-Fc, due to its dimerization capacity, exhibited a higher molecular weight of 300 kDa.

**Conclusion:**
This comprehensive methodology establishes a robust foundation for efficient cytokine trap design and production, representing a notable advancement in the quest for effective therapies for canine atopic dermatitis.

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# Unlocking DOE potential selecting the most appropriate design for rAAV optimization.

**Dr Jesús Lavado-García**[1], Mr. David Catalán-Tatjer[1], Ms. Konstantina Tzimou[1], Prof. Lars K. Nielsen[1]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:** The use of recombinant adeno-associated viruses (rAAVs) in gene therapy is currently rising in popularity, leading to a substantial increase in research on how to produce rAAVs more efficiently.

**Impact/Novelty:** This works compares four different DOE-based approaches and how efficiently these models can identify interactions and optimal solutions for crucial parameters like the ratios between plasmids and transfection reagent.

**Introduction:** The more than 2,000 gene therapies currently in active development have evidenced the need to improve their manufacturing technologies and yields and reduce costs. For rAAV production, optimization of plasmids and transfection reagent ratios is required for every new gene of interest (GOI) and serotype. Therefore, given the increasing number of therapies using rAAVs, a systematic method for fast optimization is crucial. Within the different design of experiments (DOE) approaches, we have compared rotatable central composite design (RCCD), face-centered composite design (FCCD), Box-Behnken design (BBD) and mixture design (MD) to analyze their ability to predict interactions, optimize plasmid and transfection reagent ratios and maximize rAAV productivity and culture viability.

**Methods/Approach:** Blocking was performed for 4-factors RCCD, FCCD and BB designs to reduce uncontrolled variability, mainly coming from the qPCR quantification step. Since blocking was treated as a random effect, a mixed model and the reduced maximum likelihood (REML) algorithm were used to analyze these designs. D-optimal MD was used to optimize plasmid ratio followed by an unblocked 2-factors CCD to optimize transfection reagent ratio.

**Results:** RCCD, FCCD, BBD, and MD approaches led to different optimal solutions. Blocking proved to be essential, representing >50% of the total model variability. MD led to optimization with fewer runs.

**Conclusion:** Blocking should be performed. FCCD and BBD are more time-cost effective, but MD presents advantages when analyzing interactions of plasmids.

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# Impact of MOI and Baculovirus Ratio on rAAV Production with the Baculovirus Expression Vector System

**Mr Mels Schrama**[1], Pranav Puri[2], Femke Hoeksema[2], prof.dr. Monique van Oers[1], prof.dr.ir. Gorben Pijlman[1], prof.dr.ir. Rene Wijffels[1], dr.ir. Dirk Martens[1]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to theme 1:** This research offers important insights into how specific process parameters influence the quality and quantity of viral gene therapies produced using insect cell factories.

**Novelty:** This study is among the first to examine the impact of both total MOI and the baculovirus ratio in the production of rAAVs with the Two-Bac BEVS.

**Introduction:** Recombinant adeno-associated viruses (rAAVs) play an essential role in gene therapy, serving as effective vectors to deliver therapeutic genes into target cells, thereby holding great promise for treating a variety of genetic disorders. The generation of rAAVs using the Baculovirus Expression Vector System (BEVS) has gained significant attention for its versatility and scalability as a gene therapy production platform. The Two-Bac system employs two distinct baculoviruses, each carrying essential genetic elements for rAAV generation in insect cells. One baculovirus carries the AAV genes for genome replication and capsid formation while the second baculovirus harbors a specific therapeutic gene surrounded by Inverted Terminal Repeats (ITRs) functioning as packaging signals. The BEVS dual system allows for the expression of components necessary for rAAV production, as well as the flexibility in the encapsidated therapeutic gene. This study investigates the influence of two critical parameters of the Two-Bac system, total Multiplicity of Infection (MOI) and the ratio of the two baculoviruses, on the efficiency of rAAV produced with BEVS.

**Approach:** Varying MOI values for both baculovirus constructs were employed to evaluate the effects of total MOI and the baculovirus ratio on rAAV capsid production and genome packaging.

**Results:** Our data indicate a significant dependency of rAAV production on Baculovirus ratio used to infect the insect cells. Optimizing the ratio between the two baculoviruses is thus crucial for achieving a balance that maximizes transgene packaging and rAAV particle assembly. When the ratio of baculoviruses remained constant, variations in total MOI had a minimal effect on the overall efficiency of the rAAV production process.

**Conclusion:** The optimization of MOI and the ratio of the two baculoviruses contributes to a more robust and efficient process for large-scale rAAV production, promoting advancements in gene therapy and biotechnological applications.

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# Enhancement of rAAV2 Viral Vector Yield Recovery in Upstream Lysate Material

**Dr Lorna Kelly**[1], Mr Shandel Pariag[1], Mrs Taelor Patterson[1], Miss Kirsty McManus[1]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected**
We demonstrate higher yield of rAAV2 viral vector produced in mammalian cells

**Impact**
Our work demonstrates that formation of large aggregates of cellular material can be prevented to improve viral vector recovery in our platform process

**Introduction**
At Pharmaron, driving innovation through state-of the-art high-throughput upstream capabilities, coupled with advanced analytical tools, is key to rapid process development.
Production of certain AAV serotypes as gene therapy vectors require a cell lysis step to release capsids that are not secreted from the producer cell. This releases host cell impurities that can interact with key production reagents and the capsid itself to form large aggregates which sequester the vector, affecting clarification recovery.

**Methods**
At Pharmaron, different pre-harvest treatments were trialled to overcome this rAAV production process challenge. We used the high-throughput AMBR250 bioreactor system for simultaneous exploration of five different treatments in various combinations.

**Results**
A specific combination of two of these treatments increased capsid titres by more than two-fold compared to untreated lysate and resulted in a marked increase in post-clarification recovery. Moreover, Nanoparticle Tracking Analysis (NTA) has demonstrated an overall reduction in the frequency of large aggregates following post-lysis treatments, leading to improvements in product yield and impurity profiles.

**Conclusion**
We have demonstrated that Pharmaron's high-throughput process development pipeline, Design of Experiment expertise and state-of-the-art analytical technologies can be applied to solve critical process challenges to great effect. We show that the formation of large aggregates after cell lysis can be prevented; the benefit afforded by this improves capsid titre post-clarification recoveries and levels of impurities going into Downstream processing. This ultimately increases titres and product quality for our customers and patients.

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# Mechanisms of antiviral action of the influenza defective interfering particle "OP7"

**Dr Julita Piasecka**[1], Dr. Daniel Rüdiger[1], Jan Küchler[1], Dr. Sascha Kupke[1], Prof. Udo Reichl[1,2]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:** Due to its inhibitory activity against influenza and unrelated viruses, OP7 is a potent candidate for a new broad-spectrum antiviral that can be produced in cell culture-based processes.

**Impact/Novelty:** We use mathematical modelling in combination with *in vitro* experimental data to deduce the mechanism of antiviral action of OP7.

**Introduction:** OP7 is an influenza defective interfering particle, a viral mutant carrying 37 nucleotide substitutions in viral RNA (vRNA) segment 7 (S7-OP7) [1, 2]. During co-infection, it strongly suppresses the replication of infectious standard virus (STV). To elucidate the molecular mechanisms of OP7, we developed a mathematical model of OP7/STV co-infection.

**Methods/Approach:** The G3A/C8U "superpromoter" mutation identified on OP7 was described to induce higher vRNA and cRNA levels, but its effect on mRNA remained elusive due to conflicting reports. We obtained experimental data on intracellular viral replication by RNA and protein quantification, and extracellular viral titers from OP7/STV co-infection to calibrate and validate the model.

**Results:** Our model simulations indicated higher synthesis rates of S7-OP7 vRNA and cRNA, but a decrease in S7-OP7 mRNA. We examined primary IAV transcription in the cycloheximide experiments and confirmed significantly lower accumulation rates of the S7-OP7 mRNA compared to wild-type segments. Additionally, simulations suggested that viral polymerase and nucleoprotein are not uniformly distributed between the segments, but are mainly consumed by the accumulating S7-OP7.

**Conclusion:** S7-OP7 mutations result in replication advantage and transcription reduction. The replication advantage induces the high levels of S7-OP7 vRNA in co-infection. Depletion of viral proteins by S7-OP7 is a potential mechanism of STV suppression. Our results support the development of OP7 as a broad-spectrum antiviral [3,4].

1. Kupke *et al.* (2019) *J Virol*
2. Hein *et al.* (2021) *Appl Microb Biotech*
3. Dogra *et al.* (2023) *Sci Rep*
4. Pelz *et al.* (2023) *Viruses*

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# CHO based production of vaccines, therapeutic enzymes and antibodies with bespoke glycosylation.

**Mr Bjoern Voldborg**[1,2]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to 01-Cells as Factories:**
Development of understanding through which cells and products can be engineered to improve efficacy, economy and safety of protein-based therapeutics.

**Impact/Novelty:**
Application of a novel panel of engineered CHO cells for the production of glycovariants of therapeutic candidates for the discovery of more efficient, cheaper and safer therapeutics.

**Introduction:**
Glycosylation can have significant impact on the efficacy, activity and stability of protein-based therapeutics, and the ability to produce glycoproteins with reproducible and bespoke glycosylation has been a challenge for the industry. Heterogeneously glycosylated therapeutics may contain significant amounts of ineffective, inactive and/or immunogenic glycovariants resulting in suboptimal drugs.

**Methods/Approach**
Using a panel of glycoengineered CHO (geCHO) cell lines, we have produced a large number different glycovariants of different protein-based therapeutics (vaccine, enzymes and antibodies) with bespoke glycosylation. These variants were glycoprofiled and analysed *in vitro* and *in vivo* for activity, efficacy, stability and/or immunogenicity.

**Results:**
The analysis showed very significant differences both in the recognition of different vaccine glycovariants by Ab panels derived from patients, as well as their ability to generate neutralising antibody responses and in the In Vitro Activity of enzyme glycovariants and antibodies.

**Conclusion:**
We have demonstrated that the geCHO panel can be used to identify lead drug candidates with optimal glycosylation profiles, to move forward into the development of superior protein-based therapeutics. The expectation is that this will reduce the dose needed for treatment and/or the treatment frequency, thereby increasing drug safety and efficacy as well as reducing costs, side-effects and hospitalization length and frequency.

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# Lowering culture temperature changes ubiquitinated proteins in the Endoplasmic Reticulum of Chinese Hamster Ovary cells

**Mr David Ryan**[1,2], Dr Michael Henry[2], Ms Christiana Kondylo-Sideri[1,2], Dr Selvaprakash Karuppuchamy[2], Dr Esen Efeoglu[2], Dr Paula Meleady[1,2]
[1]School of Biotechnology, Dublin City University, Dublin, Ireland, [2]Life Sciences Institute, Dublin City University, Dublin, Ireland

Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM
**Title:**

**Relevance to Theme Selected:** Investigation of the CHO ubiquitinated proteome could generate novel targets for genetic engineering of more ER-stress resilient cells, potentially increasing productivity and viable time in culture.

**Impact/Novelty:** The ubiquitinated proteome is under-represented in CHO proteomic studies.

**Introduction:** This study characterises changes in the ubiquitinated proteome following exposure of a non-producer (CHO-K1), an IgG producing (CHO-DP12) and an Erythropoietin producing CHO cell line (CHO-SK15-EPO) to a reduction in culture temperature from 37°C to 31°C. Lowering of culture temperature is employed by the biopharmaceutical industry to enhance productivity and viability. Part of the popularity of CHO cells is the ability to replicate human post-translational modifications. The post translational modification ubiquitination is an essential part of the cell's ER stress response, targeting misfolded proteins for degradation.

**Methods/Approach:** Cells were grown in suspension at 37°C and culture temperature was shifted to 31°C after 48hrs. Cells were collected at day 4 and day 7 for whole cell proteome and ubiquitinated proteome analysis. Ubiquitinated peptide enrichment was performed via immunoprecipitation of diGly peptides prior to LC-MS/MS. Proteins were identified using Proteome Discoverer and Progenesis QI for Proteomics was used for relative label-free quantitative LC-MS/MS analysis on samples from day 4 and 7.

**Results:** Lower culture temperature results in slower growing cultures that maintains viability for an extended period, allowing enhanced titre in the producer cell lines. There are 529 differentially expressed ubiquitinated proteins on day 4, and 116 on day 7 in the EPO producer, with similar trends also seen in the other cell lines.
**Conclusion:** Acquiring both whole cell lysate and ubiquitinated proteomic data allows for a greater insight into ER function, shedding new light onto pathways and processes that could be cell engineering targets.

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# Engineering a lactate sensing and controlling system in CHO cells

**Mr Alex Arrese-Igor**[1,2], Dr Elise Cachat[1,2]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
The development and application of orthogonal synthetic biology tools such as synthetic receptors or inducible promoters help engineering host systems and seek to improve their efficacy as cell factories.

**Impact/Novelty:**
In mammalian cells, no lactate-inducible system has been developed before. This leaves space for developing a biosensor that can activate downstream molecular effectors upon detection of lactate.

**Introduction:**
Lactate-prompted medium acidification in a fed-batch run is a long-standing issue for the biotechnology industry. Current solutions do not tackle the problem at its source, but rather use solutions that are not ideal for industrial-scale bioreactors, such as dynamic feeding regimes.

**Methods/Approach:**
We propose a system that detects lactate by a genetically encoded biosensor that would then trigger a reduction in the lactate production, thus limiting lactate accumulation at its source in the cell. For detection, we are developing two potential systems: a lactate-inducible promoter or a GPCR-based synthetic receptor. Given the modularity of these systems, they can easily be coupled with powerful downstream expression regulators such as CRISPR inhibition or activation to limit lactate production.

**Results:**
Current results show the development of an engineered promoter that can be repressed without lactate and could therefore be induced by lactate. We have also constructed synthetic receptors with different architectures and are testing them in a reporter cell line. Moreover, we have built a modular system for the introduction of five guide RNA sequences in the same plasmid harbouring the dCas gene, thereby facilitating the introduction of all-in-one CRISPRi or CRISPRa systems in difficult-to-transfect cell lines.

**Conclusion:**
A lactate regulation tool based on a genetically encoded biosensor and effector system would mitigate the effects of high lactate concentration as an autonomous system in the later stage of a fed-batch run.

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# Custom cell culture media increased production by 70% in a platform CHO fed-batch process

**Dr Kimberly Curtis**[1], Dr. Nan Lin[1], Dr. Zhou Jiang[1]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected: "Cells as Factories"**
Developing custom cell culture media enhances understanding of cell productivity and maximizes manufacture of therapeutic modalities.

**Impact/Novelty:**
Customized cell culture basal and feed media increased titer yield in a proprietary platform CHO cell process by 70% compared to media historically used in the process.

**Introduction:**
Customizing cell culture media can significantly increase therapeutic molecule manufacturing by maximizing the productivity of cell lines used during platform processes. It can also address hard-to-express molecules, reduce process variation, and improve product quality, all of which generate significant time and cost savings. We developed custom basal and feed media for a fed-batch platform process using a representative CHO host cell line and assessed its effect on IgG production.

**Methods/Approach:**
We performed an initial base media screen and optimization of 15 prototypes using a representative CHO clone in simple fed-batch culture and evaluated for growth and production in shake flasks. The top base media candidates were paired with a panel of 15 feed media prototypes and screened in fed-batch culture. We performed further optimization of the feed strategy and key process parameters using Ambr 15 mL bioreactors. Top conditions were validated using Ambr 250 mL and 3 L bioreactors.

**Results:**
Optimization of lead basal media prototypes yielded titer up to two-fold higher than control in simple fed-batch culture. The specific productivity of all top basal media prototypes surpassed the control. Combining the top basal media prototypes with the optimal feeds and culture parameters increased titer by 70% compared to control. During bioreactor validation, the custom media exhibited a 70% and 50% increase in titer and specific productivity, respectively, compared to the control media.

**Conclusion:**
Incorporating custom cell culture media into a commercial CHO platform process increased production by 70%, demonstrating how tailored media can significantly affect cell productivity.

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# Breaking Boundaries: From Hybridoma Adaptation to the Development of Analytical Techniques

**Miss María Jesús Leopold**[1,2], Dr. Ricardo Kratje[1,2], Dr. Marcos Oggero[1,2], Dr. Natalia Ceaglio[1,2]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
mAb-epitope platform: Production of mAb-CC1H7 from a hybridoma adapted to serum-free suspension growth, for techniques to exploit its interaction with tagged-proteins with modified Granulocyte-Macrophage Colony-Stimulating-Factor O-glycosylated peptide (mGMOP).

**Impact/Novelty:**
Epitope tagging is a vital technique for tracking recombinant proteins. mAb-CC1H7 recognizes an epitope in mGMOP and exhibits ionic strength-modulated binding, offering versatile applications and eliminating animal use concerns.

**Introduction:**
The lab-developed mAb-CC1H7 recognizes an epitope in mGMOP, with binding influenced by salt concentrations. This system is versatile for ELISA, chromatography, and western blot. Given the need for large antibody quantities, often obtained *in vivo*, this study aimed to adapt mAb-CC1H7 hybridoma to suspension, serum-free, *in vitro* culture. The performance of the resulting mAb was assessed in the above-mentioned techniques, to enhance its application in research settings.

**Methods/Approach:**
Hybridomas were progressively adapted, reducing FBS to 1.25%, subsequently cultured in serum-free medium using a direct protocol. Protein-A purified mAb facilitated techniques for detecting, quantifying, and purifying proteins labeled with mGMOP peptide, produced in CHO-K1 cells adapted to suspension. Antibodies specificity was assessed through a specific indirect ELISA and western blot. An experimental design explored the impact of anti-chaotropic salts and pH on CC1H7-mGMOP interaction. A competition ELISA for quantifying tagged-proteins was established, along with conditions for immunoaffinity chromatography.

**Results:**
Hybridoma maintained productivity, mAb-specific production rising with decreasing serum content in culture media. The mAb specificity was demonstrated by recognizing the mGMOP epitope. A competition ELISA with appropriate sensitivity and detection limits was designed. Optimal conditions ($Na_2SO_4$ 1M, pH 8) identified in the experimental design enhanced epitope-paratope interaction, elevating apparent affinity 15 times compared to the control. Under these conditions, purification achieved 80% yield and 96% purity.

**Conclusion:**
mAb-CC1H7 was successfully obtained through *in vitro*, serum-free, suspension culture, and used to develop a mAb-mGMOP system for detection, quantification and purification of proteins tagged with the O-glycosylated peptide.

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# In pursuit of a minimal genome: Establishment of a large-scale genome deletion strategy

**Mrs Melina Braeuer**[1], Linus Weiss[1], Tobias Jerabek[1], Hannah Fahrion[1], Dr. Nadja Raab[2], Dr. Nikolas Zeh[3], Dr. Simon Fischer[3], Prof. Dr. Kerstin Otte[1]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
We present a novel approach for Chinese hamster ovary (CHO) engineering aiming for drastic reduction of genome size to reduce molecular complexity.

**Impact/Novelty:**
While current cell engineering efforts aim for single gene or pathway modulation, our novel approach aims for the first time at large-scale genome deletions to create a clean production chassis.

**Introduction:**
CHO engineering approaches are always faced with the dramatic complexity of eukaryotic cells and moreover, non-essential or adverse genes for an ideal CHO production phenotype deprive cells of resources and energy. Large-scale genome reduction may therefore offer a novel route towards engineering a clean production chassis. We here present a large-scale deletion strategy enabling significant reduction of the CHO genome.

**Methods/Approach:**
Non-essential gene clusters were identified via siRNA screening and expression analyses. Single guide RNAs (sgRNAs) for genomic knockout (KO) were verified (ICE/TIDE) and transfected in different numbers and combinations with Cas9 protein. Following single cell sorting, deletion and non-deletion PCRs of gDNA/cDNA identified homozygous KO clones. Bioprocess compatibility was accessed in batch-cultivation measuring productivity, cell growth and viability.

**Results:**
We successfully established two highly efficient strategies for large-scale deletions of 200-850kb using CRISPR/Cas9 by targeting non-essential regions including the protocadherin gamma cluster coding for cell adhesion proteins and a carboxylesterase cluster coding for adverse host cell enzymes. By using a sgRNA up- and downstream of the target region, we achieved an editing efficiency of 20%, which was even exceeded by >30% efficiency using mini-pools prior to single cell sorting and the use of two sgRNAs each upstream and downstream. Finally, batch-cultivation confirmed no negative or even beneficial effects towards bioprocess parameters. With this first-in-CHO deletion size of nearly 1Mb sequence we have laid the foundation to aim for significant genome reduction in CHO.

**Conclusion:**
As genome reduced CHO cells may require less energy and resources investable into the production of recombinant proteins and adverse molecules will be eliminated, ultimately a clean host cell chassis will emerge for efficient production.

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# Transient cell line for high-titer rAAV production with low-level hcDNA encapsidation

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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:**
ELEVECTA™ transient cells — HEK293 cells genetically modified to reduce encapsidation of host cell DNA (hcDNA) — are novel cell factories for producing rAAV with reduced DNase-resistant hcDNA.

**Impact/Novelty:**
To our knowledge, this is the first description of genetically modifying cells to reduce hcDNA packaging for commercial rAAV manufacturing in a fully transient setting.

**Introduction**:
The landscape of recombinant adeno-associated virus (rAAV) manufacturing is undergoing a profound transformation, as stable producer cell lines emerge as the future for large-scale production. This trajectory, however, constitutes a nuanced journey that recognizes the pivotal role of transient transfection cell lines in the initial stages of research and development. This dual approach acknowledges the indispensable contributions of transient transfection in the early phases of developing rAAV candidates.

**Methods/Approach:**
We genetically modified parental suspension HEK293 cells to achieve low-level hcDNA encapsidation during rAAV production. Subsequent single-cell cloning resulted in a clonal cell line, which we adapted to a newly developed animal-component-free, chemically defined, and transfection-ready culture medium. After initial process development in shake flasks, performance of the ELEVECTA™ transient cell line was evaluated under controlled conditions using high-throughput bioreactor screening as well as benchtop bioreactors.

**Results:**
The ELEVECTA™ transient cell line grows in single-cell suspension mode to high density and supports high-titer rAAV production for multiple serotypes with low level hcDNA encapsidation.

**Conclusion:**
The ELEVECTA™ transient cell line is a versatile and competitive solution, adeptly meeting the market's requirements for high-titer production of top-quality rAAV products. Endowed with unique attributes, this cell line is positioned to play a substantial role in advancing the gene therapy field and in fulfilling the growing expectations for effectiveness and quality of rAAV therapies.

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# Bridging the gap: perfusion scalability for process intensification using WAVE™ 25 bioreactor

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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

Incorporating perfusion cell culture processes acts as a strategic process intensification tool. Cultured biopharmaceutical cell lines grow continuously with sustained and often enhanced productivity.

**Impact/Novelty:**
Process development labs need simplified and cost-effective processes that scale to manufacturing. We demonstrate the potential for using WAVE™ 25 bioreactor as a scale-down model for developing large-scale processes.

**Introduction:**
Using perfusion processes in biomanufacturing offers you several key advantages. Perfusion processes can improve your product yields and quality, enhance facility flexibility, reduce capital costs, and enable continuous downstream processing. We demonstrate how perfusion mode in WAVE 25 bioreactor leads to outcomes similar to those found in larger systems. Additionally, we show that a combination of standard HyClone™ cell culture media is suitable for steady-state perfusion processes.

**Methods/Approach:**
We used Cellbag™ biocontainers integrated with an internal perfusion filter. The steady-state perfusion process was operated at a constant culture volume of 0.5 L and 5 L using a 2 L and 20 L Cellbag biocontainer, respectively. We cultured a mAb-producing CHO cell line using HyClone ActiPro™ cell culture medium supplemented with HyClone Cell Boost™ 1 and Cell Boost 3 feed media at a CSPR of 20 pL/cell/d. Cells were maintained at a density of 70 MVC/mL by continuous cell bleeding.

**Results:**
We maintained the 0.5 L and 5 L perfusion cultures for 10 d in steady-state perfusion mode at a cell density approaching 70 MVC/mL. Cells cultured in WAVE 25 bioreactor showed process performance equivalent to perfusion work completed in Xcellerex™ XDR-50 single-use bioreactor connected to Xcellerex automated perfusion system.

**Conclusion:**
Our data provides compelling evidence that the WAVE 25 bioreactor—coupled with Cellbag biocontainers and using an internal perfusion filter—can be a simple, practical, and cost-effective option for developing steady-state perfusion processes.

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# Evaluation of Small Molecule Antagonists of Cellular Innate Immunity for Efficient Manufacturing of T-cell Therapeutics

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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:** Unleashing the potential of HEK293/HEK293T cells as viral vector factories using novel enhancers.

**Impact/Novelty:** Robust increase in manufacturing yields using novel small molecule Viral Sensitizers (VSE$^{TM}$), resulting in improved process efficiency and economics across cell and gene therapy (CGT) manufacturing platforms.

**Introduction:** Biomanufacturing of cell and gene therapies is a complex process, hampered by variability in yield, quality, and scalability of manufacturing platforms. Manufacturing bottlenecks represent key challenges to clinical development and commercial success.

Innate cellular antiviral defenses are an overlooked feature hindering CGT manufacturing. Introducing foreign particles and nucleic acids into living cells triggers the cells' robust innate antiviral responses, consequently limiting viral transduction, assembly and replication.

**Methods/Approach:** We provide evidence supporting the use of VSEs in CAR-T-based therapeutics manufacturing, targeting 2 distinct and complementary aspects of the manufacturing process:

a. more efficient production of quality lentiviral vectors
b. more efficient T-cell transduction processes

**Results:** VSEs were evaluated for enhanced production of lentiviral vectors from transfection-based processes and inducible lentiviral packaging cell lines. A multi-compound VSE formulation was developed, which provided a 4-5X increase in functional lentivirus titer from an adherent-based 293T platform. Similarly, VSEs exhibited activity in a suspension-based HEK293 inducible lentivirus production system, demonstrating repeatable increases of 3-fold in functional lentivirus titer.

Additionally, VSEs have improved the lentivirus transduction efficiency of primary T-cells. Screening of VSEs for increased transduction efficiency revealed several molecules which enhanced the transduction of T-cells with lentiviral vectors. Further validation of candidate molecules demonstrated a repeatable >2X increase in transduction efficiency across multiple T-cell donors, with little to no impact on cell viability post-transduction.

**Conclusion:** Together, this data supports using small molecule antagonists of cellular innate immunity for enhanced production of cellular therapies.

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# Designing a 1.5 L bench-scale model of a perfusion process

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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected: Cells as factories**
Perfusion processes are a means to introduce process intensification into mAb manufacturing and use the industry workhorse — CHO cells — to deliver the final product.

**Impact/Novelty: Scale consistency**
Applying appropriate scaling and modern tools is crucial for production consistency.

**Introduction:**
The complexity and cost associated with large-scale perfusion cell culture processes justify the use of scaled-down models that provide a reliable prediction of behavior. Establishing an appropriate model allows for higher throughput during simultaneous operation and monitoring of several cultures at different process conditions. During process development, the optimized conditions can easily be translated back to production scale and *vice versa*.

**Methods/Approach:**
We developed a 1.5 L perfusion system operating in TFF mode to support steady-state production at a constant 70 MVC/mL by applying a continuous cell bleed. To support high cell concentration, we supplied ActiPro™ cell culture media with HyClone™ CellBoost™ 1 and CellBoost 3 supplements at a CSPR of 20 pL/cell/d.

**Results:**
We observed consistent process performance concerning cell growth, cell viability, and volumetric productivity. Cells reached 70 MVC/mL on day 8 and the controlled state was maintained until day 18 with cell viability above 90%. Our results at the 1.5 L scale are comparable to a pilot-scale perfusion process we developed concomitantly in an Xcellerex™ XDR-50 (50 L) single-use, stirred-tank bioreactor.

**Conclusion:**
In this study, we show the development of a bench-scale (1.5 L working volume) proof of concept model for a 50 L Xcellerex XDR-50 bioreactor and Xcellerex automated perfusion system (APS) with comparable growth, metabolic profiles, and volumetric productivity.

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# High-throughput screening for high-performance cell culture media development

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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to theme**: Cells as factories and therapies: modernizing biologics production for emerging modalities – This study modernizes media formulation screening for production of therapeutics in mammalian cells.
**Impact/Novelty**: Introduction of a high-throughput media formulation screening workflow tailored for HEK293 cells and viral vector production with potential applicability to diverse cell lines and various bio-products.

**Introduction**: Production of biological products in mammalian cells is a complex process and not fully understood. Optimizing a chemically-defined cell culture media formulation, which typically involves more than 40 components, to achieve high yield and quality for a specific biological product can be a challenging task.

**Methods/Approach**: In this study, we successfully screened 279 diverse formulations generated by Design Expert software (a mixture design) for HEK293 cells, aimed at enhancing both cell growth and the production of Adeno-associated virus serotype 2 (AAV2). This was achieved through cell passage and transient transfection in 96-deep well plates, assisted by an automated liquid handler, and was accomplished just in three weeks.

**Results**: The performance of the selected formulations, as well as predicted formulations with high AAV2 titers, were further verified in 96-deep well plates and 125-mL shake flasks. Remarkably, our results demonstrated a more than 3-fold improvement in the titer compared to the control medium.
**Conclusion**: This streamlined process can be readily customized for the development of any cell culture media, facilitating the production of desired biological products in diverse cell lines. Notably, this can be achieved at a low cost and with a short turnaround time, without the necessity of fully comprehending cellular metabolic pathways. Additionally, the high-throughput screening yields a rich dataset that can be leveraged for evaluating potential synergistic interactions between components for further optimization.

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# Large-Scale Production of Human iPSCs with Automated Stirred Tank Bioreactors for Bioprinting Applications

**Mrs Mira Genser Nir**, Mrs Rukmini Ladi[1], Debbie L. L. Ho[2], Stacey Lee[2], Jianyi Du[2], Jonathan Weiss[2], Tony Tam[2], Soham Sinha[2], Danielle Klinger[2], Sean Devine[1], Art Hamfeldt[1], Hope Leng[2], Jessica Herrmann[2], Mengdi He[2], Lee Fradkin[2], Tze Kai Tan[2], David Standish[1], Peter Tomasello[1], Donald Traul[1], Noushin Dianat[3], Quentin Vicard[3], Kishore Katikireddy[1], Mark Skylar-Scott[2]

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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to the Theme Selected: Cells as factories and therapies: modernizing biologics production for emerging modalities**:
Our work aligns with the theme by demonstrating how hiPSCs can be harnessed as both cellular factories and therapeutic agents, facilitating a sustainable and transformative approach to regenerative therapies.

**Impact/Novelty:**
This research innovates in biologics production, introducing a scalable hiPSC culture-to-3D bioprinting process, crucial for advancing the feasibility and scalability of organ-scale tissue engineering and bioink development.

**Introduction:**
Tissue engineering at the organ scale requires scalable production of billions of human cells, especially for bioprinting applications. However, conventional 2D cell culture methods are limited in scalability due to cost, space, and handling constraints. This study focuses on optimizing the suspension culture of human induced pluripotent stem cell-derived aggregates (hAs) in an automated bioreactor system and demonstrates its scalability to larger systems.

**Methods/Approach:**
The suspension culture of hAs was optimized using an automated 250 mL stirred tank bioreactor system. We employed a Design of Experiments approach and multivariate data analysis to identify optimal culture conditions from a dataset of 15 variables, including impeller speeds, seeding density, and the use of polyvinyl alcohol.

**Results:**
At the 250 mL scale, optimal hA sizes and diameters were achieved by adjusting the impeller speed. Across three serial passages in two distinct cell lines, SCVI-15 and WTC-11, we maintained a high cell yield (1 billion cells in a 5-day culture), consistent aggregate morphology (over 72% for SCVI-15-derived hAs and 90% for WTC-11-derived hAs with diameters ≤ 300 µm, and an overall circularity of ≥ 0.73), and pluripotency marker expression above 90%. In a 1L bioreactor, the culture expanded to 4 billion cells in 4 days. The pluripotent aggregates were differentiated into derivatives of the three germ layers, including cardiac, vascular, cortical, and intestinal organoids. These aggregates were processed into wholly cellular bioinks for rheological analysis and 3D bioprinting, resulting in bioprinted tissues with high post-printing viability and the potential for differentiation into vascular and neuronal tissues

**Conclusion:**
This study demonstrates an optimized suspension culture-to-3D bioprinting workflow using an automated stirred tank bioreactor system, enabling a sustainable approach to billion cell-scale organ engineering. The next phase will focus on addressing larger-scale challenges by implementing a 10L-scale stirred tank bioreactor system.

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# Optimizing Antibody Expression through Refined Vector Element Screening in Isogenic CHO Cell Lines

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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:** Employing a site-specific integration platform to examine vector components enables the detection of genetic elements that can either enhance or reduce antibody expression in CHO cell lines.

**Impact/Novelty:** Screening of factors influencing antibody expression is usually performed transiently. However, using isogenic cell lines allows a thorough comprehension of the actual essential elements improving antibody yield and quality.

**Introduction:** CHO cells are widely used for monoclonal antibody production due to their ability to perform post-translational modifications similar to humans. However, achieving high expression levels remains a challenge. To enhance productivity, it is necessary to optimize culture conditions, media, expression vector design, and antibody gene sequences.

**Methods/Approach:** Using vector engineering, we investigated the impact of genetic elements on the expression of six antibodies in CHO RMCE-mediated master cell lines. We created expression vectors varying alternative codons, signal peptides, lambda and kappa light chain isotypes, and specific mutations in the heavy chain's constant region. Stable expression of these vectors and antibody titer measurements in batch cultivation allowed us to assess the effect of these genetic elements on antibody expression.

**Results:** Altering vector elements significantly affected antibody production. Codon-optimization in variable regions generally increased antibody expression. Some antibodies showed a ten-fold increase, while others experienced up to a two-fold rise. Additionally, the signal peptide selection also impacted expression levels. Substituting a kappa light chain isotype with a lambda light chain isotype resulted in reduced antibody expression. Modifying the codons of the constant heavy chain led to substantial decreases. Interestingly, no notable changes were observed when specific mutations were introduced or removed from the constant heavy chain.

**Conclusion:** Our study emphasizes the importance of fine-tuning genetic elements for high antibody yields, offering significant implications for the biopharmaceutical industry. These insights can help optimize CHO cell-based production systems for therapeutic antibodies.

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# Engineering mammalian cell growth dynamics for biomanufacturing

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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:** Aligning with “cell as factories” theme, we introduce an innovative approach in cell engineering that merges CRISPR/Cas9 with synthetic biology (SynBio), to control growth phases of mammalian cells during bioprocessing.

**Impact/Novelty:** This study present a unique case of study by creating mammalian cell lines capable of on-demand control of exponential and stationary phase in cultures.

**Introduction:** Achieving precise control over mammalian cell growth dynamics is a major challenge in biopharmaceutical manufacturing. Traditional methods are limited to regulate the three culture phases – exponential growth, stationary and death phases – due to their reliance on cellular response to environmental cues. Our approach seeks to achieve control of these growth phases at a cellular level by employing CRISPR/Cas9 and synthetic genetic circuits.

**Methods/Approach:** We employed a multi-level and sequential cell engineering strategy. Firstly, we targeted the death phase by using CRISPR/Cas9 to knockout (KO) pro-apoptotic proteins Bax and Bak, creating a death-resistant cell line. We then introduced a growth acceleration system, akin to a “gas pedal”, based on an abscidic acid inducible system regulating cMYC gene expression. Finally, we developed a stationary phase inducing system, comparable to a “brake pedal”, consisting of a tetracycline inducible genetic circuit regulating BLIMP1 gene. The resulting cell line featured a dual controllable system, combining the “gas and brake pedals”.

**Results:** The Bax and Bak KOs resulted in attenuation of cell death, improved cell viability and extended culture lifespan in mammalian cells. Implementing the “gas pedal” system accelerated growth, increased cell densities and controlled cell cycle, while the “brake pedal” system effectively induced growth cessation and cell cycle arrest. This dual system enabled controlled, dynamic growth phases transitions, resulting in higher cell densities and enhanced biopharmaceutical production.

**Conclusion:** This study showcases the potential of SynBio and combinatorial cell engineering in reprogramming mammalian cell behaviour.

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# Internalization of DNA:PEI polyplexes in transient transfection of HEK293 cells is mediated by Glypican-4 coalescence

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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:**
Novel insights into the mechanisms of transient transfection enabling successful internalization of DNA:PEI polyplexes, gaining knowledge for crucial biomanufacturing processes that rely upon transient gene expression (TGE).

**Impact/Novelty:**
New possibilities to address the cell density effect, one of the major limitations for a complete implementation of TGE at industrial scale.

**Introduction:**
TGE is a versatile tool for heterologous gene expression. Currently, the manufacturing of relevant biopharmaceutical products like recombinant adeno-associated viruses (rAVVs) rely upon TGE. A major limitation for a complete implementation of TGE at industrial scale is the cell density effect (CDE). The CDE is a biological phenomenon that hinders transfection efficiency at high cell densities, consequently limiting scalability and productivity. Hence, the overall cost-effectiveness of the bioprocess is compromised.

**Methods/Approach:**
HIV-1 Gag virus-like particle (VLP) production by TGE is used as a model to explore the CDE phenomenon and DNA:PEI polyplex dynamics. Techniques such as flow cytometry, fluorimetry and NTA tracked VLP production. X-ray spectroscopy and cryo electron microscopy analyzed polyplex alterations, while immunolabeling and confocal microscopy examined cell interactions with DNA:PEI polyplexes at varying densities.

**Results:**
Interaction of polyplexes and conditioned medium at high cell density revealed the presence of sulfur-containing molecules interfering with transfection. Removal of these negatively charged molecules associated to extracellular vesicles completely restored transfection efficiency. In vivo tracking of polyplex cell entrance pathways showed a complete relocation of sulphated membrane proteins, such as Glypican-4, allowing polyplex accommodation and internalization.

**Conclusion:**
The results of this work suggest that the CDE is influenced at least by an extracellular phenomenon that can be described as a competitive inhibition between free and EV-associated negatively charged molecules and the ones present in the cell membrane, required for transfection.

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# Development of a CDM for duck cell process intensification for the cultured meat industry

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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:** The development of chemically defined culture media (CDM) is critical to achieve high productivity and reproducibility in cell culture systems, primarily within the field of cultured meat.

**Impact/Novelty:** Development of a species-specific serum-free culture media for the production of cell-cultured fat as a novel food ingredient.

**Introduction:** The emerging field of cultured meat as a new alternative to livestock production has drawn attention to the culture of novel cell types. For these non-pharmaceutical applications of animal cells, specific animal compound-free and cost-effective culture media are not currently available at the necessary low costs. Here we developed and optimized two media that sustain the proliferation and adipogenesis of duck primary cells (DPC) at a lab-scale bioreactor.

**Methods/Approach:** Multiple statistical design of experiments (DoE) approaches were used for efficient and systematic exploration of multiple factors affecting the cells. By doing this, the main components driving cell growth and differentiation were identified and further optimized to improve cell culture performance.

**Results:** The developed proliferation medium sustains cell growth (>10 doublings) with a PDT of 45 hours. Using this medium as a starting point, we designed a differentiation culture medium that allowed for a more efficient adipogenesis process when compared to serum-containing media. Finally, we used both 2D and microcarrier-based cultures to characterize and optimize the performance of the media as a previous step to the intensification in a lab-scale bioreactor.

**Conclusion:** A systematic and efficient approach allowed identifying critical compounds and optimizing their concentrations, leading to the development of chemically defined media for the culture in the bioreactor of DPC.

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# Baculovirus as a versatile tool for recombinant protein expression and **mammalian cell line engineering**

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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
Efficient recombinant protein production requires the use of stable cell lines or inefficient transfection processes. Baculoviral transduction of mammalian cells (BacMam) offers cost-effective and robust gene transfer and straightforward scalability.

**Impact/Novelty:** BacMam offers several advantages over conventional approaches: (i) no need of high biosafety level laboratories, (ii) efficient transduction of various cell types, (iii) integration of large DNA fragments into genomes.

**Introduction:** Recombinant protein production still relies on plasmid transfection of mammalian cells or on time-consuming and tedious stable cell line generation. We propose the use of baculoviruses for efficient gene delivery into mammalian cells and stable cell line generation thereof.

**Methods/Approach:** In our study, we compare the transient transfection efficiency of different transfection agents and baculoviruses for transient protein production in different cell lines as well as the potential of the different strategies for the generation of stable cell lines. Additionally, we optimized the transduction by purification of the baculovirus particles and increased transcription rates by the addition of deacetylation inhibitors.

**Results:** We show that in transient transduction experiments the baculovirus achieves similar or even higher gene delivery efficiencies as compared to the best performing membrane permeating transfection agents. In addition, the protein production per individual cell, measured by GFP fluorescence, was lower in baculovirus transduced cells. However, this disadvantage was overcome by the addition of transcription enhancers such as deacetylation inhibitors. Transduction efficiency could be increased by using purified virus preparations. Of note, the baculovirus did not stress the cells or lead to enhanced cell death, as observed for higher doses of various transfection agents. Moreover, experiments addressing the stable cell line generation displayed a higher rate of genome integration after baculovirus transduction as compared to transfection with various transfection agents used in this study.

**Conclusion:** Overall, the baculovirus platform offers advantages over standard transfection reagents, not only for transient but also for stable transfection procedures, including the financial aspect. Additionally, the abundance of commercially available vectors and systems allow simple cassette generation for the production of large proteins like antibodies or particles such as AAVs and VLPs. Furthermore, the above-mentioned benefits allow efficiently generating stable cell lines.

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# Inducible expression systems for recombinant protein production in mammalian cells

**Ms Maria Toth**[1], Dipl.-Ing. PhD. Manuel Reithofer[1], Priv.-Doz. Dipl.-Ing. Dr. Astrid Dürauer[1], Univ.Prof. Dipl.-Ing. Dr.rer.nat. Reingard Grabherr[1]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected: Inducible expression systems enable stable cell lines carrying toxic proteins**
Stable cell line generation for manufacturing of viral particles requires genome-integration of toxic genes. Therefore, inducible systems are vital for the establishment of such stable cell lines.

**Impact/Novelty: Antibiotic-free inducible expression system for viral vector production**
An inducible expression system is being developed that comprises viral polymerases from bacterial as well as mammalian viruses. We propose an antibiotic-free alternative to the widely used Tet-On system.

**Introduction:**
For the production of recombinant adeno-associated virus (AAV) in mammalian cells a triple transfection using three different plasmids is commonly used. Two plasmids provide the AAV genes responsible for DNA replication and capsid proteins and the adenoviral (AdV) helper genes. The sequence encoding the therapeutic gene, to be packaged into the viral particles is provided by a third plasmid. Packaging cell lines, that stably express all AAV and AdV components have the advantage that only the plasmid, encoding the target gene, must be transfected, which markedly increases production efficiency. However, the expression of cytotoxic proteins such as the AAV gene *rep* in stable cell lines requires a tight regulation of gene expression. We propose the use of a system based on the specific interaction of a viral RNA polymerase and the corresponding viral promoter. Thus, expression is activated only when a plasmid encoding the viral polymerase is present in the cell.

**Methods/Approach:**
In our study, we investigate the feasibility of new inducible systems in HEK293-6E. Therefore, we designed and introduced different plasmids carrying fluorescent proteins under the control of the viral promoters T7, VSV and SFV. Basal and induced expression levels after co-expression of the corresponding RNA polymerase were assessed by flow cytometry.

**Results:**
When stably integrating YFP under control of a viral promoter there were no or only minimal expression levels detected. Growth rates and behaviour were comparable to HEK293-6E without YFP. Fluorescence increased upon transfection with the corresponding viral RNA polymerase, proving our concept of a packaging cell line for AAV production.

**Conclusion: Inducible systems using viral polymerases feasible in mammalian cells**
Our experiments demonstrate the activation of protein expression using viral polymerases and contribute to the development of stable cell lines for the expression of toxic proteins in mammalian cells.

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# Evaluation of Targets for CHO Cell Line Engineering and Generation of Enhanced Host Cell Lines

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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:** Genetic engineering of Chinese Hamster Ovary (CHO) cells resulted in production clones with higher yields of recombinant proteins during fed-batch cultivation.

**Impact/Novelty:** Traditionally, mammalian expression systems have been optimized targeting expression vector, bioprocess regimen and media. With the advent of CRISPR tools, genome editing enables optimization of the host cell line for biopharmaceutical production.

**Introduction:** To satisfy the growing demand for biopharmaceuticals, commercial production cell lines must express proteins at high yields with suitable product quality. This study illustrates the process of screening for advantageous gene targets and the creation of an improved CHO host cell line, optimized for the production of recombinant proteins.

**Methods/Approach:** Leveraging CRISPR technology, selected genes were successfully knocked out with an efficiency exceeding 80% in CHO cell pools. CHO knockout lines were used to establish stable antibody-expressing cell lines. Their bioprocess performance was evaluated in fed-batch cultivations. For the most promising target, a clonal knock-out cell line was generated, followed by a selection of the top performing clone as new potential CHO host cell line.

**Results:** In our investigation, one specific gene target enhanced antibody yield by 20-100% during fed-batch cultivation, depending on the recombinant protein produced. Upon creating a clonal CHO knockout cell line, 35 out of 306 clones exhibited a complete gene knockout. Of these, four clones were chosen as top performers. The leading clonal cell line demonstrated a 60% increase in final titer and a 74% rise in specific productivity.

**Conclusion:** Our data revealed that both cell pools and clones are effective for assessing gene knockout impacts. We identified a target gene whose knockout boosts final titer and enhances specific productivity. Finally, we developed a clonal knock-out CHO host cell line, which shows promise as a new platform to generate highly performing biopharmaceutical production clones.

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# T22 peptide-conjugated GagGFP-VLPs: A novel approach for targeted therapy in metastatic colorectal cancer

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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
Targeted drug delivery using T22-GagGFP-VLPs offers a promising strategy for precision treatment in metastatic colorectal cancer and CXCR4-linked diseases, addressing urgent therapeutic demands.
**Impact/Novelty:**
The innovative use of T22-GagGFP-VLP allows precise targeting of CXCR4-expressing cells, presenting a new route for intracellular drug delivery in the treatment of metastatic colorectal cancer.
**Introduction:**
The use of VLPs as nanocarriers for biomolecules presents a promising alternative in drug delivery research. CXCR4 is a cell surface receptor marker associated with metastatic colorectal cancer. T22 peptide is a known antagonist of this receptor that binds to and penetrates CXCR4+ cells efficiently.
**Methods/Approach:**
The GagGFP-VLPs were produced extracellularly in HEK293 cells and purified by anion exchange chromatography. The resulted GagGFP-VLPs, were functionalized with the T22 peptide by click chemistry reaction. The capacity of the functionalized nanoparticles to penetrate target cells was studied in vitro by internalization experiments monitored by flow cytometry and confocal images.
**Results:**
The purification process allowed to obtain the GagGFP-VLPs at a purity level of 60%. The functionalization of the VLPs with the peptide T22 by click chemistry was confirmed by DLS analysis. The specific immunoreaction signal in the western blot followed by SE-HPLC studies also confirmed VLP functionalization. The ability of the T22-GagGFP-VLPs to penetrate CXCR4+ cells was observed to be dose-dependent. CXCR4+ cells exposed to $8{,}0 \times 10^9$ functionalized VLPs/mL were 30 times more fluorescent than those exposed to un-functionalized VLPs after 24h of incubation. Thirty minutes after exposure, the uptake of functionalized nanoparticles was already evident, and the amount of intracellular fluorescence increased reaching its maximum at five hours. The non-entry of T22-GagGFP-VLPs into CXCR4- cells demonstrated the specificity of internalization via CXCR4 receptor.
**Conclusion:**
The functionalization of GagGFP-VLPs with T22 ligand has been demonstrated, thus creating opportunities for potential therapeutical applications encompassing intracellular delivery of therapeutic biomolecules.

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# Inefficient transcription is a production bottleneck for artificial therapeutic BiTE® proteins

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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected**
Complex molecules, like multi-specific modalities proved to be difficult-to-express (DTE) in the past. We present the investigation of molecular bottlenecks in stable production cell lines.

**Impact/Novelty**
Various methods were used to analyze crucial steps of recombinant protein expression to identify transcription as the bottleneck in protein production of complex molecules.

**Introduction**
Antibodies are potent biopharmaceuticals used to treat severe diseases such as cancer. Recently, more complex modalities were developed including half-life extended (HLE) bispecific T-cell engager (BiTE®) and HLE-dual-targeting T-cell engager (dBiTE) molecules. However, non-natural, complex molecule formats prove to be difficult-to-express (DTE). Due to the growing importance of multi-specific modalities, this class of proteins was investigated for bottlenecks in production.

**Methods and results**
After determination of low product titer levels of HLE-BiTE® molecule producing cell lines using Protein A UPLC, molecular steps of the protein expression pathway were analyzed for reduced efficiency using biochemical assays. Co-immunostaining of recombinant proteins and production organelles showed no protein retention or aggregation; however, lower intracellular protein and mRNA abundance of these complex molecules were identified using Western Blots and qPCR. As these data pointed towards a production bottleneck, we aimed to uncover the root cause for limited mRNA abundance. While mRNA stability, determined using an Actinomycin D assay, was not causative for reduced mRNA levels, a reduced transcription efficiency was observed for HLE-BiTE® molecule producing cell lines using an in vitro transcription (IVT) assay. Here, the artificial structure of the HLE-BiTE® molecule was not the rate limiting step for reduced IVT rate, but modulation of the primary DNA sequence led to significant improvement.

**Conclusion**
The presented analyses provide novel insight into production bottlenecks of the HLE-BiTE® and HLE-dBiTE molecule class of DTE proteins and enable optimization strategies to overcome manufacturability challenges putatively associated with complex therapeutics.

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# Unleashing the Power of Perfusion: A Breakthrough in Cell Line Development for Intensified Processes

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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
Perfusion processes require cells with unique performance characteristics. Re-purposing fed-batch clones for perfusion processes is feasible, however modifications to the cell line development (CLD) process can enhance the production of therapeutics.

**Impact/Novelty:**
To maximize the efficiency of perfusion processes cell growth and nutrient consumption must be balanced. Modification of the CLD process can lead to the derivation of more productive clones.

**Introduction:**
As the industry shifts towards continuous and intensified processing it is essential to develop cell lines that can meet the demands of these processes. High-performing perfusion cell lines need to have specific characteristics not typically considered during traditional CLD processes. Notably, cells must have high specific productivity (qP) and a moderate growth rate, enabling the maintenance of high cell densities required in perfusion bioprocesses. Optimally balanced qP and cell growth leads to perfusion cell lines that produce high volumetric productivity and yield.

**Methods/Approach:**
Although it is a common industry practice to develop cell lines via a traditional fed-batch CLD process and then adapt them to perfusion processes, this may result in suboptimal performance. Through the development of a perfusion CLD process that incorporates the use of cell line expansion and perfusion production media, scale-down perfusion models, and perfusion-specific cell line selection criteria more productive perfusion cell lines can be generated.

**Results:**
Utilizing a perfusion-specific CLD process led to the derivation of clones that outperformed their counterparts developed using the traditional fed-batch CLD processes. In a scaled-down perfusion microbioreactor, perfusion CLD derived clones reached an average volumetric productivity ~20% higher and an average qP ~50% higher than fed-batch CLD derived clones.

**Conclusion:**
Together, this provides an integrated solution for continuous and intensified processing that is facilitated by the CHOZN® $GS^{-/-}$ cell line, a suite of fit-for-purpose media, and the Mobius® Breez Microbioreactor. These new methods and tools will empower the bioprocessing community to develop higher performing clones for perfusion processes and progress from CLD to process development more efficiently.

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# CHO-Cell Expansion Optimization for Monoclonal Antibody Production

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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:** This study addresses manufacturing challenges associated with monoclonal antibody (mAb) production in Chinese hamster ovary (CHO) cells by optimizing cell expansion processes and thereby enhancing efficiency.
**Impact/Novelty:** Our research demonstrates that using high density cell banks (HDCB) significantly streamlines the expansion process, offering a sustainable approach to CHO-cell mAb production.

**Introduction:** The increasing demand for mAb emphasizes the need for improvements in production processes. This study focuses on the pivotal step of cell expansion, aiming to uncover the potential benefits of using HDCB.

**Methods/Approach:** A comprehensive evaluation of the cell expansion strategy involved testing HDCB (50 & 125x10^6 cells/mL) from two distinct cell lines in a 14-day fed-batch shake-flask experiment. Control conditions (cells banked at 15x10^6 cells/mL) were included for both cell lines. Viable cell concentration and viability were monitored during both the seed train and subsequent production phase.

**Results:** The experiment revealed that implementing HDCB exhibited similar trends in viability and growth compared to control conditions. This trend persisted throughout the seed train and production phases. This makes it evident that initiating the seed train with a larger cell quantity can lead to a substantial reduction in its overall duration. Depending on the bank's initial concentration and specific growth characteristics of the tested cell line, it is estimated to significantly improve facility scheduling by increasing flexibility.

**Conclusion:** This study confirms that optimizing cell expansion with HDCB is a viable strategy for enhancing CHO-cell mAb production. This optimization holds the potential to significantly reduce seed train duration while maintaining robustness of the cell culture growth. Consequently, this approach can improve facility scheduling, enhance flexibility, and contribute to the overarching goal of minimizing the environmental impact of bioprocessing operations. Despite these results, further exploration of optimization strategies and their broader implications for mAb production remains crucial.

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# HEK Cell line development to optimize rAAV production

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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:** Improved performance of HEK293 cells for rAAV production after single cell cloning
**Impact/Novelty:** In this work we established a platform which allows the identification of clones with better rAAV productivity during the early stages of clone expansion.

**Introduction:**
Recombinant Adeno associated viruses (rAAV) are the predominant viral vector for *in vivo* gene therapies in clinical studies. The initial phase in therapy development includes the optimization of rAAV yield. Hence, an essential component of this step is the development of a cell line generating highly potent doses of viral vectors. (51)
**Methods/Approach:**
Adherent HEK293 cells were adapted to suspension culture in serum free medium (e.g HEK TF). After readaptation the cells were seeded in nanowell plates, and a single cell cloning (SCC) was performed using the ALS CellCelector™. 400 single cell clones were picked and transferred to well plates and expanded up to shake flask vessels to test rAAV production. Additionally, the clones were evaluated during the expansion process using an automated high throughput screening approach for rAAV productivity using small scale deep well plate transfection associated with fluorescence detection potency assay.
**Results:**
After SCC, an outgrowth rate in nanowell plate up to 80% were reached after only four days post seeding. Clones form separate SCC rounds were expanded up to shake flask vessels to test rAAV production. 96 clones were selected and evaluated in comparison to the pool for rAAV2 production using a new developed small scale rAAV transfection combined with an *in vitro* potency assay. Results revealed clones with higher rAAV2 productivity compared to the pool and were further evaluated using standard AAV production in Ambr 15. The cultivation in deep well plates allowed early screening of clones which revealed a positive correlation with the test done in larger volume.
**Conclusion:**
Overall, we have developed a cell line development platform, which allows the generation of a novel host cell line improved for rAAV production. With thy system different parameters including media and plasmid can be evaluated, with an early high throughput screening for the clones.

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# Development of fed-batch-based processes for intensified rVSV-vectored SARS-CoV-2 vaccine production in suspension HEK293 cells

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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:** As an increasing number of viral vectored vaccine candidates are developed, this work addresses the need for the development of scalable and cost-effective production processes for this modality.

**Impact/Novelty:** While process intensification in virus production has mainly focused on perfusion operations, we explore fed-batch-based strategies for simpler and cost-effective r-VSV-vectored vaccine production in suspension HEK293 cells.

**Introduction:** Although perfusion operations have gained ground with high productivity and small footprints, most legacy cell culture processes are still operated in fed-batch due to lower costs and simplicity. As the accumulation of metabolites – such as lactate and ammonium – has been previously linked with a reduction in virus titers, the design of dynamic feeding strategies supporting higher cell concentrations while limiting by-product formation is critical.

**Methods/Approach:** Suspension HEK293SF cells were cultured in an Ambr® 250 modular bioreactor system. Cultures were operated in batch (B), fed-batch (FB), and fed-batch with continuous harvest (FB/CH), the latter performed semi-continuously. Addition of concentrated feed followed a pre-determined cell specific feeding rate (CSFR; pL/cell/day), which was defined based on specific nutrient consumption. A rVSV-vectored SARS-CoV-2 vaccine candidate (rVSVInd-msp-SF-Gtc) was used. Infectious viral particles (IVP/mL) were quantified by TCID50, and the productivity was evaluated based on the total yield, TY (IVP), the space time yield, STY (IVP/L/day), and the yield on media, Y/M (IVP/L).

**Results:** The FB operations sustained viable cell densities of up-to 9.5 × $10^6$ cells/mL and showed more than 5-fold increase in TY, STY and Y/M, when compared to the batch reference. Cultures operated in FB/CH reached up-to 13 × $10^6$ cells/mL, resulting in an increase of up-to 4-fold, 3.5-fold and 1.7-fold in TY, STY and Y/M, respectively, in comparison to the batch reference.

**Conclusion:** Overall, these results highlight the potential of fed-batch-based strategies as an efficient alternative for intensified viral vector production.

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# DirectedLuck®: A careful transposon design boosts expression beyond the limit

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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:** ***Transposase-based cell engineering***
Tranposase technologies are used to introduce recombinant genes into cell genomes and to generate highly productive cell lines for biologics and viral vectors. They are also applied in cell and gene therapy.
**Impact/Novelty:** ***Higher expression and speed to clinic***
An artificial transposon in combination with the DirectedLuck® transposase considerably increases expression level of various antibody formats. This system reduces the screening effort for high-quality cell lines and enables to manufacture larger quantities of material with cell pools, which speeds time to clinic.

**Introduction:**
Great care was taken to create the highly advanced DirectedLuck® transposase and transposon ITRs. However, transposons include not only ITRs and target genes, but also DNA elements that control and promote its expression, selection markers or sequence motifs that may interfere with transposition. We designed an artificial transposon with lower number of TTAA sequence motifs to minimize potential transposition between individual vector molecules. In addition, we tested DNA elements, which may enhance target gene expression, as well as different configurations of the individual transposon elements. The optimal transposon variant was tested against the traditional transposon.
**Methods/Approach:**
CHORight® platform cells were transfected with artificial and traditional transposons. Cell pools were generated and analyzed in fed batch mode. Titer, qp, mRNA level and integrated copy numbers were compared.
**Results:**
Artificial transposons showed considerably higher titer and qp than traditional transposons. In the novel transposon that carried the dhfr marker, also much higher dhfr and target gene mRNA level were observed. Expression of cell pools and clones was highly stable as expected for the DirectedLuck® technology.
**Conclusion:**
For an optimal performance of a transposase system, a concerted interaction of transposon elements (and genomic integration sites) is essential. However, since the DirectedLuck® transposase combined with artificial transposons drives expression to a new level, adjustments of the selection or media/feed platform might be required.

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# Developing inducible stable producer cell line for rAAV production via site-specific integration

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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Impact/Novelty:**
The research proposed to develop a novel inducible stable packaging/ producer cell line for rAAV production via Crispr-cas9 mediated site-specific integration.

**Introduction:**
AAV-mediated gene therapy is a quickly growing segment of the pharmaceutical market. However, the current transient transfection process to produce rAAV has many challenges, such as low productivity of rAAV from host cells, difficult scalability of the rAAV-producing bioprocess, and high levels of impurities (e.g. empty/partial capsid) materialized during production. The stable packaging/ producer cells are ideal for industrial large-scale continuous production processes, overcoming the drawbacks in the current transient transfection process and benefiting the streamlined rAAV manufacture.

**Methods/Approach:**
In this study, we proposed to use synthetic inducible promoters to control the viral component expression and develop the baseline of the HEK293 stable packaging/ producer cells via CRISPR-Cas9 mediated site-specific knock-in approach for human genomic safe harbor sites (ROSA26, AAVS1, and CCR5 locus). With a total of three round integrations, stable cell pools were developed and evaluated after each round of integration. Single clones were further characterized for each integration round.

**Results:**
Regarding the stable pools, the 5'/3' junction PCR results confirmed the site-specific integration to each locus. The genome copy result showed that Rep78/68, E2A, E4orf6, Cap, and Rep52 were successfully integrated into the host cell genome. Genome and capsid titer after induction confirmed rAAV production for stable cell pools in each round. The isolated single packaging cell line (after 2nd round integration) was able to produce AAV, although the titer is ten-fold lower than the traditional triple plasmids transfection. The out-to-out PCR, and qPCR assay results further confirmed the site-specific integration.

**Conclusion:**
The study confirms the feasibility of developing the inducible stable packaging/ producer cell line with the refactored viral vector components under the regulation of the inducible system via a site-specific integration strategy.

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# Heterogeneity in Adeno-Associated Virus Transfection-Based Production Process Limits the Production Efficiency

**Mr Brian Ladd**[1,2], Ms Sofia Tunmats[1,2], Prof Torbjorn Graslund[2,3], Prof Véronique Chotteau[1,2]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to "Cells as factories and therapies: modernizing biologics production for emerging modalities"**
This study is part of our aim to improve the production yield by increasing the capacity of the cells to produce recombinant adeno-associated virus (rAAV). This work has identified a significant limitation in the production of rAAV by HEK293 cells during the transfection process.

**Impact/Novelty:** The novelty of this work lies in the use of single cell transcriptomics to study the heterogeneity of rAAV production, supported as well by phenotypic evidence.

**Introduction:** Of the four approved and five pre-registration in-vivo viral vector gene therapies, six use rAAV, while many others are in the pipeline. These upcoming therapies are placing a burden on current production methodologies requiring improvements in overall titres and lowering of the cost-of-goods.

**Methods/Approach:** Cultures of a HEK293T cell line were sampled at various time points during rAAV9 production. A triple plasmid transient transfection system was used for rAAV9 production. The sampled cells were studied with single cell and bulk transcriptomics, intercellular rAAV9 capsids were stained and analysed by flow cytometry, and supernatant and cell lysate samples were analysed by qPCR and ELISA.

**Results:** The results showed that there is only a small fraction of cells producing rAAV9 in the culture. The single cell transcriptomic analysis highlighted that there was a significant proportion, 41%, of cells not expressing any genes on at least one of the plasmids. Among the 59% rest of the cells only roughly half of them showed high expression of all three plasmids. The intracellular staining of rAAV9 capsids confirmed these results, by showing that only ~3% of cells had significant levels of rAAV9.

**Conclusion:** The use of single cell transcriptomics, confirmed by flow cytometry, provided unique insight into production of rAAV. It highlighted the heterogeneity in this form of production and gave a means to improving rAAV titre.

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# Comparison of CHO-K1 cell lines following serum-free and suspension adaptation.

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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**

1)

Serum-free and suspension adaptation processes are fundamental to host cell line development, for use in industrial-scale biopharmaceutical production.

**Impact/Novelty**
Limited, detailed studies focus on characterisation of the molecular nature of CHO cell populations before, during and after adaptation to serum-free and/or suspension culture. Changes occurring during adaptation may profoundly impact the properties of host-derived cell lines, in terms of production and product quality attributes.

**Introduction:**
Removal of serum and ability to grow in suspension conditions are prerequisites for the use of Chinese Hamster Ovary (CHO) cells for biopharmaceutical manufacturing. The adaptation process is time- and labour-intensive and is not well-characterised in accessible literature. This study set out to assess how an adapted CHO-K1 cell line differs from parental adherent CHO-K1 cells.

**Methods/Approach:**
CHO-K1 cells (ECACC 85051005) were passaged directly into suspension culture, with serum concentration reduced stepwise from 10% (v/v). Basal medium composition was also changed from Ham's F12 to CD-CHO. 1% (v/v) anti-clumping agent addition accelerated CHO-K1 adaptation, limiting aggregation and improving cell growth and viability. A unique, serum-free, suspension adapted cell line (XS-CHO) was generated.

**Results**
We compared molecular parameters between parental CHO-K1 and XS-CHO cell lines. In addition to altered cell morphology, batch culture duration increased from 4 to 8 days and maximum viable cell concentration increased from $1x10^6$ to $1.5x10^7$ cells/mL post-adaptation. Karyotype analysis identified a decreased modal chromosome number from 19 to 18 and distinct chromosome profiles between the two populations.

**Conclusion**
Adapting CHO-K1 cells to serum-free, suspension culture exerts significant evolutionary pressure, changing fundamental cell properties i.e., cell growth and karyotype. These data suggest it would be valuable to identify "molecular indicators" associated with desirable cell characteristics and develop adaptation protocols which maintain or select for these indicators.

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# Unraveling Productivity-Enhancing Genes in CHO Cells via CRISPR Activation Screen Using Recombinase-Mediated Cassette Exchange System

**Ms Minhye Baek**[1], Dr. Che Lin Kim[2], Dr. Su Hyun Kim[1], Dr. Karen Julie la Cour Karottki[2], Dr. Hooman Hefzi[2], Dr. Lise Marie Grav[2], Dr. Lasse Ebdrup Pedersen[2], Prof. Nathan E. Lewis[3], Prof. Jae Seong Lee[4], Prof. Gyun Min Lee[1]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:** A genome-wide CRISPR activation screen was conducted to discover novel gene targets in Chinese hamster ovary (CHO) cells producing bispecific antibody (bsAb), a hard-to-express protein with low productivity.

**Impact/Novelty:** A virus-free CRISPR activation screen was performed using the RMCE system to discover novel engineering targets among silenced genes in CHO cells.

**Introduction:** CHO cells, widely used for therapeutic protein production, have undergone genetic manipulations such as overexpression, deletion, or downregulation to enhance productivity. Many genes in CHO cells are silenced, and overexpressing silenced genes using CRISPR activation technology facilitates the screening of productivity-enhancing genes.

**Methods/Approach:** We established a CRISPR activation screening platform to discover engineering targets in CHO cells. The master cell line for gRNA introduction and expression of transcriptional activation domain linked to dead Cas9 was developed using a bsAb-producing CHO cell line. The 110,989 gRNAs targeting 13,812 silenced genes in CHO cells were introduced via the RMCE system, ensuring a single gRNA per cell. The enrichment of the gRNA library was followed by successive sorting of high-producer cells using a cold capture assay. Genomic DNA from sorted high-producer cells was extracted and amplified for next generation sequencing (NGS).

**Results:** High-producer candidate genes were identified through NGS data analysis, and the transient expression of the top 10 gRNAs increased the mean fluorescence intensity of bsAb-producing CHO cells in the cold capture assay. Ultimately, overexpression of a candidate gene augmented both titer and specific productivity of bsAb in clonal cells, which proved the effectiveness of the CRISPR activation screening platform.

**Conclusion:** This study presents a virus-free CRISPR activation screening platform as a potent tool to identify novel engineering targets and enhance the production of therapeutic proteins.

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# Optimization of the Autographa californica multiple nucleopolyhedrovirus genome by CRISPR-Cas9 for biologics production

**Miss Madhuja Chakraborty**[1], Ms. Lisa Nielsen[1], Miss Jacqueline Powichrowski[1], Dr. Marc Aucoin[1]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected: Cells as factories and therapies**
Optimization of the baculovirus genome to achieve minimized genome virus(es) will facilitate the insect cell hosts to focus their resources on foreign proteins thus improving biologics production.
**Impact/Novelty:**
The function of many baculovirus genes and their impact on foreign protein production in cell culture is unknown.

**Introduction:**
The insect cell-baculovirus expression vector system is an established platform for producing recombinant proteins, vaccines, and other biologics. The commonly used baculovirus, *Autographa californica* multiple nucleopolyhedrovirus (*Ac*MNPV) has a 134kbp dsDNA genome. Identification and removal of the genes that do not affect foreign protein production can redirect cellular resources toward the expression of foreign genes.

**Methods:**
A CRISPR-Cas9-based transfection-infection assay (Bruder 2023) is used to probe the essentiality of *Ac*MNPV genes to produce foreign proteins and progeny viruses in cell culture. Briefly, Sf9-Cas9 cells are transfected with sgRNA plasmids, followed by infection with recombinant *Ac*MNPV expressing green fluorescent protein (GFP). Although the sgRNA selection criteria have been optimized to minimize off-targets, for each gene we assessed 2-3 sgRNAs to gain confidence in the resulting phenotype.

**Results:**
15 late/very late *Ac*MNPV genes are disrupted to assess their impact on late foreign protein production and infectious virus titer (IVT) in cell cultures. Further analysis of these disruptions includes analyzing the effect of the target sequence distance from the 5' end of the open-reading frame (ORF), how different target sequences impact the phenotypes, and examining off-targets. Targeting genes such as *lef1*, *pk-1*, *lef11*, and *gta* resulted in a reduced GFP population and IVT, whereas disrupting *AcOrf-19, AcOrf-22, pkip,* and *env-prot* did not change the GFP population. Moreover, with increasing target sequence distance from the 5’ end of the ORF, the knockout is similar to the control for *p47*.

**Conclusion:**
Therefore *AcOrf-19, AcOrf-22, pkip,* and *env-prot* should be eliminated from the *Ac*MNPV genome as a next step towards a minimal genome baculovirus.

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# DNA-based genomic barcoding for clonal diversity monitoring and control in cell-based complex antibody production

**Mr Niels Bauer**[1], Mr. Christoph Oberist[1], Dr. Michaela Poth[1], Prof. Julian Stingele[2], Dr. Oliver Popp[1], Dr. Simon Auslaender[1]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:** Engineered cell line sub-lineages exhibit substantial genetic and phenotypic heterogeneity. Here, we enabled clonal diversity monitoring and control using a DNA-based genomic barcoding strategy during a complex antibody CLD process.
**Impact/Novelty:** Insights about clone diversity during stable cell pool selection, imaging-independent monoclonality assessment after single cell cloning, and improved hit-picking of antibody producer clones by monitoring of cellular lineages during CLD.

**Introduction:** During the development of a novel engineered cell line various sub-lineages of cell clones occur that exhibit substantial genetic and phenotypic heterogeneity. There is still a limited understanding of the source of this inter-clonal heterogeneity as well as its implications for biotechnological applications.
**Methods/Approach:** Here, we developed a DNA-based genomic barcoding strategy for a targeted integration (TI)-based CHO antibody producer cell line development process. This technology provided novel insights about clone diversity during stable cell line selection on pool level, enabled an imaging-independent monoclonality assessment after single cell cloning, and allowed monitoring and control of cellular lineages during the CLD process.
**Results:** We observed that CHO producer pools generated by TI of two plasmids at a single genomic site displayed a low diversity (<0.1 % TI efficiency) and underwent rapid population skewing towards dominant clones during routine cultivation. Clonal cell lines from one individual TI event demonstrated a significantly lower variance regarding production-relevant and phenotypic parameters as compared to cell lines from distinct RMCE events. Using cellular barcodes as a proxy for cellular diversity, we improved our CLD screening workflow and enriched diversity of production-relevant parameters substantially.
**Conclusion:** This implies that the observed cellular diversity lies within pre-existing cell-intrinsic factors and that the majority of clonal variation did not develop during the CLD process. This work, by enabling clonal diversity monitoring and control, paves the way for an economically valuable and data-driven CLD process.

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# Optimising a novel cell desiccation and storage protocol: an in vitro and in vivo study

**Ms Lady Barrios Silva**[1]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:** a feasible technological advancement in the long-term storage and delivery of cells for biomedical and therapeutical applications.
**Impact/Novelty:** Cell desiccation is an underexplored field, with the potential to evolve into an alternative protocol to current cell preservation techniques.

**Introduction:**
Improving cell storage, transport and delivery is vital to meet the increasing demand for therapeutics and research. This study aimed to develop a protocol for cell storage in a low- or zero-water environment as an alternative to the logistical, financial, and biological (i.e. use of DMSO and FBS) drawbacks of liquid nitrogen cryopreservation.

**Methods/approach:**
In addition to the thermo-physical and morphological profiling (i.e. SEM, DSC, TGA, FTIR, rheology) of several biomaterials (e.g., gelatin, alginate, nanofibrillar cellulose), their water retention characteristics, *in vitro* diffusion/permeability potential, and their angiogenic effect (i.e CAM assay) were also studied. Response surface analysis and fractional factorial design were then employed in the desiccation and rehydration of cells including C2C12 myoblasts to identify the optimal biomaterial(s), supplementation, and dehydration-rehydration protocol to enhance survival and functional recovery; as assessed by standard cell culture techniques including metabolic activity, cell membrane integrity assay, and cell cycle profiling and functionality/differentiation assays (i.e. immunohistochemistry, flow cytometry). In addition, *in vivo* testing of the constructs was performed by means of a subcutaneous implantation/muscle wound injury model in SD wild-type rats and C57BL/6 mice.

**Results:**
Results of this study documents a full characterisation of various biomaterials- noting that matrix formulations and supplementation were critical for cell desiccation. The optimised protocol suggested that viability and functionality of the cells were maintained after 5-days post rehydration.

**Conclusion:**
Taken together, results show a potential technological advancement in the long-term storage and delivery of cells for biomedical and therapeutical applications.

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# Self-controlled synthetic cells for detection and counteraction of bacterial infections

**Mr Sushobhan Sarker**[1], Mr Omkar Desai[1], Dr Natascha Gödecke[1], Dr Hansjörg Hauser[1], Dr Mario Köster[1], Prof Dr Dagmar Wirth[1]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
The study demonstrates the development of a self-controlled cell factory that is specifically activated by physiological triggers.

**Impact/Novelty:**
Bacterial infections are usually treated systemically. This study presents a cell-based concept that enables early and local recognition and neutralisation of infections.

## Introduction:

Bacterial infections contribute to a huge mortality and morbidity each year. While antibiotics exist to treat most bacterial infections, antimicrobial resistance is rising and represents a severe problem in clinics. Moreover, infections are usually only discovered when they are already advanced making their elimination a challenge. Therefore, we aimed to develop a cell-based system that detects bacterial infections automatically at an early stage and in response produces therapeutic proteins.

**Methods/Approach:**
An infection-responsive intracellular synthetic network was developed by integrating the synthetic transactivator tTA into the endogenous Mx2 locus, which is activated by type-I/III interferon. This module was rewired to $P_{tet}$ promoter-controlled antibacterial and reporter genes. A positive-feedback module was implemented to achieve long-lasting activation of the system by IFN-β, which is electively controllable by doxycycline.

**Results:**
To mimic physiological scenarios, co-cultures of the infection-responsive sensor-actor cells and macrophages were exposed to bacteria. Both gram-positive and gram-negative bacteria induced the synthetic signalling cascade, enabling the sensitive visualization of infections in a dose-dependent manner. Implementation of a positive feedback module induced a response that was maintained even if the trigger had faded out, while doxycycline treatment reset the sustained expression to the basal level. We demonstrated that this infection-induced synthetic cascade can be utilised to secrete chemo-attractants (e.g., CCL2), which boost immune infiltration to the site of infection.

**Conclusion:**
Autonomously controlled sensor-actor systems are characterized by high sensitivity and have the potential to combat bacterial infections in early stages in various scenarios (e.g., implant surfaces). In addition, this concept may help develop cell-based diagnostic and therapeutic alternatives relating to different inflammatory diseases.

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# Optimization of scalable rAAV-production for gene therapy – leveraging at-line amino acid measurements

**Dr Milla Neffling**[1], Prasanna Srinivasan[2], John Joseph[2], Tam N T Nguyen[3], Graziella Piras[1], Prof. Anthony J Sinskey[4], Prof. Richard D Braatz[5], Jacqueline Wolfrum[2], Paul W Barone[2], Stacy L Springs[2]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
**Cells as factories** – We demonstrate how modelling the mechanism of capsid formation and addressing key nutrient needs in a data-driven manner can enhance HEK293 as "factory" for rAAV production.

**Impact/Novelty:**
Utilising mechanistic models for HEK293 rAAV production and at-line spent media analysis for data-driven process intensification and yield improvement by cell culture media and feed optimisation.

**Introduction:**
rAAV viral vectors (VV) used for *in vivo* gene therapy are commonly produced using transient transfection of HEK293 cells. Although this method is well established, challenges remain in achieving high VV titer and a high ratio of full-to-empty capsids. Our aim is to build a mechanistic understanding and knowledge of amino acid (AA) consumption to guide the development of advanced manufacturing processes. The resulting model-driven approach is used to design, predict, test, and optimize VV production processes – scaling up and transferring to a continuous process at bioreactor scale.

Media optimization can be a laborous process involving multiple DoEs. Rapid and simple analytical tools at the point-of-use are required to quickly assess key-nutrient consumption and provide feedback to optimize feed and supplement addition strategies. In this study, we performed data-driven media screening and optimization experiments leveraging a fast, at-line amino acid analyser, REBEL.

**Methods/Approach:**
For initial experiments, rAAV was produced via transient transfection in a fed-batch 300 mL mini-bioreactor and in the shake flasks. Samples were collected to identify potential AA depletion that may impact capsid titre.

**Results:**
Asn is an important AA building block and constitutes ~10% of capsid protein composition. Results show clear differences in fluxes of Asn and Asp, which were reported previously as candidate AAs for producing viral particles. Other essential AA also exhibited strong depletion, indicating opportunities to optimize cell culture conditions. With subsequent experiments, the impact of AA addition to the cell culture was assessed with the goal of improving vector titre.

**Conclusion:**
This work is relevant to addressing key challenges of rAAV production platforms via process engineering and optimization of cell culture conditions to scale-up vector production and development of continuous bioprocessing of rAAVs. The technology used uniquely enables at-line AA measurements, leading to deeper insights and informed feeding strategy optimization.

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# Spent media analysis aids in determining metabolic mechanisms of superior CAR T-cell products

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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:**
**Cells as factories** – We aim to enhance understanding on how potent, desired phenotypes of CAR T-cells differ metabolically and how potency is influenced by novel constructs and manufacturing processes.
**Impact/Novelty:**
CAR T-cell manufacturing processes enhanced by characterisation of products through spent media amino acid analysis – correlating metabolism with desired, less-differentiated and less-exhaustive phenotype CAR T-cell populations.
**Introduction:**
As the use of CAR T-cell therapies advances, testing different CAR T-cells, and associating metabolic characteristics to well-performing, desired phenotypes of CAR T-cells enables optimization of CAR constructs and manufacturing processes. Vasoactive intestinal peptide (VIP) is an emerging checkpoint pathway for T-cell function. Antagonization of VIP/VIPR axis in T-cells enhances T-cell-mediated anti-tumor function. In this work, we elucidate the mechanisms by which CAR T-cells engineered to secrete potent VIPR antagonistic peptides (CAR/VIPRa) can overcome the immunosuppression of VIP-rich tumor microenvironments.
**Methods/Approach:**
To interrogate if VIPR antagonism impacts metabolic pathways, spent media analysis was performed using 908 Devices REBEL at-line amino acid analyzer to determine overall amino acid usage differences between CAR and CAR/VIPRa T-cells. Spent media analysis revealed differences in consumption of amino acids such as alanine, glutamate, and serine. This prompted further investigation of the differences in metabolic pathway utilization and alterations in metabolites within these CAR T-cells. LC/MS metabolomics, cellular bioenergetics and RNA sequencing were used to elucidate differences between metabolic states of CAR and CAR/VIPRa T-cells.
**Results:**
We found that when the manufactured cells are antigen-stimulated, CAR/VIPRa T-cells become significantly more energetic. In contrast, CAR T-cells become less energetic and more glycolytic, which are known features of T-cell exhaustion. Further, metabolic assessment in terms of oxygen consumption and amino acid levels in spent media correlated with these findings. CAR T-cell activity, potency, and cytotoxicity assessments showed correlation with anti-tumor activity. Together, this work demonstrates that distinct metabolic features allow CAR/VIPRa T-cells, when antigen-stimulated, to have enhanced effector functions against tumors.
**Conclusion:**
At-line spent media analysis of amino acids, in combination with other assays and measurements, was used to understand, characterise and optimise CAR T-cell manufacturing process, correlate with cell performance, and develop bioprocess parameters.

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# Systematic Bioprocess Characterisation of NISTCHO, a Freely Available Producer Cell Line

**Ms Larissa Hofer**[1], Ms Jerneja Štor[1], Mr Dominik Hofreither[2], Ms Laura Liesinger[2], Dr. Veronika Schäpertöns[3], Mr Thomas Berger[3], Dr. Wolfgang Esser-Skala[3], Prof. Dr. Nicole Borth[1], Prof. Dr. Ruth Birner-Grünberger[2], Dr. Nikolaus Fortelny[3], Prof. Dr. Christian Huber[3]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
The open access producer cell line NISTCHO is meant to serve as reference material for comparison of possible process optimisation or cell engineering strategies across the scientific community.

**Impact/Novelty:**
Here we present a first characterisation of NISTCHO using different -omics techniques to serve as reference material for other researchers.

**Introduction:**
Gaining a thorough grasp of bioprocessing is crucial for maintaining product quality and ensuring process compliance. It involves creating a design space that outlines how changes in parameters affect the process and, ultimately, the product. Unfortunately, such approaches are difficult to compare across different cell lineages and subclones. Here, we use a new reference cell line comparable to industrial use production clones to fully characterise a fed-batch process on a multi-omics level.

**Methods/Approach:**
A standard fedbatch process with and without temperature shift, in a benchtop bioreactor at 0.7 L scale was analysed to obtain an extensive profile of the phenotype during exponential and stationary phases, representing the most critical stages. High-confidence measurements were integrated into our bioprocess monitoring, to detect and monitor subtle changes in nutrient consumption, metabolic by-products, product quality and cell proliferation. Additional samples were processed (but are not yet fully available) for analyses of the genome, epigenome, transcriptome and (phospho)proteome.

**Results:**
As expected the temperature shift resulted in reduced metabolic fluxes while maintaining cell viability. Changes in metabolite concentrations as well as growth rate and product quality parameters will be presented.

**Conclusion:**
The availability of a reference cell line with industrial standards of productivity provides new possibilities to the academic community to compare different optimisation and engineering strategies across labs. Here we provide a first reference data set for this purpose.

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# Increasing LVV quality via stable overexpression of VSV-G in suspension producer cell lines

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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected.**
Improving LVV quality by genetic engineering stable suspension producer cell lines

**Impact/Novelty.**
Impact of additional VSV-G overexpression on two stable packaging/producer suspension cell lines

**Introduction**
Lentiviral vectors are widely used to modify cells for *ex vivo* gene and cell therapies due to their ability to efficiently integrate their genome into the chromosomes of dividing and non-dividing cells, resulting in long-term transgene expression. VSV-G plays a crucial role in the virus's entry into target cells. Therefore, we have investigated the effect of higher VSV-G expression levels by stable transfections with the already integrated VSV-G construct. We compared virus quality and transduction ability for human CD34+ cells of the genetically modified cell lines originating from two stable packaging cell lines – GPRG and GPRTG.

**Methods/Approach**
Producer pools were transfected with concatemer using Lipofectamine 3000 under doxycycline, zeocin, and either blasticidin or hygromycin selection to stably overexpress VSV-G.

**Results**
Transduction experiments with purified and TFF-concentrated lentiviral preparations using CD34+ cells showed higher transduction efficiencies in producer cell lines compared to parental cell lines. Titer analysis on monoclonal cell lines suggests similar ranges are obtained for adherent and suspension-adapted packaging cell lines. Further, we assessed the manufacturability of different clonal lines in a 5 L bioreactor perfusion set-up. We showed that the productivity of high infectious titers within a cultivation time of 36 days was maintained.

**Conclusion**
We presented that the increase of VSV-G content in stable producer cell lines impacts LVV quality if VSV-G content is limited. Additionally, we describe a workflow for scalable serum-free lentiviral production using two stable packaging cell lines with similar titers and yields and efficient transduction of human CD34+ cells.

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# Advancing Ferritin's Potential for Antigen Presentation in Vaccine Development

**Ms. Margarida Queluz Rodrigues**[1,2], Ms. Sara Patão[1,2], Dr. Brian Kloss[3], Mrs. Mónica Thomaz[1,2], Mr. Tiago Nunes[1,2], Dr. Paula Marques Alves[1,2], Dr. António Roldão[1,2]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:** This work enhances our understanding of how ferritin nanoparticles can be engineered to present antigens, contributing to the development of a robust method for producing vaccines.

**Impact/Novelty:** Ferritin (Ft) was engineered to present antigens using genetic fusion and tyrosinase (TYR)-mediated bioconjugation, representing an innovative modular approach for vaccine development.

**Introduction:** Ft nanoparticles are versatile 24-mer protein scaffolds for antigen presentation. Genetic fusion, the preferred method for antigen display, often results in improper folding and steric hindrance. Alternatively, bioconjugation allows the maintenance of the proteins' native conformation but may suffer from low specificity and conjugation efficiency. For instance, TYR enables site-specific conjugation of tyrosine-tagged peptides to cysteine-containing proteins.

**Methods/Approach:** TYR-mediated approach was used to conjugate the SARS-CoV-2 Spike protein's Receptor Binding Domain comprising a tyrosine tag (RBD-Y), produced in insect cells via baculovirus expression vector system (BEVS), to native cysteines on Ft nanoparticles, produced in *Escherichia coli*. In another approach, the glycoprotein Gn from the Rift Valley Fever virus was genetically fused to Ft (rendering GnFt nanoparticles) using insect and mammalian cells via BEVS and baculovirus transduction of mammalian cells (BacMam) technologies, respectively. The RBD-Y-Ft and GnFt nanoparticles were analysed through SDS-PAGE, western blot, mass spectrometry, high performance liquid chromatography, dynamic light scattering, mass photometry, and transmission electron microscopy.

**Results:** TYR-mediated conjugation yielded RBD-Y-Ft monomers of anticipated weight (46 kDa); although not all have been conjugated, RBD-presenting Ft nanoparticles could be generated. In the genetic fusion approach, the assembly of monomers comprising Gn-fused Ft into 24-mer nanoparticles was limited. Nonetheless, those properly assembled exhibited the expected round shape, size (20 nm), and weight (1.3 MDa).

**Conclusion:** This work shows that engineered antigen-displaying Ft nanoparticles can be produced through bioconjugation and genetic fusion approaches, making them a rapid response and robust vaccine platform.

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# Establishment of novel CHO-MK cells and optimal culture strategy for enhancement of therapeutic protein production

Dr. Junshin Iwabuchi[1,2], **Ms Erike Widyasari Sukowati**[1], Ms. Ryoko Aoki[1], Mr. Takashi Kaieda[1,2], Ms. Kayo Nishida[1,2], Ms. Michi Kubota[1,2], Dr. Takayuki Horiuchi[1,2]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
A novel high-performance host cell line, CHO-MK, was developed to meet the needs of global therapeutic proteins. Optimization of high-seed density strategies using CHO-MK cells can enhance therapeutic protein production.

**Impact/Novelty:**
We established a novel fast-growth and high-productivity CHO cell line, CHO-MK cells. Optimization of high-seed density strategies using CHO-MK cells resulted in enhanced productivity of 10 g/L on day 5.

**Introduction:**
Chinese Hamster Ovary (CHO) cells are the most widely used cell lines for therapeutic protein production. We developed a novel high-producing CHO cell line, CHO-MK cells, derived from Chinese hamster ovary tissues (*Cricetulus griceus*). CHO-MK cells show unprecedented fast growth of <10 hours of doubling time and a growth rate twice as fast as conventional CHO cells. Taking advantage of the characteristics of CHO-MK cells, we developed a process intensification of N-1 seed culture, resulting in enhanced productivity of 10 g/L on day 5.

**Methods/Approach:**
The primary cultured cells to serum-free immortalized suspension culture of CHO-MK cells were established from Chinese hamster ovary tissues. IgG1 high-expressed clone derived from CHO-MK cells was used for each study. To conduct high seed density and enhance productivity, an enrichment step at N-1 culture was performed, followed by production culture in 2 L bioreactors.

**Results:**
We developed a novel cell line (CHO-MK cells), serum-free immortalized cells. Consistent with the short doubling time, the enrichment step at N-1 seed culture was successfully performed, allowing the seeding of production culture at high-seed density. Further, production culture with 10 x $10^6$ cells/mL seed density was conducted in 2 L bioreactors, reaching a higher productivity of 10 g/L on day 5. The results suggested that the high performance of CHO-MK cells could reach higher productivity using only a simple method of intensified N-1 seed culture, which can reduce time consumption on production culture to less than half of those in conventional culture.

**Conclusion:**
In this study, we developed fast-growth and high-productivity CHO-MK cells for producing therapeutic proteins. Optimization culture strategies of CHO-MK cells with intensified N-1 seed culture process resulted in an astonishing productivity of 10 g/L on day 5.

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# Reality-cHEK – How Innate Immunity Impacts Lentivirus Production in HEK293 and How to Address It

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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:** Using rational approaches and genetic engineering to identify and address cellular bottlenecks leads to more efficient production cell lines for lentiviral vectors (LVs).

**Impact/Novelty:** Genetic engineering of targets within the antiviral and immune response pathways identified by gene expression profiling enables the generation of high-producing HEK293 cells for the growing demand of LVs.

**Introduction:** LVs have become a highly relevant tool for gene and cell therapy applications, resulting in constantly increasing demands. One strategy to address this need is targeting antiviral and immune response pathways in HEK293 producer cells, which are posing a potential bottleneck for efficient LV production. Identifying associated genes and using genetic engineering approaches to manipulate these restrictive genes can lead to increased cellular productivity.

**Methods/Approach:** A HEK293 cell line was transfected with LV production plasmids and PEI-MAX. Samples were taken at multiple timepoints. The transcript levels of selected genes were quantified using ddPCR. Differentially expressed genes were identified, and their impact on LV production was evaluated through means of either overexpression or knockout.

**Results:** Two genes, which have been identified to be substantially downregulated compared to the mock control, have been successfully overexpressed, demonstrating a correlation between their expression and LV titer. A further gene, which was upregulated during LV production, was successfully knocked out to generate a cell line more suitable for high-yield LV production.

**Conclusion:** A rational approach was used to identify genes in the antiviral and immune response pathways of HEK293 cells that impacted LV production. Those findings show that targeting these pathways could be an effective strategy for generating high-yield LV production cells.

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# Unravelling the link between genotype and fast-growth via optimized CHO whole genome knockout screening

**Mr Jannis Marzluf**[1,2], Dr. Ann-Cathrin Leroux[1]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:** Whole genome knockout (KO) screening enables the discovery of novel engineering targets for improved Chinese Hamster Ovary (CHO) cell lines exhibiting faster growth rates.

**Impact/Novelty:** We introduce an optimized whole genome KO screen for single-copy integrations, ensuring high coverage within a six-week screening period. This approach is implemented in an industrially relevant CHO cell line.

**Introduction:** Commercial production cell lines must express proteins at high yields with suitable product quality in a short time. Here, we aim to address the bottleneck of cell growth in cell line development and manufacturing by employing an optimized method to explore the genetic mechanisms behind a fast-growth phenotype in CHO.

**Methods/Approach:** A CRISPR competent CHO cell line was generated, followed by the implementation of a lentiviral small guide RNA (sgRNA) genetic KO screen. This process aimed to refine our methodology, specifically focusing on achieving single-copy integrations and ensuring high complexity coverage to achieve high data quality.

**Results:** Our efforts yielded a robust CRISPR competent CHO cell line capable of producing reliable insertion-/deletion-(InDel) events. Through our optimized setup, we attained 80% single-copy integrations, coupled with a >5,000x complexity coverage in preliminary studies. The observed data indicated a substantial impact of CRISPR-nuclease-expression on the enrichment and depletion of growth-affecting gene targets.

**Conclusion:** In conclusion, our presented pooled CRISPR screening platform exhibits a favorable signal-to-noise ratio, proficient identification of growth-inhibiting genes, and effective recognition of lethal genes employed as positive controls. We achieve high coverage with a high single-copy integration ratio compared to previous pooled CRISPR-KO screening studies. Our next goal is to generate high-quality data in an industrially relevant setup and cell line using a whole genome library, targeting 17,761 genes with an unprecedented library coverage exceeding 5,000x. These findings offer opportunities for innovative methodologies in the broader pooled CRISPR screening field but also facilitate the engineering of superior CHO cell lines.

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# Exploring different transcriptional responses in isogenic multi-copy CHO cells upon recombinant protein production

**Ms Giulia Scapin**[1], Mr Leo Dworkin[3], Ms Daria Seergeeva[2], Ms Lise Marie Grav[1], Mr Gyun Min Lee[4], Mr Lars Keld Nielsen[2,5]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:** Optimizing mammalian cell lines engineering via multi-copy targeted integration (TI) is relevant for supporting production of novel therapeutic format.

**Impact/Novelty:** Engineering efforts to resolve protein secretion bottlenecks have not considered transcription as a co-limiting factor when increasing gene of interest (GOI) dosage.

**Introduction:** Although advances in CHO bioprocess boosted protein titers, gains in cell line engineering were limited. CRISPR/Cas tools facilitated rational CHO cell engineering through TI. However, the need to understand cell productivity complexity persists. Transcriptomics of multi-copy isogenic cell lines with low clonal variations can reveal unique gene expression pattern linked to enhanced protein production, aiding the identification of new engineering targets.

**Methods/Approach:** Up to 4 copies of Erythropoietin (EPO) or Etanercept (ETN) were integrated in CHO cells at specific sites (A and T9). After the identification of a transcriptional bottleneck in the 4-copy clones, we performed RNA-seq analysis to investigate 1) the impact of integration sites, 2) the common and 3) unique effect of increased protein production on the coding transcriptome. A new panel of multi-copy Interleukin-2 (IL-2) and Interleukin-15 (IL-15) isogenic cells with the same design were generated to further test the multi-copy TI platform.

**Results:** Integration sites affect nearby genes. Common response to increased EPO/ETN production occurs at different gene dosage with upregulation of ER, protein export and DNA replication, and downregulation of lysosomal processes. Interestingly, ETN-specific response revealed IFN-stimulated genes upregulation. RNAseL knockout didn't resolve the transcriptional bottleneck. In contrast, no transcriptional bottleneck was observed for IL-2/IL-15 expressing CHO cells, but significantly lower IL-expression than EPO/ETN.

**Conclusion:** An exact explanation on the transcriptional bottleneck occurring in EPO/ETN 4-copy clones remains elusive. Ongoing RNA metabolism pathway analysis aims to comprehend mechanisms linked to increase gene dosage, considering variations in total transgenes amount. Complementary post-transcriptional, translational, post-translational studies can provide further insights.

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# Amino acid response elements for inducible control of gene expression in Chinese hamster ovary cells

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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
Engineering Chinese hamster ovary (CHO) cells to sense amino acid availability enables the decoupling of gene expression to specific growth phases, promoting biologics manufacturing.

**Impact/Novelty:**
The glutamine-responsive promoters enable time-resolved reporter gene expression, creating opportunities to express auxiliary proteins in the production phase of recombinant protein production.

**Introduction**
Chinese hamster ovary (CHO) cells exhibit a dynamic cellular phenotype throughout the various phases of a batch culture. To adjust their metabolic state in response to changes in nutrient availability, they induce distinct transcriptional programs, such as the integrated stress response (ISR). As part of the ISR triggered by glutamine deprivation, the activating transcription factor 4 (ATF4) is upregulated, which binds to promoters containing CCAAT-enhancer binding protein-activating transcription factor (C/EBP-ATF) response elements (CARE), upregulating the corresponding genes. Using this knowledge, we envisioned an inducible promoter which responds to glutamine scarcity, opening a possibility for growth phase-dependent expression of helper proteins, enhancing recombinant protein production.

**Methods/Approach**
We cloned potential CARE with either natural or synthetic linkers, or the endogenous nutrient sensing units (NSRUs) in the case of Asparagine synthetase (ASNS), from ISR-activated gene promoters upstream of a minimal cytomegalovirus promoter (mCMV), which is inactive without CARE. Upon ATF4 binding the CARE-mCMV promoter drives the expression of an enhanced green fluorescent protein (eGFP). We evaluated its inducibility by culturing cells in glutamine-free medium and followed eGFP expression by flow cytometry.

**Results**
In glutamine-deficient medium, promoter constructs “Asns-2xNSRU” and “Eif4-4CARE” exhibited highest inducibility. Testing for ISR specificity, the experiment was repeated with an endoplasmic reticulum (ER) stress inducer Tunicamycin (Tm) not affecting glutamine levels. Expression of eGFP remained at background level, showcasing ISR specificity. CARE element dose-dependency was examined by engineering plasmids to contain half of the initial CARE elements, reducing the eGFP expression accordingly. To assess reversibility of the system, the cell medium was replenished with glutamine, yet a substantial expression of eGFP persisted, indicating that remnants of the ISR are still present 24 h after the stressor is removed.

**Conclusion**
We successfully constructed inducible promoters, activated by glutamine depletion in CHO cells lacking endogenous GS. Further work will include elaboration of the construct using genes that benefit the recombinant protein production and secretory capacity in producer CHO cell lines.

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# Scalability of spheroid-derived extracellular vesicles production in stirred system

**Mr. Thibaud Dauphin**[1], Ms. Laurence De Beaurepaire[1], Dr. Apolline Salama[1], Ms. Karine Haurogne[1], Ms. Sophie Sourice[1], Mr. Quentin Pruvost[1], Dr. Eric Olmos[2], Dr. Steffi Bosch[1], Dr. Blandine Lieubeau[1], Dr. Mathilde Mosser[1]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:** 1. Cells as factories. We present here the generation of spheroid under stirring conditions to produce small extracellular vesicles.

**Impact/Novelty:**
Assessment of scale-up strategy on spheroid formation and investigation of spheroid-derived sEV attributes produced under stirring conditions in both spinner and stirred-tank bioreactor.

**Introduction:** Therapeutic applications of small extracellular vesicles (sEV) have gained widespread interest, but efforts remain to ensure standardisation and high-scale production. Implementing processes in stirred-tank bioreactors (STR) is crucial to finely control the cell environment and enabling the process scale-up, but it remains a significant challenge for anchorage-dependent cells. Here, using a pancreatic cell line, we investigate the formation of spheroid in spinner flask and STR at a constant power input and evaluated the impact of culture mode of cell fate and sEV released.

**Methods/Approach:** 1.4E7 cells were cultured as monolayers in T-flask or as spheroids in spinner flask or Ambr®250 STR. After 24h of culture in serum-free medium, sEV were isolated using differential centrifugation, tangential flow filtration, ultrafiltration, and size exclusion chromatography. sEV size and concentration were determined by nanoparticle tracking analysis of tetraspanin-labelled particles. Protein expression was detected by western blot. sEV immune properties were assessed through mixed lymphocytes reaction (MLR).

**Results:** Culturing 1.4E7 cells in suspension reduced their specific growth rate but allowed the formation of homogeneous and highly viable spheroids. Spheroid formation is dependent of the power input, and maintaining this criterion constant across scales proved to be the optimal scale-up strategy. However, the process transfer to Ambr®250 STR altered spheroid fate due to impeller design and its impact on flow regime. Compared to a monolayer process, sEV yield decreased (2-fold) in spinner flask but increased in Ambr®250 STR (2-fold), and sEV exhibited distinct immune responses in MLR.

**Conclusion:** As sEV are the mirror of their parental cells, it is crucial to understand and master the culture environment and to fine tune each process parameter during set-up and scale-up to warrant sEV final quality and quantity attributes. We present a comprehensive framework addressing key challenges encountered in developing scalable sEV-based therapy production from anchorage-dependent cells.

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# Precise methylation of FUT8 promoter regions allows isolation of CHO cells with a fine-tuned glycoprofile

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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
The fine-tuning of glycogenes allows the use of cell lines tailored to produce proteins with a desired, specific glycoprofile.

**Impact/Novelty:**
Variation in DNA methylation levels results in differential gene transcription, which allows to fine-tune gene expression to desired levels.

**Introduction:**
DNA methylation of promoters largely influences gene expression in mammalian cells and the application of CRISPR/dCas9 tools coupled with DNA methyltransferases (dCas9-DNMT3A3L) allows to turn off gene transcription by completely methylating the promoter. However, the impact of methylation of specific positions remains unexplored. Here, we present an approach to obtain a panel of CHO cells with defined FUT8 expression after targeted methylation.

**Methods/Approach:**
dCas9-DNMT3A3L was targeted with two individual sgRNAs next to the FUT8 transcription start site, resulting in partial methylation of the promoter region. By lectin staining the presence of a population with no FUT8 expression, a population with full FUT8 expression and an intermediate population with various expression levels was detected. Next, single cells were sorted using five different gates spanning from low to high FUT8 expression. FUT8 expression of the sorted clones was analysed up to 60 days after sorting. Additionally, promoter methylation status and the presence of specific histone modifications were assessed.

**Results:**
Clones sorted for low expression inherited a homogeneous phenotype showing reduced FUT8 expression. For clones sorted for high expression, we observed again a phenotype diversification. A second round of single cell sorting allowed the isolation of homogenous cell pools ranging different FUT8 expression levels. DNA methylation and histone modification analysis showed differential epigenetic signatures between the clones indicating that localized epigenetic modulation of promoters can fine-tune gene expression in CHO cells.

**Conclusion:**
Targeted promoter methylation can not only act to shut down gene expression but can result in tuned expression if site-specific and localized changes are achieved. The use of dCas9-DNMT3A3L with a confined target space around the TSS triggers changes in histone modifications of the FUT8 gene, ultimately allowing lectin-based cell sorting of CHO cells clones with fine-tuned levels of FUT8 profiles.

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# Investigating Titre and Genome Packaging during Recombinant AAV (rAAV) Gene Therapy Viral Vector Production

**Miss Kseniia Pidlisna**[1], Dr Pamela Gamba[2], Dr Vera Lukashchuk[2], Dr Michela Pulix[2], Dr Sue Podmore[2], Prof Mark Smales[1,3]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:** We report on development of AAV bioprocessing approaches and bioprocesses to understand limitations upon genome packaging.

**Impact/Novelty:** The serotype of ITRs used for genome packaging into recombinant AAV2 or 9 capsids does not impact packaging efficiency in HEK293 cells but that anti-viral responses can limit AAV production.

**Introduction:** Of viral vector systems currently utilised in the clinic, Adeno-Associated Virus (AAV) is the leading platform. The AAV genome encodes several protein products: three structural capsid proteins (VP1–3) and four replication Rep proteins. For therapeutic purposes, the production of recombinant AAV (rAAV) is usually achieved via triple co-transfection of three plasmids into a mammalian host cell line. The three plasmids co-transfected most often contain AAV *Rep* and *Cap* genes, adenoviral genes, and the target therapeutic gene flanked by AAV ITRs (inverted terminal repeats). However, production of high yields of fully packaged rAAV (AAV capsids containing the genome of interest) remains challenging.

**Methods/Approach** We report upon limitations on genome packaging into capsids and subsequent rAAV2 or rAAV9 yields produced using HEK293 cells. Two approaches have been investigated. The first is manipulation of the ITRs utilised to flank the target gene of interest. Different constructs were prepared with eGFP as the model gene of interest flanked by alternative ITRs. The second approach was to investigate and determine cellular anti-viral responses that might be initiated upon production of rAAV in HEK293 cells and whether knockdown of key proteins in anti-viral responses impacted rAAV titre and genome packaging.

**Results:** We show that different ITRs, and a consensus ITR sequence, all give similar genome packaging efficiencies into either serotype 2 or 9 rAAV capsids and thus genome packaging efficiency does not appear to be sensitive to alternative ITRs. We also show the impact of knockdown of different anti-viral signalling pathways on rAAV production and genome packaging, and how such approaches might be used to produce modified HEK293 cell systems with improved rAAV production.

**Conclusion:** Packaging efficiency of genome into recombinant AAV2 or 9 capsids is agnostic to ITR serotype and anti-viral responses can be reduced and impact AAV production.

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# Genetically encoded sensor circuits for the development of therapies: a theragnostic approach

Ms Eunice Gonçalves[1,2], Ms Ana Isabel Almeida[1,2], Dr Miguel Guerreiro[1,2], Dr Sofia Fernandes[1,2], **Dr Ana Sofia Coroadinha**[1,2]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
Cells can be both targets and effectors in therapeutic approaches. This work explores proteolytic biosensors to develop and deliver effective gene and cell therapies using theragnostic circuits.

**Impact/Novelty:**
Novel genetically encoded biosensors triggered by proteolytic activity were generated. Our second-generation circuit provides versatile and regulatable output signals, enabling the development of targeted advanced therapeutics.

**Introduction:**
Proteases play essential roles in cell survival and death. Alterations in proteolytic systems underlie multiple pathological conditions such as infectious diseases, cancer, or neurodegenerative disorders. As such, proteases are a major focus of attention as potential drug targets or as diagnostic and prognostic biomarkers. Synthetic circuits based on proteolysis can regulate therapeutic actions in gene and cell therapies for the treatment of both acquired and hereditary diseases.

**Approach:**
As many viruses encode proteases, our first biosensors were developed targeting infectious diseases. We have successfully implemented two types of cell-based biosensors. The first generation consisted of a single genetic module providing sensor and output signal. A second improved generation of biosensors was developed with an extra module of control, where the viral protease activates a protease-conditional recombinase (ProRec) sensing molecule that activates the transcription in a second output module. GFP was used as proof-of-concept output signal in both cases. The sensing modules were conditionally-inactivated through the use of protein distortion.

**Results:**
A whole-cell biosensor for the detection and quantification of infectious adenovirus was successfully developed, with fluorescence emission (re)activation upon infection. The sensitivity and dynamic range were, however, limited.
The second-generation ProRec biosensors showed low background and high output signal. Circuits for the successful detection of several viruses were developed, namely tobacco etch virus, adenovirus, and rhinovirus.

**Conclusion:**
The ProRec biosensors show robust detection of rare events (high sensitivity) and high flexibility. ProRec module is currently being coupled with antiviral therapeutic modules to provide advanced gene theragnostic treatments.

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# Unveiling the mechanisms behind high rAAV titers in HeLaS3 platform using Genome Wide Genetic Screens

**Miss Filipa Moura**[1,2], Miss Mariana Antunes[1,2], Dr Patrícia Gomes-Alves[1,2], Dr Paula M. Alves[1,2], Dr José Escandell[1,2]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:** The generation of new improved hosts can shorten the time required to generate producer cell lines, making them an alternative in the gene therapy manufacturing landscape.

**Impact/Novelty:** CRISPR-Cas9 genome-wide genetic screens allow us to identify recombinant Adeno-Associated Virus (rAAV) productivity-related genes. By fine-tuning the genetic background of HeLaS3 cells we aim to generate a host with improved productivity.

**Introduction:** rAAVs are the vector of choice for Gene Therapy applications. However, the manufacturing platforms available still represent a bottleneck in the production pipeline due to lack of scalability and use of expensive GMP grade raw materials. Producer cell lines (PCLs), such as the HeLaS3 production platforms, are an alternative allowing the reduction of the costs implicated with the production scale up while maintaining the quality required of the final product. Nevertheless, rAAV titers in PCLs are still low to meet the commercial stage demands.

**Methods/Approach:** We aim to bring new insights into the mechanisms behind rAAV production by employing genome wide CRISPR screens in HeLaS3 based PCLs. We engineered a stable HeLaS3 PCL previously established in our lab with two typologies of Cas9 nucleases that allow CRISPR screening. Cells were transduced with genome wide sgRNA libraries using lentiviral particles and we identified specific cell productivity levels with using assembled capsids staining and FACS. Analysis of the sgRNAs present in the different populations provided us the target genes for cell line engineering.

**Results:** By implementing two CRISPR-Cas9 based screen strategies (loss and gain of function), genes behind high rAAV production were identified, enabling host engineering to generate a PCL with enhanced production.

**Conclusion:** By improving the host, this project will boost the scalability and cost-efficiency of rAAV production in the HeLaS3 system. This would, hopefully, expedite the clinical translation of gene therapy solutions.

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# Advancing T cell manufacturing through tight control of cell activation in stirred-tank bioreactors.

**Miss Constança Costa**[1,2], Margarida S. Costa[1,2], Marta H. G. Costa[1,2], Nádia Duarte[1,2], Paula M. Alves[1,2], Margarida Serra[1,2]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:**
This work aims to improve strategies for CAR-T cell production, focusing on enhancing process scalability and cell phenotype control to overcome the low efficacy of CAR-T therapies in solid tumors.

**Impact/Novelty:**
As an improvement on current alternatives for CAR-T cell manufacturing, we propose to shape cell phenotype by tightly controlling process parameters and activation stimuli in stirred-tank bioreactors.

**Introduction**
Adoptive cell therapies using Chimeric Antigen Receptor (CAR)-T cells have shown great potential for treating haematological malignancies. However, such success has not been observed in solid tumours, due to reduced tumour engraftment and suboptimal CAR-T cell persistence. Moreover, current production platforms lack the scalability required to produce clinically relevant cell numbers and have limited control over critical process parameters.

**Methods/Approach**
To address these issues, we propose an integrated manufacturing process in stirred-tank bioreactors (STB) for controlled T cell activation and expansion. Anti-CD3/CD28 functionalized microbeads were used to control the initiation/termination of the T cell activation stimuli in small-scale STB (ambr15®). Culture parameters such as agitation profile or dissolved oxygen concentration were optimized to improve cell expansion, minimize exhaustion and favour less differentiated cell subsets, which have been correlated with improved clinical outcomes. Cell quality was monitored by flow cytometry.

**Results**
We have shown that functionalized microbeads can activate T cells in STB, as corroborated by the high expression of activation markers CD25 and CD69. When expanded in STB, T cells showed a significant increase (up to 4-fold) in cell expansion when compared to static controls, accompanied by an increase in viability. This process yielded a product enriched in naïve (CD45RA+CCR7+) and central memory (CD45RA-CCR7+) subsets. T cell activation under hypoxic conditions ($pO_2$= 3% $O_2$) was also explored. The effect of this short-term preconditioning strategy on the expression of effector cytokines TNF-α and IFN-γ was evaluated, with no impact on cell proliferation or viability being observed. Three different donors were used to confirm process reproducibility.

**Conclusion**
Functionalized microbeads can activate T cells in STB, allowing for precise temporal control of activation stimuli through the adjustment of the agitation profile. These results highlight the potential of controlled activation and further cell expansion in STB to manufacture CAR-T cells with improved potency

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# Impact of oxygenation on hWJ-Mesenchymal Stromal Cells (MSCs) growth and EVs production in mixed bioreactors

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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Cell factories:** This project aims to understand the impact of oxygenation environments on MSCs to improve the production yield of extracellular vesicles (EVs) while ensuring quality of therapeutic product.

**Impact/Novelty:** The impact of MSCs culture conditions and bioproduction process on the quantity and quality of the secretome is poorly described. Here, the impact of precisely controlled oxygenation are investigated.

**Introduction:** Secretomes from human MSCs, including EVs, are emerging as a promising cell-free therapeutic. However, the scale-up and the standardization of the bioproduction process remain major challenges. This work aimed to test different oxygenation conditions on cells physiology and characterise EVs.

**Method/approach:** Wharton's Jelly hMSCs were seeded on Synthemax II at 5 cells/microcarrier in Ambr® 250 bioreactors in controlled microenvironments of hypoxia (2%, 10%) or normoxia (19% oxygen). Until day 7, cell physiology was monitored daily by counting cells after trypsinization or fluorescence staining, and checking their metabolism (glucose, lactate, glutamine, lactate dehydrogenase). Then, the cells were starved for 48 hours to produce particles which were characterized by nanoparticle tracking analysis.

**Results:** In all oxygenation conditions, no latency was observed while from day 4 there was a significant increase of cell concentration under hypoxia compared to normoxia ($p$<0.05). However, $4.75\pm1.15\times10^{11}$ particles were produced at 2% of $O_2$ versus $8.49\pm1.25\times10^{11}$ and $7.28\pm1.15\times10^{11}$ at 10 and 19% of $O_2$ respectively. Similarly, one cell produced $5.39\pm1.22\times10^{4}$ particles (at 2% of $O_2$) but $9.84\pm1.1\times10^{4}$ and $8.12\pm2.16\times10^{4}$ particles with 10% and 19% of $O_2$, respectively. In addition, different particle sizes were obtained with 172.15±4.03 nm, 187.5±7.77 nm and 143.2±0.14 nm (2, 10 and 19% of $O_2$, respectively) suggesting a significant effect of oxygenation on specific production.

**Conclusion:** Data demonstrated that hypoxia promoted cell proliferation but reduced particles bioproduction in dynamic culture. Interestingly, 10% of oxygen increased cell proliferation while maintaining high EVs bioproduction.

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# Dual Technology Intensification- Fixed Bed Bioreactor Technology Combined with Viral Sensitizers for Adherent Cell Lines

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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:** Unleashing the potential of adherent HEK293 cells as viral vector factories using novel enhancers.

**Impact/Novelty:** Robust increase in manufacturing yields using novel small molecule Viral Sensitizers (VSE™) to improve process efficiency and economics across viral vector manufacturing platforms.

**Introduction:** Capacity limitation is a key challenge in gene therapy production using adherent cell lines. Due to the nature of these cell lines, some type of 3D matrix is required for cell attachment and growth; typically, microcarriers in suspension are used. These systems have limitations related to the volume required to achieve target yield and cell adherence challenges. Furthermore, innate antiviral defenses in producer cells diminish cell productivity during viral vector manufacturing.

**Methods/Approach:** To address these complexities, Cytiva and Virica Biotech have collaborated to combine the benefits of a fixed bed bioreactor with Viral Sensitizer (VSE™) technology to maximize the productivity of adherent cell lines.

**Results:** The iCELLisÒ is a compact, single-use, fixed-bed bioreactor that provides volumetric scalability, simplifying gene therapy applications in adherent cell lines. The fixed bed fosters a low-shear, 3D growth environment which supports high cell densities in a compact footprint. Virica's VSEs encompasses a proprietary collection of small molecules that enhance the growth of viruses by transiently dampening cellular antiviral defenses. Here, we compare baseline AAV productivity to productivity using customized VSE™ formulation for adherent HEK293 cells in iCELLis bioreactors. These simple additives were introduced to the iCELLis system during transfection to reduce antiviral defenses in production cells and demonstrated a multi-fold increase in AAV productivity.

**Conclusion:** Together, this data supports the benefits of the iCELLis fixed bed format combined with VSE technology in terms of capacity improvements due to higher productivity within the same footprint.

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# Label-Free Quantitative Proteomic Analysis of CHO-DP12 and CHO-K1 Cells under ER Stress Conditions

**Miss Christiana-Kondylo Sideri**[1,2], Dr Michael Henry[2], Dr Esen Efeoglu[2], Prof Paula Meleady[1,2]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:**
In keeping with "Cells as factories", this CHO study looks at ER stress mechanisms, addresses manufacturing issues, and offers insights for improving cell factory hosts and optimizing biologics output.

**Impact/Novelty:**
The study's impact lies in uncovering previously unrecognized proteins associated with ER stress in CHO cells, offering novel insights crucial for optimizing biopharmaceutical production.

**Introduction:**
Chinese Hamster Overy (CHO) cells are the primary host for the production of biotherapeutics, such as monoclonal antibodies and recombinant proteins. The activation of endoplasmic reticulum (ER) stress, which raises unfolded protein response (UPR) levels to maintain cell viability and productivity, is frequently correlated with high recombinant protein expression. Limited understanding of ER stress in protein production hampers enhanced yields and product quality.

**Methods/Approach:**
Mass spectrometry-based (MS) proteomics and subsequent gene ontology analysis of IgG producer (CHO DP-12) and non-producer (CHO-K1) CHO cells were investigated focusing on protein folding, UPR and ERAD in response to ER stress. Both cell lines were treated with tunicamycin and proteasome inhibitor MG132. To confirm ER stress induction, western blotting with BiP and pan-ubiquitin antibodies was conducted.

**Results:**
Upon induction of ER stress, quantitative label-free LC-MS/MS differential expression analysis of control and treated cells resulted in 1052 and 1093 proteins being differentially expressed (DE) for CHO-K1 and CHO DP-12 cells, respectively. The number of DE proteins was increased with proteasome inhibition. Next, assess the phenotypic impact of identified proteins on CHO cell productivity through knockdown and overexpression assays.

**Conclusion:**
We identified novel upregulated and downregulated proteins in ER stress and ubiquitin-mediated degradation pathways, not previously linked to ER stress. The study offers insights into challenges and opportunities in biopharmaceutical production, emphasizing the complex link between CHO cell biotherapeutic production and ER stress. This knowledge is crucial for targeted interventions to boost productivity and product quality.

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# Improvement of IgG Production in Chemically defined Systems via Protein Hydrolysate Supplementation

**Mr Brandon Wrage**[1], Angel Varela-Rohena[1], Salewa Akande[1], Brittany Patterson[1]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected: Cells as Factories**
Recent focus by Biopharma companies on supply-chain sustainability. Plant hydrolysates are a sustainable solution enabling increased productivity when added to chemically-defined media and feed systems, while reducing production costs.

**Impact/Novelty:**
Metabolic flux analysis was performed to better understand the effects of protein hydrolysates supplementation.

**Introduction:**
A growing focus for the biopharma industry is the implementation of process intensification as a more efficient and cost-effective approach to the production of biologic drugs. Media development and optimization are important strategies for bioprocess optimization during the production of biotherapeutics. Each process and cell lineage can exhibit different nutritional requirements. While most CHO-based processes utilize chemically-defined (CD) media and feeds, plant-derived protein hydrolysates can serve as an easy to implement, nutritionally dense and sustainable tool to increase production yields.

**Methods/Approach:**
This study focuses on two CHO cell lines expressing industrially relevant IgG. Each cell line was grown in three chemically defined media and feed systems according to each manufacturer's recommendations. Sheff-CHO supplements were supplemented once the cells reached peak viable cell density. The performance of the new experimental conditions was evaluated in terms of cell proliferation, culture viability and protein expression.

**Results:**
These case studies demonstrate that Sheff-CHO supplements and ultrafiltered protein hydrolysates can increase IgG titers up to 25% over standard fed-batch processes. The developed methods enable use of these supplements and hydrolysates within existing processes, without the need for cell adaptation or changes to medium and feed. The individual effect of each supplement or hydrolysate can be dependent on the medium, feed or cell line. Thus, screening and dose optimization of Sheff-CHO supplements and HyPep$^{TM}$ protein hydrolysates should be considered. Cost reductions of 20-30% on a per gram basis were achieved by supplementation with plant hydrolysates.

**Conclusion:**
Impact of supplementation on product quality will be discussed, but basal media on its own imparted a greater impact on glycosylation than supplementation with plant hydrolysates. Future work will explore the effect of these supplements and hydrolysates as tools for process intensification.

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# Enhanced production of AAV2 and AAV9 in HEK293 cells through DoE-optimization of triple plasmid ratio.

**Mr Sungje Park**[1], Dr. Seunghyeon Shin[1], Prof. Haeshin Lee[2], Prof. Jae-Hyung Jang[3], Prof. Gyun Min Lee[1]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
The study aligns with the theme "Cells as Factories and Therapies" by emphasizing enhanced biologics manufacturing for gene therapy vectors through DoE-optimization of plasmid ratios in HEK293 cells.

**Impact/Novelty:**
This study fills a gap by DoE-optimization of plasmid ratios to simultaneously enhance genome titer and full capsid ratio in various AAV serotypes with different production kinetics.

**Introduction:**
The recombinant adeno-associated virus (rAAV) vectors used in gene therapy are usually produced by transfecting three different plasmids (pHelper, pRepCap, pAAV-GOI) into human embryonic kidney 293 (HEK293) cells. However, the high proportion of unwanted empty capsids generated during rAAV production is problematic.

**Methods/Approach:**
To simultaneously enhance the genome titer and full capsid ratio, the ratio of the three plasmids transfected into HEK293 cells was optimized using design-of-experiment (DoE). AAV2 and AAV9, which have different production kinetics, were selected as cell-associated and secreted model AAVs, respectively.

**Results:**
The genome titers of rAAV2 and rAAV9 at DoE-optimized plasmid weight ratios (pHelper:pRep2Cap2:pAAV-GOI = 1:3.52:0.50 for rAAV2 and = 1:1.44:0.27 for rAAV9) were 2.23-fold and 2.26-fold higher than those in the widely used plasmid weight ratio (1:1:1) respectively, without any negative impact on infectivity. Full capsid ratios significantly rose (AAV2: 5.55%, 2.04-fold increase; AAV9: 15.99%, 1.89-fold increase) compared to the 1:1:1 plasmid weight ratio. Increases in full capsid ratios were also confirmed by transmission electron microscope (TEM) analysis.

**Conclusion**
Taken together, regardless of the AAV serotype, DoE-aided optimization of the triple plasmid ratio was found to be an efficient means of improving the production of rAAV with a high full capsid ratio.

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# Optimizing T-cell expansion with a high-performance medium free of animal derived components.

**Dr. Joanna Kern**[1], Mr Kyle Zastrow[1], Ms Lauren Kapus[1], Mr Evan Zynda[1], Mrs Anna Hachmann[1]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:** A new medium free of animal sourced components for T-cell expansion was developed to increase therapeutic potential and meet CAR-T manufacturing requirements.

**Impact/Novelty:** The new formulation was developed to support the cell therapy industry's need for an animal-origin free T-cell medium that allows consistent, reproducible, and automated T-cell manufacturing.

**Introduction:** The manufacturing process of immune-based therapies consists of many steps, including T-cell isolation, activation, genetic modification, and expansion. Cell culture media are critical for these phases, and precise control over medium composition reduces the risk of possible contaminants associated with animal-sourced materials. In addition, immunotherapies are shown to be more effective when memory T-cells are in a less differentiated state. Therefore, a medium supporting expansion of T-cells with early memory phenotype could improve the clinical outcome of emerging immunotherapies.

**Methods/Approach:** Our medium was designed and optimized using Design of Experiment and metabolite analysis. Cell expansion and memory marker expression was evaluated in small scale vessels and bioreactors to demonstrate scalability.

**Results**: We conducted a series of experiments to evaluate performance of our new T-cell medium. Compared to AOF or xeno-free media from other suppliers, our medium resulted in superior T-cell yield and higher expression of memory markers. Our results show that the medium supports efficient gene editing as well as scalability in bioreactors.

**Conclusion:** Our newly developed medium is free of animal-derived components and supports the robust expansion of T-cells on a consistent and reproducible basis. It mitigates potential safety concerns associated with the use of animal sourced components and is manufactured to GMP standards. Additionally, our formulation is easy to use and available in automation-friendly formats with scalable packaging. The versatility of our medium allows for straightforward adaptation and integration into various workflows and is amenable to both process development and clinical manufacturing of T-cells.

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# Novel human cell lines, HAT, for high-yield AAV production

Dr. Yugo Hirai[1,2], **Dr Yu-Hsin Chang**[1,2], Ms. Kazuko Aizawa[2,3], Ms. Arisa Yamamoto[1,2], Mr. Ryo Asahina[1,2], Ms. Rena Moromizato[1,2], Ms. Michi Kubota[1,2], Prof. Kazuaki Nakamura[2,3], Dr. Takayuki Horiuchi[1,2]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
In this study, human placenta-derived HAT cell lines were established and developed as a novel platform for AAV vector manufacturing.

**Impact/Novelty:**
Novel HAT cells with high proliferation potential and AAV productivity provide a hopeful option for future AAV-based gene therapies.

**Introduction:**
Adeno-associated virus (AAV) has emerged as a powerful gene delivery vehicle, however, there remain challenging issues regarding productivity, scalability, and quality with current AAV manufacturing platforms. The industry is still searching for the best host cells and production methods. Human embryonic kidney 293 (HEK293) cells have served the gold standard for producing AAV vectors. There are various HEK293 progeny cell lines developed for biomanufacturing purposes, but the derivatives all originate from the same parental lineage. To provide a diversified starting host cell population, we established a panel of new human amniotic epithelial cell lines for gene and cell therapy (HAT) derived from multiple placental tissues.

**Methods/Approach:**
The primary cells were isolated from amniotic membranes of the discarded placentas after cesarean section in accordance with the ethical regulations. The cells were immortalized by Ad5 E1 regions. Through screenings of more than 500 transformed colonies, over 40 cell lines displaying high proliferation ability were adapted to animal component-free, chemically defined suspension culture, followed by single cell cloning to meet the demand of mass production at the industry level. AAV productivity of HAT cells was assessed from adherent culture, shake flasks to benchtop bioreactors.

**Results:**
HAT cells exhibited significantly excellent proliferation capacity and produced remarkably high titers of AAV vectors compared to HEK293 cells (ATCC CRL-1573) in adherent culture and commercially available VPCs 2.0 cells (Gibco) in shaking flasks. High-yield AAV production was achieved across multiple serotypes. In addition, scalable cultivation and AAV production were successfully demonstrated in the orbital shaken benchtop bioreactor system.

**Conclusion:**
Here we established HAT cells for AAV production. High AAV titers up to 3 x $10^{14}$ vg/L were achieved. Consistent high-yield AAV production was also demonstrated at bioreactor scale. Upstream process development and downstream AAV characterization are ongoing to optimize the HAT system.

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# A scalable insect cell (Sf9)-dual baculovirus system for non-viral gene therapy (NVGT) production

**Dr Adhvait Shah**[1], Dr Swetha Kumar[1], Mr Hyun-Woo Kim[1], Dr Darrick Yu[1], Dr Jeong Lee[1]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Cells as factories and therapies:**
With an objective to achieve excellent process performance, we established insect cell-based processes for high product productivity whilst being scalable to large scale systems.

**Novelty/Impact:**
We developed and optimized an upstream process for manufacturing NVGT products using the Sf9-baculovirus system, which has been used for AAV manufacture and can address disorders that AAV can't.

**Introduction:**
Sf9 cells have been employed successfully as a viable alternative to produce AAV. Recombinant AAV can elicit immune responses, thereby preventing drug redosing, and have limited coding capacity. NVGT allows re-dosing with larger payloads. We faced and overcame several challenges including variability in cell-banks and quantification of the full-length NVGT product.

**Approach:**
In our work, we have compared two approaches to infection of Sf9: One-baculovirus and two-baculovirus system. Two-baculovirus system have rep and transgene on two different baculoviruses while one-baculovirus system has the transgene stably integrated in Sf9, with a Rep baculovirus. Various parameters including, but not limited to, viable cell densities at infection, relative MOI, medium composition, pH, and temperature were optimized for two-bac system.

**Results:**
Two-Baculovirus system was superior in performance to one-baculovirus system. Therefore, we developed a novel Two-Baculovirus scalable process for product manufacture and demonstrated good performance, up to 500L. The optimized process showed 5-fold increase in product titer. Our process includes growth of insect cells, NVGT product manufacture, and concluding with single-use continuous centrifugation to harvest a concentrated cell suspension for faster purification.

**Conclusion:**
Two-Bac Sf9-baculovirus system offers greater flexibility with an ability to deliver large transgenes. Since Two-bac is a 'plug-and-play' system, time-to-clinic for new indications is drastically reduced. Our improved process requires fewer batches to meet clinical demand, thereby significantly reducing COGs making NVGT more accessible to patients.

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# Thermo-Responsive Microcarrier as A New Tool for Expansion Cell Culture

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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:** 1. Cells as factories

**Impact/Novelty:** A new microcarrier technology that enables non-enzymatic and highly uniform subculture, and a seed train strategy leveraging the microcarrier technology which allows less unit operations with reduced cost-of-goods are proposed.

**Introduction:** In the upstream bioprocess, seed train strategies that involve bead-to-bead cell transfer or proteolytic enzyme treatment have been practiced for expansion cell culture with microcarriers (MCs). However, one encounters several issues such as non-uniform cell distribution and intricate operation which can result in lower product quality and higher cost-of-goods (CoGs) than expected. To overcome these issues, we introduce thermo-responsive MC (t-MC) and propose a new cell transfer method named as “cooled bead-to-bead”.

**Methods/Approach:** Newly designed thermo-responsive block copolymer was applied onto MCs’ surface to fabricate the t-MCs. Several cell lines were tested its growth rate on t-MCs. By low-temperature treatment and stirring, cell recovery rate was investigated. To test the efficiency of the seed train strategy leveraging the “cooled bead-to-bead”, we serially passaged 3 times with t-MCs for an expansion culture. This consists of adding fresh t-MCs with pre-cooled growth media and stirring at ambient temperature.

**Results:** The t-MCs showed high growth rate and most of grown cells were well recovered from t-MCs with high viability after low-temperature treatment and stirring, while a negligible number of cells were recovered from non-t-MCs. The recovered cells were singled or aggregated which might depend on cell-to-cell junctions. Furthermore, the results of seed train expansion test with the “cooled bead-to-bead” using t-MCs revealed significantly and remarkably higher proliferation rates and higher accumulated cell number than that of traditional bead-to-bead strategy. These results indicate that the combination of t-MCs and “cooled bead-to-bead” strategy can overcome current issues on unit operations, time and CoGs.

**Conclusion:** We have introduced a new strategy for expansion culture practice by combination of t-MCs and “cooled bead-to-bead” strategy. We believe that the proposed strategy serves as a fundamental scaffold for a MC-based expansion culture that allows overall success in cell culture-based manufacturing with less unit operations, time and CoGs.

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# Accelerating CRISPR Therapies: A Scalable eVLP Manufacturing Platform for Breakneck Advances in Gene Therapies

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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:** CRISPR revolutionized the gene-based therapy field. The generation of a scalable platform to produce CRISPR-based therapies will bring more efficient, safe, and economic therapies to the clinic.

**Impact/Novelty:** To date no stable producer cells for eVLPs production have been described. Such technology will accelerate the development of CRISPR-based therapies.

**Introduction:** Base editing is one of the most promising gene editing approaches as it can precisely and efficiently correct single-nucleotide variants, avoiding double-stranded breaks in the DNA and, consequently, large indels and chromosomal rearrangements formation. Engineered virus-like particles (eVLPs) have been developed to deliver CRISPR base editors (BEs) as a ribonucleoprotein (RNP) complex to cells. These particles have two main advantages: i) eVLPs preserve the targeting capacity of viral vectors, avoiding viral genome integration; ii) the short half-life of the BE RNP complex reduces the off-target effects, while maintaining efficient on-target editing. However, as eVLPs do not replicate, it is necessary to produce a high number of particles to achieve high editing efficiency. Here, we propose to develop a stable producer cell line for eVLPs manufacturing.

**Methods/Approach:** To develop a stable producer cell line (PCL), the different genetic cassettes required for eVLPs production (gag-pro-pol, envelope protein) as well as the cargo (base editor) will be successively integrated into 293 cells adapted to grow in suspension cultures. In addition to the base editor, another element needed for base editing is the gRNA, which binds the BE protein, forming the RNP complex. However, as the gRNA varies according to the target genomic sequence, its coding sequence will not be integrated into the cells genome and will be delivered subsequently. AT7 RNA polymerase will also be stably integrated into our PCL to catalyse RNA synthesis in the cytoplasm and improve BE/gRNA RNPs formation during particle production.

**Results:** Particle productivity of the system is being evaluated comparing transient and stable production. The eVLPs potency is being evaluated by targeting a cystic fibrosis-causing variant in a cellular model of the disease.

**Conclusion:** A scalable platform to produce such particles would enable to more CRISPR-based therapies reach the clinic rapidly and efficiently.

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# Enhancing Outgrowth of Glutamine Synthetase Double KO in CHO-K1 using Directed Evolution

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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected**
This study is related to Cells as Factories as it presents an efficient selection system combined with an optimized host cell line.

**Impact/Novelty**
The host cell line has properties that were selected to increase IVCD for higher titers during bioprocessing in combination with a well-established GS selection protocol.

**Introduction**
Due to its efficiency, the Glutamine synthetase (GS)-based selection system has become the most frequently used system for large-scale production of recombinant proteins. Such cell lines are commercially available, but usually not open source. Before we had developed a double knock out cell line with both GS variants deleted. Here we improve the process relevant properties of this cell line for increased subclonability, growth rate and final cell density using a directed evolution approach.

**Methods/Approach**
Biallelic deletion of both GS variants in CHO-K1 were successfully established. Cells reached a final cell count of 2-3x$10^6$/mL in an unoptimized batch culture. To improve IVCDs, cells were subcloned by single cell sorting into 96 well plates. After 3 weeks incubation, the largest 10 colonies were pooled, expanded in TPP tubes and sorted again. These steps were repeated three times.

**Results**
The fastest growing 10 subclones after the third round were expanded and growth curves in batch culture compared to the parental GS-/- cell lines. Maximum cell density of the subclones ranged between 4-5x$10^6$/mL.

**Conclusion**
The cells were optimized for improved growth using this directed evolution approach. Next steps will include comparison of a streamlined cell line development protocol to determine efficiency of selection and achievable production titers.

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# Influence of different culture media exchange ratios on umbilical cord blood-derived Natural Killer cell expansion

Mr Gabriel Costa[1,2], **Isabel Doutor**[1,2], Prof Ana Fernandes-Platzgummer[1,2]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to the Theme Selected**
This study refines umbilical cord blood-derived Natural Killer (UCB-NK) cell expansion, providing key insights for a smooth transition from lab to large-scale bioreactor manufacturing, crucial for advancing immunotherapeutic applications.

**Impact/Novelty**
This study reveals a cost-effective 75% culture media exchange ratio and IL-2 supplementation translatable to large-scale manufacturing, while ensuring cost-effectiveness without compromising UCB-NK phenotype, cytotoxicity and glucose metabolism.

**Introduction**
UCB-NK cells have emerged as promising candidates for immunotherapy due to their inherent ability to target infected and cancerous cell, allogeneic nature, low immunogenicity, and easy accessibility. However, their clinical application demand large-scale production and transitioning from small-scale laboratory settings to bench bioreactors and, ultimately, large-scale manufacturing is crucial for unlocking the therapeutic possibilities of UCB-NK cells.

**Methods/Approach**
This study investigates the influence of varying culture media exchange (CME) percentages (100%, 75% and 50%) and IL-2 supplementation ratios on UCB-NK cell expansion, evaluating their immunophenotype, cytotoxicity and glucose metabolism. Cost analysis is employed to evaluate the economic impact of different ratios.

**Results**
Preliminary findings suggest that a 75% CME achieves a comparable fold increase to the traditional 100% CME, while preserving the characteristic phenotype and cytotoxicity profile of UCB-NK cells. Cost analysis reveals approximately 8% reduction in expenses with superior fold increase, affirming the cost-effectiveness of the 75% CME ratio. Additionally, metabolite assays further indicate minimal impact on glucose metabolism with a 75% or even 50% CME.

**Conclusions**
These results underscore the feasibility of adopting a more cost-effective approach without compromising the therapeutic potential of UCB-NK cells. To strengthen these conclusions, further investigation involving more donors, exploration of other commercially available culture media, and a comparison of static and stirred culture conditions are required. The ultimate goal is to apply the optimized culture conditions to Chimeric Antigen Receptor-NK (CAR-NK) manufacturing, advancing the field of immunotherapy.

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# Epigenetically Heritable Gene Expression Patterns Are Associated With Stress Resistance in CHO Cell Lines

**Assoc. Prof. Mark Blenner**[1], Mr. Spencer Grissom[1], Zach Dixson[1], Prof Abhyudai Singh[1]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:** This abstract describes our innovative method to determine the drivers of phenotypes relevant to CHO cell production of biopharmaceuticals.

**Impact/Novelty:** This study examines long-lived gene expression fluctuations to identify drivers of important phenotypes for CHO cell production of biopharmaceuticals.

**Introduction:** CHO cell line development (CLD) is biased towards clonal populations that perform well in the unique environment that occurs during screening. It fails to identify optimal clones that perform exceptionally well in a larger production environment and associated stress agents.

**Methods/Approach:** This research describes a workflow for identifying heritable biomarkers slowly co-fluctuating and that are associated with stress resistance and improved productivity. To identify suitable biomarkers, a population-based RNA sequencing technique, referred to as MemorySeq, was first used to identify gene expression states whose fluctuations continue for several divisions and were distinct from a bulk average noise control. These expression states are considered heritable if their variation significantly exceeded the transcriptome-wide variation in the bulk average noise control. Given the small number of cell divisions in this study, the gene expression fluctuations are likely epigenetically driven rather than the result of genetic mutations. These data were paired with differential gene expression analysis (DGEA) in the presence of stress characteristic of production conditions.

**Results:** The overlap of heritable expression states from MemorySeq and differentially expressed genes from DGEA with functional analysis may suggest genes that would bias the CLD clonal pool to better performance. The MemorySeq workflow identified nearly 200 heritable expression states and six network communities of co-fluctuating genes, characterized by cellular adhesion, response to chemicals and stimulus, and cell differentiation from GO enrichment analysis. DGEA of several manufacturing stresses revealed significant enrichment of stress induced genes among the epigenetically heritable set. Six genes associated with either higher protein secretion, negative regulation of apoptosis, or increased glycosylation were selected from this pool as possible biomarkers for screening.

**Conclusion:** Clones with high expression of one or more of these six genes were selected and expanded for fed-batch culture to verify the heritability and assess its impact on production performance.

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# Defining and Manipulating Cellular Mechanisms Underpinning DNA Transfection Efficiency to Enhance Transient Recombinant Protein Production

**Dr James Budge**[1], Prof. Mark Smales[1,2]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

Transfection techniques have underpinned ground-breaking research activities and industrial applications since its development in 1973. The ability to deliver DNA into nucleated eukaryotic cells has been essential in establishing step changing technologies such as recombinant protein production, stem cell differentiation, gene editing, gene therapy, viral particle production and cell therapies. As a result, considerable effort has been devoted to discovery of novel transfection strategies and optimisation of protocols with a view to improving transfection efficiencies and recombinant protein product yields. Whilst many commercially available transfection reagents are highly efficient in delivering DNA across the cell membrane, very few focus on the subsequent delivery into the nucleus; a necessary step in expression of a recombinant transgene. Furthermore, the process by which DNA traverses the nuclear membrane is poorly defined although two major theories behind the cellular mechanisms involved in nuclear import have been proposed. The ‘active’ import theory is that DNA is able to migrate across the intact nuclear membrane through the nuclear pore complex (NPC) in an ATP dependent process. Conversely, following delivery of DNA across the plasma membrane during transfection, cells undergo division and the nuclear membrane is temporarily degraded during mitosis. Exogenous DNA is then spontaneously enveloped into the nucleus of the resulting daughter cells in a mechanism described as ‘passive’ import.

In this study, high resolution live fluorescent confocal microscopy has been used to track plasmid DNA and subsequent gene expression during both electroporation and lipid-mediated transfection approaches in Chinese hamster ovary (CHO) cells. This has led to a more comprehensive understanding of the cellular events which promote nuclear import and consequential expression of a gene of interest. These findings have enabled development of novel approaches which employ cell cycle manipulation strategies to improve transfection efficiencies and, in particular, nuclear import to enhance recombinant protein expression yields.

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# Supplying NAD+ Precursor improves specific productivity of mAb and modulates glucose metabolism in CHO cells

**Hye-Jin Han**[1], Ms. Hagyeong Kim[1], Mr. Hyun Gyu Yu[1], Mr. Jong Uk Park[1], Ms. Joo Hee Bae[1], Mr. Ji Hwan Lee[1], Prof. Jong Youn Baik[1]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:** We focus on improving the specific productivity of monoclonal antibody (mAb) by enhancing energy metabolism efficiency using media additives.

**Impact/Novelty:** This pioneering study evaluated $NAD^+$ precursors in CHO cell cultures, suggesting their use as a supplement for high-performing cell culture media development.

**Introduction:** CHO cells serve as a major workhorse for mAb manufacturing, but they exhibit inefficient energy metabolism and low productivity compared to bacterial cells. In our prior study, we observed a strong correlation between intracellular $NAD^+$ concentration and mAb productivity in CHO cells supplemented with $NAD^+$ [1]. However, applying $NAD^+$ for mAb manufacturing is economically challenging due to its high costs. We hypothesized that enhancing of $NAD^+$ biosynthesis with $NAD^+$ precursors could increase mAb productivity. We screened $NAD^+$ precursors and identified their effects on CHO cell cultures.

**Methods/Approach:** mAb-producing CHO cells were cultured with varying concentrations (0-10 mM) of $NAD^+$ and its precursors (NA, NR, NAM, NMN). We measured cell density, viability, metabolites, and titer to assess the effects of $NAD^+$ precursors on cell culture performances. Intracellular $NAD^+$ levels were analyzed to verify the $NAD^+$ biosynthesis from its precursors. We performed qRT-PCR and ATP assay to investigate the energy metabolism.

**Results:** While 5 mM $NAD^+$ precursors reduced cell growth by 1.3-28.8%, all the $NAD^+$ precursors extended culture longevity, increased intracellular $NAD^+$ levels by 32.2-73.7%, decreased glucose consumption and inhibited lactate accumulation by 21.0-45.5%. $NAD^+$ precursors, especially NR, enhanced specific productivity of mAb, showing a 57.0% increase compared to the control.
These results demonstrate the effective role of $NAD^+$ precursors in restraining lactate accumulation and enhancing cellular mAb production capacity.

**Conclusion:** Using $NAD^+$ precursors as media additives in CHO cell cultures can be an attractive strategy for increasing energy metabolism and mAb productivity.

**Reference:**
1. Lee et al. (2023) *Biotechnol. J.*, 18: e2200570.

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# Strategies to optimize the generation of high-titer CHO production cell lines

**Dr Alexandra Baer**[1], Dr Peter Ravn[1]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
Our work suggests solutions how to efficiently establish CHO production cell lines capable of expressing biologics, including molecules of high complexity like bispecific antibodies.

**Impact/Novelty:**
We have developed a CHO DG44 cell line development workflow using rationally designed expression vectors with cis-acting elements facilitating competitive expression of different classes of therapeutic molecules.

**Introduction:**
The generation of stable CHO cell lines for biologics manufacturing is a critical step in the development of therapeutics. Our goal was to establish a CLD platform superior to a commercially available system.

**Methods/Approach:**
We have expressed different classes of molecules in stable DG44 pools and clones in fed-batch processes and examined the effect of different vector and process optimizations on titer and product quality.

**Results:**
Using our new expression vectors, we have successfully produced proteins consisting of one to three subunits, like enzymes, monoclonal and bispecific antibodies. For complex molecules, the ratio of the expressed protein chains can be adjusted by changing the order of the genes in the expression vector. Cell lines exhibit stability of expression for 60+ cell generations and clone titers have been more than doubled by process optimization. Using gene amplification with the DHFR inhibitor methotrexate, production clones with titers of 6 g/L can be generated.

**Conclusion:**
Our cell line development platform is capable of generating higher expressing clones than a commercially available system. For monoclonal antibodies titers of 6 g/L were achieved.

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# Investigation of the metabolism and fed-batch culture development of Jurkat cells

**Miss Markella Zacharouli**[1,2], Prof Veronique Chotteau[1,2]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:** This study aims to give insight into the metabolism of T-cells and develop a feeding strategy for their culture, where Jurkat cells are studied as a model.
**Impact/Novelty:**
It is critical to understand the metabolism of Jurkat cells as T-cell models which has great importance in the immunology community.

**Introduction:** Treatment with T-cells is very important for fighting cancer. Optimal cultivation of these cells is important to ensure the cell expansion with high viability and quality. This requires understanding of the cell metabolism and process development, areas which are still poorly investigated.

**Methods/Approach:** We studied the effect of the feeding of the nutrients, glucose and glutamine, and the effect of the by-products, lactate and ammonia, in Jurkat cells culture using RPMI1640 medium supplemented with serum. We identified the feeding conditions optimal for cell growth, with maintained low levels of lactate and ammonia, and investigated the effects of these by-products on the cell growth and viability.

**Results:** It was observed that the cells can use lactate as an alternative carbon source when glucose was depleted. However the cells did not consume lactate when low glucose amounts were regularly provided. The cells actively consumed glutamine, indicating a low rate of its biosynthesis, to the contrary of other human cells, e.g. HEK293. A fed-batch strategy was developed leading to a 20-fold expansion of the cells, while maintaining a high viability.

**Conclusion:** Understanding the effects of the nutrients and the by-products is an important step towards ensuring satisfactory Jurkat cell expansion and enables the development of a strategy for fed-batch culture. This work provides a better understanding of the immune cell metabolism and provide strategies for the manufacturing process development.

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# Engineering of CHO Cell Lipid Metabolism Results in Expanded ER and Enhanced Recombinant Biotherapeutic Production

**Dr James Budge**[1], Dr Tanya Knight[1], Dr Jane Povey[1], Miss Joanne Roobol[1], Mr Ian Brown[1], Dr Gurdeep Singh[2], Dr Andrew Dean[2], Ms Sarah Turner[2], Dr Colin Jaques[2], Dr Robert Young[2], Dr Andrew Racher[2], Prof. Mark Smales[1,3]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
Chinese hamster ovary (CHO) cells are the most commonly employed cells for production of biotherapeutics such as monoclonal antibodies.

**Impact/Novelty:**
Recombinant protein production is reliant on cellular processes highly dependent on lipid metabolism. Herein is the first demonstration of how engineering lipid metabolism can enhance mammalian expression cell lines.

**Introduction:**
Chinese hamster ovary (CHO) cells are routinely employed for the production of recombinant biotherapeutics such as monoclonal antibodies (mAbs) as they are able to generate efficacious, multi-domain proteins with human-like post-translational modifications. However, the emergence of new, novel format biotherapeutic molecules present new challenges for existing CHO hosts and such proteins are often considered difficult-to-express (DTE). Existing CHO expression systems must therefore be developed and updated to align with arising challenges involved in generation and secretion of mAb and non-mAb products in order to improve productivity and product quality attributes.

**Methods/Approach:**
Here we show that a global transcriptional activator of lipid biosynthesis, sterol regulatory binding factor 1 (SREBF1), and an enzyme which catalyses the conversion of saturated fatty acids to monounsaturated fatty acids, stearoyl CoA desaturase (SCD1), can be overexpressed in CHO cells to enhance cellular processes involved in production of recombinant biotherapeutics.

**Results:**
The amount of overexpression of these lipid metabolism modifying (LMM) genes is related to the phenotypes observed and, by tuning the overexpression levels of SREBF1 and SCD1, we were able to modify the cellular lipid profile, alter cellular structure and expand the endoplasmic reticulum (ER) of an existing CHO expression host. Ultimately, direct engineering of lipid metabolism machinery through overexpression of these LMM genes resulted in improved productivity of a range of different biotherapeutic products evaluated.

**Conclusion:**
Transient and stable expression of a number of model secretory biopharmaceuticals was enhanced between 1.5-9 fold in either SREBF1 or SCD1 engineered CHO host cells as assessed under batch and fed-batch culture.

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# Towards an Understanding of Donor-to-Donor Heterogeneity in In Vitro Natural Killer Cell Production

**Mr Brian Ladd**[1,2], Ms Markella Zacharouli[1,2], Dr. Per-Henrik Holmqvist[3], Dr. Stefanie Renken[3], Dr. Pontus Blomberg[4,5], Prof Véronique Chotteau[1,2]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to "Cells as factories and therapies: modernizing biologics production for emerging modalities"**
This work aims to improve the robustness of *in vitro* NK cell production by a detailed understanding of the underlying cells using single cell transcriptomics (sc-mRNA).

**Impact/Novelty:** The novelty lies in the use of sc-mRNA to better understand the production process of autologous NK cells.

**Introduction:** Autologous NK cell therapies for cancer treatments have shown great promise in recent years. However, as with any autologous cell therapy the donor-to-donor heterogeneity is a significant hurdle for the robust production of these ground breaking treatments.

**Methods/Approach:** A feeder-cell free NK cell production process using Peripheral Blood Mononuclear Cells, after apheresis generating a mixed population as starting material, was studied with single cell transcriptomics. Samples were taken from multiple donors and at several timepoints during the production, which involves NK cell proliferation. The sc-mRNA data were used to study cell-to-cell interactions, pathway enrichment, and phenotype changes.

**Results:** Studying the cell-to-cell interactions and pathway enrichment of all donors together led to the development of a donor independent map. This map could be used to better understand which interactions are consistent throughout the NK cell production process regardless of donor. Cluster annotation on identified cell types revealed differences in the expansion of certain subtypes of cells between donors, possibly revealing the cause of the observed heterogeneity.

**Conclusion:** The use of sc-mRNA provided unique insight into the cultivation and proliferation of NK cells. These insights enabled a greater understanding of the underlying interactions necessary for NK cell proliferation and possibly highlighted the cause of the donor-to-donor heterogeneity in this process. This information also provided key insights to tune the process, such as, by adding signalling molecules to strengthen beneficial interactions.

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# Building up a tailored, CHO host library via cell line engineering

**Dr Sam Zhang**[1], Dr. Yu Zhang[1], Dr. Tao Sun[1], Dr. Shuai Wang[1], Ms. Qiao Gao[1], Dr. Zichen Qian[1], Dr. Weichang Zhou[1]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:** Developing novel host cell lines to support the production of emerging therapeutic modalities of various formats and functions.

**Impact/Novelty:** Applying advanced gene editing and other molecular biological tools to engineer and obtain tailored CHO host(s)

**Introduction:** Host cell lines have been one of the most critical components of any mammalian expression system. Over the decades, the Chinese Hamster Ovary (CHO) cell line has served as the major workhorse for biologics development and manufacturing. Although the wild-type CHO-K1 host and the DHFR or GS deficiency derivatives, together with optimized expressing vector(s), medium and process, largely accommodate the need of high volumetric productivity while maintaining product quality attributes, an increasing demand of tailored host cell development is emerging for customized requests.

**Methods/Approach:** These include fine tuning glycosylation profile, productivity enhancement of recombinant proteins with complex formats, elimination of problematic host cell proteins, developing targeted integration system, creating site-specific conjugation system etc.

**Results:** In this presentation, we summarize our recent effort in developing novel host cell lines to meet specific requirements in productivity and quality with the aid of advanced gene editing tools.
**Conclusion:** We believe that establishing a comprehensive host cell library would aid the expedited development of innovative therapeutics that better meet patients' needs.

**Conflict of Interest:** No conflicts to declare.

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# Evaluation of an Engineered CHO Cell Line as novel host for Enhanced Biopharmaceutical Production

**Mrs. Kristin Thiele**[1], Dr. Ann-Cathrin Leroux, Dr. Jimit Shah, Ms. Imme van der Kamp, Mr Lukas Klein, Ms. Martina Raasholm, Ms. Carmen Leichtle, Dr. Christoph Zehe
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance:** This study introduces an engineered CHO cell line as a new cell factory for the commercial production of biopharmaceutical proteins.
**Impact/Novelty:** Evaluation of an engineered CHO knockout cell line serving as novel host for production clones with optimized cell traits and quality attributes, aiming for high-yield, cost-efficient production of new therapeutics.

**Introduction:** CHO cell lines are the leading mammalian systems for producing protein-based biopharmaceuticals. Advances in cell line development, including screening via automation, vector optimization, media and process innovations, have significantly improved yield and quality. To keep pace with the rapid development of new therapeutics, genetic engineering of cell lines for improved growth, productivity and product quality characteristics is a promising approach. This study evaluates a genetically engineered CHO cell line as a novel host for the production of biopharmaceuticals, ensuring robust, high titer cell lines.

**Methods:** Engineered CHO cells were used to generate stable pools expressing different biopharmaceuticals, which were subsequently evaluated in fed-batch processes using 4Cell®SmartCHO media and feeds. Stable clones expressing a monoclonal antibody were obtained using the 4Cell® CHO platform, then analyzed for titer, productivity, quality, growth, and viability in Ambr 15 fed-batch processes, compared to wild-type (WT) cell lines.

**Results:** The results of our study demonstrated a substantial improvement of final titer and specific productivity for all three tested products. Fed-batch results of stable pools generated based on the novel host cell revealed an average increase in final titer of 1.7-fold compared to WT pools. While cell growth was reduced (peak viable cell concentration of 0.55-fold compared to WT), an average increase of 2.8-fold was achieved for cell-specific productivity. Subsequent single cell cloning and clone evaluation in an Ambr15 fed-batch process confirmed the observations made at pool stage.

**Conclusion:** The engineered CHO cell line significantly enhanced productivity and final protein yields for all tested molecules, marking it as a promising new host for our cell line development platform.

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# Novel Directed-Evolution Approach to Engineering Cells for Significantly Improved Therapeutic Protein and Viral Vector Production

**Mr Larry Forman**[1], Dr Kathy Ngo[1], Dr Lawrence Chasin[2]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:** Our cell-engineering technology has created cells capable of significantly higher mAb productivity (via engineered CHO cells), and AAV productivity (via engineered HEK-293 cells); both with improved product quality attributes.

**Impact/Novelty:** The first major increases to specific productivity in decades; for example, a five-fold increase in mAb volumetric productivity means 80% of new mAb manufacturing capacity may not be needed.

**Introduction:** Advances in therapeutic protein manufacturing using CHO cells have mainly come from improvements in media, transfection methods, and/or screening thousands of transfected clones—not from significant increases in specific productivity. Efforts to engineer improved host cells have met with limited success, owing to limitations inherent to CHO cells. Viral vector production faces similar challenges owing to limitations inherent to HEK-293 cells. A novel cell-engineering platform based on directed-evolution was developed to address these limitations.

**Methods/Approach:** We created engineered cells with diverse increased chromosome numbers by repeated homotypic cell fusions, followed by isolating those with desired phenotypes using direct selective pressure, or by staining and screening via cell sorting. This protocol yields pools and clones with phenotypes related to the targeted biomanufacturing process.

**Results:** We have demonstrated the effectiveness of our engineering platform for several applications by creating engineered cells with the following phenotypes (with further improvements anticipated): created CHO cells with >10-fold higher endoplasmic reticulum levels for therapeutic mAb production; demonstrated specific productivity >110 pg/cell-day and volumetric productivity of 13.5 g/l in batch shake flask culture; created HEK-293 cells with enhanced virus-production machinery; demonstrated 12-fold higher AAV productivity and 55% capsid percent-full.

**Conclusion:** We have demonstrated the application of our technology for increased and improved protein production, as well as for increased and improved AAV production. It is anticipated that our cell engineering technologies could further be used to improve protein glycosylation and the production of difficult-to-produce proteins (*e.g.*, blood clotting factors and multi-valent antibodies).

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# Achieving High Monoclonal Antibody Production in Fed-Batch Cultures by Engineering Requisite Phenotypes into CHO Cells

**Mr Larry Forman**[1], Dr Kathy Ngo[1], Dr Lawrence Chasin[2]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:** Bottlenecks inherent to CHO cells have been a barrier higher specific productivity for decades. Bottlenecks are alleviated by engineering-in new and/or important phenotypes, such as >10-fold higher endoplasmic reticulum.

**Impact/Novelty:** The first major increases to specific productivity in decades; for example, a five-fold increase in mAb volumetric productivity means 80% of new mAb manufacturing capacity may not be needed.

**Introduction:** Advances in manufacturing therapeutic proteins using CHO cells have mainly come from improvements in media, transfection methods, and/or screening thousands of transfected clones—not from significant increases in specific productivity. Efforts to engineer improved host cells have met with limited success owing to limitations inherent to CHO cells. A novel cell-engineering platform based on directed-evolution was developed to alleviate these limitations.

**Methods/Approach:** Engineered cells with diverse increased chromosome numbers were created by repeated homotypic cell fusions. Cells with desired phenotypes (such as increased endoplasmic reticulum) were isolated by staining and screening via cell sorting. This protocol yields pools and clones with phenotypes relevant to the targeted biomanufacturing process. The cells were stably transfected with mAb heavy- and light-chain genes using Sleeping Beauty transposase soluble protein. Transfected cells were grown in shake flasks, and their productivity was evaluated.

**Results:** The effectiveness of our cell-engineering platform was demonstrated by creating cells with multiple desirable phenotypes, including 10- to 15-fold higher endoplasmic reticulum per cell. Specific productivities up to 117 pg/cell-day and volumetric productivity of up to 13.5 g/l were observed from fed-batch cultures. 60-generation stability was demonstrated for 83% (10 of 12) of transfected clones.

**Conclusion:** We have demonstrated the application of our cell-engineering technology for increased protein production. It is anticipated that our cell engineering technologies could further be used to improve protein glycosylation (*e.g.*, higher sialic acid site occupancy) and the production of difficult-to-produce proteins (*e.g.*, blood clotting factors and multi-valent antibodies).

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# A cell line biosensor for viral detection and quantification

**Miss Eunice Gonçalves**[1,2], Ana Isabel Almeida[1,2], Miguel Guerreiro[1,2], Sofia Fernandes[1,2], Ana Sofia Coroadinha[1,2]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**

In the emergence of infectious diseases is essential new antiviral therapies and vaccines development. Engineered cell-based viral sensing systems represent a pivotal strategy to advance the field.

**Impact/Novelty:**

Viral quantification and detection methods available nowadays hamper vaccination and antiviral therapies development. The cell-based biosensor herein presented opens doors to virology research as a robust detection method.

**Introduction:**

The increasing infectious diseases threat to public health requires more sensitive and precise viral diagnose and titration methods development. Therefore, herein a novel cell-based system, genetically encoded, for detecting viral proteolytic activity is described.

**Methods/Approach:**
This sensing system is comprised of (1) a structurally distorted Cre recombinase, through cyclization by *Nostoc punctiforme* DnaE intein splicing **(inactive state),** harboring a protease cleavable sequence as sensor, and (2) a reporter cassette encoding an inverted GFP inserted into a flip-excision system. Therefore, in the protease presence, distortion is relieved **(active state)**, resulting in stable fluorescence emission.
The cell-based biosensor was successfully implemented to detect Tobacco Etch virus (TEV) and Human Rhinovirus (HRV) in H1-HeLa cells. H1-HeLa cells genetically encoding the reporter module were co-transfected with sensor and protease modules to evaluate sensor performance. Positive and negative controls were also performed.
**Results:**

Flow cytometry analysis demonstrated 70% and 60% of $GFP^+$ cells for TEV and HRV sensor systems, respectively, comparable to positive controls. Both systems exhibited low background (less than 2.5% of $GFP^+$ cells). These preliminary results highlight the biosensor's efficacy in viral detection, showcasing minimal background activity and robust sensor performance.

**Conclusion:**

The biosensor here described is an engineered solution that will allow improved efficacy and precision on viral quantification and detection methods. Furthermore, it can be further used as a theranostic tool to other than infectious diseases, providing a platform with the potential to enhance therapeutics development for different diseases.

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# Exploring the potential of secreted products from canine mesenchymal stromal/stem cells against antibiotic-resistant bacteria

**Mrs Amira Aburza**[1], Prof Xavier Donadeu[1], Dr Gavin Paterson[1], Dr Cristina Esteves[1]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:** The use of canine mesenchymal stromal/stem cells (MSCs) secretome as potential sources of anti-infective agents**.**

**Impact/Novelty:** To exploit MSCs' conditioned media (CM) against multidrug-resistant methicillin-resistant *Staphylococcus pseudintermedius* (MRSP), a bacterium commonly associated with unresolved lethal infections in dogs.

**Introduction:** Antibiotic resistance is a growing concern worldwide, and traditional antibiotics are becoming less effective. In this project, we compared the antibacterial capacity of the CM from endometrial and adipose MSCs (EM-CM and AT-CM). Additionally, we tested the antimicrobial efficacy of CM in the presence of antibiotics, to decrease antibiotic usage in the treatment of infections.

**Methods/Approach:** CM was obtained from primary cultures of canine MSCs (EM and AT) following 48h of cells in culture in the absence of serum and antibiotics. The resulting cell-free CM containing the secretome was then used in bacterial growth assays to test the antibacterial effect alone or combined with antibiotics to which the bacterium strain is either resistant (Erythromycin) or sensitive to (Chloramphenicol). A relevant canine pathogenic isolated from skin infection was used for this purpose. quantitative polymerase chain reaction (qPCR) was used to identify antimicrobial genes.

**Results:** Bacterial assays demonstrated a significant reduction in growth with CM, even against erythromycin-resistant bacteria ($p$-value<0.01). Both EM and AT-CM exhibited similar effects. qPCR identified antimicrobial expression by both MSC types. Combining CM with erythromycin showed stronger attenuation with EM ($p$-value<0.001) than AT ($p$-value<0.01), suggesting EM's enhanced antimicrobial potential. Additionally, synergistic effects against MRSP were observed when CM was combined with effective antibiotic chloramphenicol ($p$-value<0.0001), indicating a potential strategy to reduce antibiotic usage in infections.

**Conclusion:** This project has shown that both AT and EM-MSCs express anti-infective genes and attenuate the growth of resistant bacteria. Combined with antibiotics, these cells or their products could reduce the use of medically important antibiotics. This highlights their potential as a novel tool to compact antibiotic resistance.

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# Implementation of a scalable downstream processing of immunoregulatory small extracellular vesicles

**Mr Quentin Pruvost**[1], Mr Thibaud Dauphin[1], Ms Clémentine Claire[1], Ms Valérie Lalanne[1], Mr Grégoire Mignot[1], Ms Blandine Lieubeau[1], Mr Jean-Marie Bach[1], Ms Apolline Salama[1], Ms Laurence De Beaurepaire[1], Ms Mathilde Mosser[1]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:** In this study, we seek to understand the impact of the implementation of a scalable purification process on yield, purity and efficacity of an emerging therapy based on extracellular vesicles.

**Impact/Novelty:**

- Development of a scalable downstream process for small EV-based therapy.
- Impact of downstream processing on sEV recovery, purity and properties.

**Introduction.** The small extracellular vesicles (sEV) have gained attention due to their safety, biocompatibility and efficacy, but their use as a new class of medicine is contingent upon the development of scalable processes that meet GMP standard. sEV are cell-secreted lipid bilayer particles smaller than 200 nm that carry biological materials mirroring the parental state. sEV are a heterogeneous population of particles whose composition and purity varies according to separation methods, mainly based on size. In this study, we describe the implementation of a scalable process for sEV isolation and purification.

**Methods/Approach.** sEV were produced from beta cells lines cultured in serum-free medium. At lab scale, sEV were isolated by differential centrifugations, ultrafiltration (UF) and size exclusion column (qEV). For large scale implementation, the feasibility of replacing these steps by depth filtrations, tangential flow filtration (TFF) and scalable size- and charge-based column (capto$^{TM}$ core) for purifying sEV were respectively assessed. EV size and concentration were determined by nanoparticle tracking analysis. Purity was determined by the particles to protein ratio and tetraspanin-positive particles percentage. The immunoregulatory properties of sEV were assessed through mixed lymphocyte reactions.

**Results.** Replacing the UF step with TFF slightly improved the recovery yield of sEV by cells. The use of a 100 kDa cut-off membrane instead of a 300 kDa one reduced the purity obtained without improving EV yield. The addition of 5 diafiltrations did not achieve as good a purity as that obtained after the qEV. Interestingly, purification by capto$^{TM}$core chromatography gave the same yields and purity as on qEV column. The centrifugal clarification step was successfully replaced with depth filtrations. Characterization of the immunoregulatory properties of sEV obtained from lab scale and scalable processed is currently being evaluated.

**Conclusion.** Our study highlights the main challenges encountered to design a scalable purification process for therapeutic sEV production.

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# Novel isolation and activation platform with active-release technology for scalable cell therapy manufacturing

Mr. George Prout, Maren Grün[1], PhD Hany Meås[2], Sylvie Kjær[2], PhD Artur Javmen[3], Joanna Kern[4], Hendrik Adams[5], Laurens Sierkstra[5], Eugene Kang[6], PhD Tuva Hereng[2], Heidi Vebø[2]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
The new CTS™ Detachable Dynabeads™ CD3/CD28 enable increased T cell yield and quality as well as more consistent, automated and scalable autologous or allogeneic cell therapy processes.

**Impact/Novelty:**
The CTS Detachable Dynabeads CD3/CD28 represent the new generation of Dynabeads, used for T cell isolation and activation, that now allows for active bead removal from target cells.

**Introduction:**
CTS Dynabeads CD3/CD28 has been used successfully in advancing T cell therapies through >200 clinical trials to commercial T cell drug manufacturing. The new CTS Detachable Dynabeads CD3/CD28 combine performance of CTS Dynabeads CD3/CD28 with the flexibility of an active release mechanism, enabling termination of activation signaling by releasing the Dynabeads using the CTS Detachable Dynabeads Release Buffer.

**Methods/Approach:**
T cells were isolated and activated using CTS Dynabeads CD3 /CD28 or CTS Detachable Dynabeads CD3 /CD28. Culturing was performed, both at laboratory scale and using an automated system, with CTS™ OpTmizer™ T Cell Expansion SFM. Isolated and activated T cells were expanded in media supplemented with 100 U/mL IL-2 for 7-10 days.

**Results:**
The generated data show that the CTS Detachable Dynabeads CD3 /CD28 Kit requires no upstream T cell selection due to simultaneous isolation and activation of naïve and early memory T cells. The T cells are isolated with high recovery purity and uniformly stimulated. Activated and expanded T cells preserve a young phenotype. Finally, we show that the process is successfully scaled-up and automated using the CTS™ DynaCellect™ Magnetic Separation System.

**Conclusion:**
This new bead release technology is critical to i) control activation time and preserve a young phenotype, ii) release Dynabeads prior to T cell manufacturing steps, and iii) to shorten the manufacturing process. The beads are also compatible with the automated CTS DynaCellect Magnetic Separation System.

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# 2G-UNic genetic enhancers applied in the CGT field for increasing viral titers and payload expression

**Dr Maurice van der Heijden**[1], Bart Engels[1], Annemarie de Jel[1], Chantal Tilburgs[1], Elena Gugiatti[1], Bircan Coban[1], Raymond Verhaert[1]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:** Genetic enhancer elements are developed for improved capacity of the cells used as factories for CGT production.

**Impact/Novelty:** Application of genetic enhancer technology in the CGT field will help in the development of affordable therapies and overcome production capacity limitations.

**Introduction:** 2G UNic genetic elements are routinely used in plasmid vectors to obtain high-level protein expression in stably transfected CHO cells. This study uses modified versions of these elements aiming to increase Lentivirus titres and AAV payload expression.

**Methods/Approach:** Genetic 2G UNic elements were introduced in multiple vectors of a widely used 3rd Generation LV platform and used to co-transfect HEK-293 derived suspension cells. LV production of the packaging cells was measured by different analytical methods. In a second project, 2G UNic elements were introduced in the transfer vector of an AAV system used to transfect HEK-293 packaging cells. Isolated AAV particles were used to transduce HEK cells and expression of the payload gene was measured.

**Results:** Introduction of the 2G UNic elements in single LV vectors of the co-transfected set resulted in significantly increased LV titers. Combinations of vectors with 2G UNic elements further increased the LV titers. A specialized 2G UNic element was applied in the AAV transfer vector and AAV particles were harvested. The enhancer element significantly increased payload expression in transduced HEK cells with a constitutive CMV promoter as well as a tissue-specific promoter.

**Conclusion:** The data show that 2G UNic enhancer elements could be widely applied in the cell and gene-therapy field for improving viral titres and payload expression.

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# Improving Monoclonal Antibody Production in CHO Cells for Gastric Cancer: Assessing Promoters and Vector Designs

**Dr Yesenia Latorre**[1,2], Ms. Isis Araya[2,6], Dr Carla Gutiérrez[2], Dr. María Carmen Molina[1], Dr. Claudia Altamirano[2,3,4,5]

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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:** The identification of optimal promoter combinations and vector design stands as a pivotal pursuit with the potential for substantial enhancements in antibody production.

**Impact/Novelty:** This study holds promise for a direct impact on the efficiency and quality of monoclonal antibody (mAb) production, contributing to the progression of knowledge in cellular biotechnology.

**Introduction:** Monoclonal antibody production using CHO cells faces challenges such as epigenetic silencing in specific promoters. Bicistronic transfection, known for its productivity advantages over monocistronic methods, typically employs IRES elements in vector design. While alternative designs testing independent promoters for each mAb chain offer advantages in equal expression, the accumulation of heavy chain (HC) in complex mAbs poses toxicity challenges. This study proposes two approaches for Anti-MicA production: a double promoter (prom-LC-prom-HC) or a promoter plus IRES element (prom-LC-IRES-HC), testing UCOE-CAG and RegCG/GRE/CMV promoters.

**Methods/Approach:** Vectors are designed using SnapGene software and synthesized by GenScript. After transformation into *E. coli* TOP10, purification occurs using NucleoBond® Xtra Maxi (Macherey-Nagel GmbH & Co.). Subsequently, vectors are transfected into CHO-S cells using the ExpiCHO transient transfection kit, following the manufacturer's instructions. Each transfection is conducted in triplicate. Quantification of mAb is carried out through ELISA. Western Blot and q-PCR methods are employed to confirm the presence of both light chain (LC) and heavy chain (HC) inside the cells.

**Results:** Ongoing transfections prevent conclusive results at this stage. However, preliminary findings demonstrate superior production when using the RegCG/GRE/CMV promoter compared to UCOE-CAG.

**Conclusion:** Anticipated outcomes suggest that a combination of these promoters, strategically employing the weakest for HC and the strongest for LC, holds promise as a more effective option, potentially increasing mAb productivity.

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# Enhancing Monoclonal Antibody Yield in CHO Cells for Gastric Cancer: Assessing Ratios of Global Regulators

Dr Yesenia Latorre[1,2], Ms Isis Araya[2,3], Dr Karen Toledo[1], Dr Carla Gutierrez[2], Mr Jose Rodriguez[2], Dr María Carmen Molina[1], Dr Claudia Altamirano[2,4,5,6]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:** Identify the best ratio between two global regulators for enhancing antibody production.

**Impact/Novelty:** This research holds the potential to significantly improve the efficiency and quality of monoclonal antibody production, contributing to the advancement of knowledge in cellular biotechnology.

**Introduction:** The biopharmaceutical industry, centered around monoclonal antibodies, grapples with challenges in CHO cells, characterized by toxic metabolite accumulation and inefficiencies in protein secretion. Previous attempts to enhance CHO cell production through genetic modifications targeting protein transporter and enzyme genes have fallen short of optimal outcomes. Consequently, attention has shifted to global regulators such as c-Myc and Xbp1s, known for their positive influence on CHO cell metabolism and protein secretion. Earlier studies have showcased increased productivity and growth rates in EPO CHO cell producers through the co-overexpression of c-Myc and Xbp1s. This study aims to enhance human monoclonal antibody (mAb) productivity by transiently transfecting CHO cells with varying ratios of these regulators.

**Methods/Approach:** The experimental design employs response surface methodology, utilizing Statgraphics 19 software. CHO-S cells that produced the mAb were cultured under standard conditions (37°C, 5% CO2, humid conditions. The vectors, including pCDH-puro-cMyc (addgene, #46970) and pLHCX-XBP1-mNeonGreen-NLS (addgene, #115971), were transiently transfected in different proportions using the ExpiCHO transient transfection kit, following the manufacturer's instructions. Each transfection was conducted in triplicate.

**Results:** Ongoing transfections prevent conclusive results at this stage.

**Conclusion:** Based on prior findings, it is anticipated that a higher concentration of regulators may not necessarily yield better results. Additionally, it is hypothesized that an optimal proportion between regulators may exist, negatively impacting productivity with an increase in cell viability over time, and vice versa — suggesting a trade-off between productivity and cell viability.

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# Improvement of the production of an innovator human monoclonal antibody again MICA receptor

**Mr Jose Alejandro Rodriguez-Siza**[1], Dra Karen Toledo-Stuardo[3], Dr Mauricio Vergara[1,4], Dra Maria Carmen Molina[3], Dra Claudia Altamirano[1,2,4,5]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:** This work show efforts for discovery and development of new therapeutic molecules for diseases of worldwide relevance.

**Impact/Novelty:** This work fill a gap in knowledge on media culture for a new CHO cell line improve on develop of production process of a mAb innovator with cancer therapy application.

**Introduction:** MICA is a protein expressed in adenocarcinoma cells, activating the immune response. Once this molecule is released, as an evasion mechanism, it enables it to bypass the immune system response. In our laboratory a human mAb against MICA is being developed. This mAb is produced by a stable CHO-s cell line that was developed co-transfecting two vectors with an optimized promoter that is not susceptible to silencing. We present the first progress in improving the effect of culture media on production and cell growth with the innovative mab CHO-s cell line.

**Methods/Approach:** We tried different culture media for mAb production in CHO cells: ExpiCHO expression, as a control, FortiCHO, BalanCD growth A and SFM4CHO. The cultures were carried out by triplicate in spinner flask. We measured cell density, viability, mAb production and metabolites concentration.

**Results:** We observed that FortiCHO and BalanCD growth A produce a similar maximal cell density (~8x10^6 cell/mL), but only with the latter, we obtained ~3 times more mAb titter, in comparation with the control. Using SFM4CHO, we observed the lowest cell concentration and the volumetric productivity, obtained about ~3 times less in comparation with ExpiCHO. The specific growth rate and specific productivity of mAb didn´t show significant differences for all media tested.

**Conclusion:** We conclude that the culture media BalanCD growth A has the capacity to improve the growth and mAb production for the mAb-producer CHO cell line. As a projection, It will be explored to develop different strategies of culture modality to get a higher mAb titre.

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# Rational design of inducible rep gene for rAAV packaging cell line generation in HEK293 cells

**Mrs Laia Bosch-Molist**[1], Dr. Xavier León[2], Dr. Miquel Garcia[2], Dr. Laura Cervera[1], Dr. Francesc Gòdia[1]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
By demonstrating effective rep gene induction via genetic engineering, this study addresses a critical step towards enhancing rAAV manufacturing by the generation of stable HEK293 packaging cell lines.

**Impact/Novelty:**
This study tackles a key challenge in rAAV production: scaling up robust systems through the development of stable HEK293 packaging cell lines, overcoming the hurdle of E1A-mediated rep toxicity.

**Introduction:**
As a first step for generating a HEK293-based packaging cell line for recombinant adeno-associated virus (rAAV) production, we aimed at elucidating the minimal rep gene elements with functionality to support inducible rAAV production while minimizing rep toxicity potential.

**Methods/Approach:**
First, the feasibility of rep and cap gene separation into different open reading frames towards rAAV production was studied. Then, different large and small rep constructs were designed and by a DoE approach, we identified the minimal rep elements in combination leading to maximal rAAV production under inducible Tet-On system. Finally, we evaluated several large rep genetic constructs to understand how the introduced mutations contribute to achieving tight control of rep expression.

**Results:**
It is demonstrated that newly designed Rep68min and Rep68plus constructs are the only essential sequences for effective rAAV production under inducible Tet-On system. Further, it is established that the optimal dose-response is dependent on Rep protein dosage. Finally, the study of mutations in the large rep constructs demonstrates that mutations at splicing site are crucial for maintaining tight inducible control of Rep expression.

**Conclusion:**
The minimal rep elements necessary for maximal rAAV production in inducible system are explored. The stepwise rational engineering approach used, offers a first step towards effective control of rep toxic expression for the generation of a packaging cell line for rAAV production in E1-dependent cells.

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# An improved CRISPR-Cas9 protein-based method to engineer Sf9 insect cell genes

**Mr Miguel Graça**[1,2], Dr. Nikolaus Virgolini[1,2], Dr. Ricardo Correia[1,2], Dr. Jose Escandell[1,2], Dr. António Roldão[1,2]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
The increasing complexity of biologics and production scales requires the continued development of tailor-made producer cells, for which improved tools for gene editing such as herein described are crucial.

**Impact/Novelty:**
We implemented a CRISPR-Cas9 pipeline for genetic engineering of Sf9 insect cells (IC) yielding higher editing efficiencies than other existing methods showing great promise for accelerating future studies.

**Introduction:**
Cell line development in IC has stagnated due to the limited amount of editing tools available. CRISPR-Cas9 technology has transformed genetic engineering, but so far has been applied to insect cells with limited efficiencies.

**Methods/Approach:**
A protocol for delivery of a ribonucleoprotein complex comprised of a guide RNA and the enzyme Cas9 to Sf9 IC was developed. It was then applied to knock-out caspase initiator *Sf-Dronc*, as proof-of-concept gene, aiming at alleviating cell apoptosis during a baculovirus expression vector system (BEVS) process. The resulting engineered cell lines were characterized as per their phenotype and production of recombinant adeno-associated viruses (rAAVs) and influenza virus-like particles (iVLPs).

**Results:**
A knockout rate of 67 % was achieved with the implemented protocol (vs. 12 % of other reported methods). When applied to knocking-out *Sf-Dronc*, mutants containing deletions in all four alleles of the host genome were obtained. The onset of cell viability drop following infection with baculovirus was efficiently delayed in these mutants when comparing to parental cells demonstrating that Dronc gene is required to induce apoptosis in BEVS process. *Sf-Dronc* deletion was shown to have minimal impact on production of rAAV, while production of iVLPs still requires investigation.

**Conclusion:**
This work reports the development of an efficient protocol for editing IC using CRISPR-Cas9 technology and showcases how it can be used to manipulate the kinetics of a BEVS process.

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# Use of Drosophila S2 Cells for Production of Highly Immunogenic Antigens

**Dr. Magdalena Skrzypczak**[1], Dr. Stine Clemmensen[1], Ms. Tanja Domeyer[1], Dr. Anders H. Hansen[2], Prof. Adam F. Sander[3], Dr. Louise Goksøyr[3], Dr. Cyrielle E. Fougeroux[3], Assistant Prof. Manja Idorn Haugum[4], Dr. Lasse Ebdrup Pedersen[5], Dr. Willem A. de Jongh[3]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
*Drosophila* S2 insect cells are utilized to produce high-quality proteins for the development of protein-based vaccines and therapeutics.

**Impact/Novelty:**
S2 cells produce short, paucimannosidic N-glycans. Through glycoengineering of S2 cells we aimed to obtain cells producing potentially more immunogenic N-glycans for production of vaccine candidates with increased potency.

**Introduction:**
Glycosylation, a vital post-translational protein modification, profoundly influences aspects such as folding, stability, activity, half-life, and immunogenicity. Lectin receptors expressed by antigen-presenting cells play a pivotal role in recognizing carbohydrate structures on pathogens or vaccine antigens, making them attractive targets for immune response enhancement. In our study, we investigated the potential of high-mannose and xylosylated N-glycans as cis-adjuvants for a vaccine candidate.

**Methods/Approach:**
High-mannose N-glycans producing S2 cell line was obtained using CRISPR/Cas9 for deletion of α-Man-Ia gene and xylosylated N-glycans producing S2 cell line was obtained by expression of plant xylosyltransferase gene. Glyco-modified cells were used to produce receptor-binding domain (RBD) of SARS-CoV-2 and vaccines containing this antigen were tested in mice and compared to the wild-type, paucimannose antigen. Neutralisation potency of sera was tested in virus neutralization assay. Our study also benefited from cutting-edge tools such as the CellCelector™ and C.BIRD™ microincubator, expediting the generation of monoclonal S2 cell lines.

**Results:**
RBD with high-mannose glycans induced a stronger immune response in mice compared to RBD with the wild-type paucimannosidic glycan structures of S2 cell proteins. Moreover, functionality of the antibodies elicited was confirmed in a virus neutralization assay and showed high neutralization capacity of sera from mice immunized with the high-mannose antigen. The mice study with xylosylated RBD also showed higher titers in comparison to the paucimannosylated RBD.

**Conclusion:**
In summary, these findings underscore the immunomodulatory potential of vaccine antigen glycans, emphasizing their significance in future vaccine development.

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# Large-scale Production of Extracellular Vesicles derived from hPSC for cardiac repair

Ms Ana Meliciano[1,2], Dr Ana Filipa Louro[1,2], Mr Pedro Vicente[1,2], Ms Claudia Diniz[1,2], Mr João Jacinto[1,2], **Dr Margarida Serra**[1,2]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
Cells as factories and therapies: modernizing biologics production for emerging modalities
This work shows the development of a scalable bioprocess for the production of Extracellular Vesicles derived from human induced pluripotent stem cells in bioreactors, through modulation of critical process parameters.

**Impact/Novelty:**
We developed a powerful bioprocess that ensures the scalable production of clinically relevant numbers of hiPSC-EV, enhancing both EV yields and functionality, thus potentiating their use in cardiac repair.

**Introduction**
The development of effective extracellular vesicle (EV)-based therapies for cardiac repair is hindered by the challenge of efficiently scaling up their production. Addressing upstream obstacles involves the selection of the parent cells and their large-scale expansion. We previosly identified human induced Pluripotent Stem Cells (hiPSC) as a promising source of native EVs for cardiac regeneration. In particular, we elucidated the miRNome and bioactive signatures of hiPSC-EVs and provided evidence about their mechanism of action. Herein, we developed a scalable bioprocess for hiPSC-EVs production by combining 3D hiPSC culture approaches with stirred-tank bioreactor (STB) technology, while fine-tuning critical process parameters to boost EV secretion without compromising their quality attributes.

**Methods/Approach**
hiPSC were expanded as 3D aggregates in STB, operated in perfusion. EV were isolated from conditioned medium via density gradient ultracentrifugation and characterized for the presence and topology of specific EV-associated markers, yield, particle size distribution, and morphology. The bioactivity of EV was evaluated through in vitro cardiac fibrosis and angiogenesis models using human cells. Small RNA-Seq was performed to identify differentially expressed miRNA between hiPSC-EV produced in STB and the conventional 2D monolayer static culture.

**Results**
The STB bioprocess yielded a remarkable 6-fold increase in EV isolated per mL of conditioned medium compared to 2D culture. The hiPSC-EV produced in STB displayed a cup-shaped morphology, tested positively for EV markers, and were efficiently taken up by recipient cells. Functionally, hiPSC-EV exhibited enhanced pro-angiogenic and anti-fibrotic activities. These functional outcomes were further confirmed by correlating effects at the transcriptomic level through small RNAseq. Importantly, bioprocess scalability was successfully validated in 2L scale using a cGMP-compatible bioprocess.

**Conclusion**
We developed a powerful bioprocess that ensures the scalable production of clinically relevant numbers of hiPSC-EV in STB, enhancing both EV yields and functionality, thus potentiating their use in cardiac repair.

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# Synthetic biology and Inducible genetic circuits: A pathway to next generation Biotherapeutics

**Dr Rajinder Kaur**[1], Ms Sheryl Lim, Dr. Alfonso Blanco, Dr. Ioscani Jimenez Del Val
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

Synthetic biology and Genetic circuits has enabled tunable gene expression in production cells and play a crucial role in controlling and manipulating cellular function in various cellular systems including Chinese hamster ovary (CHO) cells. CHO cells are the workhorse for production of complex proteins such as monoclonal antibodies (mAbs). The site specific integration and inducible promoters has opened another arena to modulate the mAb quality and critical quality attributes (CQAs). Genetic consist of DNA sequences and regulatory elements, allowing precise control over gene expression and cellular behavior. These circuits can be engineered to respond to specific signals or triggers, enabling precise temporal and spatial control of gene expression. This capability is particularly beneficial in optimizing protein expression and increasing product yield. Tunable gene expression incorporating synthetic regulatory elements, such as inducible promoters or transcriptional activators/repressors, gene expression can be modulated in response to external factors, such as small molecule inducers or changes in environmental conditions. IGCs (Inducible genetic circuits) allows fine-tuning of protein production, optimizing productivity while minimizing cellular burden. It enables the optimization of manufacturing processes by fine-tuning protein expression levels, improving product quality, and reducing metabolic burden. Additionally, genetic circuits can be used to enhance cellular functions and address bottlenecks in protein production pathways, leading to increased product titers and improved process robustness. CRISPR-Cas9 genome editing technology has enabled the precise engineering of CHO cell lines with specific genetic circuits, allowing for more sophisticated and controlled gene expression. Furthermore, advancements in synthetic biology tools and computational modelling have facilitated the design and optimization of genetic circuits, accelerating the development of biopharmaceutical production processes. In conclusion, IGCs offer powerful tools for controlling and modulating gene expression in CHO cells. These advancements provide opportunities for improving biopharmaceutical production processes, enabling higher yields, improved product quality, and increased process robustness.

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# Data-driven reprogramming towards plasma cell phenotype to enhance recombinant protein production

**Dr. Ieva Berzanskyte**

Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
We align to “cell as factories” and “modernizing biologics production” by using a synthetic data-driven approach to improve the protein production capability of industrial cell lines.

**Impact/Novelty**
We aim to maximise protein production capacity of the key industrial cell line, CHO, by directly reprogramming towards hamster plasma cell using algorithm-predicted transcription factors based on hamster plasma cell transcriptome.

**Introduction**
The efficiency of large-scale production, particularly for complex products, still lags behind the potential of *in vivo* antibody-producing cells. In this project, we aim to improve CHO cell line capabilities for protein production by reprogramming them to exhibit a more plasma cell-like phenotype, characterised by a high specialisation in antibody production. To achieve this, we employed a direct reprogramming approach using the Mogrify algorithm, which incorporates high-quality transcriptomic data for both the donor cell type (CHO line) and the target cell type (hamster plasma cell) and analyses it within the context of a background dataset and knowledge of the gene regulatory network derived from independent databases.

**Methods/Approach**
We developed a protocol to enrich for hamster plasma cells and obtained transcriptomic data from an abundant population using single-cell RNA sequencing. To facilitate data-based predictions for non-model species, plasma cells were also isolated from well-annotated human and mouse species. By applying the Mogrify algorithm, we predicted transcription factors that could direct a CHO cell line towards acquiring an antibody-secreting plasma cell phenotype. Finally, we are overexpressing the target transcription factors and assessing the production of a panel of industrially-relevant biologics.

**Results**
Obtaining a hamster plasma cell transcriptome allowed us to predict hamster-specific transcription factors required to transition from CHO (epithelial phenotype) to plasma cell phenotype. We ran Mogrify predictor algorithm on CHO to mouse/hamster/human plasma cell fate, and compared the differences in gene networks, allowing us to extract hamster-specific information. The data shows that CHO cells lack lymphocyte background as well as phagocytic, endocytic and Golgi apparatus-related gene networks when compared to plasma cells. The algorithm proposed an optimal novel combination of transcription factors that are being tested for reprogramming efficiency and, subsequently, protein production improvement.

**Conclusion**
Our study showcases that CHO cell lines have a measured potential to upregulate pathways required for high productivity, as exemplified by the plasma cells. Reprogramming CHO cell lines towards a plasma cell-like phenotype offers promising avenues for enhancing protein production capabilities in industrial settings.

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# Perfusing a mixture of four cell lines – insight into cell line divergence

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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:** Several therapies benefit from a mixture of antibodies to harness synergistic effects. Instead of mixing antibodies post-production, we mix cell lines to study behaviour in a more cost-efficient production mode.

**Impact/Novelty:** A mixture of two antibodies was developed for treating Covid19 (REGEN-COV). Besides infectious diseases, antibody mixtures can be used for treatment of cancer. Cost-efficient production will revolutionize treatment.

**Introduction:**
The production of monoclonal antibodies is a well-known concept for treating diseases. For several conditions, the use of several antibodies is recommended due to synergistic effects that assure an effective disease treatment. Due to high costs of separate process development and GMP validation of each batch, a single process is desirable.

**Methods/Approach:**
We mixed four isogenic cell lines generated through targeted integration and producing four different antibodies in perfusion and cultivated the mixture for 30 days. We assessed the cell line population by analysing the composition of the genetic material.

**Results:**
In contrast to batch cultivation, in which both the isogenic cell lines and the mixture grew similarly, and cell line ratios stayed stable over time, the ratio in perfusion diverged. However, divergence profile was reproducible, which allows for correction of drift at the inoculation stage, if process conditions are held constant.

**Conclusion:**
Due to a need for antibody mixtures, more cost-efficient processes need to be developed. Mixing several cell lines will avoid the need for many GMP validation processes and circumvent high costs, if regulatory authorities allow for it. Cell lines can be cultivated in batch mode or perfusion mode with the need for additional process optimization.

# Poster Presentations

# Product Quality

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# Characterization and Risk Assessment of Polysorbate-Degrading Hydrolases in Monoclonal Antibody Products

**Mr. Stefan Schneider**[1], Mr. Linus Weiß[1,2], Ms. Melanie Maier[2], Dr. Nikolas Zeh[2], Dr. Valerie Schmieder-Todtenhaupt[2], Mr. Daniel Heinzelmann[2], Dr. Moritz Schmidt[2], Prof. Dr. Kerstin Otte[1], Dr. Daniel Lakatos[2], Dr. Simon Fischer[2]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Product Quality**
This study tackles enzymatic polysorbate degradation in biological drug formulations, a key issue in the biopharmaceutical industry.

**Impact/Novelty**
Our research provides a functional characterization of polysorbate-degrading hydrolases detected in purified monoclonal antibody products, aiding the removal of critical HCPs during biopharmaceutical production.

**Introduction**
Enzymatic polysorbate degradation, mediated by host cell proteins, poses a significant challenge in the biopharmaceutical industry. We aim to systematically overexpress and characterize CHO cell-derived hydrolases that degrade polysorbate in monoclonal antibody formulations.

**Methods/Approach**
We used a sensitive LC-MS/MS method to identify polysorbate-degrading hydrolases in seven different purified monoclonal antibody products. These hydrolases were overexpressed in a lipoprotein lipase knockout CHO-K1 host cell line for detailed characterization, including polysorbate-degradation activity, pH-dependent activity testing and localization study.

**Results**
Using LC-MS/MS, we analyzed seven purified mAb products and identified several hydrolytic enzymes, with significant variation among products. A high correlation was found between mRNA expression and hydrolase occurrence for some hydrolases. A knockout of LPL in CHO cells led to slower PS degradation, suggesting LPL's major role but also highlighting the involvement of other hydrolases. Most of the remaining CHO hydrolases were secreted into the supernatant when overexpressed in a non-producing CHO-K1-LPL-KO host cell line, partially explaining their presence in purified mAb products. Detailed analysis showed that some overexpressed hydrolases had significant PS degradation activity and a common catalytic triad distinguished active from inactive hydrolases. Furthermore, pH screenings of the purified enzymes revealed unique pH-dependent activity profiles for each hydrolase. Varying pH-dependent PS degrading activities were observed in three purified mAb products, indicating the presence of various residual hydrolases with unique pH optima.

**Conclusion**
Our research provides a comprehensive characterization of polysorbate-degrading hydrolases in purified mAb products. We've developed a risk matrix based on these findings, which serves as a guide for identifying critical HCPs in bioprocess development.

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# Creating robust calibration models for Raman spectroscopy to monitor and control cell culture condition

Mr Wataru Kobayashi[1], Mr Hiroaki Yamanaka[1], Ms Ayaka Suetsuna[1], Mr Natsuki Fukui[1], **Mr Soichiro Shimoda**[1]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:** We utilize Raman spectroscopy to precisely track and regulate component levels in cultures, ensuring consistent results and stable quality.

**Impact/Novelty:** We've established an innovative method for Raman calibration model creation, which enables a model predictive control with precise nutrients monitoring and feeding.

**Introduction:** Raman spectroscopy is used to monitor component concentrations in cell cultures in real time. Traditionally, this technique needs numerous training batches for each set of culture conditions to build a calibration model, complicating the development of a reliable model. We've developed a new approach that constructs calibration models without relying on actual culture data, simplifying the process. Moreover, by integrating this with our control system, we've successfully implemented a model predictive control (MPC) system that enables real-time monitoring and nutrient control using Raman spectroscopy.

**Methods/Approach:** Initially, we pinpointed the unique Raman spectrum peaks corresponding to individual components such as glucose, lactate, glutamine, glutamic acid, ammonia, and antibodies by using developed algorithm. We used the spectra peaks that did not overlap to build our calibration models. To gather uncorrelated data, we prepared mixed samples containing each of these components. Ultimately, we developed calibration models capable of measuring concentrations of glucose, lactate, and antibodies. These models are now ready for integration with the MPC system to manage nutrient levels in both 5L and 50L scale cultures.

**Results:** The findings indicate that our approach can generate a calibration model that is effective across various scales. Using the shared calibration models, we were able to accurately monitor concentrations of glucose, lactate, and antibodies with Raman spectroscopy in both 5L and 50L cultures. Furthermore, we showed that our calibration model is compatible with our control system, allowing for the maintenance of constant glucose levels at different scales with great precision.

**Conclusion:** We've effectively developed strong calibration models without needing actual culture data. Merging these models with our control system, we've attained precise and highly accurate monitoring and control across two distinct culture scales. This study's outcomes lead us to anticipate that our system will consistently replicate a stable culture environment at various scales, cell lines and culture media.

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# Heparin-binding motif mutations of human diamine oxidase allow the development of a first-in-class histamine-degrading biopharmaceutical

**Dr Elisabeth Gludovacz**[1], Kornelia Schuetzenberger, Marlene Resch, Katharina Tillmann, Karin Petroczi, Markus Schosserer, Sigrid Vondra, Serhii Vakal, Gerald Klanert, Jürgen Pollheimer, Tiina A. Salminen, Bernd Jilma, Nicole Borth, Thomas Boehm
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
Protein engineering is a valuable tool to improve the characteristics of a biopharmaceutical, like the in vivo half-life and aggregation, and can thereby ensure its efficacy and safety.

**Impact/Novelty:**
Our study represents the first step towards the development of a first-in-class biopharmaceutical to effectively treat diseases characterized by excessive histamine concentrations in plasma and tissues.

**Introduction:**
Excessive histamine concentrations in plasma and tissues cause unpleasant to life-threatening symptoms in mast cell activation syndrome, mastocytosis or anaphylaxis. Anti-histamines are often insufficiently efficacious. Human diamine oxidase (hDAO) can rapidly degrade histamine and therefore represents a promising new treatment strategy for conditions with pathological histamine concentrations.
Recombinant hDAO (rhDAO) is cleared from the circulation in rats and mice within less than five minutes due to heparan sulfate proteoglycan-mediated cellular internalization. This is highly unfavorable for a therapeutic protein that is intended to degrade plasma histamine.

**Methods/Approach:**
Positively charged amino acids of the heparin-binding motif were replaced with polar serine or threonine residues. To prevent aggregation the unpaired cysteine 123 was mutated.

**Results:**
Heparin-binding motif mutations resulted in a strong reduction in binding to heparin and heparan sulfate. The double mutant rhDAO-R568S/R571T showed minimal cellular uptake. The short α-distribution half-life of the wildtype protein was eliminated, resulting in a significantly reduced clearance and a 6-hours half-life in rodents. The ability to degrade histamine was not impaired.
Aggregate formation was inhibited by replacement of cysteine 123 by alanine, without affecting the enzymatic activity or the in vivo plasma half-life.

**Conclusion:**
Mutations in the proposed and now proven heparin-binding motif converted rhDAO wildtype protein into a candidate for a first-in-class histamine receptor independent biopharmaceutical for the rapid and complete elimination of excessive histamine. In addition, the inhibition of aggregate formation will potentially enhance the manufacturability, lower the immunogenicity and enhance the safety of rhDAO.

# 162
# Rapid glycosylation profiling of capsid proteins of COVID-19 and AAV viruses

Dr Yongjing Xie[1], **Dr Michael Butler**[1]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme:** Viral serotypes and variants were characterised by a novel rapid method of glycan profiling.

**Impact/Novelty:** Glycan profiling identified an evolutionary tree of Covid spike protein variants during the pandemic and the basis for tissue tropism in AAV serotypes.

**Introduction:** Novel spike protein variants of the SARS-CoV-2 virus emerged during the Covid pandemic due to adaptive amino acid mutations. However, variations in glycan profiles of the heavily glycosylated virus surface could contribute to the observed changes in infectivity.
Adeno-associated viruses (AAV) are the leading platform of vectors for gene therapy. Each serotype exhibits a different tissue tropism and immunogenicity. Selecting the most suitable AAV serotype is critical for efficient gene delivery to target cells or tissues. Divergence between serotypes is due mainly to the hypervariable regions of the AAV capsid proteins but heterogeneity of capsid glycosylation is largely unexplored.

**Methods/Approach:** A novel protocol to N-glycan profiling of spike proteins from 7 variant Covid viruses enabled us to map evolutionary changes that occurred during the Covid pandemic. Protein capsids of 9 AAV serotypes were also analysed.

**Results:** Cluster analysis was employed to classify the glycan profiles of 7 Covid spike variants into different similarity groups. This showed that the Omicron variant was significantly different from its counterparts. The Delta variant showed substantial similarity to Gamma and Kappa variants, while the Alpha and Beta variants showed the closest similarity to the originally-derived virus.
Glycan profiles of 9 AAV serotypes were investigated. These showed comparable profiles but a conspicuous feature was the high abundance of mannosylated N-glycans, (up to 83%), but with relatively lower abundance of fucosylated and sialylated structures.

**Conclusion:** An evolutionary framework of Covid spike protein variants was mapped from a series of glycan profiles. Capsid proteins of AAV serotypes showed profiles that could explain differences in tissue tropism.

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# High-Throughput Raman Spectroscopy Titer Prediction in Pharmaceutical Manufacturing: PAT as a Tool for Analytical Reduction

**Dr João Medeiros Garcia Alcântara**[1], Dr Mengyao Li[1], Mr. Jean-Michel Kessler[1], Dr. Annemarie Schoenfeld[1], Dr. Benjamin Sommer[1], Dr. Olivier Graf[1], Dr. David Garcia[1]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:** Process Analytical Technology (PAT) is a powerful tool for analytical reduction and acceleration in pharmaceutical manufacturing, with Raman spectroscopy emerging as an important PAT tool due to its non-invasive and chemical-specific measurements.

**Impact/Novelty:** Here, we present a modelling approach which, based on spectra acquired by a high-throughput Raman spectroscopy platform can predict titer across several biomolecules with a prediction average deviation of less than 9%.

**Introduction:** We collected the spectra of various pharmaceutical biomolecules using a Raman spectrometer coupled with a flow-cell to which samples were delivered by a high-throughput liquid handling platform.

**Methods/Approach:** Using a cross-validation approach, we trained a multivariate model based on support vector regression for predicting titer. An optimization algorithm was used to improve the parameters of the pre-processing pipeline, which further improved the accuracy.

**Results:** The general model developed using this approach accurately predicted titer across multiple pharmaceuticals and was validated using an independent test set. Moreover, the optimization of the preprocessing pipeline using an optimization algorithm and cross-validation approach emphasizes the importance of efficient data processing in Raman spectroscopy analysis. The optimization approach could be extended to other analytical targets, further reducing the need for multiple analytical assays in product characterization.

**Conclusion:** Our work highlights the potential of Raman spectroscopy in pharmaceutical manufacturing, and the crucial role of PAT as a tool for analytical reduction. Specifically, each Raman spectra was acquired over 5min, while the analytical method it substitutes would take at least 10min. All in all, Raman spectroscopy can reduce the number of analytical assays in product characterization, saving resources, accelerating the analytical workflow and therefore a faster process development.

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# A Novel Strategy to Reduce Polysorbate Degradation? Influence of Cultivation Media on CHO Hydrolase Expression

**Mr Linus Weiss**[1,2], Ms. Elena Bollgönn[2], Dr. Nikolas Zeh[2], Ms. Melanie Maier[2], Mr. Andreas Unsöld[2], Prof.Dr. Kerstin Otte[1], Dr. Simon Fischer[2]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
Residual hydrolytic host cell proteins (HCPs) are jeopardizing the final drug product quality, as they can degrade the stabilizing surfactant polysorbate (PS).

**Impact/Novelty:**
PS degradation is tackled at various stages of the biopharmaceutical development process. To date, controlling expression of hydrolytic host cell proteins via cell culture media design remained unexplored.

**Introduction:**
As a crucial surfactant, PS is deployed in the final formulation buffer to stabilize biologics – often monoclonal antibodies – and thus ensuring the safety and efficacy of the drug product. However, it was seen in some described instances that Chinese hamster ovary (CHO) HCPs possessing hydrolytic activity could persist the purification process of the biotherapeutic and were found at trace levels in the final drug product. Over time, even those tiny amounts of hydrolases can possibly cleave PS ultimately posing a risk for the occurrence of (sub)-visible particles.

**Methods/Approach:**
Various CHO cell lines were cultivated in different media formulations, followed by PS degradation studies, qPCR and HCP quantification via Western Blot, ELISA and quantification of His-tagged hydrolases.

**Results:**
During a head-to-head cultivation of CHO cell lines in various cultivation media, we revealed strongly differing PS degradation properties when assaying the harvested cell culture supernatant. This phenomenon seemed to be lipoprotein lipase (LPL) related, since PS degradation was substantially reduced in LPL knockout cells even in media that showed pronounced PS degradation. Consequently, PS degradation clearly correlated with LPL protein abundance in the supernatant. In order to elucidate the connection between media and varying LPL levels, we analyzed media dependent cellular LPL expression and could already rule out differences in transcription as the root cause and focused on media dependent effects on LPL protein expression, secretion and stability. In addition, media compositions were correlated to LPL expression to identify possible stabilizing or destabilizing agents. These efforts aimed at elucidating the root cause for observed media related influences on PS degradation in CHO cells.

**Conclusion:**
The understanding of the influence of cell culture media components on hydrolase expression and hence varying PS degradation offers a novel solution to mitigate eventual PS degradation in CHO cell processes. Our results suggest that by rationale media design we might also be able to reduce the activity of other critical PS-degrading hydrolases in the future.

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# Interplay of Heavy Chain introns influences efficient transcript splicing and product quality of mAbs

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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:**
This work highlights how introns in the mAb heavy chain can influence correct or incorrect transcript splicing resulting in correct or aberrantly spliced protein.
**Impact/Novelty:**
This recently work deepens our understanding of intron mechanisms and how these can be designed within manufacturing plasmid expression cassettes to maximum effect for development of correctly formed mAbs.

**Introduction:**
Introns are included in genes encoding therapeutic proteins for their well-documented function of boosting expression. However, mis-splicing of introns in recombinant immunoglobulin (IgG) heavy chain (HC) transcripts can produce amino acid sequence product variants. These variants can affect product quality; therefore, purification process optimization may be needed to remove them, or if they cannot be removed, then in-depth characterization must be carried out to understand their effects on biological activity.

**Methods/Approach:**
In this study, HC transgene engineering approaches were investigated and were successful in significantly reducing the previously identified IgG HC splice variants to <0.5%. Subsequently, a comprehensive evaluation was conducted to understand the influence of the different introns in the HC genes on the expression of recombinant biotherapeutic antibodies.

**Results:**
The data revealed an unexpected cooperation between specific introns for efficient splicing, where intron retention led to significant reductions in IgG expression of up to 75% for some intron combinations. Furthermore, it was shown that HC introns could be fully removed without significantly affecting productivity.

**Conclusion:**
This work paves the way for future biotherapeutic antibody transgene design with regard to inclusion of HC introns. By removing unnecessary introns, transgene mRNA transcript will no longer be mis-spliced, thereby eliminating HC splice variants and improving antibody product quality.

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# New Cell Culture Analyser Technology at Large Scale: Case study of implementation, issues and resolution

**Dr Jesús E. Martínez-López**[1], Dr Josiah O'Sullivan[1], Dr Gearóid Duane[1], Dr Andrew Kelly[1], Ms Anne Marie Molloy[1]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:** Cell culture analyser's (CCA) are ubiquitous in the biopharma industry from early process development to commercial manufacture. To guarantee the robustness of manufacturing processes, it is key to understand any change in CCA manufacturers and/or technologies and consequent potential impacts on process control strategies.

**Impact/Novelty:** Understanding of equivalency during CCA equipment transitioning is crucial to process performance and product quality, in particular for scale dependent parameters.

**Introduction:** CCA1 was used for process pH measurement in a monoclonal antibody production process. Due to obsolescence of this instrument, transition to a newer model "CCA2" that uses different sensor technology was necessary. A CCA equivalency testing study was performed at bench scale prior to implementation at commercial manufacturing scale.

**Methods/Approach:** Quality control standards and in-process samples were evaluated in a lab scale cell culture study to determine if CCA differences had any practical difference for in-process parameters or productivity.

**Results:** A lab scale study did not identify any practical difference for in-process parameters or productivity, however, when assessed at manufacturing scale, CCA2 was not equivalent to CCA1 where unexpected metabolic profile conditions were observed with direct impact on production bioreactor process outputs.

**Conclusion:** Gas hold up variation driven by scale differences was identified to impact the measurement of cell culture pH using CCA2. This led to unexpected metabolic changes in the production bioreactor at manufacturing scale that were not evident in the bench scale study. A pH offset determination and implementation was needed to restore normal process performance. The output of this work demonstrates that scale differences must be considered in any CCA changes to ensure no impact to cell culture process outputs.

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# Fine-Tuning Post-Translational Modifications in Mammalian Cells: Exploring Media Optimization and Gene Editing Strategies

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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:** By employing gene editing and media optimization strategies, the study demonstrates the ability to modulate protein product charge variants and glycosylation, providing a tool for customizing recombinant protein attributes.

**Impact/Novelty:** This work not only advances our understanding of glycan engineering through gene editing but also highlights the importance of metal control in media for consistent cell culture processes.

**Introduction:** Mammalian cells, widely employed for therapeutic protein production, require precise control over post-translational modifications, to tailor recombinant protein quality attributes. This work demonstrates the ability to modulate cellular post-translational modifications, notably glycosylation and charge variants, with gene editing or media optimization approaches.

**Methods/Approach:** We exert control of metal bioavailability in CHO cell culture using “metal-targeted” chemical additives. Based on mathematical model predictions, specific chemical agents were employed to design buffers that alter metal availability in culture media. For manipulating glycosylation, overexpression, and CRISPR Cas9 mediated disruption of several genes were performed in HEK293 cells.

**Results:** Basic and acidic charge variants of a model antibody could be altered by varying the concentration of the specific chemical agents. This effect can be attributed to interactions between the chemical additives and protein-processing enzymes. With glycoengineering, the developed cell line exhibited the production of recombinant glycoproteins with altered glycans containing predominantly bi-antennary sialylated N-glycan structures.

**Conclusion:** When transition metal availability needs to be stringently controlled, especially in the presence of raw material metal impurities, researchers can customize media formulations with various ratios of chemical buffers and metal ion supplementation based on their specific final product processing goals. Our glycoengineering can provide a broader platform to produce glycoproteins with altered glycoforms in HEK293 cells. It is useful to develop cellular platforms to produce recombinant proteins with alternative, potentially humanized glycan patterns, allowing for appropriate non-immunogenic and pharmacokinetics of therapeutic protein products.

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# Advancing Raman model calibration for perfusion bioprocesses

**Mr Louis Hellequin**[1]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:**
Raman spectroscopy in industrial process analytical technology (PAT) toolkits is becoming more prevalent for assessing critical quality attributes (CQAs) in biopharmaceutical processes, particularly in continuous ones.

**Impact/Novelty:**
As its industrial use increases, there is a growing need for a more efficient Raman spectroscopy calibration method for the pharmaceutical industry.

**Introduction:**
Raman spectroscopy is increasingly employed for synchronously overseeing multiple process parameters. Nevertheless, hurdles persist in establishing rigorous and distinctive calibration models, largely due to the scarcity of sufficient analyte variation for effective model training, and significant cross-correlation arising from diverse media constituents and process-related artifacts.

**Methods/Approach**
A Raman calibration workflow for perfusion processes was systematically developed, enabling rapid and highly accurate model calibration. Harvest samples from different batches of CHO cell cultures at various time points were used as harvest libraries. An offline setup using a flow-cell was selected to perform model calibration through a spiking approach. Model optimization was then performed using different data pre-treatment options, in combination with traditional partial-least square, as well as other non-PLS regression models such as ridge, linear, XGBoost and neural networks.

**Results:**
Raman spectroscopy was proven to be able to predict sugars with similar chemical structures as well as various other metabolites independently in process-relevant concentrations. The differences and similarities of the diverse machine learning regression models show the optimization potential of Raman spectroscopy with respect to various metabolites that are important for product quality control.

**Conclusion:**
Machine learning algorithms and model optimization have the potential to increase Raman model robustness and performance which was demonstrated with experimental validation runs. However, model optimization tools do not substitute a systematic spiking approach which is crucial to achieve robust Raman predictions.

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# Investigating the effect of linker peptides on the fragmentation of Fc-fusion protein in mammalian cells

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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Impact/Novelty:** This study suggests the application of linker peptides to reduce the fragmentation of Fc-fusion protein in mammalian cells, which has not yet been elucidated to the best of our knowledge.

**Relevance to Theme Selected:** The adoption of empirical linker peptides in this study can reduce fragmentation of Fc-fusion protein, which is one of the critical quality attributes for biotherapeutics produced in mammalian cells.

**Introduction:** Fc-fusion proteins are often observed as fragmented form in mammalian cells, and this issue has sparked interest in protein production. Many studies have been attempted to reduce the fragmentation in mammalian cells such as lowering cuture temperature, protease inhibitors supplementation, cell enginering, and vector enginerring with manipulating linker peptides.

**Methods/Approach:** The Fc-fusion proteins with different empirical linker peptides were produced in Expi293F™ and ExpiCHO-S™ cell-based TGE systems. The cleavage sites of the fragmented protein were identified by *N*-terminal sequencing. The fragmentation pattern of Fc-fusion proteins was analysed using conditioned medium harvested at the end of TGE, and the effectiveness of protease inhibitors was investigated.

**Results:** When Fc-fusion protein with original linker peptide were produced, Fc-fusion protein from HEK293 cells was cleaved at only one site in linker peptide region, whereas it was non-specifically cleaved at multiple sites in CHO cells. The application of other empirical linker peptides could successfully reduce fragment products in HEK239 cells, not in CHO cells. Analysis of lysates from CHO cells transfected with Fc-fusion protein confirmed that the Fc-fusion protein was expressed in its intact form. Based on the results of *in vitro* incubation assay with conditioned culture media and cultivation supplanted with protease inhibitors, it was expected that cleavage would occur in the process of the secretion pathway.

**Conclusion:** The fragmentation pattern of Fc-fusion protein produced in mammalian cells can be influenced by linker peptide types and host cell lines.

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# Considerations for Selecting Hydrolysates for use in Cell Culture Process

**Mr Jagadeesh Kanagala**[1], Mr Madhava Ram Paranandi[1]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to theme selected:**
Assessing product quality is important when selecting complex hydrolysates for use in biomanufacturing.

**Impact/Novelty:**
The case study demonstrates the approach for selecting complex additives for cell culture process improvement.

**Introduction:**
Hydrolysates have been used in cell culture media for cell growth and productivity enhancement. To finalize hydrolysate supplementation strategies, a comprehensive understanding of their impact on growth, productivity and product quality is required. This was achieved by performing structured experiments at small-scale to identify top performing hydrolysates.

**Methods/Approach:**
In this case study, the effect of addition of two different hydrolysates one SOY (SH) and two COTTON (CH) during fed-batch culturing was assessed on cell culture performances. Studies were conducted in shake flasks first and the data was further confirmed in 5L bioreactors.

**Results**:
The study was started to understand the impact of individual hydrolysates in medium. The SH at 10 g/L increased the cell density by 25 % and productivity by 12% whereas CH at 10 g/L inhibited the growth by 50%. However, due to increased Qp, titer was same as SH. In the next study several conditions were screened for different concentrations of SH and CH in medium and also feed. Lower concentration of SH (5 g/L) yielded similar growth profiles and productivity as higher concentration (10 g/L). Addition of SH in feed has no impact. Addition of lower concentration CH (5 g/L) improved cell growth, however Qp was reduced. In CH conditions also, there is no advantage of hydrolysate in feed. It was observed that in all the CH conditions higher impurities (HMW and fragments) were observed. In this study combination effect of two hydrolysates was also assessed, which had no positive impact.

**Conclusion:**
Based on cell growth, productivity and product purity SOY hydrolysate at 5 g/L in medium was finalized for further scale-up.

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# Protein database modification to construct CHO SWATH-MS spectral library for quantitative profiling of high-risk HCPs

**Dr Tomoko Matsuda**[1,2,3], Prof. Norichika Ogata[1,2,3], Ms. Mari Yukimaru[3], Ms. Ryoko Tani[3], Dr. Hideo Takahata[3], Prof. Takeshi Omasa[1,2,3]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:** HCPs are process-related impurities that copurify with biologics produced in Chinese hamster ovary cells and could impact drug quality.

**Impact/Novelty:** We have successfully reduced the false-negative detection of high-risk HCPs by modifying the protein database to construct the CHO SWATH-MS spectral library.

**Introduction:** HCPs can affect the quality of biologics and need to be managed. While ELISA is a common technique used to quantify total HCP levels, SWATH-MS provides additional information on individual HCP identification and quantitation. In this study, we propose a method for database modification used to construct a spectral library for managing HCP in the newly established CHO-MK cell lines, which shows rapid cell growth and mAb production.

**Methods/Approach:** The protein lysates from cultured mAb-producing CHO-MK cells were tryptic digested and subjected to data-dependent acquisition (DDA) MS analysis. Downstream processing CHO-MK cell culture was tryptic digested and subjected to SWATH-MS in the TripleTOF 7600 (SCIEX). The raw DDA data was searched against the unmodified and modified public CHO database. The modified database was created as follows: if a peptide that was supposed to be produced by trypsin digestion was shared by more than one protein, the peptide was retained only in the high-risk protein sequence, and the remaining protein sequence was masked.

**Results:** SWATH-MS measurements using a spectral library constructed with the modified CHO protein database detected more proteins and fewer false negatives for high-risk HCPs than in measurements using a library with the unmodified public database.

**Conclusion:** Our result indicates that not only the quality and comprehensiveness of the protein database used for DDA searches but also the redundancy of peptides in the protein database affects the accuracy of protein detection by SWATH-MS.

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# A novel bioassay for predicting the immunogenicity of biologics in pre-clinical stages of development

**Miss María Jesús Leopold**[1,2], Dr. Agustina Gugliotta[1,2], Dr. Marina Etcheverrigaray[1,2], Dr. Eduardo Mufarrege[1,2]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:**
The present study constitutes a significant advance in biotherpeutic development as it accurately predicts the drug's immunogenicity at the pre-clinical stage, a critical quality attribute of the product's safety.

**Impact/Novelty:**
We developed an *ex vivo* experimental protocol based on human PBMCs to accurately predict product immunogenicity with high correlation with clinical trials and compliance with regulatory guidelines.

**Introduction:**
Protein immunogenicity is a critical attribute that can compromise product safety and efficacy. Consequently, reliable studies that predict the immunogenicity of biosimilars and biobetters are extremely required in the pre-clinical stages of product development.
In particular, rhIFN-a is highly immunogenic. For this reason, in this study, we developed an *ex vivo* protocol based on human peripheral blood mononuclear cells (PBMC) for the immunogenicity prediction of rhIFN-a long-lasting variants, designated here as IFN-3NM47-Nter, IFN-2NM47/95, and IFN-3NM47.

**Methods/Approach:**
rhIFN-a variants were produced in CHO-K1 cells and purified by immunoaffinity chromatography. PBMC were isolated from 12 donors and HLA-DRB1 allotypes were determined by Luminex technology. Monocytes were isolated and differentiated into immature dendritic cells (iDCs) and incubated with each rhIFN-a version. Upon maturation, antigen-pulsed DCs were co-incubated with autologous T cells. T cell responses were assessed through cytokine profiling.

**Results:**
T cell activation assays showed distinctive response patterns for each rhIFN variant and HLA-DRB1 allele. Also, a comparative analysis highlighted 3NM47/95 as the most immunogenic, while IFN-3NM47-Nter exhibited the lowest immunogenicity. A similar proportion of responders (33%) was observed for IFN-2NM47/95, IFN-3NM47 and IFN-WT. This data is correlated with previously reported results.

**Conclusion:**
Considering the results presented here, IFN-3NM47-Nter appears to be a promising candidate for antiviral therapy. In addition, the high correlation between the results shown here and those previously reported from clinical studies highlights the usefulness of this experimental platform as a screening tool to predict the potential susceptibility of patients to develop undesired immune responses to biologics.

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# Cell-free Raman spectroscopy in Ambr® bioreactor system utilizing automated flocculation

**Mr. Tobias Habicher**[1], Mr Nicolas Maguire[1], Dr. Thomas Wucherpfennig[1], Ms. Carina Guelch[1], Dr. Jochen Schaub[1]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme selected:** Simplified calibration and fluorescence cancelling for Raman spectroscopy as PAT tool in Ambr® bioreactors.

**Impact/Novelty:** Utilizing cell flocculation tackling two major challenges in Raman spectroscopy has not been described yet.

**Introduction:** Raman spectroscopy has shown great potential in bioprocess development and manufacturing. However, a major hurdle is the time-consuming calibration of predictive models. In addition to the effort involved in reference analytics, the generation of calibration spectra often requires ongoing cultivations. Cell-free calibration can reduce the workload needed. Additionally, it enables the use of the common excitation wavelength 785 nm on samples which would otherwise exhibit excessive cell-based fluorescence. In our approach we're using the automated bioreactor system Ambr®250 to tackle these two major challenges.

**Methods/Approach:** We analyzed fluorescence levels in cell-containing and cell-free supernatant samples. Specifically, we studied how the flocculating agent pDADMAC affected the Raman spectra of cell culture supernatants, which had significant cell-based fluorescence. To simplify calibration, we created artificial calibration spectra by sample modification.

**Results:** We observed a significant reduction in fluorescence in samples exhibiting high cell-based fluorescence, regardless of the cell separation method employed. Furthermore, we determined that the flocculating agent had minimal impact on Raman spectra. Additionally, we found that the analytical range of the model could be extended with minimal effort through sample modification.

**Conclusion:** Our approach can successfully decouple calibration from cultivation using cell-free samples. This allows for easy calibration and more variation in calibration data through sample modification. Calibration measurements can be conducted during idle times of the Ambr®250 integrated liquid handler without interfering with running cultivations. Automated in-process cell flocculation for generation of cell-free samples cancels cell-based fluorescence, allowing full utilization of the 785 nm wavelength and leading to reduced effort in calibration.

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# Without a Trace: Multiple Knockout of Host Cell Hydrolases to Tackle the Polysorbate Degradation Challenge

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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:**
Residual hydrolytic host cell proteins (HCPs) are jeopardizing the final drug product quality, as they can degrade the stabilizing surfactant polysorbate (PS).

**Impact/Novelty:**
Knockout (KO) of multiple PS hydrolyzing HCPs in Chinese hamster ovary (CHO)-K1 cells with subsequent cell line characterization.

**Introduction:**
Enzymatic hydrolysis of PS by HCPs poses a major challenge for the biopharmaceutical industry. The degradation of the protein-stabilizing PS can reduce the stability of the biological drug product and can lead to the occurrence of visible particles. Tackling strategies are manifold and encompass the entire bioprocess. However, the most effective approach to mitigate this effect is the genomic KO of these critical HCPs within the CHO-K1 host cell line.

**Methods/Approach:**
HCPs which have been validated to degrade PS were knocked out in lab scale using established technologies followed by single cell sorting to obtain monoclonal host cell lines. Next, KOs were verified via sequencing and deletion PCRs. For characterization of host and production cell line performance bioreactor runs were applied.

**Results:**
A list of several PS hydrolyzing CHO HCPs was knocked-out in a CHO-K1 host cell line. During the sequential knockout of increasing numbers of hydrolases, fed-batch fermentation data informed clone selection for subsequent knockout of further hydrolase targets. In the finally established host cell line several enzymes selected from carboxylesterases, lipases and phospholipases were genomically removed. The suitability as host cell line was verified in a cell line development campaign including generation of monoclonal antibody producing cell lines, bioprocess performance assessment and product quality analysis. Further, reduced PS degradation potential was investigated using PS degradation assays.

**Conclusion:**
Based on a comprehensive list of PS degrading CHO HCPs these target hydrolases were selected for specific removal in CHO-K1 host cells to obtain an enhanced biopharmaceutical cell factory. As other strategies to reduce PS degradation might be developed molecule specific, the multi-hydrolase KO represents an additional solution as the hydrolases are removed at its origin.

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# Immunocompetent human 3D CNS cell models to advance ATMP development

**Miss Catarina M. Gomes**[1,2], Dr. Gabriela Silva[1,2], Dr. Stephan Holtkamp[3], Dr. Maria João Sebastião[1], Dr. Daniel Simão[1], Dr. Mark Trautwein[3], Dr. Patrícia Gomes-Alves[1], Dr. Catarina Brito[1,2]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
A major hurdle in ATMP development is addressing the impact of the manufacturing process on host immune response to the therapeutic cells and gene therapy vectors.

**Impact/Novelty:**
Establish an immunocompetent 3D CNS cell culture model in a miniaturized and higher throughput culture setting, which allows for parallel assessment of rAAV immunogenicity in a human context.

**Introduction:**
To ascertain the safety and potency of ATMPs, human-relevant experimental models in which early target tissue response can be addressed are fundamental. In CNS disorders, ATMPs may lead to the activation of both astrocytes and the resident immune cells, microglia, that can trigger inflammatory responses. Here, we present a 3D immunocompetent human CNS cell model that recapitulates neuroinflammatory hallmarks and can be a useful tool in ATMP development.

**Methods/Approach:**
We explored a hiPSC- and perfusion stirred-tank bioreactors (STB)-based neural 3D cell culture methodology pioneered by our team, in which neuron-astrocyte interactions are recapitulated in iNSpheroids. iNSpheroids were generated in 200 mL STB and transferred to a miniaturized and higher throughput STB. iNSpheroids were co-cultured with hiPSC-derived microglia (iMGL) and after optimization of medium composition and cell ratio, co-cultures were exposed to recombinant adeno-associated virus serotype 2 and 9 (rAAV2 and rAAV9), candidates for gene therapy.

**Results:**
The iNSpheroid culture was successfully transferred to the miniaturized STB by optimizing critical process parameters, maintaining cell viability and expression of neuronal and astrocytic proteins (e.g., MAP2 and VIM, respectively) during culture. The iMGL incorporated the iNSpheroids, with typical ameboid to ramified morphologies and retained microglial proteins (e.g., TMEM119) and function (e.g., phagocytosis) during the 10-day culture. Seven days after transduction with rAAV2- and rAAV9-eGFP, 30-40 % of the iNSpheroid cells expressed eGFP. iMGL showed no eGFP expression. The iNSpheroids mounted an immune response, with upregulation of IL-6 and TNF transcripts and chemokine secretion (e.g., CXCL10). Curiously, iMGL-iNSpheroids displayed quicker kinetics and potential role in restoring homeostasis. Further functional impact of rAAV transduction is under analysis with emphasis on microglial response.

**Conclusion:**
We advocate that the iNSpheroid cultures are suitable screening tools for addressing neuroinflammatory responses to ATMPs and can contribute to accelerating the development of emerging therapeutic modalities.

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# Amplification and fragmentation free Nanopore sequencing enables direct identification of adeno-associated virus (AAV) ssDNA

**Ms Alice Karlsson**[1], Mr Maximilian Karlander[1], Mr Martin Ryner[2], Dr Luigi Grassi[3], Dr Claire Dobson[3], Dr Janet Saunders[3], Dr Fiona Cusdin[3], Dr Lekan Daramola[3], Dr Diane Hatton[3], Dr Magdalena Malm[1], Prof Johan Rockberg[1]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
Analysis of encapsidated genetic material in recombinant AAVs is key for safe and efficacious gene therapies. We aim for a direct and unbiased untargeted sequencing analysis of packaged ssDNA.
**Impact/Novelty:**
We have established a novel workflow with sample preparation and a tailored bioinformatics pipeline for AAV Nanopore sequencing, which allows for direct analysis circumventing amplification and fragmentation sources of bias.

**Introduction:**
AAV is the most popular gene therapy platform with several approved therapies. These are commonly produced in HEK293 cells through transient triple-plasmid transfection (pTransgene, pHelper, pRepCap), which can result in contaminating DNA being packaged. Here, we aim to analyse encapsidated DNA through a less biased sequencing approach.

**Methods/Approach:**
AAVs were produced by triple-transfection of HEK293T and purified by AAVX. A preparation protocol was designed enabling extraction of capsidated ssDNA followed by direct adapter ligation utilising the dsDNA ends of digested ssDNA ITR regions before sequencing. Tailored analysis was designed to avoid loss of rare sequences by masking low quality reads or regions to provide efficient mapping. AAVs with two transgenes (TG) of different length were analysed, and findings were validated using ddPCR and electron microscopy.

**Results:**
Full length reads of both transgenes were detected indicating successful encapsidation. The pTransgene backbone was the most common contaminant, accounting for 4% (short TG) and 22% (long TG) of the sequence reads. Additionally, the analysis allowed for detection of rare contaminants from pHelper, pRepCap and host genome. A/G and G/A transitions were the most common substitutions.

**Conclusion:**
Our simplified genome preparation method combined with tailored analysis enable a direct and less biased characterisation of the AAV DNA and pinpoint contaminations of plasmid backbone in up to 22% of the sequence reads.

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# A case study for ultrafiltration of high concentration mAb – Challenges and mitigation strategy

**Mrs Anindita Das**[1], Mr Kishor Galani[2], Mr Abhishek Bhatt[3], Mr Rijikappoor Nagoorkani[4], Mr Madhava Paranandi[5]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to theme selected:**
Impact of functional parameters in ultrafiltration unit operation have a significant role on intermolecular interaction for concentrated antibody formulations required for subcutaneous administration.

**Impact/Novelty:**
The case study provides a method for reduction of intermolecular interactions for high concentration antibody, by modulating load conditions of ultrafiltration process in combination with strategy for addition of excipients.

**Introduction:**
Ultrafiltration process is used for concentrating antibody to >150mg/mL which is required for subcutaneous injectables. A critical consideration during development is lower volume with higher dosage and lower viscosity. The key challenges associated with this process are polarization effect, viscosity and opalescence which occurs due to intermolecular interactions at high concentration. In this study a detailed strategy is developed addressing these challenges and provide a scalable solution for achieving concentrated antibody formulation with low viscosity.

**Methods/Approach:**
During the optimization experiments process parameters like feed channel, load composition, load concentration, TMP, cross flow rate and protein concentration for buffer exchange were evaluated. Additionally, several excipients were screened for reducing viscosity. Small scale cassettes were used as available with vendors screened during the optimization.

**Results**:
During this study it was observed that when protein is concentrated to >50 mg/mL there is significant increase in intermolecular interaction leading to decrease in flux decay and termination of process at concentration >100 mg/mL. Addition of excipients like amino acids in the process and modulating the load concentration has significant impact on reduction of the intermolecular interaction. Selecting appropriate feed channel, TMP and cross flow rate reduction in accumulation of protein molecules on membrane surface was achieved.

**Conclusion:**
Conditioning of load with excipients and decreasing the duration of initial concentration, increases the delay in achieving the $C_{GEL}$ concentration which contributes towards achieving concentration >200 mg/mL with viscosity of <10cP required for patient convenience pertaining to subcutaneous injectables.

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# Well Characterised Panel of Bovine Embryonic Stem Cells for Cultivated Meat Applications

**Ms Niamh Hyland**[1], Ms Elena Silverstein[1], Dr Max Pickup[1], Dr Joe Kelk[1], Dr Britt Tye[1], Dr Deepika Rajesh[1]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme** Product Quality – Functional Relevance and Emerging Technologies
Ensuring fully characterised, quality bovine embryonic stem cell (ESC) lines which are suitable for manufacturing at scale and product development is vital for sustainable, nutritional, and ethical cultivated meat production.

**Impact/Novelty:**
Establishing of a panel of stable bovine ESC lines, that align with the principles of slaughter free meat production, provides a readily available source for key players in commercial production of cultivated meat

**Introduction:**
Bovine embryonic stem cells (ESCs) lend well to the cultivated meat industry due to their ability to expand indefinitely whilst retaining pluripotency, karyotypic stability and differentiation potential to generate cell types essential for creating the structure and organoleptic properties of meat. Stable well-characterised bovine ESC banks with intact traceability are a crucial asset for reliable downstream scale up, process and product development. Roslin Technologies have successfully banked a panel of robust bovine ESCs harbouring favourable genetic traits making them suitable for large scale cultivated meat production.

**Methods/Approach:**
- Pluripotency status of the bovine ESCs was confirmed via qPCR and flow cytometric analysis
- The ability to spontaneously differentiate to all germ layers was demonstrated via tri-lineage differentiation assays
- Directed differentiation of bovine ESCs to mesodermal progenitors and further to muscle and fat
- RNA sequencing to confirm pluripotency signatures and identify key pathways in bovine ESCs

**Results:**
Our bovine ESCs displayed the presence of key pluripotency markers SSEA-4, Oct3/4, Nanog and Sox-2. In addition, they retained tri lineage potential and pluripotency characteristics over multiple passages. RNA Seq data will be used to validate and identify species specific pluripotency signatures that are key for banking and scale up. Efforts are ongoing to generate MSCs, adipocytes and myocytes from banked bovine ESC.

**Conclusion:**
The availability of a well characterised panel of bovine ESCs from Roslin Technologies offers a model for understanding bovine pluripotency, assisted reproductive technology and would fill an unmet need within the wider cultivated meat industry.

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# Unlocking the Power of Antibodies: The Impact of a sugar analogue on Core-Fucosylation

**Dr Martina Grabner**[1], Ms. Melanie Nguyen[1], Mrs. Katherine Hellman[2], Dr. Aline Zimmer[1]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
The glycosylation profile of therapeutic antibodies plays a crucial role in their biological activity, and reducing core fucosylation through the use of fucose analogues emerges as a promising strategy to enhance cytotoxic efficacy without requiring cell line engineering.

**Impact/Novelty:**
A novel fucose analogue was assessed.

**Introduction:**
Rituximab is an important therapeutic antibody used to treat various cancer diseases. A known method to boost the antibody response is the reduction of antibody core-fucosylation which leads to enhanced antibody-dependent cellular cytotoxicity (ADCC). 5-Thio-L-Fucose (ThioFuc) was investigated as a media and feed supplement for modulating the fucosylation profile of therapeutic proteins and, thereby, improving the resulting effector functions.

**Methods/Approach:**
To assess the impact of ThioFuc, glycan analysis of five different therapeutic proteins produced by a diverse set of Chinese hamster ovary cell lines was performed at several time points during fed batch experiments. Furthermore, FcγRIIIa binding affinity and ADCC activity was assessed by surface plasmon resonance analysis and a cell-based reporter bioassay, respectively.

**Results:**
In general, ThioFuc treatment resulted in reduction of core-fucosylation and partial incorporation of ThioFuc in place of core-fucosylation. Comparing the different cell lines, a clone dependent impact of ThioFuc was observed. Using rituximab as model, an efficient dose and time dependent reduction of core-fucosylation up to a minimum of 5% was obtained by ThioFuc. Besides a concomitant increase in the afucosylation level up to 48%, data also revealed up to 47% incorporation of ThioFuc in place of core-fucosylation. In accordance with the glycan data, antibodies produced in the presence of ThioFuc revealed an enhanced FcγRIIIa binding up to 7.7-fold. Furthermore, modified antibodies proved to exert both a 1.5-fold enhanced ADCC efficacy and 2.6-fold enhancement in potency in comparison to their native counterparts – both of which contribute to an improvement in the ADCC activity.

**Conclusion:**
This new fucose derivative has the potential to serve as a valuable tool in drug development processes by decreasing core-fucosylation.

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# Dial-A-Sugar: Characterisation of Inducible Genetic Circuits to Control mAb Glycosylation

**Ms Sheryl Li Yan Lim**[1], Dr Apostolos Tsopanoglou[1], Dr Rajinder Kaur[1], Dr Alfonso Blanco[2], Dr Ioscani Jiménez del Val[1]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected**
Glycosylation heavily influences the safety and efficacy of therapeutic glycoproteins. The ability to tightly control the glycosylation process will help ensure that the quality of biopharmaceuticals is optimal and consistent.

**Impact/Novelty**
Dial-A-Sugar aims to control the glycosylation process by engineering CHO cells with inducible transcriptional circuits to produce mAbs with targeted glycosylation profiles and, ultimately, yield safe, efficacious, and cost-effective therapies.

**Introduction**
Monosaccharide composition of glycans bound to the Fc fragment of monoclonal antibodies is widely reported to influence their safety and therapeutic efficacy. Absence of α6-linked core fucose greatly enhances antibody-dependent cellular cytotoxicity (ADCC), while increasing levels of β4-galactosylation enhance ADCC and complement-dependent cytotoxicity (CDC). ADCC and CDC are key effector functions that determine the oncolytic activity of therapeutic mAbs.
The Dial-A-Sugar framework aims to control the levels of mAb α6-fucosylation and β4-galactosylation by regulating the expression of α-1,6-fucosyltransferase (Fut8) and β-1,4-galactosyltransferase (β4GalT1) via linearly inducible transcriptional circuits (lineariser). However, before deploying the lineariser circuits to control glycosylation, optimisation for fold-induction, linearity of the dose response, and orthogonality must be conducted.

**Methods/Approach**
Two negatively autoregulated lineariser circuits (TetR_Lin and PhlF_Lin) with highly favourable fold inductions and linear dose responses were evaluated. The lineariser circuits contain a fluorescent protein reporter (eGFP or mCherry) and a repressor protein gene (TetR or PhlF, respectively). Performance of both linearisers was evaluated by site-specific integration into host cell genome (CHO-VRC) through a Cre-Lox landing pad system. Once established, the lineariser cells were cultured under several concentrations of small molecule inducers and assayed for fluorescent reporter protein expression using flow cytometry.

**Results**
Previous deployment of TetR_Lin circuit via random integration resulted in compromised basal expression with two regions of linear response between Dox concentration and eGFP expression. Therefore, a Cre-lox landing pad was used to control the location of gene integration and copy number ensuring stoichiometric equivalence of the repressor and reporter genes. Improved linear responses were achieved in both circuits, minimising basal level of fluorescent reporter expression while maximising fold-induction and linearity of the linearisers.

**Conclusion**
The lineariser characterisation experiment seeks to replicate the response achieved with Fut8 and B4GalT1 to successfully tune and control glycosylation on mAbs, further advancing technology in obtaning consistent product quality profiles.

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# Well-Characterised Porcine iPSCs and c-Myc quantification for cultivated meat applications.

Dr Max Pickup[1], Ms Niamh Hyland[1], Ms Sarah Ho[1], Dr Jorge Santoyo Garcia[1], **Dr Madeleine Carter**[1], Dr Pritika Singh[1], Dr Britt Tye[1], Dr Sheena Fraser[1], Dr Joe Mee[1], Dr Deepika Rajesh[1]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Theme: Product Quality – Functional Relevance and Emerging Technologies**

Assessing the level of residual reprogramming factors in iPSCs for cultivated meat, especially c-Myc, is crucial for downstream product development due to unknown impact on health upon human consumption.

**Impact/Novelty:**
iPSCs are an important source for differentiation and biomass production for cultivated meat applications. Quantification of exogenous reprogramming factors is crucial to meet regulatory requirements for downstream cultivated meat products.

**Introduction:**
At Roslin technologies we have successfully developed a bank of Porcine iPSCs. These cells display tri-lineage potential, retain pluripotency makers, adapted to high cell density expansion and capable of downstream differentiation into cell types relevant for cultivated meat. C-Myc is a known proto-oncogene, the quantification of human and porcine c-Myc levels in iPSCs provides an insight on its role in differentiation and supports regulatory requirements for cultivated meat applications.

**Methods/Approach:**
We have generated porcine iPSCs using a non-integrative reprogramming method. Characterisation of pluripotency and trilineage potential has been performed using flowcytometry and qPCR assays. In addition. we have developed an assay for determining quantity of exogenous and endogenous c-Myc levels using qPCR and digital PCR.

**Results:**
Porcine iPSCs displayed pluripotency markers SSEA-4, Oct3/4 and Nanog. In addition, they retained tri-lineage potential and pluripotency characteristics over multiple passages. Porcine iPSCs also successfully generated MSCs and adipocytes. The levels of both exogenous and endogenous c-Myc at banking and expansion were initially quantified using qPCR and the assay was further optimized using digital PCR, in nanograms per gram of cell mass.

**Conclusion:**
Providing well-characterised Porcine iPSCs along with a robust assay for quantification of c-MYC provides an insight for evaluating the role of c-Myc at various stages of reprogramming, banking and scale up, as well as regulatory filing for use in cultivated meat products.

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# SialMAX: maximising biopharmaceutical α-2,6-sialylation in CHO Cells

**Miss Cristina Abascal Ruiz**[1], Dr Mina Ghahremanzamaneh[1], Dr Alfonso Blanco[2], Prof. Ioscani Jimenez del Val[1]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected**
Controlling and improving sialylation is vital for product quality as it enhances monoclonal antibody functionality, optimizing therapeutic outcomes. Precise control ensures consistent, high-quality manufacturing, minimizing variations and promoting efficacy.

**Impact/Novelty**
Innovating mAb manufacturing, we strategically shift sialylation, substituting α-2,3 with α-2,6-sialic acid. This enhances mAb properties, pioneering precision optimization in biopharmaceutical manufacturing, positioned at the intersection of innovation and patient-centric care for excellence.

**Introduction**
The Global Biopharmaceutical Market is projected to reach unprecedented sales of US$554.03 bn by 2028, emphasizing the urgent need for advancing therapeutic strategies. Monoclonal antibodies (mAbs), serving as indispensable treatments for diseases like cancer and autoimmune disorders, face challenges arising from antibody variability, particularly in glycosylation. Our research delves into the intricate landscape of glycosylation, spotlighting sialylation as a key factor that significantly influences the therapeutic mechanisms of mAbs.

**Methods/Approach**
Building upon the innovative GalMAX technology, this project strategically targets α-2,6-sialylation in CHO cells by eradicating α-2,3-sialylation through CRISPR/Cas9 genome editing. The ST3GAL3, ST3GAL4, and ST3GAL6 genes, encoding α-2,3-sialyltransferase, have been targeted for knockout using CRISPR/Cas9, and plasmid construction has been executed with USER cloning, thus setting the stage for a sophisticated glycoengineering approach.

**Results**
Progress in our research involves the removal of the ST3GAL3, ST3GAL4, and ST3GAL6 genes, a crucial advancement in attaining optimal α-2,6 sialylation in CHO cells. We have introduced a novel lectin-assisted confocal laser scanning microscopy technique for the screening and phenotypic characterization of clones. This more efficient approach not only streamlines the phenotypic assessment of 2,3 K.O. lines but also guarantees effective screening and characterization of diverse cell lines with varied Glyco-profiles, facilitating subsequent phases of our project.

**Conclusion**
As we move towards the characterization phase, our next steps involve the isolation and purification of the product, followed by mass spectrometry-based glycan analysis. This innovative approach holds substantial promise for elevating the therapeutic potential of monoclonal antibodies, setting a new standard in precision glycoengineering and significantly impacting the landscape of biopharmaceutical manufacturing.

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# Development of innovative orthogonal analytical tools for in-depth size determination and separation of AAVs

**Ms Sabrina Leigheb**[1], Catarina Maia[2], Verena Buchacher[1], Maral Rahimzadeh[1], Patricia Pereia Aguilar[3], Astrid Dürauer[1]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:**
The size determination in AAVs manufacturing is a crucial Critical Quality Attributes to determine to ensure the product safety. Therefore, fast and robust methods need to be investigated.

**Impact/Novelty:**
Asymmetrical Field-Flow-Field Fractionation and Size-Exclusion Chromatography combined with Light Scattering detectors, and Capillary Electrophoresis combined with Taylor Dispersion Analysis have been developed to assess the in-depth size determination of AAVs.

**Introduction:**
In the AAVs manufacturing, challenges in achieving consistent quality for clinical application are still present. The state of art of AAVs characterization is Analytical Ultracentrifugation (AUC) which requires long turnaround and high volume.
In our study, Asymmetrical Flow-Field-Flow Fractionation (AF4) and Size-Exclusion Chromatography (SEC) have been established and, by the combination with Light Scattering detectors, such as Dynamic Light Scattering (DLS) and Multi-Angle Light Scattering (MALS), it has been possible to obtain an in-depth size and shape determination of the viral particles.
In addition to that, Capillary Electrophoresis (CE) in combination with Taylor Dispersion Analysis (TDA) has been developed as orthogonal method for the size determination of AAVs. Nanoparticle Tracking Analysis (NTA) and cryo-Transmission Electron Microscopy (cryo-TEM) have been used as reference methods.

**Methods/Approach:**
For the development of CE-TDA and AF4-MALS, preliminary studies have been conducted with standard non-biological material. Subsequently, Standard AAVs capsids have been used to assess the reproducibility and accuracy of the developed methods.

**Results:**
For development of AF4-MALS, the injection volume and the particle concentration are the crucial parameter for the obtainment of the expected size. In CE-TDA, the mobilizing pressure throughout the capillary is the central criterion for the correct size detection of AAVs. NTA has not been able to provide either the particle size nor the concentration.

**Conclusion:**
In our study, we developed three fast, robust and reproducible methods for the size determination of AAVs particles. SEC and AF4 combined with MALS and DLS enabled the obtainment on the particle size and shape of AAVs. The CE-TDA allows the size determination with a low sample volume.

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# Tailoring Critical Protein Quality Attributes with Feeds

**Mrs Kate Hellman**[1], Martina Grabner
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected**
Protein glycosylation impacts safety, efficacy and half-life of therapeutic proteins and is considered a critical product quality attribute. The feeds discussed in this poster modulate protein glycosylation.

**Impact/Novelty:**
Highly concentrated cell culture feeds that improve growth, viability and titer while shifting galactosylation and sialylation on a protein of interest.

**Introduction:**
A defined glycan profile is critical for regulatory approval of biosimilars, as manufacturers must target the originator molecule's glycosylation profile. In the instance of new active pharmaceutical ingredients (e.g. bi- or trispecifics, fusions, etc.), certain glycosylation profiles (e.g. high sialylation) are crucial to confer anti-inflammatory properties.
Controlling glycosylation on a protein of interest is important and can be achieved by multiple parameters. One method is to introduce glycosylation modulating raw materials into the production system.

**Approach:**
We created a cell culture feed toolbox consisting of four feeds; one base feed, and three glycosylation modulating feeds (galactosylation increasing, galactosylation decreasing and sialylation increasing.) The feeds are highly concentrated, pH neutral, non-animal origin, and stable for thirty days at room temperature. These feeds enable high titers and enhanced culture longevity and can be mixed with the base feed to target specific glycosylation profiles in fed-batch or perfusion process.

**Results:**
The feeds can be used independently or mixed with the foundation feed to titrate the effect on galactosylation and sialylation and thereby fine tune glycosylation.

**Conclusion:**
We demonstrated that the feed toolbox can create significant shift in galactosylation and sialylation on multiple proteins of interest. These feeds are a valuable resource for innovators and biosimilar developers.

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# AAV2 capsid fusion of large protein domains: multivariable insertion sites breakdown

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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
This work furthers gene therapy by uncovering AAV capsid sites that sustain functional large protein insertions, unlocking innovative pathways in vector design, and expanding biotechnological possibilities.

**Impact/Novelty:**
Our study unveils hotspots for large protein insertions, expanding AAV tools. These findings broaden insertable protein domains in the AAV capsid, enabling more innovative vector systems.

**Introduction:**
Adeno-associated viruses (AAV) are promising gene delivery systems and several capsid engineering strategies have been pursued. Current capsid design approaches rely on small peptide insertions for vector retargeting and reduced immunogenicity. However, integrating large proteins poses challenges due to the unpredictable nature of protein folding and complexity of capsid assembly. This study exposes capsid engineerable hotspots for large protein insertion, unlocking its use beyond traditional approaches.

**Methods/Approach:**
We employed a capsid mosaic approach, integrating a mCherry protein and an enzymatic protein (>200a.a.) into VP1 capsid protein (453/587 residues) and VP2 (138 residue), respectively. A systematic breakdown of the different capsid insertions was performed to elucidate insertion site versatility and modified capsid behavior. Engineered AAVs were evaluated for production yields, affinity purification, particle functionality, and physical properties namely: thermostability, heparin-binding, and immunogenicity.

**Results:**
Results revealed consistent outcomes across all tested insertion sites for both mCherry and enzymatic protein. No impairments in total particles and genome incorporation were observed, showcasing the AAV capsids' ability to support insertions without compromising production. Affinity purifications showed a 50%-60% recovery across all conditions, demonstrating that capsid modifications did not impair vector processing. Only a decrease in the fused protein functionality was observed, with the 453/138 sites showing a 3-fold reduction.

**Conclusion:**
Our work shows the AAV capsid robustness to host substantial domain insertions, with the 453/138 residues emerging as permissive sites. These findings establish foundational knowledge for extending AAV capsid manipulations, enabling the development of novel vector systems.

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# TheraPRO® CHO Media System: Streamlining Protein Production for Accelerated Antibody Therapeutics

**Dr. Jyoti Rawat**[1], Dr Josi Buerger[1], Ms Ioanna Zormpa[1], Dr Ivan Carubelli[1], Mr Dipankar Borgayari[1], Mr Jacob McCowen-Smith[1], Ms Lucy Tate[1], Ms Aymaan Rahman[1], Mr Harinath Makanaboina[1], Dr Kenneth Low[2]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to theme selected (Product Quality):**
TheraPRO CHO Media system enhances product titers and guarantees superior product quality: low aggregation, robust charge variants, and high level of intact IgG. This results in increased total product yield.

**Impact/Novelty:** We conduct a stress test on monoclonal antibody dissociation in TheraPRO CHO media, pushing parameters to their limits. Despite this, the media system consistently produces high levels of intact IgG.

**Introduction:**
The TheraPRO® CHO Media System represents a significant breakthrough in the dynamic realm of antibody therapeutics, streamlining protein production for improved efficiency and adaptability. This chemically-defined, animal component-free formulation stands out for its effectiveness and contribution to workflow optimization, characterized by user-friendly attributes. Specifically designed for the CHOK1SV GS-KO cell lines, this system offers multiple advantages, positioning it as the industry's preferred choice. Prioritizing high production yield, it achieves up to double the protein titer production compared to rival products. The system's scalability empowers enhanced control from cell line development to manufacturing, facilitating a streamlined approach to reduce time-to-market.
In bioproduction, antibody stability is a critical concern, with dissociation posing a common challenge. Maintaining antibody integrity is vital to prevent reduced efficacy, potential loss of function, aggregation, and product wastage. Factors like pH variations, temperature fluctuations, and mechanical stress during production contribute to antibody dissociation, necessitating careful consideration in process optimization.

**Methods/Approach:**
This study demonstrates the production of intact antibodies using TheraPRO CHO Production Media in fed-batch cultures ranging from 0.1 to 500L. Product quality data reveals high purity, consistency, and minimal aggregation. Additionally, TheraPRO CHO Production Media and Feed undergo stress testing under conditions known to induce antibody dissociation. Resilience testing involves using a dissociation-prone GS-CHO cell line, harvesting a 10L bioreactor with suboptimal sample handling, and utilizing undersized filters during purification.

**Results:**
Throughout the dissociation stress test, the antibody maintained structural integrity, underscoring the ability of TheraPRO CHO Production Media to uphold high product quality across the bioproduction pipeline.
**Conclusion:**
The TheraPRO CHO Media system not only elevates product titers but also ensures superior product quality, as validated by a dissociation stress test.

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# Know your AAV - Quality Control starts during Process Development

**Ms Maren Lehmkuhl**[1], Dr. Mareike Schulz[1], Julia Engel[1], Dr. Tim Steffens[1]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
Production of AAV as new therapeutic substance is of high priority in the pharmaceutical industry. Therefore, suitable analytical procedures for the determination of critical quality attributes need to be established.

**Impact/Novelty:**
A novel analytical process to analyze diverse quality parameters during USP is described. Compared to common methods like ELISA and ddPCR it offers several advantages.

**Introduction:**
Adeno-associated viruses (AAVs) are used as gene delivery systems for therapeutic applications. The genome packaging of the virus capsids, displayed by the ratio of full and empty capsids, represents a main criterion of AAV quality. Here we show the combination of AAVX affinity chromatography and size-exclusion chromatography with multi-angle light scattering (SEC-MALS) analysis for the determination of the full/empty ratio during process development of AAV8 as early quality control.

**Methods/Approach:**
Crude media samples containing AAV8 were purified by affinity chromatography using HPLC-DAD-FLD applying custom-made analytical columns. The AAV fractions were used for the analysis by SEC-MALS after buffer exchange and filtration.

**Results:**
After affinity chromatography, purified AAV8 fractions showed SEC-MALS chromatograms with low background, displaying a main monomeric AAV8 peak. The numbers of total, full and empty AAV particles were determined, resulting in the full/empty ratio. The value of the full/empty ratio in crude and purified samples using ELISA and ddPCR, showed no deviation as compared to the purified samples after affinity chromatography. Additionally, SEC-MALS analysis delivered two other quality parameters, the molar mass and the radius of the AAV.

**Conclusion:**
We showed an effective method to analyze the full/empty ratio from crude samples during USP. The performance of the affinity chromatography was verified against common analytical methods to ensure that no change in the full/empty ratio occurs. The additionally determined parameters molar mass and radius of the AAV particles represent further quality criteria for the AAV development, displaying the advantages of SEC-MALS compared to other analytical methods.

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# Impact of carbon dioxide ingress in DS on Gene Therapy Drug Substance's pH and Quality

**Dr Marie Haufroid**[1]
[1]Ucb, Leuven, Belgium

Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
Carbon dioxide ingress in the drug substance (DS) sample container during shipment with dry ice can lower the pH of the sample buffer, therefore altering the quality attributes of samples.

**Impact/Novelty:**
This study aims to understand the kinetics of $CO_2$ ingress and to identify appropriate container (primary and secondary) and conditions to minimize the impact of $CO_2$ ingress on DS quality.

**Introduction:**
Gene therapy bulk DS and samples are regularly shipped on dry ice to maintain storage temperature below -60°C. Exposure to dry ice could increase the partial pressure in $CO_2$ in the containers and lower the pH of the sample during shipment. Acidification could adversely impact quality attributes of the DS and the analytical assays conducted to verify those quality attributes.

**Methods/Approach:**
The study was initiated by measuring the impact of pH on DS quality attributes (percentages of high molecular weight species (HMWS) and DNA-containing capsid). Subsequently, an assessment to reduce $CO_2$ ingress was performed using different materials of DS sample containers and conditions exposed to dry ice. The pH of each container and condition was measured using a microprobe and then compared to control samples.

**Results:**
In a preliminary study, assessing the impact of pH from 7.50 to 4.50 on quality showed a trend in decreasing percentage of DNA-containing capsids and an increase in HMWS, indicating that acidification can impact quality attributes of DS. To prevent acidification by $CO_2$ ingress during shipment, various container materials and protection/sealing methods were screened.

**Conclusion:**
Shipment of DS samples on dry ice can alter the quality and stability of the product of interest. To minimize this risk, the most suitable container and protection/sealing methods for shipment of DS on dry ice were identified.

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# Novel Continuous Production System for Standardized Extracellular Vesicles from immortalized human Mesenchymal Stromal Cells

**Dr Klaus Graumann**[1], Ms. Melanie Reininger, Dr. Roland Prielhofer[1], Ms. Claudia Lindner[1], Dr. Ingrid Hartl[1]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
Reproducible and productive manufacturing is key for clinical and commercial success of therapeutics. We provide next-generation product and process development technology for high-quality EV-based therapeutics.

**Impact/Novelty:**
Our holistic approach combining immortalized cell lines, innovative bioprocessing and orthogonal analytical methods addresses some of the key issues for broader success of vesicle-based therapeutics.

**Introduction:**
Cell-based therapies are inherently complex and their broader translation into clinical efficacy has been slow over the past years. Reasons behind are the complexity and variability of cells, and difficulties in scalable manufacturing. This holds also true for extracellular vesicles (EV) which offer great promises for many applications in Regenerative Medicine.

**Methods/Approach:**
We have established a novel manufacturing setup based on telomerized MSC (MSC/TERT), a perfusion bioreactor, and standardized isolation steps which ensure the molecular integrity of the EV fraction. Continuous harvesting of conditioned media was tested (up to 36 days), and we could produce EV preparations with relevant biological functionalities and significant productivities. The MSC-EV functionality could be reproduced from independent bioreactor runs. Also, we have established the analytical basis for better understanding the composition-function relationship. Different cell lines, harvest time points and downstream steps have been assessed.

**Results:**
We found a range of growth and productivity performance with the different MSC/TERT lines. Moreover, EV characterization data (e.g. miRNAs and marker proteins) from preparations have been correlated with anti-inflammatory and anti-fibrosis bioassay data. The collected database is supporting the selection of EV preparations for testing in proof-of-concept organoid models and pre-clinical *in vivo* disease studies.

**Conclusion:**
Our newly developed production system for MSC-EVs and our systematic approach will support the understanding of critical process parameters and critical quality attributes of EV-based products. Therefore, it will help accelerate the standardization and translation of EV-based product candidates into (pre-)clinical testing.

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# Characterization of Antibody N-glycosylation from a Freely Available Producer CHO Cell Line: NISTCHO

**Mr Thomas Berger**[1], Dr. Veronika Schäpertöns[1], Ms. Larissa Hofer[2], Ms Jerneja Štor[2], Prof. Nicole Borth[2], Prof. Christian Huber[1]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected: (30 words)**
Tight product quality monitoring of monoclonal antibodies (mAbs) encompasses a critical step to ensure product quality and safety. We present a unique methodology for precise quantification of N-linked glycosylation abundances.

**Impact/Novelty: (30 words)**
CQAs are typically analysed at the end of bioprocess only, due to workload and time limitations. With our efficient workflow, we can now detect changes in N-glycosylation during the bioprocess.

**Introduction:**

Understanding the relationship between critical process parameters (CPPs) during the bioprocess and the resulting critical quality attributes (CQAs) in the product molecule is essential for production of cheaper and safer drugs. Here, we use advanced bioanalytical methods to investigate the effects of different production parameters on N-glycosylation (critical CQA) of the resulting monoclonal antibody.

**Methods/Approach:**

Ion-pair reversed-phase HPLC was directly hyphenated to high-resolution mass spectrometry (IP-RP-HPLC-HRMS) to detect changes in the N-glycosylation profile of an intact mAb product under different production conditions. Unique bioinformatic R-based in-house software applications were used to: (1) remove potential hexose bias which is typically not accounted for and to (2) quantify the actual abundances in the relative N-glycan distribution at different timepoints with/without temperature shifts during the bioprocess. This approach provides a robust, sensitive and time efficient method for monitoring N-glycosylation in mAb production.

**Results:**

Characterization of mAb during the bioprocess demonstrates differences in the N-glycan patterns at different time points and varying temperatures. This data elucidates CQA-relevant changes during the bioprocess, allowing for precise timing of end-of-batch to obtain the highest product quality.

**Conclusion:**

Our method reduces time and sample preparation efforts. Such methodology identifies the critical process stages that have the highest impact on product quality. The generated data provides a reference standard for NISTCHO to understand the interactions between CQAs and CPPs in industrially relevant producer CHO cell lines.

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# Evaluating Intracellular Protein Expression Levels of Antibody Chains in CHO Cells to Identify Productivity Bottlenecks

Ms Maja Semanjski[1], Ms Urša Šipec[1], Ms Petra Sečnik[1], Ms Nina Pirher[1], **Mr Lovro Kramer**[1]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
This methodology has the potential to enhance the optimization of antibody production, especially for multi-specific antibodies requiring high and balanced expression levels of multiple chains.

**Impact/Novelty:**
In this study, we present a novel protocol for evaluating the intracellular protein expression levels of Heavy and Light chains of antibodies within the secretory pathway of CHO cells.

**Introduction:**
Successful cell line development for monoclonal or multispecific antibodies relies on achieving high and balanced expression of polypeptide chains. Typical methods of assessing mRNA levels via quantitative PCR techniques provide valuable information but fail to consider post-transcriptional processes that contribute to the final secreted molecule.

**Methods/Approach:**
Our aim was to gain a deeper understanding of productivity bottlenecks. To accomplish this, we combined secretory proteome enrichment, achieved through membrane enrichment to enrich for organelle-rich cell proteome, with mass spectrometry-based proteomics.

**Results:**
Through our approach, we successfully confirmed that typical protein markers of the secretory pathway were enriched compared to whole cell proteomes. Interestingly, our findings revealed that although mRNA levels of antibody chains in production cell lines correlated well with intracellular protein levels in the secretory pathway for some investigated recombinant antibodies, this was not always the case. This discrepancy indicates the presence of potential bottlenecks in translation, early intracellular trafficking, maturation, and secretion processes. Furthermore, our method allows for the evaluation of expression levels of endogenous CHO proteins involved in biologics production, providing valuable insights into the overall cellular machinery.

**Conclusion:**
Overall, our study demonstrates the utility of evaluating intracellular protein expression levels to identify productivity bottlenecks and improve our understanding of the complex processes involved in cell line development for recombinant biologics.

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# Mesenchymal Stromal Cells retain their hematopoietic support capacity post cryopreservation without the need for revitalization

**Miss. Ana Lúcia Tiago**[1,2,3], Miss. Maria Catarina Carreira[1,2,3], Prof. Cláudia Lobato da Silva[1,2,3], Dr. Ana Fernandes-Platzgummer[1,2,3]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
Quality and function are deeply connected in the development of cell-based products. Improving MSC functional assays will deepen understanding of key quality attributes vital for the development of emerging therapies.

**Impact/Novelty:**
Human platelet lysate as an alternative to fetal bovine serum for MSC feeder layer establishment. MSC's hematopoietic support capacity is maintained following cryopreservation, suggesting that revitalization might not be necessary.

**Introduction:**
MSC hold great therapeutic potential, but a robust and good manufacturing practices-compliant manufacturing process is still lacking. Functional assays are essential as MSC potency is known to fluctuate with culture, handling, and storage conditions, particularly cryopreservation.

**Methods/Approach:**
A hematopoietic support assay, used as a functional assay, assessed the fitness of freshly thawed (FT) and revitalized MSC (48h in culture). Adipose tissue-derived MSC were thawed and plated for 8h at 60k cells/cm^2, 48h at 30k cells/cm^2 and 7-10 days at 3k cells/cm^2 (control) in DMEM with 5% human platelet lysate. A 7-day co-culture with umbilical cord blood-derived hematopoietic stem/progenitor cells (UCB-HSPC) and the confluent MSC feeder-layers was initiated. Subsequently, HSPC were characterized.

**Results:**
The fold increase in HSPC number for FT and 48h conditions was comparable to the control. Their immunophenotype and clonogenic potential were similar across conditions. This suggests that the potency of MSC and their ability to support HSPC expansion remained unaffected upon cryopreservation, and the 48h recovery period in culture, described as sufficient for MSC to regain their characteristics, resulted in no significant differences.

**Conclusion:**
Cryopreservation and thawing of MSC do not significantly affect their ability to form feeder-layers and support the expansion of HSPC *in vitro*. This suggests that the hematopoietic support assay can be optimized to save time in the establishment of the feeder-layer using FT MSC and thus streamlining its integration into a time- and cost-effective cell therapy manufacturing process and serving as a potency assay for MSC.

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# Technical insight into product testing for AAV: Advancing quality assessments

Mr Stuart Wright[1], **Dr Lisa Blackwood**[1], Mr Lorenzo Tudini[1]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:**
Developing orthogonal assays is required when assessing Product Quality for AAV therapeutics. Identity, purity, potency and safety are required to be assessed by the regulators.

**Impact/Novelty:**
The goal was to develop multiple format assays to fully characterise AAVs, including bioassays, and physicochemical assays, utilizing state of the art instrumentation.

**Introduction:**
From a market-oriented point of view, the multifaceted nature of adeno associated virus (AAV) serotypes and full:empty ratios presents a substantial challenge. As a result, adopting a one size approach becomes insufficient due to the intricate complexities involved. AAV serotypes, though predominately uniform in size, present differences in different physiochemical characteristics such as density and isoelectric point. Despite their minimal variations, a package of analytical techniques offers high-resolution characterization of several serotypes.

**Methods/Approach:**
Due to product complexity, an orthogonal approach was used to provide the most complete testing package to ensure sufficient data was generated to properly characterize the Quality Target Product Profile defining the basis for the specific Critical Quality Attribute of the product.

**Results:**
A broad package of assays was developed allowing for a greater understanding into the characteristics of the different AAV serotypes. From bioassays, such as infectivity, to full:empty capsid ratio by anion exchange chromatography.

**Conclusion:**
The AAV testing package's innovative approach not only will allow scientists to comprehensively assess and collect product's Critical Quality Attributes such as identity, purity, potency and safety but at the same time, it will enhance the understanding, improving the decision-making during critical phase of the development and manufacturing process in accordance with regulatory guidance that will facilitate the release of new gene therapy treatments ensuring the best level of product safety and quality.

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# Characterization of cell lines with enhanced antibody dependent cell cytotoxicity

**Dr Tiffany Mclamarrah**[1], Dr. Zach Goldblatt[1], Jennifer Tedstone[1], Victor Cairns[1], Dr. Jack Scarcelli[1]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:** For generation of antibodies possessing enhanced ADCC, modulation and or monitoring of various product quality attributes (glycosylation, antibody integrity) is key.

**Impact/Novelty:** Mutations in antibody Fc regions in conjunction with engineered cell lines lead to enhanced ADCC and altered product quality.

**Introduction:** Fucosyltransferase 8 (Fut8) plays a significant role in immune system regulation in mammalian cells through the fucosylation of the N-glycan core of IgG1 antibodies, inhibiting antibody dependent cell cytotoxicity (ADCC). Functional knock-out (KO) of Fut8 in CHO-K1 cells leads to the production of afucosylated monoclonal antibodies (mAbs), increasing the potency of certain therapeutic antibodies for cancer and other diseases.

**Methods/Approach:** Fut8 KO cell lines were generated by transfecting two single guide RNAs (sgRNAs) targeting exon 9 of the Fut8 gene and the Cas-CLOVER mRNA. Gene edited clones were isolated, expanded and functionally screened for target knock-out. Following screening, edited cells were analyzed by low resolution next generation sequencing (NGS) to identify insertions/deletions (indels) in the target locus and confirmed using fragment analysis by capillary electrophoresis (FA-CE).

**Results:** Successfully edited clones were transfected with plasmids expressing two standard mAbs with varying substitutions in the Fc region to determine both the productivity of the Fut8 KO cell lines compared to the wild-type parental cell line and the efficacy of the Fut8 knock-out through glycan analysis of the produced antibody. Potential Fut8 -/- clones were initially screened for fucosylation of the glycocalyx using the lectin, Lens culinaris agglutinin (LCA), and were subsequently analyzed using NGS and FA-CE, thus producing several clonal host cell lines containing indels in exon 9 of the Fut8 gene. Expressive Fut8 -/- pools were further analyzed for productivity, stability, and product quality (fucosylation, glycosylation, aggregation, charge variants, etc.) of the standard mAb. Fut8 -/- pools expressing antibody variants produced 100% afucosylated antibodies at high productivities. Differing substitutions in the Fc regions yielded varying product quality attributes and cell line performance.
**Conclusion:** This work demonstrates a novel platform for producing antibodies with enhanced ADCC through cell engineering to knock out Fut8 to use in pipeline production of standard mAbs.

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# Elucidating the impact of host cell protein populations on monoclonal antibody production

**Ms Jodie McAvinue**[1], Dr Stanislas Helle[1], Dr Cillian McCabe[2], Dr Kevin Turner[2], Dr Edel Durack[3], Prof Sarah Hudson[1]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:** Product Quality
Host cell proteins (HCP) are process-related impurities in monoclonal antibody production that can reduce the mAb yield in the feedstream, impact safety and induce post-translational modifications that impact product efficacy.

**Impact/Novelty:**
Previous publications have focused on shake flask and glass bioreactor studies. This publication focuses on investigating host cell proteins produced at 15mL, 60 mL and 2L scale.

**Introduction:**
HCP's are identified as a critical quality attribute of mAb product formulations. Additionally, they have been shown to promote degradation of polysorbates, a surfactant used in formulation buffers. This degradation can compromise protein stability in the final drug product. The current clearance operation for HCP's is performed during the downstream process, which accounts for approximately 80% of manufacturing costs. By identifying the parameters that affect HCP production from Chinese Hamster Ovary (CHO) cell lines and their subsequent effect on purification, better yields, higher purity and lower cost mAb production processes can be developed.

**Methods/Approach:**
A commercially available adherent CHO cell line was adapted to serum free suspension culture using a newly tested rapid adaption method. This cell line was cultivated and process parameters were altered in 60mL shake flasks, 2L bioreactors and 15mL Ambr15 micro-bioreactors. The supernatant was harvested and an eFASP protocol was used to identify the most abundant host cell proteins using LC QTOF mass spectrometry.

**Results:**
During adaption, the viability percentage decreased dramatically to 40%, upon applying chemical and physical anti-clumping techniques this increased to 85% after 20 days with a viable cell density of 3 x $10^6$ cells/mL. HCP's were identified and categorised by subcellular location, molecular function and interaction with the product, particularly troublesome HCP's that are known to evade chromatography steps such as heat shock protein, peroxiredoxin-1 and clusterin.

**Conclusion:**
A commercially available CHO cell line was adapted from adherent to serum-free suspension culture in 20 days, more rapidly than previously reported gradual adaption methods. Host cell proteins were identified with an emphasis on more troublesome HCP's that are known to interact with the product and were compared based on scale, agitation, feeding strategy, pH, dissolved oxygen and temperature.

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# Contactless Biomass Monitoring for Bioprocessing Using bio-reflectance Based Sensors

**Dr Lindsey Male**[1]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

Recent advances in sensing technologies have made significant strides in enhancing the monitoring and control of critical quality attributes in cell culture processes. However, the industry often relies on labour-intensive off-line measurement methods, which not only carry the risk of contamination but also provide infrequent sample points.

The integration of traditional sensors for biomass monitoring becomes even more challenging in single-use bioreactors due to form factor and size constraints, as well as the crucial requirement for sterility. In response to these challenges and to enable real-time biomass monitoring, this work introduces the OPTURA SPY sensor, a state-of-the-art contactless solution based on bio-reflectance principles. The OPTURA SPY sensor is utilised in conjunction with various bioreactor formats including single use bags, benchtop scale bioreactors and shake flasks, demonstrating its versatility as a process monitoring tool. By mitigating contamination risks and enabling continuous monitoring, the OPTURA SPY sensor facilitates intelligent monitoring, allowing the user to optimise processes and supporting data-driven decision-making.

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# Can IgG glycosylation be controlled through process parameters? A systematic analysis

**Mr Javier Bravo-Venegas**[1], Mr Jose Rodriguez-Siza[1], Prof Maria Molina Sampayo[2], Prof Marcela Hermoso[2,3], Prof Julio Berrios[1], Prof Claudia Altamirano[1,4]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance of the Theme Selected** Controlling glycosylation is key for its fundamental role modulating the monoclonal antibody's biological activity. The appropriate strategy, as process parameters(PP)-manipulation, could significantly improve the molecule's therapeutic properties.

**Impact/Novelty** Although many works have studied the impact of PP on glycosylation, this is the first that allows easy comparison between them clarifying whether it is a valid control strategy.

**Introduction** Glycosylation, according to Quality by Design initiative, must be understood as function of Critical PPs(e.g., temperature, pH, dissolved oxygen…). Consequently, we ask if is possible to achieve a relevant impact on glycosylation considering the limited range in which a PP can be manipulated for a mammalian cell? And even, if is possible that an external factor could affect a complex process localised in specific cellular compartments, across membranes and buffered environments?

**Methods/Approach** A systematic review and mathematical analysis was performed to more than 35 articles, all the possible in the field. The denominated Glycosylation-Indexes(GIs) were calculated and used as normalization/standardization tool to compare between works.

**Results** Surprisingly none of the PPs-manipulation were translated in a relevant displacement of the GIs. Generally, it was found that the unpredictable impact is less than 10% or there is no at all for the galactose, fucose or NANA-content. Although many of the conditions implies a change, that could be statistically significant, in the glycoforms content; that does not mean that the overall characterisation, the GIs, of the population changes, and therefore the impact on biological activity is likely to be low or non-existent. Counterintuitively, the reduction in temperature, a strategy widely used to increase production, did not translate into a reduction of quality.

**Conclusion** Contrary to expectations, the impact of PP in glycosylation is clearly limited and is independent of the cell type or culture modality. This finding illustrates how a rational/predictable control strategy would not employ such approach.

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# High throughput screening for product quality attributes early during mammalian cell line generation

**Dr Katharina Köther**[1], Dr. Michaela Strotbek[1], Dr. Anne Sydow[1], Dr. Simon Fischer[1], Mr Sebastian Püngel[1], Dr. Chi-Ting Ho[1], Mr Jürgen Fieder[2], Ms Annika Held[1], Dr. Benjamin Renner[2], Ms Corinna Ruedi[1], Ms Katja Lameli[1], Ms Rebecca Krieg[2], Ms Birgit Sonntag[1], Dr. Patrick Schulz[2], Dr. Joachim Bär[1], Dr. Dennis Blank[1]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

Background and novelty
Mammalian cell line development typically starts with pool generation and is followed by single cell cloning, starting with thousands or hundreds of clones and several rounds of clone ranking and selection until the final clone can be nominated. Selection and clone ranking is usually based on cell growth, productivity, monoclonality, stability, and product quality attributes. The latter gaining more and more importance – highlighting the need to look at product quality attributes as early as possible. However, as we start with hundreds or thousands of clones in small scale volume there is a constant challenge in the number of samples generated but also in the volume available for analysis. Next to high throughput screening of titer and cell growth we were able to screen for product quality attributes like high mannose, or specific glycosylation patterns in the early expansion stage roughly 4 weeks after single cell cloning.

-Experimental approach
Cell line generation for an IgG expressing cell line was performed. Initially pools were generated and used for single cell cloning. Approximately two weeks later all clones were screened for growth and titer and an initial ranking was performed according to the clone performance. Selected clones were expanded and roughly four weeks after single cell cloning supernatants of the different clones were taken and analyzed for product quality attributes like mannose, and galactosylation. This early determined product quality was compared to product quality attributes of the corresponding clones after fermentation assessment of the final clones.

-Results and discussion
Hundreds to thousands of clones will undergo single cell cloning during mammalian cell line generation. This high number of samples as well as the small scale volume available at this stage is the challenge to identify methods for analysis of the product quality as they need to be high throughput and low volume. In our approach we compared the product quality profile during early expansion phase (roughly 4 weeks after transfection) to product quality after fermentation assessment and could show that although we have no exact match in product quality but observe comparable mannose levels as well as galactosylation levels in the different stages. This observation allows us to use early screening for (critical) quality attributes and identifying unsuitable clones very early in the process, improving selection of clones with a desired product quality profile.

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# Screening of excipients as potential stabilizers for oncolytic vesicular stomatitis virus (VSV) drug product

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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

The prevention of particles in drug products is an important part of the formulation development as particles in pharmaceutical products might represent a risk to patients.

**Impact/Novelty:**
The use of various excipients in combination with other excipients in formulation development for oncolytic viruses in the ATMP field can prevent particle formation in the final drug product.

**Introduction:**
The field of ATMPs offers innovative ways to develop new drugs against diseases. Oncolytic viruses as mono- or combinatorial-therapy offers new venues for the treatment of cancer patients. The replication-competent oncolytic viruses VSV-GP and modified variants of it replaced the neurotropic envelope VSV-glycoprotein (VSV-G) to the non-neurotropic glycoprotein of the lymphocytic choriomeningitis virus (LCMV-GP). The stabilization of these VSV as drug product without particle formation is a challenge. In the pharmaceutical industry only few non-ionic surfactants are in used. While these surfactants are known to prevent particle formation efficiently are not yet well understood in their interaction behaviour with the viral surface. It was already identified that some of these surfactants reduce the infectivity of VSV-GP and cannot be used in the drug product formulation. To generate more knowledge about surfactants which reduce particle formation, different excipients were investigated for VSV projects.

**Methods/Approach:**
The effect of excipients in different concentrations on the infectious virus titer ($TCID_{50}$) of VSV-GP and their ability to prevent particle formation (visible with VI and subvisible with FlowCam® or MFI®) in stress models were investigated.

**Results:**
We identified different excipients with no negative impact on the infectivity of VSV-GP. Importantly, these candidates were able to prevent visible and subvisible particles in different stress models.

**Conclusion:**
Some of tested excipients are promising candidates for preventing particle formation and might provide a path forward for future commercial formulations for oncolytic viruses like VSV-GP.

# Poster Presentations

# Data, Cells and Processes

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# Fast Raman spectroscopy model generation for bio-pharmaceutical process monitoring and control.

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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

In recent years, increased regulatory demands from the FDA and EMA have led to the adoption of process analytical technologies (PATs) in the biopharmaceutical industry. Raman spectroscopy is one such technology that has been used for process monitoring and control. To generate a reliable model dataset for online prediction monitoring and process control, a sufficient data set with enough process variability needs to be generated. However, until recently, this was a laborious and time-consuming process, as Raman spectrometers were limited to pilot scales in an online measurement setup. The implementation of Raman spectroscopy in the AMBR250 system has significantly facilitated and sped up the model building process. Nevertheless, transferability between scales needs to be carefully evaluated for individual processes. This poster/talk focuses on the technical challenges associated with Raman spectroscopy, describes a general potential workflow for data selection, and provides an overview of the potential challenges during online process monitoring and control.

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# Soft-sensor for monitoring CHO cell pools producing SARS-CoV-2 spike protein in a fed-batch process

**Mr Juan Sebastian Reyes Davila**[1], Dr Lan Pham Phuong[2], Dr Olivier Henry[1], Mr Raul Molina[3]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:** It is relevant to field 3 as Artificial Intelligence is applied to bioprocess monitoring so as to track hard-to-detect variables throughout a fed-batch process.

**Impact/Novelty:** Novel soft-sensor architecture for one-step-ahead prediction of multiple variables in a fed-batch process by relying on bioreactor online data (DO, pH, gassing, temperature, integral DO, base).

**Introduction:** In fed-batch cell cultures, process monitoring is instrumental to maintain favourable conditions and ensure optimized nutrient feeding. Since some critical process variables are only measured off-line with low sampling rate, utilizing routine current day bioreactor online data to aid in next day predictions is a promising strategy for enhanced bioprocess control.

**Methods/Approach:** Building a Soft-sensor architecture for predicting next day sampling data: Online bioreactor data and initial measured sampling data along with an initial hidden state are received as inputs to an artificial neural network (ANN). This ANN outputs the next discrete time prediction of each sampled data (Total glucose consumption (TGC), titer, viable cell density (VCD), lactate, ammonia) along with an updated hidden state to be used in the next iteration. This process is repeated for all days during the production process by using previous output predictions along with daily online data as inputs in the next iteration. Model was trained with 17 fed-batch cultures spanning three different pools and tested with four distinct fed-batch cultures.

**Results:** Titer, VCD, TGC, lactate, and ammonia tracked across the 17-day process with low error (normalized root mean square error and normalized mean absolute error below 0.5) and high $R^2$ values (above 0.9). Glucose uptake rates were also predicted and showed good agreement with experimental measurements further offering opportunities for online glucose control.
**Conclusion:** The presented model architecture can effectively soft-sense complex variables, hinting at its potential for real-time monitoring of hard-to-detect variables, especially in data-rich processes.

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# Comprehensive modeling of cell culture profile using Raman spectroscopy and machine learning

**Dr Hiroki Tanemura**[1], Dr Ryunosuke Kitamura[1], Ms Yasuko Yamada[1], Dr Masato Hoshino[1], Mr Hirofumi Kakihara[1], Dr Koichi Nonaka[1]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:** This research is focused on modeling cell culture techniques using large datasets of Raman spectroscopy data. These techniques allow for a comprehensive measurement of components related to cell culture.

**Impact/Novelty:** This study demonstrates the efficient use of Bayesian optimization in creating Raman models for cell culture. It aids in optimizing media and processes and predicts various parameters for biopharmaceutical development.

**Introduction:** Chinese hamster ovary (CHO) cells are commonly utilized in the production of antibody drugs. Monitoring and controlling metabolite levels during CHO cell culture is crucial for generating high quantities of antibodies that meet specifications. Continuous analysis methods utilizing Raman spectroscopy have gained attention, but constructing accurate measurement models for numerous components is time-consuming.

**Methods/Approach:** In this study, a comprehensive and automated method was developed using machine learning with Python to construct a Raman model for various components. Data collection was efficiently conducted using miniaturized bioreactors, and pre-processing and spectral-range optimization were automated and accelerated using Bayesian optimization. Models for each component were constructed using linear regression, ridge regression, XGBoost, and neural network techniques.

**Results:** Bayesian optimization and each machine learning technique efficiently improved model accuracy compared to standard regression. This automated modeling method enables real-time tracking of over 100 parameters for process optimization and control. Models were even generated for low concentration stress marker proteins, showcasing potential in quality prediction.

**Conclusion:** This research demonstrates the effectiveness of Bayesian optimization and each machine learning technique in creating comprehensive Raman models for cell culture. This optimization technique not only facilitates the quick optimization of media and processes but also enables the prediction of various parameters crucial for biopharmaceutical development.

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# Towards the automated analysis of protein N-linked glycosylation pathways via an open-source computational framework

**Mr Konstantinos Flevaris**[1], Dr. Pavlos Kotidis[1], Prof. Kiyoko Aoki-Kinoshita[2], Prof. Cleo Kontoravdi[1]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
This work proposes a mechanistic modelling framework to guide cell-line development strategies with respect to *N*-linked glycosylation targets for improved product quality.

**Impact/Novelty:**
This work develops a novel end-to-end automated framework for the model-based analysis of glycosylation pathways made available to experimentalists with minimal modelling experience.

**Introduction:**
In recent years, several models have been developed to successfully predict recombinant protein glycoprofiles in Chinese Hamster Ovary (CHO) cell cultures. However, their wide adoption within the bioprocessing community remains elusive. This can be attributed to the high level of domain knowledge required to model glycosylation and the limited accessibility to such models due to the use of proprietary software. Thus, the quantitative analysis of glycosylation in biomanufacturing settings would greatly benefit from open-source tools that allow experimentalists to generate and test new hypotheses *in silico* while having minimal modelling experience.

**Methods/Approach:**
The proposed framework keeps manual decisions from the user to a minimum and accounts for the uncertainty in parameterising the glycosylation model. In particular, a minimal network that can explain the observed glycoprofiles is generated. Given this information, a kinetic model of glycosylation is assembled in an automated way. This model extends the predictive capabilities of state-of-the-art mechanistic models to include the simultaneous prediction of the glycoprofiles of multiple proteins. Finally, the estimation of the Golgi enzyme concentration levels is achieved via Bayesian inference.

**Results:**
A case study to simultaneously predict the glycoprofiles of recombinant IgG and host cell proteins (HCPs) in CHO cells is considered. The results indicate a considerable effect of HCPs glycosylation on the mAb glycoprofile. Additionally, posterior distributions for the enzyme concentrations required to match predicted and experimental glycoprofiles are obtained.

**Conclusion:**
The proposed automated framework facilitates better-informed decision-making in the targeted manipulation of glycosylation pathways towards non-immunogenic glycoproteins for effective treatments.

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# CHO Cell Culture with Digital Twins: A Practical Roadmap for Implementation

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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
The contribution utilizes machine learning and modeling approaches to harness large datasets for creating a digital twin to optimize a cell culture process.

**Impact/Novelty:**
This contribution combines the high topical concept of digital twins and machine learning algorithms with animal cell cultivation, breaking new ground in process optimization and digitalization.

**Introduction:**
Digitalization, automation and artificial intelligence are currently revolutionizing industries, research, and daily life. In biotechnology these topics offer huge opportunities for optimizing or developing bioprocesses to face the numerous current and upcoming challenges. A major goal is an increase in sales or yields, but also the saving of resources, time and money. One of the emerging concepts of digitalization is the digital twin, which here describes the complete digital representation of a real bioprocess, including all process chains. An automated data flow between the real and digital object, as well as the possibility of many *in silico* simulations before real testing in the lab, are the main benefits.

**Methods/Approach:**
The aim is to optimize an animal cell culture process using a monoclonal antibody producing *Chinese Hamster Ovary* (CHO) cell line by digitizing and implementing a digital twin of this bioprocess. This includes building an appropriate digital infrastructure, generating diverse data sets, and developing suitable models and machine learning algorithms to suggest optimal process parameters.

**Results:**
In this contribution machine learning tools were successfully used to improve antibody titers significantly, and also initial steps towards digital twin implementation were established.

**Conclusion:**
The combination of digital twins and machine learning is paving the way for further digitalization of biotechnology as well as increasing productivity and improving the efficiency of bioprocesses.

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# Leveraging prior knowledge to validate a bioreactor model across scales and cell lines

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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Bioreactor digital twin:**
We focus on the development of a platform model for biopharmaceuticals production, a general model that can be easily adapted for diverse products at multiple scales.

**Bioreactor platform model:**
We aim to advance digital workflows for building accurate cell culture digital twins, minimizing data requirements for new assets, and extending the usual range of validation and testing.

**Introduction:**
Mammalian cell cultures provide a crucial platform for biopharmaceutical production. High-fidelity predictive cell culture models have great potential to accelerate and derisk process scale-up and technology transfer. However, generating accurate bioreactor models is not trivial, model calibration is complex and time-consuming.

**Approach:**
We configured and calibrated a mechanistic bioreactor model, including cell culture phenomena and gas-liquid mass transfer with data from shake flask and GS-CHO cell line, using a systematic sequential parameter estimation strategy to obtain the relevant model parameters. The effects of temperature and pH shifts were also evaluated during the model calibration.

**Results:**
Using the first calibrated bioreactor model, we adjusted the necessary model structure and parameters to validate it for other cell lines, GS-CHO null and CHO-S, and different scales, 250mL Dasbox and 5L STR. As expected, more adjustments were required for CHO-S, a cell line with divergent behaviour. On the contrary, the Dasbox and STR data could use the same model, and no further calibration was necessary. Approaches were tested to leverage prior knowledge to develop a general cell culture model with elements transferable across different scales and clones.

**Conclusion:**
We developed a strategy to use data from different scales to validate cell culture models with a reduction of at least 80% in validation time. The evaluation of model behaviour with different cell lines and at different scales increases the usability range of the model and gives insights into bottlenecks for building a platform model.

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# Integrated culture system automatically collects data and controls cultivation in digital twin optimized condition.

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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:** Digital twin built from past cultivation data is used to explore the optimized condition in silico. We evaluated the optimized condition predicted by Digital Twin with our automated culture system.

**Impact/Novelty:** Our integrated system not only controls the cultivation under the optimized condition predicted by digital twin, but also enables automatic data collection from bioreactors, inline sensors, and offline analysers.

**Introduction:** In the bioprocess manufacturing, to keep cell culture in the optimized condition is pivotal. However, it is difficult and laborious to search the optimized condition by DoE. Furthermore, controlling culture condition and data collection required for the optimization are still operated manually in many cases. We tried to solve these problems by combining the digital twin simulation and integrated automation system.

**Methods/Approach:** First, we built digital twin for our CHO cell line. The digital twin model was built by combing the past cultivation data and our metabolic network model. With the digital twin, the optimized condition for the better titre was explored. Based on the optimized condition, we ran several evaluation batches. To operate the cultivation automatically, we performed cultivation with the system where bioreactors, offline analysers, additional sensors, and our control system were integrated.

**Results:** With the integrated system, we programmed the optimized operation and performed automatic cultivation. Once the cultivation was started, operators only did daily sampling. The data were automatically collected and integrated. Compared to the optimized cultivation and the conventional cultivation, the titre of optimized cultivation was 30% higher than that of conventional one after two weeks of cultivation.

**Conclusion:** Here we demonstrated that our digital twin and integrated system were able to yield higher performance in process development. This system not only improved titre, but also drastically reduced the man-hours. In addition, the system can be added on the existing culture system because our system has various drivers to communicate with different instruments. We expect that our system is applicable to various cultivation systems, and even applicable to other modalities such as stem cell cultivation.

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# Genome-scale modeling of CHO cells unravel the critical role of asparagine in feed media

**Dr Kuin Tian Pang**[1,2,3], Mr Yi Fan Hong[1], Dr Fumi Shouzi[4], Shunpei Furomitsu[4], Mr Matthew Myint[1], A/Prof Ying Swan Ho[1], Dr Yaron Sibelberg[5], Dr Ian Walsh[1], Dr Meiyappan Lakshmanan[1,6]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
CHO genome scale model is utilised to characterise the intracellular metabolic states of CHO cells cultured in different combination media to identify key amino acid to improve cell culture performance.

**Impact/Novelty:**
Critical role of asparagine in the feed media is identified as anaplerotic sources and in-silico simulations were performed to ascertain their optimal ratios to improve cell culture performance.

**Introduction:**
Amino acids, including asparagine, aspartate, glutamine, and glutamate, play important roles as nitrogen sources in mammalian cell cultures, crucial for purine and pyrimidine synthesis and as anaplerotic sources fueling the tricarboxylic acid (TCA) cycle for ATP generation. Despite extensive studies on glutamine and glutamate in CHO cell cultures, the roles of asparagine and aspartate, especially in feed media, remain underexplored.

**Methods/Approach:**
CHO genome scale model was utilised to characterize the intracellular metabolic states of CHO cells cultured in different combinations of basal and feed media to understand the traits of asparagine/aspartate-dependent feeds. Subsequently, in-silico simulations were performed to ascertain the optimal ratios of asparagine and aspartate to improve cell culture performance. Finally, based on the model simulations, we reformulated the feed media by tailoring the concentrations of asparagine and aspartate.

**Results:**
In-silico simulations indicated that increasing either aspartate or asparagine consumption showed similar ability to fuel TCA cycle and thereby improving VCD and titer, however experimental data reveal a CHO cell preference for asparagine. Maintaining an optimal Asn/Asp ratio of around 3 emerges as a key factor for achieving optimal CHO cell culture performance, providing valuable insights for bioprocess optimization in biopharmaceutical production.

**Conclusion:**
Critical role of asparagine and asparate in feed media as anaplerotic sources was investigated. Our study suggest that Asn/Asp ratio is a key factor for achieving optimal CHO cell culture performance.

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# Demonstrating Key Processes of the T Cell Therapy Workflow in Chemically Defined Media.

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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:** High-performing chemically defined (CD) media for standard cell therapy workflow is compatible with scale-up applications and multiple expansion systems.

**Impact/Novelty:** CD media are more compatible with scale-up, clinical applications, and regulatory submissions than traditional media.

**Introduction:** Although technologies used in the generation of immunotherapies are diverse and rapidly evolving, they share the goal of efficiently generating sufficient cell numbers with high viability and consistent clinical quality. The elimination of undefined media components such as serum-derived proteins reduces variability, facilitating safe and efficacious translation for clinical use. CD media are uniquely amenable to consistent and reproducible scale-up due to the lack of undefined media components, enabling manufacturers to reliably make products of high quality and reduced variability when transitioning from preclinical development to cGMP manufacturing of cell-based therapies.

**Methods/Approach:** Thawed human PBMCs were activated with αCD3/αCD28 beads, expanded in G-Rex cell culture vessels and Quantum hollow fiber bioreactors in PRIME-XV T Cell CDM with IL-2, and assessed for functionality. IL-2-independent expansion, expansion post-thaw from DMSO-free media without an initial wash step, and lentiviral transduction were likewise performed in G-Rex vessels in chemically defined conditions.

**Results:** PRIME-XV media support DMSO-free cryopreservation, wash-free thawing, cell activation, lentiviral transduction, and subsequent scalable expansion of human PBMC-derived T cells in G-Rex Cell Culture Systems and in Quantum hollow fiber bioreactors. Our data indicate that the expanded cells maintain robust functionality in response to antigenic stimulation, and the omission of a post-thaw wash step streamlines the manufacturing process and simplifies production scaling.

**Conclusion:** Compatible with multiple culture and activation methods and a variety of cell sources, PRIME-XV FreezIS DMSO-Free and T Cell CD Media work in tandem with CTGrade recombinant human cytokines to provide a powerful toolset for T cell therapy manufacturing.

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# EEM coupled with second-order data for monitoring SARS-CoV-2 Spike protein in mammalian cell cultures

Mr Javier Villarraza[1,2], Ms Kimey Denise Mendoza[1], Dr Agustina Gugliotta[1,2], Dr Ernesto Garay[1,2], Dr Marina Etcheverrugaray[1,2], **Dr Claudio Prieto**[1,3], Dr María Celeste Rodríguez[1,2,4]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
A simple, rapid and cost-effective method based on fluorescence excitation-emission matrix (EEM) coupled with chemometric models was developed for monitoring recombinant Spike (S) production in HEK cell line culture.

**Impact/Novelty:**
EEM coupled with MCR-ALS was proposed as a tool for visualizing changes in the bioprocess. Thereafter, multiway spectral data were applied for S concentration prediction in fermentation samples.

**Introduction:**
PAT initiatives proposed by the FDA and then adopted by both EMA and ICH, aim to minimize the impact of production process variations on product quality.
In our laboratory, a protein subunit COVID-19 vaccine based on S has been designed.
Here, PAT/QbD approaches enable both monitoring and predicting S protein production in bioprocess.

**Methods/Approach:**
First, a univariate reference method (ELISA) to quantify S protein in fermentation samples was optimized through DoE and then, validated. Second, EEMs (656×45) were acquired directly in fermentation samples on a LS-55 spectrometer. Each EEM was pre-processed to remove spectral interference. The dataset (n=24) was split into calibration (n=17) and validation (n= 7) data sub-set. Third, chemometric modelling (MCR-ALS and PARAFAC) was performed employing MVC2 MATLAB code. Fourth, the accuracy of the obtained second-order methods was assessed by elliptical joint confidence region (EJCR) test.

**Results:**
MCR-ALS provides an adequate tool for monitoring the progress of different cell culture system. Two components were identified (>97% cumulative variance), the first at the lower excitation region (aromatic amino acid of S) and the second at higher excitation regions (that may correspond to NADH). Thereafter, both MCR-ALS and PARAFAC were applied to predict S concentration in fermentation samples. Good calibration models were obtained. In accordance with the results, the ideal point (1,0) was contained in the elliptical EJCR domain, demonstrating the method accuracy.

**Conclusion:**
An accurate, precise and cost-effective second-order method for both monitoring and evaluating S protein in cell culture was achieved.

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# Blending of chemically-defined liquid media for efficient screening of medium components in antibody production

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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:**
Data, cells and processes

**Impact/Novelty:**
A systematic workflow that enables the development of culture media and the screening of medium components by blending chemically-defined media products was proposed.

**Introduction:**
Culture media are important in the production of biopharmaceuticals such as therapeutic antibodies. In general, when identifying the optimal medium for each cell line, targeted components are added to the current medium, and the culture performance is evaluated. However, this approach is not well-suited for comprehensive screening of components due to complexities in preparation. In contrast, blending media can be expected to solve the problems. In this research, the systematic workflow of media blending was investigated in a case study on medium development for a recombinant cell line producing mAbs in suspension culture using animal/protein-free chemically-defined media for CHO cells.

**Methods/Approach:**
In the process of blending media, the information of components in the media obtained with mass spectrometry was taken into consideration. Moreover, a reasonable criterion was studied for selecting the appropriate blending conditions. The criterion was used to ensure that similar conditions were not repeatedly tested and to minimize the correlation between the components. Finally, a suspended batch culture with a recombinant cell line producing mAbs was conducted under the determined blending conditions.

**Results:**
The blending conditions determined by the proposed workflow resulted in $|r| < 0.7$ in 87% of the combinations. The batch culture conducted under the selected conditions resulted in Viable Cell Concentration (VCC) ranging from 8 to 20 million cells/mL at harvest. A regression model with VCC was constructed, and the components that explain variations in VCC were identified.

**Conclusion:**
A new systematic workflow for efficient medium component screening through the blending of chemically-defined media products was proposed, and its effectiveness was demonstrated.

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# Beyond the Bench: Revolutionizing Cell Line Development with Data Driven Digital Solutions

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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:** This work highlights an ensemble of data driven digital tools which leverage available cell line development data to accelerate cell line selection.

**Impact/Novelty:** Application of multiple data-driven tools including multivariate data analytics (MVDA) and shallow or deep machine learning applications (ML) to reduce number of clones assessed in cell line development.

**Introduction:** Biopharm Process Research (BPR), within the Drug Substance Development group at GSK, is responsible for understanding and de-risking the CMC characteristics of the biopharmaceutical portfolio; to deliver by first intent, cell lines suitable for commercial manufacture. In the typical process of identifying these cell lines, it is imperative to conduct extensive screening during the cell line development process, demanding considerable time and resources.

**Methods/Approach:** We have developed a series of data-driven digital tools to deploy across the cell line development process; offering a systematic approach to cell line selection based on desired phenotypic characteristics.

**Results:** The application of multiple data-driven tools in BPR, leading to a substantial reduction in the number of clones progressed for screening in early stages of cell line development as well as in the assessment in the production process stages. In addition, this digitally optimised approach has not only enabled efficiencies in the cell line development process but is also fully aligned with different variants of GSK's proprietary, high-performance CHO expression platform.

**Conclusion:** The benefits of these tools have now been realised in BPR which has been under-pinned with extensive data capture automation and an associated mindset shift in experimental design and data formatting, to enable the value of advanced data analytics.

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# Quantitative influenza A virus proteomics: impact of host cell line and virus seed

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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected**
Quantitative data from proteomics, transcriptomics and mathematical modelling was used to capture potentially limitations in virus replication.

**Impact/Novelty**
A mass spectrometric assay for absolute quantification of five influenza A virus proteins was developed to monitor virus replication in three cell lines for cell-adapted and non-adapted virus seeds.

**Introduction**
Novel assays for quantitative proteomics combined with mathematical modelling may help to identify limitations of yields in vaccine manufacturing. Quantitative approaches could support cell line selection for manufacturing of next-generation viral vaccines.

**Methods/Approach**
Shake flask infections with non-adapted and adapted IAV seed in suspension MDCK, AGE1.CR and HEK293 cells were performed. Hemagglutinin (HA), neuraminidase (NA), matrix protein 1 (M1), nucleoprotein (NP) and non-structural protein 1 (NS1) of IAV were quantified by mass spectrometry by adding isotopically labelled peptides.

**Results**
Virus replication in all cell lines resulted in an exponential increase in the intracellular level of the viral proteins starting 2-4 hours post infection (hpi) and reaching a maximum at 6-8 hpi. Before virus adaptation, M1, NP and NS1 were equally abundant for the different cell lines at 1E+08 copies/cell, followed by HA (5E+07 copies/cell) and NA (1E+07 copies/cell). After virus adaptation, virus replication started earlier but maximum levels were constant. NS1, known as efficient antagonist of host cell response, was the most abundant protein with a maximum copy number of 1E+09 copies/cell for all cell lines, followed by M1 and NP (5E+08 copies/cell), HA (3E+08 copies/cell) and NA (1E+08 copies/cell).

**Conclusion**
Virus adaptation led to similar intracellular viral protein levels in host cells from different species. Viral replication started earlier without change of maximum virus titers. Results point to host cell-specific but yet unidentified factors limiting virus packaging and virus release.

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# Developing new host reporter cell line for rapid screening of targets for increasing mAb productivity

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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:** Use of previous RNA-seq data to select screening candidates for up- or down-regulation to create host cell line with improved productivity.

**Impact/Novelty:** Novel reporter cell line for rapid screening of impact of differentially expressed proteins on intracellular expression and secretion of monoclonal antibody.

**Introduction:** Chinese hamster ovary (CHO) cells are commonly used for therapeutic proteins production. Major challenge of cell line development is to obtain stable high-producing clones. Therefore, we developed new CHO host cell line that was selected based on the growth characteristics as well as productivity and secretion potential. This new host was used as a base for development of reporter cell line for rapid target screening.

**Methods/Approach:** New host cell line was generated by productivity assessment of ATCC's CHOK1 CCL-61 cell line using transient split-GFP antibody expression. Best subpopulations were pooled together to create new host cell bank. It was used to develop a reporter cell line by PinPoint recombination of split-GFP monoclonal antibody and single cell cloning on Beacon®. In this reporter cell line, we will transiently overexpress or knock down previously identified proteins to test their potential impact on mAb productivity.

**Results:** New host cell line showed improved productivity potential compared to original cell line as evaluated by mAb expression on clones' level. Final reporter cell line was selected based on intracellular fluorescence and secreted molecule levels of split-GFP antibody and the fluorescence stability over time.

**Conclusion:** We established split-GFP mAb high-throughput assay for rapid evaluation of intracellular and secreted mAb in one single experiment. It led to generation of new host cell line with better expression than parental cell line. Reporter cell line with split-GFP mAb was developed to be used to rapidly test impact on monoclonal antibody productivity of modified expression of different protein candidates.

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# Lifecycle DOE - Leveraging Cell Culture Development Data for Late Stage Success

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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

Design of Experiment (DoE) approaches are integral to pharmaceutical process development, particularly since the Quality by Design (QbD) initiative by the European Medicine Agency in 2009. However, Design of Experiment (DoE) approaches have traditionally been planned and analyzed independently. This siloed approach has often made it challenging to crosslink individual data sets at the end of the development process to generate a comprehensive, holistic picture. Recognizing this limitation, we propose a novel workflow to create a Life-Cycle-DoE (LDoE) that incorporates data-based process knowledge in the form of design augmentations.
The LDoE approach allows for the integration of an existing model with new experiments. This method enables flexible design adaptations and improves model predictions by utilizing all available data. It aligns with the QbD initiative, supporting model-based knowledge transfer, calculation of limits for critical quality attributes, and data-driven risk assessment. It also facilitates the definition of the characterization range for the process characterization study. The focus is not on developing new statistical methods, but on combining existing ones to establish a holistic development model.
We applied this approach to the development of a late-stage cell culture process. All commercial process development activities were conducted in LDoE format, resulting in a robust process model that supported scale down model (SDM) development and process characterization studies (PCS). The PCS development efforts were halved, and over 500 analytical samples were saved.
In conclusion, our LDoE approach offers a practical solution to data integration challenges in process development. By leveraging big data and emerging digital technologies, we can guide, enhance, and expedite the development of superior processes.

DoE approaches for process development are not new in the pharmaceutical industry. They are widely used during the product process development lifecycle, especially after the introduction of the Quality by Design (QbD) initiative by the European Medicine Agency in 2009. A central step in the QbD process is a risk assessment to identify potential critical process parameters, for instance, in a failure mode and effect analysis (FMEA). For this step, knowledge from the process development of a product is used.
However, Design of Experiment (DoE) approaches have traditionally been planned and analyzed independently. This siloed approach has often made it challenging to crosslink individual data sets at the end of the development process to generate a comprehensive, holistic picture. Recognizing this limitation, we propose a novel workflow to create a Life-Cycle-DoE (LDoE) that incorporates data-based process knowledge in the form of design augmentations.

The LDoE approach allows the connection of an existing model with new experiments in a combined design. This innovative method enables flexible design adaptations according to the needs of subject matter experts during the process development. Simultaneously, it improves model predictions by utilizing all available data. Our proposed LDoE approach aligns with the QbD initiative. It allows for a model-based knowledge transfer within the product lifecycle, it supports the calculation of appropriate limits for the critical quality attributes since preliminary experiments were performed in an aligned way. The FMEA can be performed in a data-driven way, enhancing the accuracy and reliability of the risk

assessment. Moreover, using a LDoE allows an easy definition of the characterization range for the process characterization study. This approach does not focus on the development of new statistical methods. Instead, it smartly combines existing methods with the focus on establishing a holistic development model that incorporates the combined knowledge of all development data.

In this study we show the application of this approach for development of a late stage cell culture process. All late stage development activities were conducted in LDoE format, thus a power full process model was available during development, and could be used to support scaled won model development and and process characterization studies (PCS). Development efforts for the PCS were only half, and more than 500 analytical samples were spared.

In conclusion, our approach provides a practical solution to the challenges of data integration in process development. By utilizing big data from development and emerging digital technologies, we can guide, enhance, and accelerate the development of better processes.

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# Harnessing HTP Automation Technologies and Modelling Approaches to Uplift mAbs/BsAbs Production through HID Process

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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:** Utilizing big data sets from automated high-throughput platforms for modelling purposes provides deeper understanding to control the product quality and uplift the titre.

**Impact/Novelty:** The high-throughput automation platforms such as AMBR250 and other miniaturized systems can provide generation of high-quality data for in-silico models which can decrease the time to drive biologics to patients.

**Introduction:** We have taken another initiative to further bring innovation to our recently launched intensified fed-batch process. We have integrated a fully automated high-throughput miniaturized cell culture systems using Raman spectroscopy and model for feedback loop control of glucose and two-part feeds driving towards big data analytics.

**Methods/Approach:** A panel of five different CHOK1SV GS-KO® CHO cell lines expressing monoclonal and bispecific antibodies were evaluated with intensified fed-batch process both in AMBR250 and other miniaturized cell culture systems. Raman spectroscopy was used for the measurement of glucose and amino acids. Nova and mass spectrometry were used as orthogonal methods for the measurement of these metabolites.

**Results:** In this study, we were able to develop two different miniaturized cell culture models and we were able to show the uplift in the titre ranging from 35 % to 85 % depending on the BsAb or mAb expression through PAT controlled intensified fed-batch process. The comparability of both titer and product quality including charge variant, glycan, size variant and aggregates up to 200 L scale were successfully demonstrated. For the Raman modelling RMSEP values below 1 g/L and 100 mg/L were targeted for glucose and amino acids. The data was used to drive predictive models for a variety of applications including screening of clones, media compositions and process parameters.

**Conclusion:** In summary, transformative impact of coupling HTP automated systems with modelling approaches is successfully demonstrated in this study for intensified fed batch cultures.

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# Integrating industry leading datasets with genome scale metabolic models to direct CHO cell metabolic engineering.

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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**

This work demonstrates how mechanistic & hybrid modelling, multi-variate data analysis and machine learning can be combined with an industry leading bioprocessing dataset to direct successful CHO cell line engineering.

**Impact/Novelty:**

This is the first-time genome-scale models have been applied in this way to engineer mammalian cell lines. Novel targets were selected, leading to statistically significant improvements in cell line performance.

**Introduction:**
Chinese hamster ovary (CHO) cells are the leading platform for therapeutic protein production, meaning increasing the efficiency of these cell lines is vital to meet demands and reduce costs. CHO cell genome-scale metabolic models (GeMs) possess the power to revolutionise cell line efficiency by their ability to predict whole cell metabolism in silico, allowing for model directed metabolic engineering strategies to be implemented. Despite their power, the industrial application of GeMs to improve CHO cell hosts has yet to be fully realised.

**Methods/Approach:**
In this work, a novel methodology is presented that demonstrates how GeMs can be effectively utilised in an industrial setting to direct the design of CHO cell genetic engineering strategies. The latest CHO cell GeM (iCHO2441) is coupled with an industry leading GSK dataset, which includes time course process, metabolomic and transcriptomic data from 22 industrial cell lines with a range of performance attributes. A GSK cell line specific GeM is generated, which is used to direct cell engineering strategies following two key methods: 1. data driven analysis of predicted metabolic fluxes and 2. effect of host cell proteins on phenotype predictions.

**Results:**
These two methods identify a diverse range of cell engineering strategies to potentially improve GSK's host cell line, including metabolic engineering targets in cholesterol metabolism, oxidative phosphorylation, and deletion of host cell proteins. These strategies have been experimentally validated in house at GSKs R&D facility, demonstrating statistically significant improvements in product titres.

**Conclusion:**
Overall, this work presents some of the first efforts to utilise GeMs to direct CHO cell metabolic engineering in an industrial context, utilising big datasets and a widely applicable novel modelling workflow, that is demonstrated experimentally to improve cell line phenotype.

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# Unveiling secrets: Pathway analysis provides molecular insights into stable lentiviral packaging and producer cell lines

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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
Molecular analytics of lentiviral packaging cell lines is vital for optimizing production yields for gene therapy applications.

**Impact/Novelty:**
We describe the first molecular and bioinformatics investigation of stable lentiviral GPRTG packaging and producer cell lines to enable informed process optimization.

**Introduction:**
Lentiviral vectors (LVV) stand out in cell and gene therapy applications as they can infect both dividing and non-dividing cells, permanently transform target cell genome and allow stable transgene expression. LVV production usually involves transient plasmid co-transfection, however, stable integration of genes required for lentivirus expression could enable a reproducible and cost-effective production. Recently, the generation of the stable LVV packaging cell line GPRTG (suspension and adherent) from HEK293T was reported[1], however, detailed characterization of molecular changes due to stable and inducible viral gene expression is currently not available.

**Methods/Approach:**
GPRTG packaging and producer cell lines as well as HEK293T cell line were cultured under various conditions, RNA isolated and transcriptome analysis performed. Differential gene expression was analysed using Ingenuity Pathway Analysis software.

**Results:**
Comparing the transcriptomics profile from GPRTG packaging cell line (not induced) to the parental HEK293T cell line, we have identified that pathways involved in apoptosis, transcription, translation as well as immune response pathways were activated. After induction of the GPRTG cell line (tet-off system), pathways related to energy production and immune response were affected. Additional comparisons as suspension versus adherent culture conditions, induced versus not-induced producer cell lines, and suspension with FBS versus without FBS enabled a detailed understanding of GPRTG cell lines.

**Conclusion:**
Our comparative transcriptome analysis of GPRTG cells enabled a deeper understanding of molecular changes induced by stable viral gene expression. These findings not only enhanced our fundamental knowledge of stable packaging and producer cell lines, but also provided insight instrumental for optimizing LVV packaging cell lines using cell line engineering tools.
[1]Throm et al., 2009

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# Assessing genetic plasticity – Comparative analysis of HEK293 cells in response to evolutionary pressures

**Mr Georg Peter Smesnik**[1], PhD Nikolaus Virgolini[1], Dr. Astrid Dürauer[1], Prof. Dr. Nicole Borth[1]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:**
A systematic examination of biological data from human embryonic kidney cells (HEK293), generated by whole-genome and -methylome sequencing provides insights into host cell behavior under changing environmental conditions.

**Impact/Novelty:**
Investigating the impact of evolutionary pressures on HEK293, reveals novel insights into genetic and epigenetic variability of this cell system which could impact viral vector manufacturing.

**Introduction:**
HEK293 have become a preferred expression system for the production of viral vectors, in particular recombinant adeno-associated virus particles (rAAV). Despite being widely used, there have been limited efforts to comprehend genome and epigenome instabilities in HEK293. Such instabilities are typical characteristics of other immortalized cell lines such as CHO and may have an influence on product quality and titers. This study aims to improve current understanding of the genome and epigenome stability of HEK293 in response to various culture conditions which might have implications for manufacturing processes.

**Methods/Approach:**
Adherent HEK293 cells were adapted to suspension growth using various commercially available serum-free media formulations. After adaptation, whole-genome and -methylome deep sequencing was performed on adapted and parental cells, as well as a commercially available reference HEK293 suspension cell line (HEK293-6E). Sequenced reads were then aligned to the human reference genome, to assess genome stability, structural variants, as well as DNA methylation patterns. Finally, an overall assessment of genome and methylome variation across all cell lines will be made and compared to previously published data.

**Results:**
Comparative analysis of the different cell lines reveals that changes occur at all investigated levels over the course of adaptation. Changes in the methylome landscape of individual cell lines could be observed, as a response to the adaptation process to suspension growth conditions.

**Conclusion:**
The data obtained from this characterization provides new information on the degree of genome and epigenome (in)stability of HEK293 during adaptation processes. This insights can be leveraged for improvements in directed cell line development and in the optimization of production processes.

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# Biacore™ 8K+ surface plasmon resonance system as a tool in cell line development

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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected: Data, cells and processes:**
Biacore™ 8K+ surface plasmon resonance (SPR) system can be a valuable tool in cell line development (CLD) processes.

**Impact/Novelty:**
We demonstrate how Biacore 8K+ can be used in CLD for mAbs and bsAbs.

**Introduction:**
High-throughput titer measurement is important for an effective CLD process. For titer selection early in the workflow, the chosen technology must allow low sample consumption and high sensitivity. For bsAbs, protein A (ProtA) assays can be complemented with custom sandwich assays to quantitate the correct bsAb in the presence of product-related impurities. By combining a bispecific sandwich assay with a ProtA assay, bsAb purity can be obtained and used for clone selection.

**Methods/Approach:**
We used Biacore 8K+ in CLD campaigns for standard mAbs and bsAbs with screening at the static and deep-well suspension culture stages. CLD campaigns were performed using site-directed integration into a CHO-K1 cell line. ProtA and sandwich assays were used to screen multiple plates with CHO clones at different stages to select high-performing clones. Performance of selected clones was further assessed in shake-flask fed-batch cultures.

**Results:**
We demonstrate that Biacore 8K+ is useful for titer screening during CLD. The Biacore 8K+ instrument together with ProtA sensor chips offers a ready-to-use solution for CLD of standard mAbs for unattended titer assessment of eleven (11) 96-well plates in 8–10 h with a dynamic range of ~ 0.05–10 mg/L. For bsAbs, assessing both the level and purity of the correct bispecific form was critical to identify high-performing producer clones.

**Conclusion:**
Biacore 8K+ is a valuable tool to identify desired production clones during CLD processes. The technology offers novel opportunities for early characterization and selection of bsAb-producer clones.

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# From Cells to Culture: Modelling the Dynamic Behaviour of CHO Cell Culture for Biotherapeutic Production

Mr Alejandro Avilan Garzon[1,2], **Dr Bruno Ebel**[1], Dr David Pfister[2], Prof Eric Olmos[1]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:** By leveraging experimental data and advanced modelling, this study showcases the potential for optimizing cell culture processes and improving the production of complex proteins, in a cost-effective and efficient manner.

**Impact/Novelty:** This study presents a novel method to model the internal and external dynamics of metabolites and products during cell culture based on a multi-scale, mass conservative and physically meaningful approach.

**Introduction:** Monoclonal antibodies are composed of carbon, hydrogen, oxygen, nitrogen, and sulfur atoms derived from substrates like glucose, and amino acids. The current models often overlook the atom-level balances. Therefore, the aim of this study is to develop kinetic models that focus on the mass conservation of C.H.O.N.S atoms. The model considers the transfer of substrates and products across cell membranes, intracellular reactions, cell growth, and cell death. Simplifications of the metabolic network are proposed by focusing on the elementary composition of each species. The mAb production is modeled as a polymerization reaction, and cell growth derives from the cell division mechanism. The model considers the accumulation of toxic metabolites and substrate limitations.

**Methods/Approach:** The developed model is based on the mass balance of atoms at the cell level. It inherently accounts for the accumulation of metabolites within the cell. To assess the various underlying hypothesis, data from CHO cell cultures were generated experimentally. The kinetics of various compounds, including glucose, glutamine, lactate, ammonia, mAb, and lactate dehydrogenase, were analysed in both the extracellular and intracellular environments. The amino acids concentration was also determined, using HPLC-MS.

**Results:** The results show that the developed model accurately predicts cell growth, substrate consumption, and production rates in batch and fed-batch cultures. The model's predictions of intracellular metabolite accumulation were validated using experimental data. The model offers flexibility in terms of the internal reactions network, allowing for a more or less detailed network depending on the desired level of description and available data.

**Conclusion:** The proposed kinetic model provides a detailed description of the intracellular dynamics in CHO cell culture. It accurately predicts cell growth, substrate consumption, and production rates. It offers flexibility in terms of the internal reactions network and can be adjusted based on the desired level of description and available information.

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# Integrating metabolomics and proteomics for CHO cell bioprocess improvement

**Mr. Luke Johnston**[1], Miss Darina Stoyanova[1], Dr Mark Rendall[2], Dr Jeff Keen[2], Dr Karl Burgess[1]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:** Combining omics technologies to characterise industrial CHO cell lines. Employing multivariate data analysis and metabolic pathway analysis to identify new biomarkers and cell engineering targets for more effective CHO bioprocesses.

**Impact/Novelty:** Utilisation of cutting-edge LC-IMS-MS method for improved metabolome coverage. High-throughput 10-minute proteomics method for rapid analysis. We also propose cell engineering strategies to reduce formate overspill metabolism in CHO.

**Introduction:** Chinese hamster ovary (CHO) cells are the leading host cell chassis for biopharmaceutical bioproduction. CHO cell metabolism is complex and many facets remain poorly understood. A major focus area is the production of inhibitory bi-products which negatively impact cell growth, cell viability or recombinant protein production. A significant metabolic bi-product is formate (formic acid), produced in excessive quantities far exceeding cellular demand, which results in cell growth inhibition. The underlying cause of formate overspill production in CHO cells, and the mechanism by which it inhibits growth, remain elusive.

**Methods:** We apply a combination of metabolomics and proteomics to reveal new insights into formate production and it's inhibitory mechanisms by utilising LC-IMS-MS, $1^{H}$ proton NMR and LC-MS methods. Metabolic pathway enrichment analysis and multivariate statistics were used to identify metabolome and proteome changes induced by formate overspill. A molecular network was constructed to investigate the key changes which occur in the presence of excess formate.

**Results:** Significant effects ($p<0.05$) on CHO cell growth were found by addition of various biologically relevant formate concentrations in comparison to control cultures. The integration of metabolomics and proteomics data revealed profound changes in amino acid and energy metabolism by addition of excess formate. Differentially expressed metabolites and proteins ($log_2$ fold change > 2) and PLS-DA variable importance in projection ((VIP) >1.0) scores could classify the changes between control and formate treated cultures. Several differentially expressed proteins were investigated to identify new targets to reduce formate overspill production via cell engineering.

**Conclusion:** Integration of state-of-the-art metabolomics and proteomics reveals mechanist insights into the cause of excess formate production and the biological means by which it inhibits CHO cell growth. These results tease new targets for bioprocess improvement and showcase the power of omics to enhance bioprocess understanding.

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# Advancing Downstream Processing of AAVs: From Mechanistic to Hybrid Modelling in Anion Exchange Chromatography

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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
We will present modelling strategies for chromatographic downstream processes of AAVs for gene therapy to enable online monitoring as PAT tool and gain process understanding.

**Impact/Novelty:**
We have established mechanistic and hybrid models for anion exchange chromatography in AAV production and shown the potential of hybrid modelling with online data to better represent the complex conditions.

**Introduction:**
Gene therapy using adeno-associated viruses (AAVs) holds great promise, but the high manufacturing costs associated with AAV production necessitate a paradigm shift towards increased efficiency and understanding of downstream processes. A critical step in this process is the polishing step, where anion-exchange chromatography is used to separate empty and filled AAV particles.

**Methods/Approach:**
Mechanistic modelling provides a theoretical framework that allows us to understand the underlying principles and dynamics of complex bioprocesses. In this work, the Yamamoto model is used to gain insight into the complex interactions during anion exchange chromatography. However, exploring the limitations of purely mechanistic models, we take a novel approach by integrating process data and build hybrid models. For that, a conventional chromatographic workstation was extended by a Multi-Angle-Light-Scattering (MALS) detector.

**Results:**
The Yamamoto model is a good strategy when dealing with classical separation problems, but it can be improved by including process data to build a hybrid model. With additional detectors such as the MALS, more process information on the critical quality attributes can be gained and used for online monitoring.

**Conclusion:**
This work is a step towards making AAV production more efficient, cost-effective and as we move towards advanced bioprocessing, the integration of novel modelling strategies sets the stage for a better understanding of purification processes.

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# Genome-Scale Modelling to Identify Lactate Reduction Strategies in CHO Cell Cultures

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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:** We present a modelling framework to integrate transcriptomic and metabolomic data to better understand the host. From this understanding we identify causes and mitigation strategies for lactate accumulation.

**Impact/Novelty:** We test experimentally the carnitine shuttle system as a sink for excess pyruvate. We also show the benefits of feeding NAD+ precursors on redox balance and reduction in aerobic glycolysis.

**Introduction:** Lactate production in cell culture is wasteful, diverting resources away from the TCA cycle and oxidative phosphorylation towards aerobic glycolysis. In some batches, a late lactate spike may be observed, causing negative cell phenotypes and base addition to maintain pH set point.

**Methods/Approach:** We have analysed transcriptomic and metabolomic data from in-house cell lines to assess the differences between control conditions vs high lactate producing conditions. We integrated these data types into a genome-scale model to compare different conditions. We identified NAD+/NADH ratio as a key factor in lactate accumulation, as well as oxidative stress and fatty acid metabolism. We selected a metabolite, nicotinamide (NAM), to be supplemented to reduce lactate production. In addition to NAM, we identified carnitine as a metabolite that could reduce lactate accumulation when added to cultures.

**Results:** We tested these two metabolites in an Ambr-15 system. Carnitine was able to reduce lactate accumulation during late stages of high lactate producing conditions, however it did not impact the lactate profile of the control conditions. NAM was able to reduce yield of lactate on glucose by an order of magnitude, as well as switch to lactate consumption two days before control cultures, however NAM reduced cell growth significantly.

**Conclusion:** The carnitine carbon shuttle is a potential storage location for excess carbon in high lactate conditions. In addition, modifying the NAD+ synthesis pathways are a valuable target for addressing aerobic glycolysis.

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# Accelerated Development of Novel Cell and Gene Therapies through Automated Production of Viral Vectors

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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
Viral vectors like rAAV and lentiviruses are commonly used in Cell and Gene Therapies (CGTs) as they can infect difficult-to-access cells and alter gene expression.

**Impact/Novelty:**
CGTs revolutionize medicine by providing personalized treatments for a wide range of diseases. These therapies can restore gene function and transfer new genetic material to a patient's cells to treat, prevent, or cure diseases.

**Introduction:**
CGT R&D faces several challenges, including functional complexity, registering, monitoring, controlling, and evaluating production processes, and streamlining data management. Despite these challenges, the industry has shown enormous potential. Efficient production of one of the main actors of CGTs, viral vectors, is a crucial step in CGT R&D.

**Methods/Approach:**
Streamlined data management of highly complex, automated, and high-throughput processes is crucial for optimizing processes and workflows and for accelerating the identification of therapeutic CGT candidates. We have therefore developed a platform that helps register viral vectors and monitor, control, and evaluate all laboratory production processes. It helps streamline the development process, reduce costs, and improve efficiency. It also helps with data analysis and workflow challenges posed by increased assay throughput and organizational growth.

**Results:**
We have developed a digital platform that facilitates the development of next-generation bio-manufacturing processes such as CGTs. The platform is modular, scalable, and easy to integrate, allowing flexible configuration for specific workflows like proprietary expression and purification protocols, emerging discovery workflows, or analytical technologies.

**Conclusion:**
The platform is purpose-built for digitalizing next-generation process design and achieving operational excellence in the development of biotherapeutic drugs. Designed to enforce data integrity and compliance and to streamline the development of originator drugs and biosimilars, it is globally used by pharmaceutical giants and renowned CMOs to increase the efficiency of their development processes.

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# Structured Knowledge Management Platform for Bioprocess Development

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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:**
Data-driven process optimization is key to bioprocess development. We present a structured knowledge management solution covering the entire workflow from cell line development to the final drug product.

**Impact/Novelty:**
Centralized structured data across multiple groups is critical for integrated bioprocess development with advanced data analytics, machine learning (ML) and artificial intelligence (AI) approaches, and efficient reporting to regulatory agencies.

**Introduction:**
Growing adoption of high-throughput and process analytical technologies (PAT) and laboratory automation led to a substantial increase in the volume of data to be captured, processed, and analysed during bioprocess development. We showcase an E2E structured knowledge management platform that supports the entire bioprocess development workflow.

**Methods/Approach:**
The goal of bioprocess development is to generate robust processes to produce a biotherapeutic at desired quality and scale. We designed a data platform applicable to all proteins (e.g., IgGs, ADCs, bispecifics, enzymes), RNA & DNA therapeutics and vaccines (e.g., mRNA, DNA vaccines, ASOs), and cell and gene therapeutics (e.g., AAVs, CAR-T cells). In close collaboration with groups from leading biopharmaceutical and biotech companies, we validated the platform's design for support of complete development workflows.

**Results:**
The platform automates cell line development, assesses numerous scale-down upstream processes (USP), manages USP up-scaling, supports downstream process (DSP) development, and facilitates analytical and formulation development. It enables lineage tracking of all intermediates and batches. Analytical and process data, raw materials, equipment details, and molecule and cell line information are automatically tracked with the batches, enabling systematic assessment, robust process understanding, and quality risk management.

**Conclusion:**
The platform enhances process and product understanding and control. Having accumulated structured data can serve as a powerful foundation for learning, modeling, and data analytics, such as mechanistic modeling, ML and AI, to gain process knowledge and perform predictions.

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# Accelerating Upstream Process Development Through Automated Data Processing, Analysis, and Dynamic Model Assembly

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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
This work pertains to processing, analysis, and enhanced interpretation of upstream bioprocess datasets. Augmented by high-throughput technology, the proposed approach has potential to enhance decision making while accelerating process development.

**Impact/Novelty:**
The devised workflow systematically addresses the key challenges associated with the processing and analysis of cell culture datasets, culminating in streamlined deployment of dynamic hybrid process models during bioprocess development.

**Introduction:**
The commercial success of prospective biotherapeutics is largely contingent upon a timely and efficient development campaign. Implemented through the Quality by Design framework, mathematical and statistical process models yield significant influence through intrinsically linking product quality with prominent process parameters. However, deployment of dynamic process models in a development context is often impinged by the manually intensive tasks associated with processing and analysing cell culture datasets. Automating menial tasks within a systematic workflow enables a shift in focus towards actionable insight, contributing towards accelerated development.

**Methods/Approach:**
A systematic data processing and analysis workflow is devised, based upon the general requirements for a fed-batch CHO cell culture process. The workflow initially addresses outlier identification and missing value imputation. Extended feature engineering enables enhanced exploration of state and process variables in a dynamic context, when coupled with multivariate analysis techniques. To support hybrid model development, recursive elimination techniques are applied to identify the most appropriate input parameters for data-driven elements, while cell specific kinetic rates are estimated from experimental data for subsequent model training and validation.

**Results:**
Utilising high-throughput CHO cell culture data, the devised systematic workflow yields a significant reduction in the time and resource requirements for dynamic model assembly and deployment. Furthermore, enhanced interpretation of the available cell culture data through multivariate approaches enables identification of experimental factors with influence on process performance.

**Conclusion:**
Automated strategies for data processing, analysis and model deployment are aptly posed to advance the application of mathematical models during process development, contributing towards enhanced efficiency and accelerated development strategies.

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# Time-Resolved Mass Spectrometry-Based Multi-Omics Characterisation of NISTCHO Cellular Function In Response To Nutrient Influx

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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected**
"Big data" approaches, such as multi-omics-based characterisation of cellular function, show great potential in aiding academic and industrial endeavours to improve therapeutics production.

**Impact/Novelty**
The utilisation of state-of-the-art host cell-omics to supplement conventional, medium-based measurements as proxies for cellular function will further the understanding of producer CHO cell lines.

**Introduction**
To meet the growing demand for recombinant biotherapeutics employed today, producer cell lines are under ever-constant development to improve their productivity, stability and product quality. Accurate analysis of protein expression and post-translational modification of proteins by reversible phosphorylation can effectively decipher vital cellular processes and their regulation.

**Methods/Approach**
The development of a novel, fast, and representative multi-omics sampling approach for suspension cultures allowing analysis of the host-cell proteome, phosphoproteome, metabolome, and secretome was carried out. To demonstrate the application of this approach, a fed-batch culture of the openly available producer cell line NISTCHO was consequently investigated for the impact of feeding on cellular function. Sampling took place in both the exponential and stationary growth phases to reflect the most critical stages of the bioprocess. Results were integrated into the parallel monitoring of cell proliferation and viability, as well as nutrient consumption and metabolic excrements.

**Results**
Analysis of bioprocess parameters and host cell-omic datasets of multiple time points before and after scheduled feeding of NISTCHO revealed distinct responses to altered nutrient availability. This work provides time-resolved and highly comprehensive profiling of the acute impact of feeding on the cellular landscape, including the proteome and phosphoproteome.

**Conclusion**
A novel mass spectrometry-based multi-omics method can offer an in-depth analysis of cellular function in various product and/or process-relevant biological settings. The advanced characterisation of an academically available producer cell line will drive research in recombinant protein production and bioprocess engineering.

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# Rapid and high-throughput cell count & viability assessment from label-free images using CellAi software.

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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
The CellAi software is an image classification method that aims at enhancing the cell analytic field by extracting cell features using a deep learning and machine learning approach.

**Impact/Novelty:**
The CellAi software provides a transformative method to measure cell viability, with a label-free approach and a more sustainable workflow.

**Introduction:**
Cell count and viability measurements are two critical tests that are essential to track cell health and growth in the development and manufacture of protein and cell-based therapeutics.
This operation is often performed manually or using semi-automated dye-based methods. This process is slow, low throughput, and error prone. A method that enables precise measurements with minimal investment of time and resources can significantly impact the efficiency of bioprocessing workflows. Here, we describe a simple, accurate, rapid, sustainable and automation-friendly approach to measure cell count and viability from label-free brightfield images using Artificial Intelligence (AI) neural networks.

**Methods/Approach:**
We demonstrate that our CellAi software allows high-throughput measurement of cell count, confluency, and viability from label-free images of cells in suspension. Images of Chinese Hamster Ovary (CHO) and Mesenchymal Stromal Cells (MSC) were captured with industry relevant plate imagers, and morphological features were extracted and analyzed for cell characterization.

**Results:**
CellAi count and viability measurements were benchmarked against industry gold standard techniques like Trypan Blue and live/dead fluorescent staining, showing high accuracy of results across a wide range of tested variables. The CellAi software solution enables users to analyze cells with minimal sample manipulation and does not require the addition of toxic chemical reagents. Cells can be imaged in transmitted light mode directly in multi-well expansion formats, generating instantaneous results. An added benefit is the opportunity to multiplex with additional analysis, delivering shorter and simpler lab protocols.

**Conclusion:**
The label-free CellAi software will ultimately provide a transformative approach for high-throughput, fast informative and sustainable cell analysis, extracting rich cell insights without the use of staining (more than 1,000 features are extracted and analyzed). This approach is relevant across diverse biomanufacturing workflows, and has the potential to maximize insights from single samples in critical cell therapy development and manufacturing processes.

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# Digital twin – a tool for improving mAb productivity in continuous CHO cell culture

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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:** A bioprocess digital twin could provide an in-depth, real-time simulation of the cell culture and recombinant protein production as well as the N-glycosylation process.

**Impact/Novelty:** Design and implementation of a multi-scale in-silico model to simulate the CHO cell culture bioprocess in the continuous bioprocessing platform for improvement of productivity.

**Introduction:** Chinese hamster ovary (CHO) cell culture has been the main process in the biopharmaceutical industry to produce a variety of biologics. The development towards more sophisticated, model-based, and real-time monitoring and control methods is aiming for enhanced consistency, efficiency, and quality in biopharmaceutical production. A bioprocess digital twin could provide an in-depth, real-time simulation of the cell culture and recombinant protein production as well as the N-glycosylation process.

**Methods/Approach:** A continuous CHO cell culture platform including a 1.5L bioreactor with ATF cell retention system and advanced PATs is set up for perfusion experiment. The in-silico counterpart, a multiscale model, is established and used to optimize the process design by integrating the flowsheet modeling, kinetic modeling and genome-scale modeling.

**Results:** The multiscale modeling is calibrated and validated by cell culture data in shake flasks and bioreactor. The model allows for precise manipulation and optimization of the cell culture environment, leading to improved control over the quality and yield of the produced proteins.

**Conclusion:** A multi-scale digital twin model was created to simulate CHO cell culture in continuous bioprocessing, predicting critical performance indicators like cell growth, product titer, and protein productivity from process parameters. A genome-scale model, including glycosylation reactions, forecasted the N-Glycan profile of proteins. Validation through perfusion experiments optimized the bioprocess parameters, enhancing protein titer and productivity.

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# Simulation of the cryopreservation process and its effects on CHO-K1 cells by a hybrid model

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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:**
The simulation of the cell cryopreservation process uses hybrid modelling which blends mechanistic finite element modelling of well-understood process phenomena with data-driven approaches leveraging high throughput flow cytometric datasets.

**Impact/Novelty:**
The availability of accurate cryopreservation process models can reduce R&D costs as well as support process optimization for high recovery efficiencies by reducing the experimental requirements through augmentation with simulations.

**Introduction:**
Cell cryopreservation is a standard operation during cell banking which is necessary to minimize genetic drift or cell differentiation. Despite its widespread use in both industry and laboratories, the development of new genetic modified organisms, new types of products in addition to the move toward process intensification have highlighted the limited understanding of the cryopreservation process.

**Methods/Approach:**
A hybrid model describing the cryopreservation process for a CHO-K1 cell line was developed to describe the cellular response to the process input variables (i.e, cooling rate and freezing cell density). The output variables (i.e., ratios of post-thawed subpopulations of viable, early-apoptotic, apoptotic and necrotic cells as well as changes on their mitochondrial potential) are correlated by a data-driven approach to the temperature distribution calculated by the mechanistic 3-D modelling of the relevant heat and mass balance equations. The heat transfer equation is solved in MatlabR2022a by the finite element method and the outputs are quantified by cell counting by the trypan blue exclusion method and by flow cytometry by using a multi-staining method of DRAQ-7, YOPRO-3 and JC-1 dyes.

**Results:**
The temperature profile measured for a cryovial with 1 mL of PBS for a range of cooling rates indicated that, by admitting a forced convection on a cylinder body in cross-flow, the relative prediction residuals would be mostly below 1 %, deviating only in the latent heat release region, in which the residual could reach approximately 10 %. The temperature profiles calculated by the mechanistic model feed-forward into the data-driven component of the model framework in order to predict the cellular response to the process conditions.

**Conclusion:**
Hybrid modelling represents an innovative solution for complex bioprocesses and the most recent results for the heat transfer model shows a good prediction capacity, with deviations occurring mainly in the latent heat release region.

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# Generate Data, Use Data – Advanced Spent Media Analytics and Data Analysis

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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:** The combination of high-resolution LC-MS with multi-variate data analysis (MVDA) tools allow us to use large amounts of data for in-depth bioprocess characterizations.

**Impact/Novelty:** Historically, spent media analytics was used to characterize bioprocesses based on known ingredients. Now, unknown molecules, like reaction products, are also considered and allow holistic views on processes.

**Introduction:** Cell line and bioprocess developments are often co-dependent to achieve the same goal: Provide enough product to meet the demand of an application area. Of course, those approaches are important to meet the increasing demand e.g. in viral vector manufacturing. But, to enable broad applications of viral vector-based therapies, higher yields and lower cost of goods are desirable. Advanced analytical data generation in combination with data analysis tools represent another step towards this goal.

**Methods/Approach:** A LC-HRMS system (Orbitrap Exploris240, ThermoFisher) was applied to generate secretomic data from HEK media and cell culture processes during AAV production. These datasets were analyzed using the MVDA software SIMCA (Sartorius) and Compound Discoverer (ThermoFisher).

**Results:** A sample-to-result pipeline for (spent) media analytics was established. We applied high-resolution LC-MS analytics to generate secretomic datasets from cell culture processes for AAV production. Using MVDA tools for detailed data analysis and statistical modelling, significant differences between multiple samples and even batch-to-batch variations became apparent and were visualized through e.g. PCA-plots and heatmaps. Additionally, multiple distinguishing components were identified by database comparisons.

**Conclusion:** The presented approach provides a solid basis for data driven decision making during cell line and bioprocess development. Our processed data can be used to optimize conditions for cells and provide medium with effective nutrient supply based on given performance criteria like growth or titer. Furthermore, results of each new sample can be used to create a constantly growing knowledge base, enabling the investigation of potential biomarkers.

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# Development of a dissolved CO2 sensor in true single-use format

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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected: Dissolved $CO_2$ control strategy in cell culture processes**
The $CO_2$ control strategy in a single-use (SU) bioreactor, to help maximize titer and final-product quality, is based on a dependable and accurate in-line dissolved $CO_2$ measurement.

**Impact/Novelty: Dissolved $CO_2$ sensor in true SU format**
Sensors that are fully integrated into a SU bioreactor bag facilitate the workflow for sensor handling and operation at end-users' sites and mitigate the risk of breaching the sterile integrity of SU bioreactors.

**Introduction**
The manufacturing of advanced biotherapeutics such as monoclonal antibodies and viral vectors for cell therapies starts with an upstream bioreactor process. Advantages of SU technology have resulted in an increasing demand for reliable dissolved $CO_2$ sensors in true SU format.

**Methods/Approach**
Currently, it is mainly reusable dissolved $CO_2$ sensors that are used for measurements in 3D SU bioreactors. For sterilization purposes, reusable sensors are autoclaved within SU probe sleeve adapters prior to their aseptic installation into a gamma irradiation-sterilized SU bioreactor.

**Results**
We present a dissolved $CO_2$ sensor technology concept in true SU format:

- Transfer of the Severinghaus measurement principle of a reusable sensor into a true SU sensor format.
- A sensor's form factor that leads to safe integration into SU bioreactors.
- Post-gamma measurement characterization data after 36 months' dry storage.
- A thixotropic electrolyte gel that leads to secure and clean sensor operation.
- Separation of high-cost components from the SU sensor by integrating them into a sensor head designed for repeated use.

**Conclusion**
Reusable sensors do not offer the full advantages of sensors in true SU format that users appreciate. A dissolved $CO_2$ sensor in true SU format fulfills user requirements such as convenient operation and reliable measurement results for efficient process control purpose.

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# Comprehensive meta-analysis of the CHO coding transcriptome

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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
Our work utilises multiple large omics datasets to advance the understanding of fundamental molecular processes across existing CHO cell lines.

**Impact/Novelty:**
We demonstrate a completely new approach to leverage the potential of published RNA-sequencing datasets and gain insight into transcriptomic programmes of various CHO cell lines.

**Introduction:**
The advent of RNA-sequencing has provided a more profound understanding of CHO cells. Typically, such datasets are generated to elucidate one specific biological question or experimental layout and the potential to put them into greater context is left unused. With numerous RNA-sequencing datasets accumulated in the public domain, we seize the opportunity to conduct a large-scale meta-analysis of the CHO coding transcriptome to study gene expression across various cell lines and culture conditions.

**Methods/Approach:**
We have integrated 15 RNA-sequencing datasets containing 293 samples, spanning a wide variety of biological phenotypes. We processed the data by employing a consistent and reproducible workflow, underpinned by cutting-edge bioinformatic tools. Subsequently, our analyses ensure robust normalisation and in-depth exploration of the data.

**Results:**
Our findings unveiled a substantial set of genes exhibiting consistent expression across all examined cell lines and biological phenotypes as well as a similarly large set of genes that was found to be consistently quiescent. Further investigation of these observations was undertaken through additional omics data from DNA methylation and histone modifications.

**Conclusion:**
Our work shows that CHO is characterised by expression of a given set of genes, with a lower number of genes exclusively expressed in certain lineages only. This indicates that the precise expression level of these genes is the driver for differences in phenotypes, rather than the activation of genes that are not amongst this group. This meta-analysis serves as a valuable resource for advancing our understanding of CHO cells.

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# Integrating Kinetic Modelling with Process Measurements through Bayesian Filtering

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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:** A computational tool that predicts the CHO cell culture behaviour under temperature downshift to mild hypothermia.

**Impact/Novelty:** A mathematical tool that describes cell metabolism perturbations in response to flexible temperature downshift regimes, with the potential to optimize process temperature conditions and enhance product titre.

**Introduction:** The use of temperature downshift to mild hypothermia is a widely employed strategy in industry for enhancing the productivity of Chinese Hamster Ovary (CHO) cell cultures. The shift to sub-physiological temperature slows down cell growth but prolongs culture longevity typically leading to higher product titres. Kinetic models have been developed across different cell lines, temperature shifts, and experimental conditions to investigate the effect of mild hypothermia on CHO cell culture. However, a comprehensive understanding of the underlying mechanism remains elusive. The dynamic behaviour of cellular responses is notably cell line- and product-specific, further influenced by the timing and extent of the temperature downshift, posing challenges for process knowledge transfer.

**Methods/Approach:** We introduce an adaptive Bayesian filtering approach, the Ensemble Kalman Filter (EnKF), that estimates metabolic states and model parameters simultaneously by reconciling real-time process data with the kinetic culture model. Importantly, the algorithm quantifies uncertainty therefore enabling process control for improved process robustness and reliability.

**Results:** EnKF showcases the ability to investigate the potential temporal evolutions of model parameters through updating the kinetic model with new observations available. It represents a flexible and reliable real-time estimation approach that can be generalised to any system or scale.

**Conclusion:** To summarize, online estimation of parameter and states for CHO cell culture offers promise in optimizing temperature shift regimes for improved product titre and quality.

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# The Leap-In Transposase Platform: Past, Present and Future

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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected (Cell processes and development)**
Fast and Efficient Cell Line Development mediated with Leap-In Transposase®: Leap-In transposases are highly valuable for stable cell line development and cell engineering for therapeutic protein production and gene/cell therapy.

**Impact/Novelty:**
Novel Transposases for Cell Line Development: ATUM has discovered and engineered a pair of novel orthogonal transposon/transposase systems: the Leap-In® transposases.

**Introduction:**
Leap-In transposases generate stable transgene integrants with an array of unique characteristics: 1) single copy integrations at multiple genomic loci, primarily in open chromatin segments; 2) there is no payload limit, so multiple independent transcriptional units may be combined into a single construct for integration; 3) the integrated transgenes maintain their structural integrity; 4) maintenance of transgene structural integrity ensures control of expression of each open reading frame for multi-subunit proteins, thereby guaranteeing the correct chain ratio in every recombinant cell.

**Methods/Approach:**
The Leap-In Transposase® is an engineered enzyme that catalyzes the integration of a transposon and your gene of interest into TTAT sites in the target genome. The technology enables a specified sequence of interest to behave as a transposon, a mobile genetic element, which can efficiently transpose between vectors and chromosomes via a “cut & paste” mechanism. During transposition, the Leap-In Transposase recognizes transposon-specific inverted terminal repeat sequences (ITRs) located on both ends of the transposon vector and moves the contents from the original sites and efficiently integrates them into TTAT chromosomal sites.

**Results:**
The distinctive characteristics of Leap-In mediated stable integrations result in unique and valuable consequences: 1) Leap-In mediated stable pools exhibit productivities rivaling/exceeding clonal productivities achieved by random integration approaches. The reason behind the high pool productivity is that the clonal distributions, in Leap-In mediated pools, is strongly biased towards high producers. This greatly reduces the number of clones that need to be screened from Leap-In mediated stable pools. When the Leap-In system is combined with monoclonality assurance by the VIPS technology (Solentim) <100 monoclonal clones are sufficient to isolate several high producers; 2) Leap-In mediated stable pools are highly comparable by both productivity and product quality with the derivative stable clones. This offers the opportunity to utilize stable pools to generate representative material for POC studies, initiate process development and use stable pool derived material for tox manufacturing.

**Conclusion:**
In summary, Leap-In technology is a readily available, efficient and safe tool designed to improve productivity and product quality and shorten development timelines. The valuable attributes of the system and its performance characteristics will be discussed through cell line development and engineering examples.

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# Effect of critical process parameter on site specific N-linked glycosylation of VRC01 produced in CHO

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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:** This work leverages inhouse N-linked glycosylation data to develop a mathematical model to study and predict the diversity of glycans present at different N-linked glycosylation sites on a glycoprotein.

**Impact/Novelty:** This study provides mechanistic insights into the effect of bioreactor pH and glycosylation site on the type of glycans found at that site.

**Introduction:** Fc glycosylation has been shown to effect antibody aggregation, half-life, and activity [1]. About 20% of IgG in human serum contain glycosylation sites in the Fab region. The Fab glycans were recently shown to affect aggregation, efficacy, and half-life [2]. Hence, Fab glycans must also be considered as critical quality attributes.

**Methods/Approach:** VRC01 contains glycans in the Fab and Fc regions. VRC01 was produced in CHO cells grown at different pH conditions to study the effect of pH on site specific glycosylation. A CSTR based glycosylation model was adapted to include multiple glycosylation sites.

**Results:** Different glycan fractions were observed on the two glycosylation sites. Fc region glycans were fully fucosylated but the Fab region glycans had 70% afucosylation. More complex glycans were found in the Fab region. Bioreactor pH was found to impact fucosylation in the Fab region without impacting the Fc region. Galactosylation was affected in the Fc region but not in the Fab region. The mathematical model was regressed to the data. The regressed parameters showed poor binding of the galactosyltransferase to the Fc region and poor binding of fucosyltransferase to the Fab region.

**Conclusion:** The effect of pH on site specific glycosylation of VRC01 was experimentally and mathematical studied to gain deeper mechanistic insight into the site-specific N-linked glycosylation process.

1. Liu, L., J Pharm Sci, 2015. **104**(6): p. 1866-1884.
2. Van De Bovenkamp, F.S., et al., The Journal of Immunology, 2016. **196**(4): p. 1435-1441.

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# Impact of Mesenchymal Stromal Cells Expansion Conditions on their Hematopoietic Support Capacity: A Transcriptomic Analysis

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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
Big data analysis revealed mesenchymal stromal cells (MSC) variations predicting their impact on hematopoietic stem/progenitor cell expansion, underscoring the importance of comprehensive understanding of cell features for high-quality cell-based products.

**Impact/Novelty:**
Human platelet lysate was studied as a xeno(geneic)-free alternative to fetal bovine serum for MSC expansion. Functional and transcriptomic differences were identified and critical factors influencing hematopoietic support were uncovered.

**Introduction:**
Cord blood is a valuable source of hematopoietic stem and progenitor cells (HSPC) for pediatric transplantation. MSC-based co-cultures are used to expand HSPC ex-vivo for adult application. To ensure xeno-free conditions during cell-based products production, we studied the impact of fibrinogen-depleted gamma-irradiated human platelet lysate (hPL) on MSC's capacity to expand HSPC.

**Methods/Approach:**
Three bone marrow donors' MSC were expanded using hPL- or FBS-supplemented medium under three regimens: Adapted – cells adapted to a second medium after isolation; Re-adapted – cells that returned to the isolation medium after adaptation; and Direct – cells maintained in the isolation medium. Co-culture systems were established, and transcriptomic analysis was conducted to compare hPL-MSC and FBS-MSC.

**Results:**
MSC expansion media reversibly affected their properties. Transcriptomic profiles differed between hPL-MSC and FBS-MSC, with 13% of genes differentially expressed, impacting cell signaling, extracellular matrix, and chemotaxis. Importantly, only 1-2% of genes showed differential expression between direct and adapted/re-adapted conditions. hPL-MSC had a diminished capacity to support HSPC proliferation in-vitro, yielding 2.4 fewer CD34+ cells after 7-day expansion. Top-down and bottom-up approaches indicated that cell-cell signaling, TGF-beta, PI3K-Akt, and Wnt signaling pathways influenced MSCs' hematopoietic support capacity. hPL-MSC showed higher expression of genes encoding inhibitor factors of HSPC proliferation and lower expression of promoter factors.

**Conclusion:**
This study highlights the impact of translational requirements (i.e. adaptation to GMP) on MSC function. Genes influencing hPL-MSC's hematopoietic support capacity were identified, providing insights for optimizing co-cultures and restoring MSC properties under xeno-free conditions.

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# Unlocking Multiomics Potential with a High-Throughput Data Generation Protocol

**Mr David Catalán-Tatjer**[1], Dr Nicholas Cowie[1], Mr Kristian Jensen[1], Dr Jesús Lavado-García[1], Prof Lars Nielsen[1,2]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:**
High-throughput cultivation platforms have been available for years, yet none are coupled with a multiomics characterization platform, impeding the rapid generation of reliable datasets for metabolic characterization.

**Impact/Novelty:**
Computational advances shifted metabolic model bottlenecks to dataset generation. Our high-throughput platform, using the ambr15 system, produces robust datasets from 48 bioreactors, enhancing metabolomics, proteomics and fluxomics while minimizing variability.

**Introduction:**
The high cost and complexity of individually generating proteomics, metabolics and fluxomics data for the same samples have hindered the development of metabolic models. Having robust data sets has become crucial for building accurate models that could be used for later bioprocess development. This requires a significant amount of time and resources. Although high-throughput devices have streamlined large-scale sample analysis, the bottleneck remained in sample generation. Here, we have developed for the first time a method able to generate data corresponding to 12 different cell lines in quadruplicates (48 bioreactors) for fluxomics, proteomics and metabolomics in 4 days.

**Methods/Approach:**
We assessed CHO-derived cell lines in a controlled oxygen and pH environment using the ambr15 system. Cell count, proteomics, endometabolomics, and exometabolomics samples were collected at specific timepoints, automatically spiking with labelled glucose. For time optimization and reproducibility, tasks were automatically overlapped using Gantt charts.

**Results:**
We ensured the quality of our data by fitting a flux map of all CHO-derived tested cell lines, creating kinetic models of pathways such as glycolysis, Embden-Meyerhof-Parnas or pentose phosphate. With our generated data we identified potential bottlenecks and differences among our cell lines.

**Conclusion:**
Transitioning to this high-throughput protocol is crucial for reaching the next milestones in metabolic modelling. We demonstrated the ability of our protocol to characterize cell behaviour at fluxome, proteome, and metabolome levels in 48 reactors.

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# In-depth metabolic characterisation at different phases of cell culture with a turnkey CE-ESI-HRMS workflow

**Dr Milla Neffling**[1], Dr. Erin Redman[2], Dr. Will Thompson[2], Dr. Kenion Blakeman[2], Dr. Stephanie Klaubert[1], Awab Nehela[1], Dr. Scott Mellors[2]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
Streamlined experimental design, data collection, and data analysis for increased understanding of cell cultures used in biopharmaceutical manufacturing, identification of predictive biomarkers to enhance bioprocess modelling efforts and overall understanding.

**Impact/Novelty:**
Enabling access to highly multiplexed time-aligned measurement of key metabolic shifts, including quantitative data to drive process modelling, and understanding. Simple, streamlined sample preparation, CE-ESI-MS data acquisition and data handling.

**Introduction:**
Understanding of cell culture metabolism in biologics manufacturing has increased tremendously in the past decade; however, the lack of feasible methods of high-coverage, quantitative information on the interplay between nutrients and metabolites has hindered the efforts to fully characterise processes in the different biological phases. Several published models support cell growth phase but lack the kinetic information to be applicable to the phases that follow. We discuss a method for measuring hundreds of metabolites in dozens of pathways that streamlines sample preparation from spent media and cells, enables fast data-analysis times and supports quantitative analysis of key metabolites to gain far richer and more reliable data sources for process characterisation, modelling and understanding.

**Methods/Approach:**
The approach defines streamlined sample preparation using a novel ZipChip-based Capillary-Electrophoresis-Electrospray-Ionisation-High-Resolution-Accurate-Mass (CE-ESI-HRMS) platform, from 1) spent media and 2) cell samples. The method includes automated sample preparation, stable isotope standards, and CE-ESI ZipChip-ThermoScientific Exploris 240 mass spectrometer for data collection and metabolite quantification. The experiments include a CHO mAb-producing cell line in fed-batch culture with either a bolus or a dynamic, continuous glucose feed. With daily sampling, we aimed to establish time-course correlations to metabolism, identify key metabolic indicators for growth prior to and after the lactate switch, as well as to relate cell-specific productivity to the different phases. mAb product quality attributes were assessed with an established ZipChip-MS analysis.

**Results:**
The streamlined workflow enabled sample preparation from cells and spent media in <10 min, in a fully automated manner for the spent media; data acquisition in 10 min per sample capable of measuring hundreds of metabolites, and streamlined quantitation for key bioprocess metabolites.

**Conclusion:**
We demonstrate a turnkey, high-fidelity metabolite profiling approach for quantitative analysis of a wide panel of bioprocess metabolites, with room to mine the data for quantification of additional metabolites retrospectively.

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# A mechanistical understanding of Producer Cell Line (PCL) approaches for AAV production using Metabolomics

**Dr Swetha Kumar**[1], Dr Adhvait M Shah[1], Dr. Laura Parisi[2], Dr. Bailin Zhang[2], Dr. Lilu Guo[2], Dr. Darrick Yu[1], Dr. Madhuresh Sumit[1], Dr. Jeong Lee[1]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**From Big data to Better Cells and Processes:**
In the era of 'Big Data', we have employed metabolomics in our biopharmaceutical manufacturing process to enable data-driven process design and optimization.

**Impact:**
Using the power of 'Big Data', we can identify process switch-points, challenges and biomarkers that can guide scientists to design bioprocesses with superior cellular performance and process productivity.

**Introduction:**
With more than 30 CGT FDA product approvals, gene therapy is emerging rapidly and provides much promise in the treatment of rare neurological diseases. To have a state-of-the-art process that helps improve patient access to life-saving care, it is imperative that we gain a mechanistical understanding of manufacturing processes that increase productivity while maintaining acceptable product quality, particularly AAV based processes that are most employed for gene therapy manufacture.

**Approach:**
Using an in-house developed PCL, we employed metabolomics to identify differences in cellular response and AAV production upon wild-type Ad5 (wtAd5) infection. The conditions were run as biological replicates in batch and perfusion modes, at different cell densities in Sartorius Ambr250® systems. Supernatant samples collected at multiple time-points over the first 48 hours post infection were analysed on Flex2, Cedex BioHT, LC-MS/MS for metabolome profiling. AAV, Ad5 titer were quantified upon harvest.

**Results:**
Differences in metabolite profiles between conditions with different AAV productivities, from pathways that are both cell specific and wtAd5 infection specific, were observed. Functional analyses, including KEGG and Gene Ontology (GO), of the metabolome data suggests accumulation of metabolite by-products that could potentially inhibit AAV production in batch cultures, removal of which could lead to increased productivity.

**Conclusion:**
We hypothesize that by gaining a deeper understanding of cellular response to wtAd5 infection, we can manipulate AAV processes to higher productivity and product quality.

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# Driving by CHO genome-scale metabolic models (GEMs) towards digital bioprocessing

**Assoc. Prof. Dong-Yup Lee**[1], Dr. Seo-Young Park[1], Mr. Dong-Hyuk Choi[1], Mr. Jinsung Song[1], Mr. Sun-Jong Kim[1]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Impact/Novelty:** Advanced multiple layered/scaled CHO-GEM enhances the prediction of condition-dependent cellular behaviors. By empirically or mechanistically linking CPPs with model components through mathematical representations, this approach ensures more reliable industrial applications.
**Introduction:** Despite recent advancements in CHO-GEMs and algorithmic innovations, the industrial application of these models is still in its early stage. Current models struggle to fully capture condition-specific cellular regulations impacting culture performance. As the field progresses towards digitized and dynamically managed culture processes, the integration of high-throughput omics and real-time cultural data becomes imperative, facilitated by cutting-edge analytical technologies.

**Methods/Approach:** To address the limitations of current CHO-GEMs, we propose an enhanced model that covers and integrates regulatory elements across multiple layers and scales. To modularize this regulatory component at the molecular scale, we mechanistically describe the multistep glycosylation of *N*-linked glycans. By hybridizing this with the CHO-GEM, we establish links the nutritional environment, CPPs, and product quality. Furthermore, deep learning algorithms are employed to update kinetic information at the reaction scale, yielding CHO-specific $k_{cat}$ values within the ecFBA framework.

**Results:** The CHO-GEM is intricately linked with key process variables within the GEM-FBA framework, parameterizing major operational variables such as pH and temperature. Canonical functions express the dependency of flux variables and enzymatic turnover rate ($k_{cat}$) on pH and temperature during cultures. This approach guides experimental design for cell line engineering, enhancing process efficiency, adaptability, and operability under dynamic conditions for digital bioprocessing.

**Conclusion:** CHO-GEMs emerge as indispensable tools shaping the digital twins of bioprocesses, accurately tracking, forecasting, and navigating cellular behaviors and metabolic states. By seamlessly integration with data-driven AI models and sophisticated control algorithms, a future is envisioned where these models autonomously orchestrate bioreactor runs, steering towards optimal culture trajectories. This paradigm shift promises enhanced efficiency and adaptability in the evolving landscape of digital bioprocessing.

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# RAMAN spectroscopy and off gas analysis data - the foundation for smart bioprocess development

**Dr Wolfgang Paul**[1], Thomas Dahomais[1], Quentin Demeyere[1], Damien Legros[1], Bassem Ben Yahia[1]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme elected:** Bioprocess data of inline sensors (e.g. RAMAN spectroscopy, off gas analyser etc.) used for monitoring and controlling become a foundation for advanced understanding of cell culture bioprocesses.

**Impact/Novelty:** RAMAN spectroscopy and off-gas analysis data enable the foundation for advanced and accelerated development of bioprocesses, but the data can also be used for next generation modeling.

**Introduction:** Inline sensors as process analytical technology (PAT) are commonly used for monitoring and controlling bioprocesses. RAMAN spectroscopy and off-gas analysis are used in manufacturing to monitor and control of specific metabolites, e.g. glucose and lactate. Real-time analysis of key metabolite profiles during the cell culture processes to feed process models open the space for accelerated efficient bioprocess development. The data from off-gas analysers in cell culture used for oxygen uptake rate (OUR) and carbon dioxide evolution rate (CER) can be used for characterizing the metabolic state and changes during cultivation. In addition, the online data can be used to feed hybrid modelling approaches, e.g. soft sensors and advanced data analysis.

**Methods/Approach:** Cell culture production runs were performed with Chinese Hamster Ovary (CHO) cell lines expressing monoclonal antibodies. RAMAN spectroscopy and off gas analysers were used in 10L and 2L bioreactor runs. Data are used for modeling to provide metabolic profile of the cell culture and compare the inline- and atline sensor data. Models robustness and accuracy will be discussed by comparing modelized data to cell culture performance data. The benefits of these data sets for advanced data analysis for bioprocess development will be shared.

**Results:** The RAMAN spectra models and off gas analysis data offer the prospects of enhancing advanced understanding, efficacy, and predictability in the development of bioprocess parameters.
**Conclusion:** The RAMAN spectroscopy and off-gas analysis data enable the next step of smart bioprocess development in terms of process understanding, automation, and supporting of scale-up/down models for bioreactors.

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# Accelerating high-performance and customizable media development through multivariate data analysis and in silico modeling

**Dr Seo-Young Park**[1], Dr. Dong-Yup Lee[1], Mr. Jisung Song[1], Mr. Dong-Hyuk Choi[1], Mr. Sun-Jong Kim[1], Mr. Chul-Hwan Park[1]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:** Our model-driven systematic framework utilizes advanced analytics, transforming vast datasets into media designs, revolutionizing biotherapeutics production for enhanced efficiency and accelerated timelines.

**Impact/Novelty:** Our three series of research have become evident that this cutting-edge technology of 'Digital Twin', the model-driven approach and *in silico* simulations, effectively applied for media development, maximizing production to its fullest potential.

**Introduction:** The prevalent use of CHO cells in biologics production has led to an increased interest in perfusion or continuous processes for higher productivity. This intensification requires concentrated feed media, influencing cell growth, productivity, and quality. Designing such formulations poses challenges, especially pertaining to amino acid stability and solubility. Addressing the bottleneck in the ideal cell culture medium, we systematically investigated the impact of basal and feed media as well as dipeptides feeds on culture performance.

**Methods/Approach:** We devised a systematic framework for advanced media development, encompassing four steps: culture data collection, multivariate data analysis, *in silico* flux balance analysis with CHO genome-scale metabolic model, and knowledge-based targeting of media components. To demonstrate its practical effectiveness, we conducted three comprehensive case studies: (1) Investigating the behavior of two distinct antibody-producing CHO cell lines under different basal media, (2) Assessing the growth dynamics of a CHO-K1 cell using two basal and two feed media, (3) Exploring the impact of dipeptide in feed media and its concentrations on antibody productivity.

**Results:** Synergistic components/additives in basal media (coenzyme Q10), feed media (glutamate, asparagine), and dipeptide feeds (glutamine) were successfully targeted to debottleneck energy imbalance, byproduct accumulation, and excessive TCA cycle intermediates utilization, respectively, and confirmed increased cell growth and final titer.

**Conclusion:** This approach expedites the biotherapeutics production at high yields by comprehensively understanding cellular behavior in diverse media environments. It significantly reduces the timeframe and mitigating the need for labor-intensive trial-and-error experimentation.

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# Optimizing CD4+ T Cells Long-term Expansion Process in Stirred-tank Bioreactors: Impact of the Dissolved Oxygen

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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:**
The development of T cell-based therapy products requires the development of bioprocesses to produce a large quantity of high-quality viable T cells in a controlled environment.

**Impact/Novelty:**
The approach presented here illustrates the advantages of controllable bioreactor-based culturing systems for the development of scalable T Cell production processes.

**Introduction:**
T cell lymphocytes play a central role in the adaptive immune response. They are an essential tool of adoptive cell therapy for the treatment of chronic viral infections and malignant diseases. However, the development of cell-based therapy products generally requires the production of a large quantity of high-quality viable T cells in a controlled environment.

**Method/Approach:**
Stirred-tank bioreactors can offer a suitable environment for the culture of T cells by providing homogeneous distribution of nutrients and gases, along with the maintenance of cells and molecules in suspension with a high process control capability. In this study, we tested the suitability of BioBLU® 0.3c Single-Use Bioreactors controlled by a DASbox® Mini Bioreactor System in the long-term expansion of CD4+ T cells as well as the impact of different oxygen tensions on cell proliferation. Given that 100% dissolved oxygen (DO) in the bioreactor corresponds to 20.9% atmospheric oxygen level, incubation at two different oxygen tensions (70% or 20% DO) was carried out.

**Results:**
The control of several growth parameters during cell expansion resulted in an efficient proliferation of highly viable and functional CD4+ T cells after 16 days of culture. The results suggested a tendency towards a positive impact of lower DO levels on CD4+ T cell proliferation rates.

**Conclusion:**
These results demonstrate the substantial potential of the DASbox Mini Bioreactor System used in combination with BioBLU 0.3c Single-Use Bioreactors to optimize T cell culture conditions.

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# Experimental design with Bayesian statistics to improve stem cell differentiation in small bioreactors

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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
The emerging field of cell-based therapies reveals a high need for novel and flexible experimental design tools for development of scalable and robust stem cell cultivation and differentiation processes.

**Impact/Novelty:**
Brownie Bee is a new, in-house developed, open-source, easy-to-use Bayesian statistics tool that completely anonymises user and data, thus being applicable to experimental design improvements in academia and industry alike.

**Introduction:**
Differentiation of stem cells follow complex protocols that are often extensive and expensive, as they require tightly controlled media compositions and continuous adjustment of physiochemical culture conditions. Standard DoE approaches are thus suboptimal for covering cultivation and differentiation parameters over time in sufficient detail. An attractive alternative is an iterative approach with ensured maximal learning per conducted experiment.

**Methods/Approach:**
To establish an early pancreatic endoderm differentiation in an 8-fold, instrumented 250 ml stirred tank bioreactor setup that enables future process optimisation, we used Bayesian Optimization to ensure optimal usage of a limited experimentation economy. Specifically, the python package ProcessOptimizer (with the user interface called Brownie Bee) was applied for easy modelling and design of experiment. Utilising Gaussian processes, a surrogate model is formed from the obtained data points of each iteration. This enables decisions for future experiments to be driven by optimal learning per experiment, or improved probability for a good result; the results from these experiments enable fitting of an updated model, to form the foundation for the next experiment series.

**Results:**
Upon three rounds of iterations with selected input- and output parameters (inoculation density, agitation speed and -strategy vs. yield and quality at an early endoderm stage), we had established a small-scale bioprocess with improved yield and quality compared to our standard process.

**Conclusion:**
Corresponding results were obtained upon upscaling to our standard system, thus validating Brownie Bee as a suitable and easy-to-use experimental design tool for future stem cell process development.

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# Model-based optimisation of glycosylation control in antibody-producing CHO cells

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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to theme selected**
Mechanistic and Hybrid modelling is used to assist experimental strategies for developing cell lines that produce mAbs with targeted glycosylation profiles.

**Impact/Novelty**
In this project, computational modelling will be integrated with cutting-edge synthetic biology tools to ensure production of monoclonal antibodies with consistent and optimal glycosylation profiles for safe, efficacious and cost-effective treatments.

**Introduction**
Glycosylation is a critical quality attribute of monoclonal antibodies (mAbs) because it defines their safety, serum half-life and efficacy. Even small changes in bioprocess conditions can lead to significant changes and have a negative impact on safety and efficacy of mAbs. Controlling biopharmaceutical processes to produce mAbs with optimal and consistent glycosylation is imperative and computational modelling have been widely used to facilitate this goal. In the present work, a mathematical model describing N-linked glycosylation in CHO cells is proposed as a component for achieving real-time control of mAb glycosylation.

**Methods/Approach**
In this work, a dynamic mathematical model is developed, that describes the glycosylation process based on an extensive reaction network, which yields to highly complex glycan structures that have been observed in CHO cells. The model considers cisternal maturation and approximates the Golgi apparatus as a single Plug Flow reactor. The kinetic expressions in the present model were expanded by accounting for the structural and kinetic mechanisms by which these enzymes act. Optimization-based methodologies have been implemented for the estimation of enzyme concentration profile parameters.

**Results**
The parameters that define enzyme distribution were estimated by seeking the minimum amount of total enzyme to obtain the reported oligosaccharide profile. With the obtained parameters for optimisation-based method, the model has been shown to closely reproduce the experimentally observed glycan distribution profile of CHO cells. Moreover, enzyme distribution was found to follow expected patterns.

**Conclusion**
The mathematical model presented herein describes the glycosylation process in CHO cells based on a reaction network that includes all possible reactions. Using the parameters from optimisation-based methods, the model generates results that are in good agreement with experimental data and previously reported observations. Moving forward, this mathematical model can be integrated with models for cellular metabolism to identify the critical process parameters and aid in the control and optimization of biopharmaceutical processes.

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# Antibody Humanization with Predictive Design

**Dr Mario Pereira**[1], Dr Sridhar Govindarajan[1], Dr Mark Welch[1], Dr Jeremy Minshull[1], Dr Claes Gustafsson[1]
[1]ATUM, Newark, United States

Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected: Data, Cells and Processes**
Case Studies - Fully humanized antibodies with improved titer and binding

**Impact/Novelty:**
A Rapid & Comprehensive Approach to ML based Humanization

**Introduction:**
ATUM's antibody humanization platform combines rational and empirical antibody humanization approaches. Humanized antibodies are designed in silico using our proprietary humanization and optimization algorithms and made synthetically by fusion of humanized antibody variable regions with human antibody backbones.
ATUM's integrated services and efficient workflows accelerate your project timelines. We improve antibody functionality and developability simultaneously, so that you finish with a high-affinity, high-specificity antibody with developability properties for process development, scale-up and manufacturing.

**Methods/Approach:**
Testing 96 humanized variants

- ATUM's in silico platform identifies and removes T-cell epitopes and sequence liabilities such as N-linked glycosylation sites, unpaired cysteine residues and potential amino acid modification sites. The sequence is then paired with the closest human framework.
- ATUM's humanized antibodies are consistent with the World Health Organization's criteria.
- Variants are tested for target binding and affinity.

Machine learning iterative testing
Combinatorial Matrices: ATUM typically synthesize and express

- 6 heavy chain (HC) variants X 4 light chain (LC) variants, or
- 8HC X 6LC, or
- 12HC X 8LC matrices

The exact format will depend upon the sequence identity of the starting antibody and is developed in collaboration with ATUM scientists.

**Results:**
Case studies show fully humanized antibodies with >2-fold improvement in titer and ~25-50 fold improvement in binding affinity

**Conclusion:**
We show fully humanized high-affinity, high-specificity antibodies with developability properties for process development, scale-up and manufacturing.

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# METHODS FOR CLONE GENERATION USING FLOW-BASED REPORTER SYSTEM AND HIGH THRUOGHPUT BEACON PLATFORM.

**Mr Victor Cairns**[1]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:** Rapid small-scale clone generation employing flow-based reporter for enrichment and high throughput clone screening.

**Impact/Novelty:** Variety of CLD workflows can be employed combining the power of Beacon platform with an enrichment reporter system for improved clone generations.

**Introduction:** A long-standing challenge in the biotechnology and pharmaceutic industry has been the need for a rapid Cell Line Development (CLD) process capable of screening a significant number of clones for the ability to produce high amounts of drug substance with the desired product of interest.

**Methods/Approach:** To this end, several methodologies have been developed previously which include fluorescent activated cell sorting, suspension/screening of clones in semi-solid matrices, as well as advanced instrumentation capable of extremely small-scale expression assessments. Described here is our novel cell screening platform, which employs both a flow-based cytometric reporter system for productivity (FLARE) and a high throughput nanoscale clone generation and screening platform (BEACON) resulting in significantly improved CLD workflows and the identification and isolation of high producing clonal candidates.

**Results:** FLARE (FLow-cytometric Attenuated Reporter Expression) system uses an alternate start cell surface reporter which serves as a specific productivity surrogate. FLARE can be used analytically to identify productivity differences in both pools and clones. It can also be manipulated via sorting (BDInflux) isolate individual cells (clone generations) and pooled populations (bulk sorting) to yield either pools or clones with enriched productivities. It has proven to be a powerful tool for both enriching populations and yielding high producing clonal cell lines. The Beacon high-throughput nano-scale cloning platform which employs Opto-Electric Positioning technology. The Beacon can import, culture, assay, and export cells with a capacity of up to 7000 clones in a single 4-5 day workflow. It brings significant improvements to monoclonality assurance, clone screening throughput, as well as resource/timeline reductions.

**Conclusion:** Here we will review the ways in which these two technologies (Beacon & FLARE) can be used in tandem to improve our CLD process and workflows for increased productivity, reduced resources, and improved timelines.

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# Digital bioprocessing enables fed-batch design for intensification of rAAV production in insect cells

Cristina Rosa[1], Nikolaus Virgolini[1,2], Miguel Graça[1,2], Ricardo Correia[1,2], **Dr Ines Isidro**[1,2]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to theme selected:**
We employ digital bioprocessing to design a novel fed-batch process based on limited data from a batch operation, thus enabling fast and low-cost bioprocess optimization by leveraging data-driven approaches.

**Impact/Novelty:**
Digital bioprocessing platforms have a high potential to improve cell and gene therapy production. This is the first model-based optimization of rAAV production in insect cell bioprocesses.

**Introduction:**
Recombinant adeno-associated viruses (rAAV) are promising vectors for human gene therapy, which can be produced using insect cell bioprocesses. However, the manufacturing capacity for rAAV is currently insufficient to meet the demand of clinical trials. Digital bioprocessing approaches offer a cheap environment to fast-track bioprocess intensification.

**Methods/Approach:**
In this work, we implemented a digital platform to simulate and design a novel fed-batch process for rAAV production in Sf9 insect cells based on data from only three batch bioreactor runs. Specific cell growth and consumption rates for major nutrients during the exponential growth phase were derived from experimental concentration profiles of the batch cultures. The fed-batch medium composition and feed rate were optimized using mass balance equations, with the estimated growth and consumption rates as parameters, to minimize changes in major nutrient concentrations in the culture medium, aiming thus to extend the exponential growth phase while avoiding nutrient depletion. Micronutrients were added to the feed medium based on literature information.

**Results:**
The fed-batch strategy was implemented experimentally in 0.5 L stirred tank bioreactors, with an exponential feed rate profile increasing from 0.24 to 1.68 mL/h, and a feed concentration of 150, 88, and 23 mM for glucose, glutamine, and glutamate, respectively. Major nutrients were, in general, nearly constant during the fed-batch exponential growth phase, and both the peak cell concentration and the rAAV titer doubled in the fed-batch strategy when compared to the control batch conditions.

**Conclusion:**
Digital bioprocessing enabled the design of a fed-batch operation with limited experimental effort. Subsequent rounds of model refinement, digital optimization, and experimentation will enable better predictions and higher productivity gains. Such approaches can be translated to other production systems and are especially relevant for cell and gene therapy production processes, where strategies for intensification are still not well established.

# Poster Presentations

# Transitioning from Development to Manufacture

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# Addressing the question of cellular heterogeneity - Microfluidic single-cell cultivation for mammalian bioprocesses

**Dr Julian Schmitz**[1], Mr. Boris Yermakov[2], Dr. Oliver Hertel[1], Dr. Nadiya Romanova[1], Prof. Dr. Thomas Noll[1], Prof. Dr.-Ing. Alexander Grünberger[2]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme selected:**
Cellular heterogeneity is a highly complex and poorly understood phenomenon in mammalian bioprocesses. However, it is expected to negatively affect bioprocess robustness and scalability during bioprocess development.

**Impact/Novelty:**
Investigating cellular heterogeneity more thoroughly will result in a better understanding of its influence on bioprocesses. Consequently, bioprocess development can incorporate these insights for more stable bioprocesses.

**Introduction:**
Key performance indicators during mammalian bioprocesses are mostly analysed by average measurements, thus cellular heterogeneity cannot be detected, although it affects bioprocess robustness and productivity. However, so far cellular heterogeneity has not been analysed systematically due to the lack of appropriate tools.

**Methods/Approaches:**
We developed a microfluidic single-cell cultivation (MSCC) device that facilitates the analysis of single cells. While implementing live-cell-imaging enables the investigation of heterogeneity with a high temporal resolution, the application of microfluidics allows a precise control of the cultivation conditions. This way bioprocess relevant environments can be installed.

**Results:**
Applying MSCC heterogeneity in the division time of isogenic CHO cells could be revealed. Morphology was heterogeneous as well, as cellular diameter showed aberrations throughout the population. Additionally, polyploidy of several cells was detected which led to distinct mass increase.
Furthermore, the expression of GFP was examined and variations in expression level were detectable. Likewise, the cell cycle progression of CHO cells was notably prone to heterogeneity. Especially for evaluating biphasic bioproduction strategies these observations are very important.
Changing environmental conditions like alterations in osmolarity led to heterogenous cellular stress response. This indicates that process modes like fed-batch cultivation might particularly promote cellular heterogeneity.

**Conclusion:**
Our results show that cellular heterogeneity is omnipresent in CHO cells and affects bioprocess key performance indicators. Prospectively, emulating fluctuating bioprocess environments like pH gradients will enable MSCC to not only address cellular heterogeneity but also bioprocess robustness.

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# Advantages of CHO cell process intensification in state-of-the-art single-use bioreactors

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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
Robust process transfer and control strategies are required to safe-guard efforts made in recent years with upstream process intensification to scale-up into production scale.

**Impact/Novelty:**
Transfer of an N-1 perfusion process from development to production scale. Focus on the control of CPP's in the latter one, particularly oxygen supply, perfusion rate and pressure.

**Introduction:**
Process intensification for mammalian biomanufacturing, especially applying perfusion, gained substantial attention throughout the last decade. The concepts and strategies developed in small-scale and bench-top bioreactors will require scale-up and process transfer into larger production scales more frequently in the future. To enable and facilitate the seamless implementation of these demanding cell culture processes at such scales, any bioreactor must provide precise process control and adequate power input.

**Methods/Approach:**
An industrial relevant CHO-DG44 cell line expressing a mAb was used. N-1 perfusion processes were conducted in an Univessel® 10 L single-use and scaled-up into a Biostat STR® 50 L Generation 3 bioreactor (Sartorius). Both experiments were equipped with an ATF module (Repligen). Robustness of the process performance in the 50 L scale was investigated using an 8-fold gassing box for accurate gas supply and an automated pinch-valve for pressure control at high gassing rates.

**Results:**
Data of N-1 perfusion processes in development and production scale and demonstrate their scalability with respect to cell growth and process performance. Accurate oxygen control could be guaranteed with precise gassing control steering the required oxygen transfer. With high gassing rates comes the risk of foaming and thus potential pressure build-up which is where the automated pinch valve increases safety and dismisses operator's interaction.

**Conclusion:**
Ultimately, this study fosters the ongoing implementation of process intensification at production scale that is highly required to fulfill the upcoming needs towards biomanufacturing.

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# In-situ Monitoring of Dissolved Carbon Dioxide in Bioprocessing: Two Years of Research and Application Insights

**Mr Giovanni Campolongo**[1]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Dissolved Carbon Dioxide Critical For Scale-Up/Scale-Down**
The researches presented underscore in-situ $DCO_2$ as a Critical Process Parameter for scalable, consistent biopharmaceutical processes, ensuring quality and productivity from R&D to production.

**Novel Sensor Technology Enables Real-time $DCO_2$ Monitoring**
The advent of solid-state optical sensor technology for dissolved carbon dioxide revolutionized bioprocessing efficiency by enabling reliable in-situ monitoring of this critical parameter.

**New Sensor Technology Sparked a New Research Wave**
Following the 2021 introduction of innovative $DCO_2$ sensor technology, substantial research has emerged on real-time management of this Critical Process Parameter in bioprocessing. This summary synthesizes key findings, focusing on the impact of managing dissolved carbon dioxide in various bioproduction processes, scales, and bioreactor types.

**Methods/ Approach Summary**
This contribution presents research and practical examples demonstrating $DCO_2$'s influence on cell cultures and bacteria. Highlights include:

- In-line versus off-line $DCO_2$ monitoring in CHO cell culture for monoclonal antibody production, featuring experiences with single-use bioreactors at different GMP production scales from a CMO industry in Hungary.
- Real-time monitoring of dissolved carbon dioxide, alongside dissolved oxygen, in hybridoma cell culture from a Technical University in Germany.
- The use of (soft) sensors for producing novel biopharmaceutical modalities such as plasmids, proteins, and viral vectors, from an Applied Science University in Switzerland.
- Analysis of Escherichia coli's response to elevated carbon dioxide from an Austrian University.

**Results: Proven Impact of $DCO_2$ Real-time Monitoring**
The different research results provide a comprehensive overview of the advancements resulting from $DCO_2$ monitoring with novel optical technology in bioprocessing, emphasizing its significance in improving cell viability, increasing product yield, and achieving consistent process scaling.

**New Sensor Technology Contributes to Maintain the "Golden Profile"**
In-situ $DCO_2$ control is key to ensure scalable and consistent cell cultures/ fermentations from R&D to production. The examples presented emphasize the necessity of real-time $DCO_2$ control to maintain the 'golden profile' – a stringent control strategy that preserves the benefits identified in R&D all the way through to production. This is now possible thanks to recently introduce optical solid-state sensor technology.

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# PAT to Optimize the Cost, Consistency and Yield of Cultivated Meat Production

**Dr Charlotte Hughes**[1], Mr Aniekan Esenam[1]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected: In-line process analytical technologies as tools for efficient, reproducible scaling of cultivated meat.**
An examination of how in-line process analytical technologies (PAT) could help the cultivated meat industry efficiently transition towards production scale.
**Impact/Novelty: Technological insights to aid the growth of an emerging industry.**
The cultivated meat industry is currently in its infancy with regards to commercialisation. We aim to deliver a comprehensive analysis of technologies available that could help to ease the transition towards biomanufacturing.
**Introduction: The global need for future protein demands a sustainable solution.**
As the global population is projected to reach 9-10 billion by 2050, there is a pressing demand to source sustainable protein for the future. In recent years, cultivated meat produced through cellular agriculture has emerged as a promising solution. The global market for cultivated meats is predicted to grow, with 150 companies currently operating worldwide and regulatory approval already secured by two companies in Singapore and in the United States; however, the industry is currently limited by high production costs and process scalability.
**Methods/Approach:**
One way to reduce production costs is to implement a Quality-by-Design (QbD) approach during product development, scaling and manufacturing. Using in-line Process Analytic Technologies (PAT) in a QbD approach enables precise, real-time analysis and control of process parameters defined as critical to the quality of the final product. Therefore, in-line PAT offers a paradigm shift in biomanufacturing that could improve the efficiency, consistency and yield of biomass production for the cultivated meat industry.

**Results: In-line PAT are proven, transferable tools for biomanufacturing.**
Through a series of industrial case studies, we explore how the application of in-line PAT using a QbD approach could revolutionize cultivated meat production. First, the integration of in-line PAT allows for a streamlined and cost-effective process optimization. By continuously monitoring critical process parameters such as dissolved $CO_2$, dissolved $O_2$, and pH, and key performance indicators such as cell density and viability, we can identify and rectify deviations promptly, minimizing resource wastage and reducing overall production costs. Second, maintaining consistency in cultivated meat production is crucial for consumer acceptance and market success. In-line PAT ensures reproducibility by offering real-time feedback, enabling fine-tuning of the bioprocess to maintain consistent product quality across batches. Finally, drawing upon inspiration from related fields, we suggest how PAT could aid the development of complex-structured meat products by the cultivated meat industry.

**Conclusion: In-line PAT can help the cultivated meat industry to efficiently transition towards commercial scale**
We highlight PATs ubiquitous versatility and transferability throughout all stages and scales of cultivated meat production and aim to introduce and inspire this emerging industry to technologies that could help them to thrive.

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# Single-Use Centrifugation – Aiming at Sustainable and Scalable Harvest Procedures

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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected**:
Increasing sustainability and efficiency of bioprocesses not only enable advanced biopharmaceutical manufacturing but are key during process development. Therefore, seamless transferability and in-depth process understanding facilitate agile bioprocess design.

**Impact/Novelty:**
Combining technological novelties and in-depth bioprocess understanding, especially regarding process-related impurities, as well as their impact on the harvest performance, enable the evolution of next-generation harvest approaches.

**Introduction:**
Key strategies for sustainable bioprocesses are the reduction of waste amounts and resource depletion, coming along with economic benefits. Especially during the design of a next-generation harvest process these strategical aspects are considered.
The seamless transferability of bioprocesses from development to commercial scale is discussed to be mandatory for fast and efficient drug development in the future. Therefore, using the same harvest technology from pre-clinical development to commercial production may provide cutting-edge advantage in the race for lean and cost-effective development timelines.

**Methods/Approach:**
In line with these main drivers for next-generation bioprocesses, the present study aimed at implementing a single-use separator into an existing single-use harvest method to close the gap between the harvest procedures for commercial manufacturing and pre-commercial facilities. In parallel, the entire manufacturing process was analysed regarding the origin, identity, and reduction capabilities of cellular particles by combining filter screenings and in-depth process understanding.

**Results:**
Three primary clarification technologies were benchmarked to each other in a head-to-head comparison: Technical feasibility, harvest process performance and processability of the harvested cell culture fluid during downstream purification were analysed for each approach. The capability of the secondary clarification to reduce process-related impurities was assessed by screening different depth filters.
Besides the improved technical alignment between clinical and commercial facilities, the implementation of centrifugation-based clarification showed improved process performance and reduced resource usage. Furthermore, the improved cell culture analysis added valuable input towards in-depth understanding of particle formation during bioprocesses.

**Conclusion:**
The implementation of the single-use separation technology may enable the development of more sustainable and easy-to-transfer harvest processes for next-generation bioprocessing. Thereby, it is only the first step in a series of attempts to improve existing harvest procedures. Finally, the combination of in-depth process understanding, seamless transferability and optimized process design will help to evolve a next-generation harvest approach for modern bioprocesses.

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# Mechanistic and hybrid modelling in bioreactor digital twins

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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
Bioreactor digital twins can help enable scale-up and technology transfer. This work includes the development and validation of a high-fidelity model to serve as a basis for a digital twin.

**Impact/Novelty:**
The proposed model is based on a mechanistic and hybrid approach. It includes a novel cell culture dynamics model and enables a closed-loop feed control strategy based on biocapacitance.

**Introduction:**
Biocapacitance probes combined with bioreactor digital twins can enable monitoring of the biomass dynamics during the culture period, supporting operational decisions and process optimization. This presentation focuses on the development and validation of a digital twin for an industrial CHO cell culture using a biocapacitance-based feed regulation to produce a monoclonal antibody.

**Methods/Approach:**
The model proposed as a basis for the digital twin has a mechanistic structure and includes original kinetic expressions developed and tested to define specific observed phenomena, such as cell death due to senescence. The model includes integrated data-driven elements (ANNs) to model the kinetics of poorly understood phenomena.

**Results:**
The calibrated model is able to predict the fed-batch process with a good degree of accuracy under different feeding strategies and is used to explore process robustness for various initial seeding conditions. An analysis was conducted to identify the extent of model transferability to a different cell line. The model structure resulted transferable for the most part, and parameter re-estimation has led to values close to those estimated in this work, thus significantly reducing the effort in the model validation phase.

**Conclusion:**
The proposed model is found suitable to support optimal feeding strategy design and to explore the robustness of the biocapacitance-based feeding strategy under validated process conditions.

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# Integrated clone selection and process development approaches for early TOX material production

**Mr Jeremie Kerbrat**[1]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
Acceleration of early process development for early transfer to TOX material production.

**Novelty:**
We look at the benefit of combining key CLD and process development activities over the usual sequential approach.

**Introduction:**
There is often a need to move quickly to non-clinical Tox studies in order to enable the most efficient and timely progress to IMPD submission for FIH studies. We are investigating various approaches to integrate and accelerate key activities to take NBE projects from DNA to Tox as quickly as possible, whilst ensuring acceptable productivity, product quality and 'manufacturability' of the molecules coming through.

**Approach:**
The acceleration of a NBE transfer from Clone selection to TOX was assessed, combining previous knowledge with process intensification methods.
After feeding strategy screening over a substantial number of clones in Ambr15 within our CLD labs, the prioritized clones and conditions were used in one Ambr250 bioreactor run within Process labs, and some process intensification methods applied. Performances in Ambr15 and Ambr250 were compared, and early process intensification assessed.
Bioreactor performance indicators and product quality attributes were generated across all systems and between departments, using digital data capture and visualisation tools. This combined dataset was analysed ahead of rapid transfer to 200L scale batches for the production of Tox material.

**Results:**
Data for multiple clones was generated at Ambr15 and Ambr250 scale across departments, enabling a simultaneous assessment of clone performance, process performance, product quality and manufacturability. A screen of process intensification tools has been integrated to this approach.

**Conclusion:**
This combined dataset allowed us to move directly to the successful production of tox material at 200L scale in accelerated time while achieving increased product titres compared to a platform approach. Perspectives for further improvements are discussed.

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# Towards Computational Fluid Dynamics based methodology to accelerate bioprocess optimisation

**Mr Vishal Kumar Singh**[1], Dr. Ioscani Jimenez Del Val[2], Prof. Jarka Glassey[3], Dr. Fatemeh Kavousi[1]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:** The study highlights the integration of *in silico* models and experimentation for efficient and scalable bioprocess development.

**Impact/Novelty:** An integrated Computational Fluid Dynamics and Cell Reaction Kinetics (CFD-CRK) modelling framework has been applied for high throughput parameter optimisation for a CHO cell culture process in Ambr®250.

**Introduction:** Bioprocess development is a time-constrained activity aimed at harnessing the full potential of culture performance in an ambience that is not natural to cells. Finetuning of the operating parameters are often accomplished via time-consuming experiments. A unified CFD-CRK modelling approach has been developed to optimise the mass transfer and aeration impacts on process performance.

**Methods/Approach:** A transient multiphase water-air Ambr®250 bioreactor system has been studied in Ansys CFX to predict the volumetric mass transfer coefficient ($k_La$) profiles at varying agitation rates (300 rpm to 650 rpm) and aeration rates (0.01 vvm to 0.04 vvm). Impeller rotation was modelled using Moving Reference Frame (MRF) method. The gas bubble size distribution was modelled using MUSIG (Multiple Size Group) population balance model and Luo breakup and Prince and Blanch coalescence models were used to model the bubble breakage and coalescence. Mesh refinement studies were carried out to reduce the simulation error. Validated Ambr®250 model was extended to incorporate cell reaction kinetics using multicomponent species transport model to predict the process performance.

**Results:** The $k_La$ simulation results aligned well with the literature data with a root mean square error of 0.17. The response surface of operating parameters rendered the sweet zone of operation for agitation and aeration rates as 475 rpm and 0.02 vvm respectively.

**Conclusion:** CFD-CRK *in silico* optimisation approach allows for reduced experimentation with faster process development. Overall, this approach provides a more de facto representation of the actual bioproduction.

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# Generalizing cell culture models from fed-batch to continuous using metabolic knowledge

**Miss Mariana Monteiro**[1], Stacy Bediako[3], Gregory Nierode[3], Pavlos Kotidis[2], Cleo Kontoravdi[1]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:** We introduce a versatile modelling framework for performance prediction between fed-batch and continuous modes, which also aids in control and soft-sensing of hard-to-measure metabolites.

**Impact/Novelty:** We overcome extensive experimentation by endowing bioreactor models with metabolic knowledge, enabling them to predict across different modes of operation without requiring kinetic parameter estimations.

**Introduction:** Continuous biomanufacturing has the potential to significantly enhance the productivity and consistency of therapeutic protein production. However, the use of existing cell lines typically optimized for fed-batch presents a challenge, particularly in selecting *a priori* satisfactory continuous operating parameters. Current methodologies rely on extensive experimentation, which is time- and cost-intensive. This motivates the need for model-based methods that can reduce experimental load by predicting performance, leveraging experimentation only for final validation. However, existing models are overparameterized to experimental conditions, and increasing their generality requires more data and consequently more experimentation. Thus, neither approach is satisfactory as they do not address the underlying problem of requiring extensive experimentation.

**Methods/Approach:** We propose building general reactor models through embedding first-principles knowledge. By incorporating cell metabolism in a nonparametric model of the production reactor, we can predict key process parameters like metabolite concentrations. By introducing mode-specific constraints, our formulation predicts across different modes of operation without requiring kinetic parameterization, thereby reducing the need for extensive experimentation.

**Results:** We present an evaluation of our model by comparing its predictions against both fed-batch and continuous industrial cell culture data. Our results show that this formulation can be used to estimate performance when transitioning from fed-batch to continuous. This extra knowledge can also be used for soft-sensing of hard-to-measure metabolites and optimal culture stopping point estimation.

**Conclusion:** We propose a flexible model formulation that can be used to reduce experimentation costs, soft-sense metabolites and predict culture stopping-points.

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# Scale-Down Model Qualification for the Commercial Production of a Fusion Protein

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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:** Scale-down models are valuable tools for late-stage process development. They are useful for gaining process knowledge which can be implemented into manufacturing-scale bioprocesses for commercial production of therapeutic modalities.

**Impact/Novelty:** Scale-down models are useful predictive tools that can be used to provide technical support for investigations, as well as to manipulate product quality and enhance process control at manufacturing scale.

**Introduction:** Qualification of representative scale-down models (SDMs) is a regulatory requirement for characterisation of biomanufacturing processes. High-throughput mini-bioreactor systems have accelerated timelines for early-stage process development however, bench-scale models remain a robust, scalable tool for late-stage process development. These models are imperative to process development and process characterisation, technical support for investigations and process improvements at manufacturing-scale. This work demonstrates the qualification of bench-scale models as representative systems for at-scale *n-1* and production bioreactor steps for the commercial production of a fusion protein.

**Methods/Approach:** Scale-down satellite bioreactors utilising varied scaling parameters were run from six commercial scale batches. A two-one-sided t-test (TOST) analysis was used for equivalence testing between scales along with statistical evaluation of process performance and quality attributes for comparability.

**Results:** In-process trends, titre and product quality attributes were comparable between bench and commercial manufacturing bioreactor scales. Geometric scale differences required modulation of gassing strategies to provide comparable product heterogeneity outputs between scales.

**Conclusion:** Bench-scale models were successfully qualified against manufacturing-scale *n-1* and production bioreactor steps for the commercial production of a fusion protein. The SDMs tested were evaluated as statistically and practically equivalent to at-scale bioreactors in respect of in-process trends and product quality attributes. Additionally, this work demonstrates that modulation of gassing strategies between scales can effectively mimic at-scale $pCO_2$ profiles to provide improved comparability of product quality attributes.

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# Cell culture media optimization to achieve high AAV productivity

**Dr Benben Song**[1]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected**
This study aligns with the growing demand to improve manufacturing efficiency for emerging modalities to lower expenses, making them accessible to a broader range of patients.

**Impact/Novelty**
It enhances overall process efficiency and scalability, positioning the manufacturing process for long-term success in the competitive landscape of gene therapy.

**Introduction**
The most common process for producing adeno-associated virus (AAV) relies on transient transfection of therapeutic genes using suspension HEK293 cells. Multicomponent cell culture media play a fundamental role in AAV productivity and product quality. We recognize the need for an optimized medium to support scalable production of AAV vectors with high productivity in suspension HEK293 cells.

**Methods/Approach**
Using the backbone of our existing R&D media library, we developed a new medium formulation to support multiple HEK293 cell lineages and to maximize peak viable cell density (VCD) and transfection efficiency. We evaluated performance based on peak VCD, viability, transfection efficiency, and production of multiple AAV serotypes with commercially available HEK293 cell lines and multiple transfection reagents.

**Results**
The optimized medium showed 2-fold increase in peak VCD compared to the control medium, surpassing 10 x $10^6$ viable cells/mL with a substantial reduction in lactate production. Experiments in multiple HEK293 cell lines, with multiple transfection reagents, and using a GFP reporter plasmid demonstrated transfection efficiencies over 80% and AAV5 titers over $10^{11}$ viral genomes (vg)/mL for all conditions tested. In shake flasks, benchtop bioreactors, and large-scale bioreactors the medium displayed fully scalable processes with excellent AAV5 and AAV8 titers using three different HEK293 cell lines.

**Conclusion**
In this study we demonstrate substantial AAV productivity increases by optimizing cell culture media. The improvement is reproducible with multiple AAV serotypes and scalable to large-scale bioreactors.

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# Tackling The Blue: Modifications in Culture Media To Reduce Formation Of Blue Coloration in Bioprocesses

**Mr Andreas Unsoeld**[1], Ms. Elena Bollgoenn[1], Ms. Dr. Amandine Calvet[1], Mr. Dr. Michael Loeffler[1], Ms. Dr. Lisa Junghans[1], Mr. Sebastian Puengel[1], Mr. Dr. Erik Arrango Gutierrez[1], Mr. Dr. Daniel Lakatos[1]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
The results of this study can be directly applied to cell culture media development and optimization to improve affected bioprocesses in the future.

**Impact/Novelty:**
The visible blue coloration observed from time to time during the purification process (on membrane filters and capture columns see picture 1) is an anomaly in the current bioprocesses and is discussed whether it could have a negative impact on the final product. Based on that, it was crucial to explore the potential root cause for the formation of the blue compounds.

**Introduction:**
In recent years, low molecular weight compounds causing blue coloration in bioprocesses, mainly downstream, have been described to occur occasionally in CHO culture supernatants (see picture 1)
But what is causing the formation of the blue compounds? Is it possible to influence the levels?
These questions were investigated in a study based on isotopic labelling experiments. In the cell culture experiments, two important amino acids Cysteine (CYS) and Tryptophane (TRP) could be identified as the key drivers for the formation of the blue compounds.

Picture 1: Blue compound on capture column

**Methods/Approach:**
To verify the impact of the two amino acids on the blue coloration, a Design of Experiment (DoE) cell culture study was conducted in Ambr®250 cultivation system with varying levels of the two amino acids.

**Results:**
The results of the Ambr®250 study confirmed that different levels of CYS and TRP play a major role in the formation of the blue compound during the cell culture process which has a direct impact on the levels before purification.

**Conclusion:**
This study highlights the importance of CYS and TRP in the formation of the blue compound during bioprocesses. In addition, it will be demonstrated how to optimize the balance of the two in the cell culture media to reduce the formation of the blue compound before purification without negatively impacting the cell culture process performance.

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# FOLI-REC: Recombinant equine chorionic gonadotropin hormone for veterinary use

Mr Sebastián Antuña[1], Ms María Belén Tardivo[1], Mr. Carlos Javier Villarraza[2,4], Dra. María Celeste Rodríguez[2,4], Mr. Pablo Mussio[2,4], Dr. Diego Fontana[1,2,4], Mr. David Saucedo[1], Dr. Luciano Cattáneo[3], Dra. Natalia Ceaglio[2,4], **Dr. Claudio Prieto Claudio Prieto**[1,2]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
This work describes the development and transfer to the animal health market of the first worldwide recombinant equine Chorionic Gonadotropin, novel product that aims to replace the current animal-derived product.

**Impact/Novelty:**
A novel recombinant product for artificial insemination protocols in ruminant (cattle, sheep, goat) and swine was developed and registered for commercial use in LATAM.

**Introduction:**
Up to now, the eCG products available in the vet market are eCG preparations from blood of pregnant mares (PMSG). However, the inherent drawbacks and associated risks of such animal-derived products, coupled with the removal of PMSG WHO international standard from the Pharmacopeia, necessitated a shift toward a more contemporary and animal welfare-friendly biotechnological solution. This work endeavours to replace the antiquated technology with a cutting-edge bioproduct.

**Methods/Approach**:
Suspension-adapted CHO cells were genetically engineered to constitutively express reCG, obtaining a stable clone for the production of the hormone using CD serum-free medium. Process intensification and scaling up was performed, establishing the production of the reCG in 50 L bioreactor in perfusion mode following a “push-to-low approach”. Downstream processing involving chromatographic and filtration steps was developed alongside analytical tool validation for molecular characterization. Quality by Design (QbD) principles were applied to create an optimized liquid formulation of the reCG drug product with a predefined quality profile. Field trials were subsequently conducted to ascertain optimal dosages for various livestock species.
**Results:**
A stable reCG-producing clone has been successfully characterized for industrial scale production in a perfusion bioreactor utilizing serum-free medium. A robust and cost-effective bioprocess with high productivity, aligning with the scale of the ruminant reproduction market, has been established . Developed in-process controls and quality control methods are apt for monitoring production and analysing product quality. In vivo activity of reCG has been demonstrated, confirming its efficacy in the target animals. Appropriate doses have been determined for *Bos indicus* and *Bos taurus*.

**Conclusion:**
A comprehensive process was successfully developed to replace the current animal-based production method, being significantly more environmentally friendly, robust, and reproducible.

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# Scalable, serum-free viral vector production in a high cell density bioreactor

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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Transitioning from development to manufacture – How do science and innovation translate into highly efficient biomanufacturing?**

**Impact/Novelty:** case studies illustrating the ability of cultivating various cell lines in serum- and xeno-free conditions.

**Introduction:**
Serum and xeno-free media have emerged as crucial components in cell culture systems, particularly in the fields of advanced therapy medicinal products (ATMP). Traditional culture methods often utilize serum, which contains undefined and variable components that can introduce inconsistencies and potential safety concerns into cell culture systems. In response to these challenges, serum-free and xeno-free media formulations have been developed to provide defined, consistent, and animal-derived component-free environments for cell growth

**Methods/Approach:**
This presentation will illustrate how structured fixed-bed bioreactors can be used as a flexible upstream solution accommodating a variety of cell lines and products in serum free and xeno-free conditions, while achieving linear scale-up.

**Results:**
An overview of different case studies with HEK293 and Vero cells used for the production of different viral applications.

**Conclusion:**
While challenges such as initial setup costs and optimization of culture parameters may exist, the benefits of fixed bed bioreactors in serum-free cell culture are evident. Their scalability, efficiency, and compatibility with serum-free media position them as a viable solution for large-scale production of cells for therapeutic purposes. As the field of regenerative medicine and cell-based therapies continues to advance, fixed bed bioreactors offer a promising platform for meeting the growing demand for standardized, safe, and clinically relevant cell culture systems.

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# Dissolved Carbon Dioxide in Addition to Dissolved Oxygen for In-situ Monitoring of Hybridoma Cell Culture

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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**$DCO_2$ Is Key for Viable Cell Density**
In hybridoma cell bioprocessing, in-situ monitoring of DCO2, DO, pH, and stirring speed enables maintaining targeted viable cell density.

**In-situ $DCO_2$ Measurement Enables Real-time Monitoring of an Additional CPP**
Optical In-line dissolved $CO_2$ measurement enhances industrial-scale process control by enabling an additional Critical Process Parameter (CPP) monitoring.

**Introduction: Advancing Bioprocess Control through Comprehensive CPP Monitoring**
Biotherapeutic production at industrial scales faces challenges due to the inherent variability of cell culture processes. The implementation of Process Analytical Technology (PAT), as advocated by regulatory agencies, enhances rapid development and scale-up of bioprocesses. Real-time monitoring of Critical Process Parameters (CPPs) like dissolved $CO_2$ ($DCO_2$), dissolved oxygen (DO), pH, and stirring speed are crucial to keep in target Key Performance Indicators (KPIs), such as Viable Cell Density (VCD).

**Methods/ Approach**
In-situ monitoring of $DCO_2$ and DO was performed using advanced optical sensors in a state-of-the-art bioreactor. These sensors were directly connected to the bioreactor control systems for real-time process adjustments. The study involved cultivating hybridoma cells in a controlled fed-batch process, with mentioned CPPs regulated in-line. Off-line measurements of Viable Cell Density (VCD) and glucose concentration complemented the dataset. Real-time monitoring of $CO_2$ and $O_2$ provided insights into cellular respiration and metabolic activities. Adjusting agitation speed effectively regulated the mass transfer of $CO_2$ and $O_2$, with in-line measurements enabling prompt adjustments at the bioreactor, reducing the limitations of off-line sampling.

**Results: $O_2$, $CO_2$ and pH Regulation**
The process began with $CO_2$ supplementation for pH control, later switching to NaOH addition. Nutrient levels, $O_2$ consumption and $CO_2$ production were key factors in determining feeding and agitation speed strategies for the different growth phases. Adjusting agitation speed helped manage $CO_2$ outgassing. All the CPPs monitored in real-time resulted in rapid correction of same critical process parameters preventing negative impacts on viable cell density.

**Conclusion: In-situ Monitoring of DCO2 in addition with DO**
The use of in-line sensors for $DCO_2$ and DO monitoring proved effective in real-time CPP management. This approach, including pH and stirring speed, facilitated bioprocesses rapid adjustments, thereby improving viable cell density control. This demonstrated the value of comprehensive in-situ CPP monitoring.

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# Optimized CHO cell Feed Medium without Copper Precipitation

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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected**: Transitioning from development to manufacture - Formulation of modified feed media with better copper stability leads to optimized CHO cell growth in bioprocessing industry.

**Impact/Novelty:** Precipitation of copper in feed media is a common problem. Development of feed media formulations should aim at better copper solubility and reduced precipitation.

**Introduction:** Copper being is an essential element and critical component in cell culture media development. It is important for copper to be biologically available, so media development strategies should aim at growth media and feed development to achieve this. In the Bioprocessing industry, feed media developed for CHO cells is known to have copper precipitation, resulting in lower cell productivity. Developing feed media with better copper solubility provides a method of reducing and/or preventing copper precipitation.

**Method/Approach**: A study was designed to investigate the performance of CHO cells cultured with four feed media formulated with different forms of copper. These formulations were centered around minimizing chemical interaction between components, like the redox chemistry between copper ion and cysteine. Two cell lines, DG44 and R18 were evaluated by culturing in one basal media with four feeds (8 conditions) in fed-batch culture method.

**Results:** The testing of all newly developed modified feeds have shown comparable performance in viable cell density (VCD) and viability compared to the original feed**.** One of the four newly developed feed media has demonstrated better performance than the original feed and was able to address the precipitation issue.

**Conclusion:** An alternate custom feed media was developed to overcome the precipitation of copper normally seen with such media.

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# Effects of cell residence time on IgG1 production in small-scale perfusion cultures of CHL-YN cells

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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
4. Transitioning from development to manufacturing

**Impact/Novelty:**
Small-scale perfusion culture using a gravity separator that uses convection to shorten cell residence time improved the specific $IgG_1$ production rate of CHL -YN cells.

**Introduction:**
In perfusion culture, cells are subjected to shear stress in ATF operation, or left in an uncontrolled environment in a gravity separator. To solve these problems, a gravity separator using convection was developed in this study. The separator is located inside a culture tank, and convection flow occurs in the separator by the cooling of the upper part of the separator to shorten the cell residence time. It takes only 9.4 s for a cell to enter and exit the separator. We compared the perfusion cultures between this and the gravity separator systems.

**Methods/Approach:**
$IgG_1$ producing CHL-YN cells were inoculated in a small-scale perfusion culture device (30 mL) BWB-05NA2-C (ABLE, Japan) with a gravity separator using convection using the EX-CELL®Advanced HD serum-free perfusion medium (MERCK). The cell viability, viable cell and metabolite concentrations (glucose, lactate, glutamine, glutamate and ammonium) were analyzed. The $IgG_1$ concentration was analyzed using a sandwich ELISA.

**Results:**
The specific $IgG_1$ production rate from Day 6 to Day 9 was 8 times higher in the case of the separator with convection than that with only gravity, and 1.3 times higher than that with ATF under the same conditions except that the culture volume was 2 L. In addition, the specific glutamine consumption rate for the same period was 2 times higher than that in the case of gravity separator.

**Conclusion:**
In small-scale perfusion cultures of CHL-YN cells, shortening cell residence time and improving conditions in the cell separator can improve specific $IgG_1$ production rate. This small-scale culture method could be the downscaled model for 2L perfusion culture.

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# Minimal clone to clone variation removes need for clone screening and clone specific bioprocess optimizations.

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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected**
This research aims to accelerate the transition from development to manufacturing.

**Impact/Novelty**
Our work suggests that it may be possible to entirely skip clone screening and bioprocess optimization without sacrificing productivity or product quality. This should greatly speed up the transition from research to manufacturing.

**Introduction**
In the pharmaceutical industry, there is an urgent need to speed up the drug development process to reduce the time from discovery to market. Mammalian cells are the preferred choice for the production of complex proteins; however, their development is time-consuming and necessitates extensive clone screening. Current conventional methods employ random integration for gene insertion, yielding high protein production but fostering clone-to-clone variation. This necessitates exhaustive screening to identify clones meeting desired criteria. Our project focuses on demonstrating that recombinase based targeted integration offers a robust tool, that minimizes clone-to-clone variation to a degree that enables predictive production conditions, thereby substantially reducing the time for clone screening and bioprocess optimization from 12 to 3 months.

**Methods/Approach**
To showcase the universal applicability of optimal process conditions, three cell lines engineered with targeted integration expressing different proteins were assessed, and they were compared to their respective random integrated cell line.
The production conditions of the cell lines were assessed in a fed-batch bioprocess with a full-factorial Design of Experiments (DoE), where different parameters were evaluated. The experiments were run using the ambr15 high-throughput bioreactor system, with product titer as the key output factor.

**Results**
Our DoE shows that clone picking and clone specific bioprocess optimization can be omitted without sacrificing productivity. This can reduce transition from research to production by several months.

**Conclusion**
This strategy establishes a predictive and robust expression system, that significantly reduces the time and cost associated with clone screening and bioprocess optimization, irrespective of the protein of interest.

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# Shortening the cell line development (CLD) timeline with an improved clone stability assay

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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Transitioning from development to manufacture:** The goal of this study is to accelerate and verify clone stability assessments. Clone productivity trends and stability outcomes were compared using two different approaches.

**Impact/Novelty:** Weekly productivity assays generate more data points through 60 generations than a traditional stability assay that only assesses productivity every 20 generations. In addition, weekly productivity assays would shorten the overall process.

**Introduction:** CLD is a month's-long process with significant time dedicated to assessing expression stability. Traditionally, stability is evaluated by an end-point fed-batch assay by thawing multiple generations of each clone. However, this process is laborious and compares only 3 to 4 time points per clone, which creates data gaps between every ~ 20 generation time points. Furthermore, the end-point fed-batch assays demand additional days for the completion of the study. Here, we tracked clone productivity weekly during the 8 weeks (wk)/60 generations of subculturing and compared the results to end-point fed-batch assays.

**Methods/Approach:** We examined the productivity of multiple clones/molecules by weekly and end-point fed-batch assays. For the weekly assay, clone productivity was measured in an abbreviated fed-batch during the 8 wk subculturing, and clones were cryopreserved every ~ 20 generations along the way. The productivity of each clone at ~ 0, 20, 40, and 60 generations was then assessed in a 14 d fed-batch assay and stability results were compared with the weekly titer trending.

**Results:** All tested clones showed similar growth and productivity profiles in the weekly titer trending assay as compared to the end-point fed-batch results, regardless of stability status.

**Conclusion:** Weekly productivity assessments generate more data as compared to the traditional stability assessment. In addition, the weekly assays shorten the overall time compared to the traditional end-point fed-batch assays.

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# Developing a platform for single cell cloning of the Pro10™ cell line for AAV manufacture

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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Impact/Novelty:**
We have developed a new process to generate monoclonal Pro10™ cell lines from single cell printing to cryobanking and characterization.

**Introduction:**
Pro10™ is an industry-leading suspension cell line that can produce high-yield AAV vectors in serum free conditions. Here we address the challenges of generating single cell clones from the Pro10 cell line and monoclonality assurance to meet regulatory requirements.

**Methods/Approach:**
We have established a single cell printing and imaging process using the Cytena C.Sight single cell printer and the Synentec NyOne cell imager. By screening a panel of cloning media we defined optimised conditions to scale up single cell clones of the Pro10™ cell line to 96 deep well plate and beyond.

**Results:**
We have developed a platform to generate monoclonal Pro10™ cells for AAV manufacturing. With various imaging techniques incorporated into the process, monoclonality of the stable cell lines may be traced and validated. We believe selected clones may be further expanded into cultures in mini bioreactors ready for cryobanking and characterization.

**Conclusion:**
This cell line development process potentially allows us to generate monoclonal Pro10™ cell lines with high efficiency and assured monoclonality.

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# Implementation of Quality by Design Principles for Influenza A Virus Production

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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
To meet rising demands for potent and safe vaccines embedding quality into manufacturing processes is pivotal, and establishment of Quality by Design (QbD) approaches is key for streamlined virus production.

**Impact/Novelty:**
In a proof-of-concept study, QbD principles and state-of-the-art technologies were used to achieve robust process performance and to mitigate risks in cell culture-based influenza A virus (IAV) production.

**Introduction:**
Selection and optimization of parameters influencing product yields, efficiency and quality to meet regulatory compliance in biologics production presents a complex challenge. In recombinant protein manufacturing, the application of QbD strategies ensures compliance with critical quality attributes (CQA). Given the many options for design and optimization of processes not yet implemented in viral vaccine production, further efforts are urgently required.

**Methods/Approach:**
Failure mode and effect analysis (FMEA) was performed to identify critical process parameters (CPP) for an IAV production process in two monoclonal suspension MDCK cell clones (C113 and C59). A design of experiment (DoE) approach using a reduced central composite design followed by multivariate data analysis was applied to investigate robustness of a cell culture process established in an ambr15 microbioreactor system. Results were validated at 2 L scale in a single-use Univessel Biostat vessel. Maximum total virus titer ($HA_{max}$) served as CQAs.

**Results:**
The FMEA approach revealed pH value, dissolved oxygen concentration, viable cell concentration at time of infection, and multiplicity of infection as CPPs with highest risk priority number (>15). Here, ambr15 cultivations of clone C113 and center point conditions resulted in a $HA_{max}$ of 3.31 ± 0.03 $log_{10}$(HAU/100 µL), while for clone C59 3.02 ± 0.04 $log_{10}$(HAU/100 µL) were achieved (n=4, error of HA-assay 0.15 $log_{10}$(HAU/100 µL)). Following multiple linear regression analysis, design spaces with an estimated 1% risk of failure and robust set-points could be determined for each clone.
At 2 L scale, clone C113 showed $HA_{max}$ of 3.25 ± 0.03 $log_{10}$(HAU/100 µL); for clone C59, 2.99 ± 0.08 $log_{10}$(HAU/100 µL) were achieved (n=3). With a cultivation using CPPs at the edge of the identified design space, similar or even slightly improved $HA_{max}$ could be reached.

**Conclusion:**
The DoE approach allowed identification of design spaces for each cell clone. Scale-up to a 100x increased working volume confirmed high process robustness and processes operation at the limits of the design space still meet CQAs. Overall. this approach holds promise for accelerated process development and support of regulatory approval in viral vaccine production.

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# Optimizing Recombinant Adeno-Associated Virus (rAAV) Production: Unraveling the Impact of the N-1 Seed Train

**Dr Johanna Wagner**[1], Dr Sinisa Petrik[1], Mr Matthias Boscher[1], Mr Jared Babic[1], Ms Beatriz Larena Carnio[1], Ms Dila Bitnel[1], Ms Anita Heinlein[1], Ms Christine Zach[1], Mr Sebastian Ritter[1], Mr Matthias Gora[1], Mr Tobias Klötzler[1], Dr Alexandra Schoberth[1], Mr Karl Heller[1], Mr Ahmed Youssef[1]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:**
By addressing factors impacting scalability, efficiency, and productivity, the N-1 seed train represents a critical stage in transitioning bioprocesses from development to large-scale manufacturing.

**Impact/Novelty:**
Examining the N-1 seed train deepens insight into factors influencing early-stage rAAV production, offering opportunities to refine conditions, enhance scalability, and elevate overall productivity.

**Introduction:**
Recombinant adeno-associated virus (rAAV) vectors have emerged as versatile tools for gene therapy applications, demanding efficient and scalable production processes to meet market needs. The N-1 seed train, a critical precursor in bioprocessing, plays a pivotal role in influencing the final yield and quality of rAAV vectors.

**Methods/Approach:**
Our aim is the development of a robust suspension platform process based on our proprietary HEK293 cell line, that minimizes batch variability and builds quality into product by design. Our study delves into a comprehensive investigation of the impact of the N-1 seed train on rAAV production, shedding light on key parameters influencing process performance and product characteristics. Using our HEK293 cell line, we assessed various systems for cell growth, including shake flasks as well as rocking, and stirred tank bioreactors, at both small and large scales.

**Results:**
By dissecting the N-1 seed train's role in cell expansion, we identified critical factors that contribute to enhanced cell growth, viability, and finally rAAV vector production. In this context, we examined potential constraints within the culture medium by analyzing samples derived from spent medium across various N-1 systems.

**Conclusion:**
Our results not only enhance our understanding of the complex relationship between the
N-1 seed train and rAAV production but also provide valuable strategies for improving scalability, reproducibility, and cost-effectiveness. The insights obtained from this study carry significant importance in advancing the development and manufacturing of rAAV vectors, ultimately enabling their broader application in gene therapy and biomedicine.

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# Condensing the Cell Line Development Processes – Nimble Strategies for Increasing Efficiency

**Dr Clare Lovelady**[1], Dr Jie Zhu[1], Dr John Patrick Mpindi[1], Dr Kerensa Klottrup-Rees[1], Dr Sarah Dunn[1], Dr Diane Hatton[1]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:** This work aims to improve early development efficiency by consolidating steps, reducing excess & integrating automation tools, enabling rapid clone selection for effective scale-up.

**Impact/Novelty:** We present a streamlined early development process, with accelerated development timelines, enabling more life-changing medicines to reach patients.

**Introduction:** Generation of clonal manufacturing cell lines remains a time-consuming and resource intensive step in biotherapeutic development. With increasing pressure to be first to market, more programs look to accelerate the delivery of material to support clinical trials, stretching development resources. A nimble strategy will enable higher project throughput with efficient use of resources.

**Methods/Approach:** Here, we present a condensed cell line development (CLD)-light process- identifying the minimum steps required for effective CHO cell line generation. By reducing the number of cell lines screened, shortening and consolidating screening steps, intensifying data collection & taking data-backed risks, we can save on resource across early development, whilst minimising the impact on final clone performance.

**Results:** Taking a holistic view of early development, we have identified bottlenecks, and by reviewing historical data, have assessed the potential impact of removing or delaying activities. By condensing screening whilst intensifying the sampling strategy we can capture more clone data in less time to maximise the decision-making power. In addition, implementing automated liquid handling systems, with integrated data tracking, upload & data analysis tools has facilitated efficient data review & rapid decision making. A comprehensive risk analysis was completed to assess the risks, build in data-driven checkpoint mitigations and maximise the likelihood of success.

**Conclusion:** Ultimately, this strategy required us to reframe the early stages of biopharmaceutical development, to deliver clonally-derived cell lines which meet project needs but simultaneously enable us to meet the needs of more projects.

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# Detecting Microbial Cell Culture Contamination by Measuring Nicotinic Acid to Nicotinamide Ratio At-Line

**Dr Kenion Blakeman**[1], Ji Young Anderson[2], Scott Miller[2], Alice Ng[3], Jiayi Huang[3], Meenubharathi Natarajan[3], Yie Hou Lee[3], Rohan Williams[3], Paul Barone[4], Jacqueline Wolfrum[4], Stacy Springs[4]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
Measuring the ratio of nicotinic acid to nicotinamide provides a rapid and sensitive method to detect microbial contamination of cell cultures in less than 24 hours.

**Impact/Novelty:**
The REBEL analyzer, already used in process development laboratories for amino acid measurements, accelerates the detection of a metabolite-based biomarker for sterility of cell and gene therapy processes.

**Introduction:**
Cell and gene therapies have emerged as viable alternatives for diseases that are not effectively treated by small molecule and biologic therapies. Cell therapy processes offer incubation periods as short as 7 days, but current sterility testing of 7-14 days presents a bottleneck. The ratio of nicotinic acid to nicotinamide has been demonstrated as a biomarker for detecting microbial contaminants within 24 hours of incubation. These measurements are currently challenging to obtain at line. The REBEL analyzer provides at-line quantitation for 33 targets in under 10 minutes per sample but requires optimization for the nicotinic acid and nicotinamide measurements.

**Methods/Approach:**
Cell cultures with MSC cells were prepared with known levels of microbial contamination and sampled between 0 and 24 hours of incubation time for analysis. The REBEL analyzer hardware was optimized for this application by improving the sensitivity of the miniaturized ion trap mass spectrometer detector and improving temperature control of the capillary electrophoresis separation. Automated algorithms were optimized for simultaneous measurements of both analytes.
**Results:**
Hardware and automated algorithm optimization resulted in detection limits of approximately 5 - 10 µM in undiluted spent media samples. The ratios of nicotinic acid to nicotinamide will be presented for a time course cell culture experiment using E. coli k12 spiked in MSC cells sampled at 3-hour intervals.

**Conclusion:**
The REBEL analyzer provides a solution for measuring the ratio of nicotinic acid to nicotinamide in cell and gene therapy processes and provides the basis for a rapid analytical method for assessing cell culture sterility.

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# Development of a robust process for pilot scale AAV production using a transient transfection system

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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:**
4. Transitioning from development to manufacture – How do science and innovation translate into highly efficient biomanufacturing?

**Impact/Novelty:**
We demonstrate a robust scale up strategy using small-scale bioreactor in combination with computational modeling for pilot scale AAV production. The strategy could reduce manufacturing cost and shorten development timeline.

**Introduction:**
Adeno-associated virus (AAV) is a promising delivery vehicle for gene therapies. Although the production of AAV has been thriving, many challenges in upstream development remain, such as low productivity and inconsistent performance. Here, we demonstrate a solid case of titer enhancement and robust scale up strategy using a triple transfection system.

**Methods/Approach:**
A starting process for AAV production was established in shake flask through host cell selection, transfection system selection, and plasmid optimization. The risk during scale up was assessed by both a small-scale bioreactor system and advanced modeling technology.

**Results:**
The starting process in shake flask system achieved ddPCR titer >$1x10^{11}$ vg/mL after optimization. The HEK293 transfection system was found sensitive to shear in stirred tank bioreactor during the scale down model development with Ambr250. Thus, the scale dependent shear stress was assessed using computational fluid dynamics (CFD) model. After confirming the production performance in 5L scale, the best working condition for 50L pilot scale was proposed and validated with three successful runs.

**Conclusion:**
The scale up platform we developed will facilitate new AAV programs meeting the market demand, reducing manufacturing cost, and shortening development timeline.

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# Accelerating Clinical Readiness in Gene Therapy

**Dr Marie Clincke**[1], Dr Richard Liu[1], Dr Claudia Paindelli[1], Ms Priyanka Gupta[1], Ms Zeynep Bor Tekdemir[1], Ms Marie Jubault[1], Mr Douglas Blanton[2], Mr Alexandre Valancin[1], Dr Kyle Zingaro[1]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
Integration of early science developments into CMC development using quality by design approaches and high throughput systems can translate into an efficient development of effective manufacturing process in Advanced Therapies.

**Impact/Novelty:**
In this work we disclose the challenges, approaches and solutions associated to the introduction of new materials to transform an innovative development into a high yield industrial process.

**Introduction:**
Here we present the productivity and time benefits of embedding early research prior knowledge and innovation into CMC process development through implementation of high throughput systems and quality risk management.

**Methods/Approach:**
Development performed using a research grade process identified a promising productivity increase through the introduction of new materials into the production step of a transient transfection unit operation. Process ranges were optimized using AMBR250. Scale-up runs were executed to confirm the industrial fit and ability to meet the quality target product profile (QTPP).

**Results:**
The introduction of new raw materials could present a higher risk of release of extractables & leachables and present a risk to the patient if remaining in the final drug product. A material of contact compatibility assessment was conducted leading to the successful design of a new operation step. Small scale development runs confirmed higher productivity with the introduction of the new materials. Those studies led to the optimization of the concentration and the time of addition. Successful scale up was achieved at 50L scale demonstrating a 3X improvement in productivity. The integrated CMC development allowed to accelerate upstream process development plan and to faster initiate downstream process development.

**Conclusion:**
Capitalization on developments from research for CMC process development with the end in mind in a fast-evolving and regulatory environment can shorten First in Human ready process development timelines (~30%) while increasing productivity.

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# Microfluidic perfusion reactors used for cell line stability evaluation

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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:**
This poster will describe an innovative way to age clones in order to assess their genetic/phenotypic stability until the limit of in vitro cell age.

**Impact/Novelty:**
Clones are usually passaged in expansions that require laborious manual intervention. Here, cells are grown in 2 mL microfluidic automated perfusion bioreactors, ideal for screening of clones for perfusion processes.

**Introduction:**
The Mobius Breez biosystem is a microfluidic automated perfusion bioreactor. This device enables to run perfusion cell cultures at a working volume of 2 mL only. This poster will describe a specific use case for cell line aging and cell line stability evaluation. This information is essential for the clone selection process.

**Methods/Approach:**
Different approaches were tested to evaluate clonal stability. In one of them, each bioreactor was inoculated with a specific clone that was maintained in the systems for about 90 days to increase the population doubling level in order to mimic the in vitro cell age at the end of a cell culture production run. During this period, three timepoints were used to increase the cell density and measure titer and product quality attributes. This data was then compared to see if cell performance had significantly drifted during cell aging.

**Results:**
The system was able to maintain these perfusion cell cultures for 90 days with minimal manual intervention. Titer and quality attributes were compared at different time points and compared with traditional stability evaluation methods. The capabilities demonstrated by the Mobius Breez at early stage to evaluate clone robustness and performance seems very promising to accelerate upstream process development.

**Conclusion:**
The Mobius Breez biosystem is a viable tool to grow cells in perfusion and it could be used in a simplified workflow (no open operations under a laminar flow hood) to age cells and evaluate their productivity over a duration of almost 90 days.

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# Advancing Intensified Process Development for rAAV production through Ambr250

**Ms Paula Mateus Semedo**[1], Ms. Zeynep Bor Tekdemir[1], Mr. Daniel Sanchez[1], Mr. Douglas Blanton[1], Ms. Priyanka Amba Gupta[1], Dr. Kyle Zingaro[1], Dr. Nic Preyat[1]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected**
While process intensification is seen as a great contributor for cost reduction in rAAV manufacturing, limited efforts have been made to develop reliable and cost-effective screening models for process development.

**Impact/Novelty**
The use of perfusion at small-scale for high-yield rAAV production is limited in the existing literature. We provide evidence in support of using Ambr®250 for the development of intensified processes.

**Introduction**
Gene Therapy (GT) is an innovative and rapidly expanding field developing solutions to treat serious genetic disorders. rAAVs are associated to some of the earliest GT clinical successes due to their effective and safe profile for the introduction of genetic modification *in vivo*. The manufacturing of rAAVs, however, remains associated with numerous challenges. This includes the implementation of optimal cell culture conditions compatible with plasmid transfection at a high cell density. Process intensification from batch to perfusion-mode aims to prevent nutrient depletion and accumulation of growth-inhibiting compounds when cells are cultured at a high density. The goal of our study was to identify the process parameters leading to improved process productivity with high cell density.

**Methods/Approach**
Utilization of the Ambr®250 High-Throughput system (Sartorius) allows parallel cultivation capacity as well as a highly automated process. This system was used to test different transfection and perfusion parameters simultaneously.

**Results**
We present data from several perfusion runs demonstrating a higher productivity when compared with the baseline process and share insights gathered during perfusion implementation.

**Conclusion**
The Ambr®250 High-Throughput system proves to be a valuable approach for small-scale rAAV perfusion process development, providing rapid upstream optimization capabilities.

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# Intensified, Integrated, and Connected Processing for mAbs Purification: From Robust Process Development to Agile Biomanufacturing

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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
The work demonstrates how strategic process development, innovative processing strategy, and use of smarter equipment & process control/automation, can help achieve faster, flexible, highly efficient and biomanufacturing with smaller facilities.

**Impact/Novelty:**
Connected processing on a single automation and hardware platform enables running large scale manufacturing operations in parallel, achieving reduced footprint & intermediate storages, and >35% faster processing increasing plant capacity.

**Introduction:**
In a fast-growing biopharmaceutical world, fast paced, flexible, and cost-effective manufacturing has become an inherent requirement for industries to cater to the demand of new therapeutics for complex diseases. Taking the agile approach requires strategic implementation of smarter equipment/consumables with process intensification (PI) to catch up with the ever-evolving capacity requirements, and the pace of manufacturing.
Connected processing (PI strategy) optimizes biomanufacturing, enabling parallel execution of multiple operations for faster, more cost-effective production. The work presented here showcases optimized intensified platform at small-scale for DSP of mAbs and then it's further scale up to GMP 200L scale with connected purification with Resolute® BioSC pilot.

**Methods/Approach**
A 10L upstream batch was used to develop a DSP process in the lab using ProteinA capture, virus inactivation, Sartobind Q, and CMM HyperCel in series. The choice of the consumables and process parameters enabled seamless transition to connected process. The methodology was successfully scaled up to 200L using the Resolute® BioSC modular system. A cost and footprint analysis further demonstrated the efficiency gains of connected processing.

**Results**
For both lab and manufacturing scale, protein A using twin columns gave step recovery>94% and purity>97% by SE-HPLC. Implementation of Sartobind Q membrane chromatography in a flow through mode enabled higher flux and capacities as well as reduction in HCP/HCD. CMM HyperCel ensured direct loading from Sartobind to eliminate intermediate storage while performing consistently (purity>98.5%, recovery>95%).
Connected processing for 200L scale led to >35% reduction in DSP time against the batch process boosting the productivity. Resolute® BioSC chromatography system eased automation of 4 critical steps in GMP and reduced the DSP manufacturing footprint by ~2x.

**Conclusion**
In summary, this work demonstrates a highly integrated and scalable biomanufacturing platform that utilizes connected processing to achieve significant productivity gains, cost savings, and maintain the quality and consistency required for a DSP process.

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# Combinatorial Effects of Temperature and Osmolality on Cell Culture Performance

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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to theme selected:**
This study implemented a quality by design approach to investigate the impact of critical process parameters on cell culture performance.

**Impact/Novelty:**
Specifically, the study investigates the effects of simultaneous changes to culture temperature and osmolality on the extracellular accumulation of host cell proteins (HCPs), monoclonal antibody (mAb) yield and cell growth.

**Introduction:**
Advances in mAb production technology and manufacturing strategies have led to increased yield, which is, however, accompanied by increased levels of HCPs. Several factors have been reported to influence the generation and composition of HCPs in the supernatant. Among these are the culture temperature and osmolality. Changes in culture temperature affect cell growth and viability. Meanwhile, osmolality has a significant impact on cell growth and metabolism.

**Methods/Approach:**
We adopted a design of experiments approach implemented in MODDE12 (Sartorius) that investigates a temperature range from 32°C to 36.5°C and osmolality ranging from 410mOsm/kg to 500mOsm/kg which were manipulated on day 5 of culture. The resulting design was implemented, and samples were analyzed for viable cell density, mAb concentration and HCP concentration. The supernatant was harvested and purified and HCP: mAb ratio was quantified.

**Results:**
The integral viable cell density (IVCD) increases as temperature increases and decreases as osmolality increases. Meanwhile, hypothermia sample at controlled osmolality has high IVCD,
similar with hyperosmolar sample at controlled temperature. Interestingly, there is minimal variation in the average specific growth rate and specific productivity rate among all samples. Furthermore, most samples show decreasing HCP:mAb ratio from day 7 until harvest day. Notably, the hypothermia samples with an osmolality of less than 455 mOsm/kg exhibit the lowest final HCP:mAb ratio.

**Conclusion:**
The results may suggest that osmolality and temperature have a positive synergistic impact. Lower host cell protein concentration in cell culture could potentially ease downstream burden without compromising antibody yield.

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# Accelerating process transfer into a new large-scale cell culture facility through CFD and DoE

**Mr Chandrahas Sampelli**[1], Dr Christoph Hold[1], Dr Michael Thiele[1]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
Well-characterized bioreactors accelerate the transfer of commercial processes into a newly constructed manufacturing facility.

**Impact/Novelty:**
The direct use of construction CAD models in the MSTAR CFD software allowed the creation of digital twins and the characterization of the bioreactor of the new plant prior to commissioning.

**Introduction:**
Bioreactor vessel characterization is essential not only for equipment qualification, but also for defining optimal operating conditions to enable successful technology transfer and scale-up. Since vessel characterization is time and cost intensive, it is desirable to minimize the number of experiments. However, this limits the resolution of the operational vessel design space.

**Methods/Approach:**
A structured workflow is used to set up single-phase computational fluid dynamics simulations based on solving the Lattice Boltzmann equations in commercial software. Simulation at design points yields values for power input and mixing time, which are fed into response surface models that require at scale verification at only limited operating points.

**Results:**
Reactor models are created that cover the entire operating space and provide good correlations between simulation and experiment. This greatly reduced the experimental effort and saved time.

**Conclusion:**
The developed workflow proved to be an efficient way to improve vessel characterization in terms of both effort and accuracy, allowing for accelerated process transfer and scale-up into a newly constructed 15.000 L cell culture plant.

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# Development of a universal Raman spectroscopy model for real-time monitoring of cell culture production parameters

**Dr Laetitia Macon**[1], Dr Sylvain TRIGUEROS[1], Ms Gaelle BORDES[1]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:** Raman spectrometry has proven its usefulness to control cell culture production processes as well as optimize CHO cells performances. However, transferring models developed at lab-scale to industrial remains a challenge.
**Impact/Novelty:** Scaling up cell culture processes often involves different performances compared to lab-scale (i.e., cell growth, productivity). Monitoring and controlling key parameters with Raman spectroscopy could reduce these differences between scales.
**Introduction:** Biotherapeutics are produced by CHO cells lines. To monitor the production, samples analysis and control of key culture parameters must be done daily, with risk of contamination, high cost of measuring equipment, production of waste and presence of the operator. Our Raman spectroscopy universal model was generated with multiple and different cell lines and processes to monitor key cell culture parameters and generate process knowledge on any cell culture process (at lab-scale). In the context of transfer at larger scale, our objective is to evaluate the accuracy of the universal model versus an API (Active Pharmaceutical Ingredient)-dependent model.
**Methods/Approach:** (1) Probes emitting monochromatic light are immersed in bioreactors during biotherapeutics production with CHO cells. (2) The interaction of the laser with the sample provokes light scattering and molecule vibrations leading to their excitation or de-excitation. (3) The scattered light travels back inside the Raman analyzer through the probe to reach a Notch filter that will keep it with a frequency (hvs) different to the incident light one (hvi), called Raman scattering. (4) Raman scattering reaches a CCD detector and data are then displayed on a computer for analysis of the results in the form of a spectrum. (5) Finally, chemometrics tools such as PLS-regression are used by the Data Science team to treat spectra and build a robust and predictive Raman cell culture key parameters (Glucose, Glutamine, VCD, Lactate…)

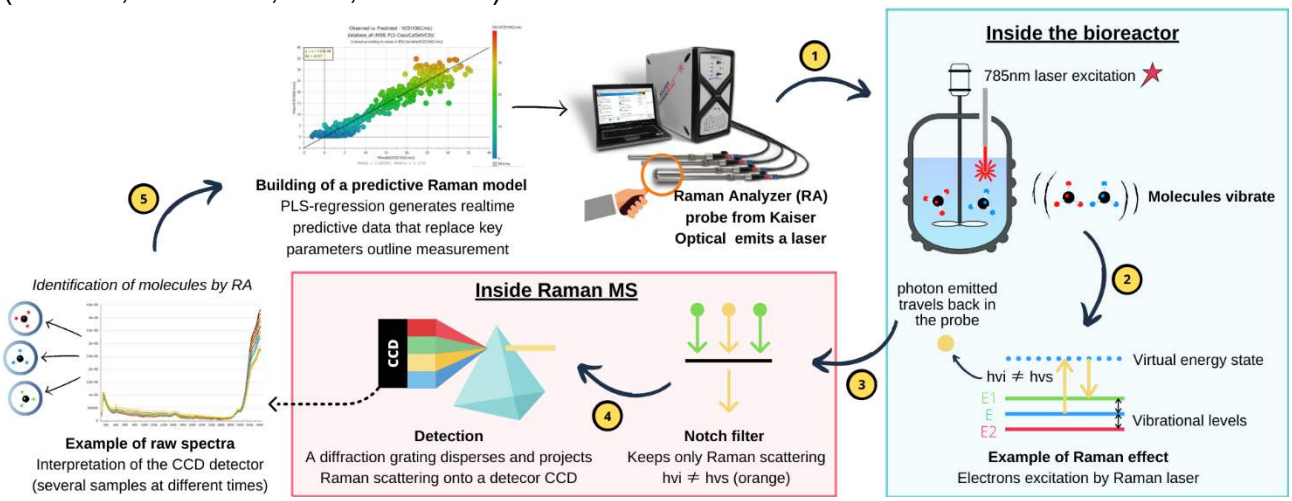

Figure 1 - Raman technology implementation PAT workflow

**Results:**

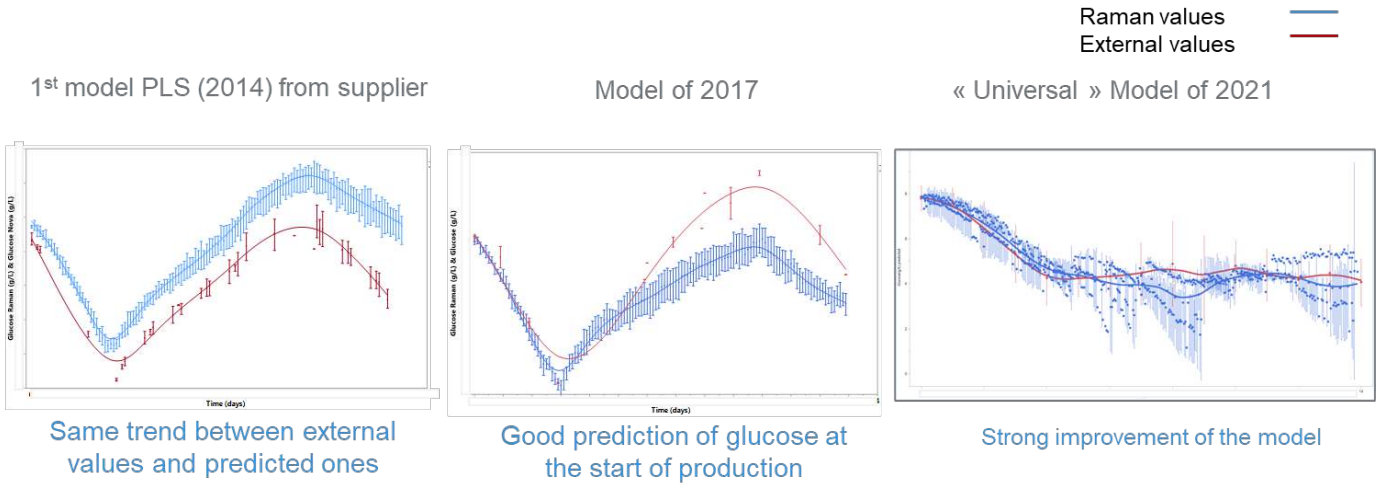


Figure 2 – Glucose concentration with external measurement method VS Raman prediction

| Model type | Nb. Prediction | Glucose | | Viable Cell Density | |
|---|---|---|---|---|---|
| | | RMSEP | $R^2$ | RMSEP | $R^2$ |
| Universal model | 96 | 0.74318 **(a)** | 0.92 | 5.51903 **(b)** | 0.51 |
| API model | 96 | 1.08413 **(c)** | 0.83 | 4.17842 **(d)** | 0.77 |

Table 1 – Performance of the Universal and API models (PLS) for the prediction of the glucose concentration and the Viable Cell Density

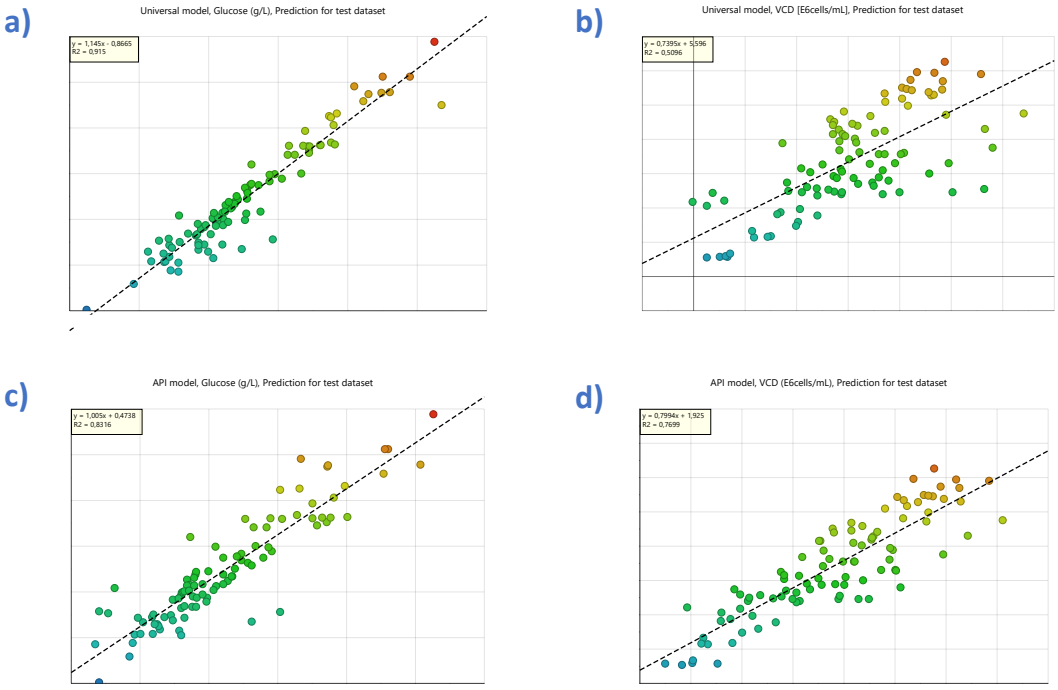


Figure 3 – Comparison of prediction between Universal VS API models

**Conclusion:** According to the type of biomarkers, both models should be used to increase process knowledge. Glucose is better predicted by the Universal model while VCD is better predicted by the API specific model. The Universal model could be used on early stage APIs as the dataset is not large enough to build a specific model (like a platform approach) while API specific models could allow new ways of working at larger scale.

303

# TheraPRO® CHO Expansion Media accelerates antibody production with user-friendly design for streamlined protein production.

**Miss. Ioanna Zormpa**, Dr Ivan Carubelli[1], Aymaan Rahman[1], Harinath Makanaboina[1], Kenneth Low[2]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected: (Transitioning from development to manufacture):**
The use of TheraPRO® CHO Expansion Medium helps to accelerate process development by being compatible with high throughput cloning platform and can be used at different scale for bioprocesses.
**Impact/Novelty:** having a ready-made bottled media system that can be used from transfection to GMP scale ensure reproducibility, ease of use and cost saving.

**Introduction:**
TheraPRO® CHO Media System is an advanced, chemically defined solution for efficient antibody therapeutics production. Compatible with GS-CHO cell lines, it enhances scalability and user-friendliness. Tailored to GS-KO cell lines, it outperforms competitors, achieving up to twice the protein titre production with high quality. The scalable process ensures greater control from cell line development to manufacturing, reducing time-to-market.

**Methods/Approach:**
Media is tested in Cell Line Development processes, including post-transfection and post-cloning using Beacon® Optofluidic system. Recovery post cloning is measured as the percentage of pens with successful single-cell cloning and growth. Recovery post transfection is determined by confluence percentage in 96-well plates. Cell stability is evaluated after aging for 20 subcultures, followed by a Fed Batch process in Ambr15 comparing growth and productivity of cells from high and low generations. Product quality is assessed through analytics like HPLC-based titre, SDS Page purity, aggregate analysis, N-Glycan UPLC, and Ic-IEF charge variants.

**Results:**
When used within the Beacon® Optofluidic system, TheraPRO® CHO Expansion Medium ensures a 50% average “in Pen” recovery 4 days post cloning. When used as an export medium after Cloning, it shows >90% viability and >2x10^6 cells/ml in a 4 days subculture regime. When cells were grown for about 20 subculture, stability was maintained for both growth, titre and product quality profile by less than 30% variation between low and high generations.

**Conclusion:**
TheraPRO® CHO Expansion Medium accelerates process development, ensuring compatibility, scalability, reproducibility, and cost savings for efficient antibody therapeutics production.

# 304
# Transcriptomic Features Reveal Molecular Signatures Associated with Recombinant Adeno-associated Virus Production in HEK293 Cells

**Dr Seongkyu Yoon**[1], Ms Yongdan Wang[1]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to theme selected (04. Transitioning from Development to Manufacture)**: The presentation deals with the challenges and solutions applied from lab-scale development to clinical scale manufacturing of a novel advanced therapy medicinal product (ATMP) in the field of virus-based immunotherapies.

**Impact/novelty**: The clinical manufacturing process we developed offers solutions for coping with the most critical safety requirements related to immuno-virotherapies besides supplying 1200+ clinical doses from one 200 L scale.

**Introduction:** Since market approval of the first oncolytic viral (OV) therapy in 2015, immuno-virotherapies are advancing with increasing importance and unprecedented speed. More than 50 clinical OV studies have already been completed, far more are currently ongoing in different clinical phases, either as monotherapy or increasingly as combination therapy. We developed a non-oncolytic immuno-virotherapy against solid tumors based on a Lymphocytic Choriomeningitis Virus (LCMV) currently being at preclinical stage. Unlike with oncolytic viruses, infection with LCMV does not directly destroy tumor cells by cell lysis, it builds-up a persistent innate and adaptive immune response directed against the tumor.

**Methods/Approach:** Virus-based immunotherapeutics are live replicating biological entities produced, stabilized, and stored at titers that easily exceed viral vaccine titers by several orders of magnitude. They vary widely in type, shape, size, size distribution and particle-to-plaque ratio. Suitable up- and downstream manufacturing technologies are required able to cope with the characteristics of live virus particles. As it applies for all parenteral products, both manufacture and storage of highly concentrated virus drug substance and drug product have to comply with highest safety and stability standards required for parenteral products.

**Results:** We established a clinical manufacturing process at clinical scale in a BSL-2 facility using fully disposable technologies for upstream and downstream manufacturing. Upon virus amplification in human cells in a stirred single-use bioreactor (SUB), two major technologies are applied for multi-stage downstream purification, hollow fiber-based tangential-flow filtration for clarification and final formulation, and monolithic chromatography for virus concentration and efficient removal of process-related impurities. The virus formulation allows for long-term liquid frozen storage without appearance of visible particles or any significant increase of subvisible particles.

**Conclusion:** Challenges during process development and scale-up have been manifold and will be discussed together with technological solutions applied to enable safe and economic clinical manufacturing of this novel immuno-virotherapy.

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# Need for Speed AND Quality: Smart Automation Selects Better Producer Clones Faster

**Dr Karsten Winkler**[1], Ms. Berit Brosemann[1], Ms. Andrea Franke[1], Ms. Anika Bauer[1], Ms. Lisa Schneid[1], Ms. Denise Resch[1], Mr. Daniel Rehm[1], Dr. Susanne Seitz[1], Dr. Annett Hillemann[1], Dr. Thomas Rose[1], Dr. Volker Sandig[1]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:** ***Automation streamlines development processes***
Selection of the right producer clone is a labor-intensive and time-consuming process. Automation streamline the entire process and enables an earlier transition to manufacturing.

**Impact/Novelty:** ***Earlier selection of clones with high productivity and desired quality***
An automation system was specifically designed to facilitate clone screening. It includes an efficient bioprocess downscale model and enables high-throughput analysis of critical protein quality attributes for a large number of clones. The system delivers better producer clones and accelerates development timelines.

**Introduction:**
The DirectedLuck® transposase system reliably delivers cell pools and clones with highest expression level. Therefore, the screening focus can be shifted towards product quality. However, targeted product characterization remains challenging and is usually limited to a small number of lead clones. We have developed an automated system that supports this screening process for large numbers of clones.

**Methods/Approach:**
A liquid handling robot based on the Biomek-i7 was combined with shaking incubators and instruments for measuring cell counts, metabolic parameters, viability and product titers. The integrated workflow ranges from 384-well plates to 96-deep well plates. This enables fully automated fed batch processes with on-demand feeding and high-throughput sampling for product characterization.

**Results:**
Producer clones generated using the automated system performed better than those generated using the traditional approach. In addition, automation enabled faster identification of clones that met the desired quality profiles in terms of biosimilarity, heterodimer content, or charge variants. The best clones showed an exceptional expression level of up to 14 g/L in a non-intensified and non-optimized fed batch process.

**Conclusion:**
The design of the automated system enables fast selection of clones that fit well into the manufacturing platform. Selected clones are extremely productive and match the desired quality profiles. Since product quality and expression are maintained at larger scales, the automation reduces USP development effort and speeds time to clinic.

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# From lab automation towards model-based process control and effective process development

**Dr. Wolfgang Sommeregger**[1], Mrs Nataša Gutmann[1], Mr. Marcus Kirschner[1], Mrs. Angélique Schmid[2]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:**
High throughput process development based on quality by design concepts requires effective software support in lab automation, data acquisition, model integration as well as for process maintenance during the experiments.
**Impact/Novelty:**
Software platform to effectively automatize high-throughput experiments seamlessly integrating data, real-time models for monitoring and control, and external analysis tools coupled with user-friendly process maintenance to save time and resources.
**Introduction:**
During bioprocess development, high data amounts are generated and obtained from various devices like bioreactors, additional sensors, and offline or at-line analysers. Often high-throughput experiments on multi-bioreactor systems are used for fast process development. Optimal process control demands real-time application of models, while further statistical evaluations are performed afterwards. Effectively handling such experiments requires a high degree of process automation, along with essential software support facilitating user-friendly process maintenance to significantly save time and resources.
**Methods/Approach:**
First, we present a software solution to automize all lab processes and support vendor-independent plug and play connection of all bioprocess entities, including control-relevant components like pumps, scales, or valves. Users can easily create recipe-based multi-reactor experiments and e.g., simultaneously perform actions like sampling generation in all bioreactors. All process data is automatically contextualized and available for user-defined models. For statistical process evaluation, relevant process parameters are published into a separate database table and are accessible by external data analysis tools for extensive process comparison.
**Results:**
We focused on an upstream bioprocess from seed to fed batch, and harvest/clarification. Using our software platform, it was possible to connect up to 90% of all data sources. We demonstrate how parallel high-throughput experiments on multi-reactor systems can comfortably be automated. Key process parameters such as total oxygen sparged are calculated automatically and monitored in real-time. Furthermore, we established automated process control e.g., for daily glucose feeding doses based on online and offline metabolite analysers.
**Conclusion:**
Bridging from automated vendor-independent data acquisition to flexible multi-experiment online monitoring and control can not only save time in the lab. Less human intervention yields to improved data integrity and ensures process robustness. Moreover, flexible online data access at any time and from various tools offers valuable insights into the ongoing experiment and enables effective process development.

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# Economic modelling of magnetic bead-based mAb manufacturing

**Dr Nils Brechmann**[1], Dr. Kristofer Eriksson[1], Mr. Torbjörn Pettersson[1]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to theme selected:**
The modelling of new DSP application is highly useful to draw conclusions on implementation and helps to save significant resources when translating technology into the manufacturing environment.

**Impact/Novelty:**
A yearlong modelled mAb manufacturing based on two different magnetic bead-based processes is investigated with their impact on COG and production throughput.

**Introduction:**
Today´ mAb marked is highly innovative, driven by the competition of emerging biosimilars. Manufactures must become more efficient and streamlined to outcompete their competitors. Previously we have shown that magnetic bead-based processing can be beneficial for the manufacturing of mAbs. But understanding the impact of a new technology on a longer production stint is essential and helps to draw conclusions under which conditions they should be implemented.

**Methods/Approach:**
In the presented study we have modelled two yearlong manufacturing stints based on magnetic separation-based antibody capture. The model was fed with key findings from the experimental data and assumptions based on a database. The output was then subject to scrutiny with regards to COG/g and annual throughput. This results in a benchmarking of the two magnetic processes against the legacy manufacturing.

**Results:**
Both modelled processes show great improvements when benchmarked to the legacy processes, reducing the COG of up to 38 % and the fixed investment of up to 17 %, while at the same time increasing the production throughput up to 40 %. More impressively, the magnetic based processes match the productivity reached by the USP, while the legacy process provides a productivity loss of around 150%.

**Conclusion:**
In conclusion, the modelled magnetic based processes show significant improvements when benchmarked against today's legacy process. Especially, when the USP dictates single high cell density harvest, the magnetic separation-based DSP provides a highly valuable option.

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# Enhancing Downstream AAV Processing: Advancements in Robust Filtration and Chromatographic Operations

**Dr Rute Castro**, Dr André Nascimento[1,2], Dr. Sónia Mendes[1,2], Dr. Tiago Faria[1,2], Dr. Ricardo Silva[1,2], Dr. Franziska Bollmann[3], Dr. Axel Thiefes[3], Dr. Marc Noverraz[4], Dr. Piergiuseppe Nestola[4], Mr. Ažbe Žnidaršič[5], Dr. Cristina Peixoto[1,2]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:** Generate alternatives to the current AAV purification scheme and increase the robustness and efficiency of critical stages in the downstream process of AAV by screening and study several materials and/or critical process parameters.
**Impact/Novelty:** Application of the design of experiments methodology to evaluate materials and purification strategies to overcome the current bottlenecks of AAV downstream process.

**Introduction:** The natural features and flexibility of Adeno associated virus (AAV) render them an attractive solution for a myriad of biotechnology and clinical applications. To give a proper answer to AAV current and future demands, improving production process efficiency, robustness and scalability are much required. Taking this into consideration, and focusing on the downstream process of AAV, we have selected critical stages of the AAV purification scheme to promote process intensification and productivity.
**Methods/Approach:** AAV harvest clarification, tangential flow filtration (TFF), and purification by chromatography were evaluated. Several filtration and purification materials and/or process parameters were screened. With the support of a design of experiment methodology the best materials and operating conditions were selected at small scape being the results confirmed at 1 liter production scale.
**Results:** A complete AAV purification scheme was developed and improved. In the harvest clarification step, a double-layer cellulose-based depth filter was capable of clarifying AAV immediately after cell lysis showing complete AAV recovery while facilitating directly a subsequent sterilizing filtration step through excellent clearance behavior. For the TFF, the use of a high throughput system enabled to establish a scalable TFF process with high impurities removal and high AAV recoveries. Finally, a two-step monolithic-based chromatography workflow was established to capture AAV and separate empty from full particles.
**Conclusions:** Having applied a structured screening methodology, supported by advanced analytical tools we were able to screen different purification materials and conditions to generate a downstream process capable of obtaining high full AAV recoveries and high protein and DNA clearance. With the work herein developed, alternatives to the current AAV harvest clarification, TFF and purification by chromatography can be applied to increase the efficiency, scalability, and throughput of AAV manufacturing.

309

# Enhancing Protein Titer in CHO Cells with a Novel Protein-Free Two-Part Feed System

**Mrs Sarya Mansour**[1], Dr. Gino Stolfa[1], Dr. Anna Barbara-Hachmann[1], Mr. Alex Fox[1], Mr. Nick Drury[1], Mr. Ryan Boniface[1]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:** By combining metabolomic analyses with high throughput processes, we have developed a novel product that consistently demonstrates enhanced titer performance in multiple CHO-K1 cell lines.

**Impact/Novelty:** We have successfully developed a two-part feed that significantly outperforms our legacy products and raises the bar for expected performance in bioproduction cell lines, maximizing titer and reducing manufacturing costs.

**Introduction:** Chinese Hamster Ovary (CHO) cells are the most widely used mammalian host in biological research, particularly for the expression of human therapeutic proteins. There is growing demand for a feed system that can support the nutritional requirements of high cell density cultures as well as yield higher protein titer. Chemically defined feeds can enhance protein titer in a broad range of cell lines ideal for industrial applications and can provide consistency, unlike non- defined formulations which can cause variations between batches and affect protein quality.

**Methods/Approach:** Using metabolomic analyses, Design of Experiment (DOE), High Throughput Screening (HTS), and bioreactor testing, we have developed a two-part chemically defined feed system that is designed to increase protein titer for CHO-K1 cell lines.

**Results:** We evaluated the performance of our two-part feed system in different industry relevant CHO K1 and CHO-K1 GS cell lines in benchtop bioreactors. Our results show 23-65% higher titer compared to internal feeds in a small-scale model and 60-64% higher titer compared to internal feeds in a benchtop bioreactor in CHO-K1 and CHO-K1 GS cell lines.

**Conclusion:** Our newly developed two-part feed system supports high titer protein production and increased cell specific productivity compared to other commercially available feeds in multiple CHO-K1, and CHO-K1 GS cell lines.

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# Generation of High-Throughput N-1 Perfusion

**Dr Stefan Wieschalka**[1], Lisa Stepper[1], Sarah Kallemeier[1], Sophie Moormann[1]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
To ensure efficient biopharmaceutical mammalian manufacturing processes, a seamless transition from cell line development to upstream manufacturing is crucial. This involves identifying the manufacturing clone in appropriate high-throughput systems. When aiming for intensified processes with a preceding n-1 perfusion step, it is advantageous to select a clone based not only on production performance but also suitability for the cell concentration step in the prestage bioreactor.

**Impact/Novelty:**
We evaluated small-scale high-throughput systems that mimic n-1 stage perfusion, to find a cost-effective and highly predictive solution for clone selection for intensified manufacturing processes.

**Introduction:**
To achieve sustainable production of high-volume biopharmaceutical products, intensified bioprocesses are recommended. Since the performance of producer cell lines can vary between intensified and non-intensified approaches, selecting the manufacturing clone in the anticipated process setup is beneficial. However, screening a large number of clones for N-1 perfusion suitability is limited by the availability of high-throughput perfusion systems and resource consumption (i.e., working hours and consumables, such as filters or media).

**Methods/Approach:**
In this study, we aimed to evaluate existing small-scale N-1 perfusion systems available on the market and prototype models from literature. Our goal was to implement a suitable system in our development procedures, mimicking cell retention and media exchanges, and to define selection criteria for this process step.

**Results:**
To generate predictive data, needed supporting the direct transfer from a clone selection to clinical supply scale, we compared ambr® 250 perfusion, ambr® 15 cell settling experiments and partially automated spin tube cultivations. We evaluated these systems based on raw material expenses, labor costs, and suitability for the intended purpose.

**Conclusion:**
Each system has its advantages and disadvantages. While ambr® 250 perfusion is costly to run, settling experiments suffer from cell loss, and working with spin tubes requires extensive labor input. We suggest using off-the-shelf systems for specific process optimization and robustness studies, rather than as screening systems. Further development of an automated spin tube model is promising for future use.

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# Further Accelerating Biologics Development from DNA to IND: Journey from COVID-19 to All Biologics Programs

**Dr Sam Zhang**[1], Dr. Kee Wee Tan[1], Mr. Pengfei Ji[1], Dr. Hang Zhou[1], Dr. Weichang Zhou[1]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:** Developed and applied innovative strategies to accelerate CMC cell line and process development with a high level of confidence.

**Impact/Novelty:** Showcased typical biologics programs can be delivered under a much-accelerated CMC timeline, leveraging the learning from COVID-19 antibody projects.

**Introduction:** Since the COVID-19 outbreak, unconventional cell line development (CLD) strategies have been taken to enable development of SARS-CoV-2-neutralizing antibodies at expedited speed.

**Methods/Approach:** We demonstrate that stable pools generated under GMP conditions exhibited similar productivity and product quality at different scales and batches, enabling rapid initiation of phase I clinical trials; clones with comparable product quality as parental pools were subsequently screened and selected for late-stage development and manufacturing. Stability study on the critical path with a shorter duration has been proved to greatly reduce the time required for final clone determination and NGS-based viral testing has been routinely used for rapid conditional release of MCB for GMP production.

**Results:** Furthermore, these approaches have been successfully implemented in some non-COVID, biologics programs with challenging timelines, resulting in much shortened development time from DNA to IND of less than 10 months.

**Conclusion:** With demonstrated product quality and consistency, we believe that innovative strategies used for COVID-19 can be widely applied in other typical biologics programs for greater speed to clinic.

**Conflict of Interest:** No conflicts to declare.

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# Downstream process development of Adenovirus-like particles (ADDomer) for snakebite therapy

**Dr Rute Castro**[1], Dr Raquel Arinto-Garcia[1,2], Ms Cláudia Paiva[1,2], Mr Emerson Dilla[3], Mr Justin Sargunas[4], Dr Mafalda Moleirinho[1,2], Prof. Paula Alves[1,2], Dr Cristina Peixoto[1,2], Dr António Roldão[1,2]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:**
This study showcases how bioprocess engineering enabled the design of a scalable, GMP-compliant setup for ADDomer purification.

**Impact/Novelty:**
The innovative purification process herein implemented can leverage biomanufacturing of ADDomers, which hold promise for development of new snakebite therapy modalities.

**Introduction:**
ADDomer is a protein-based nano-scaffold that enables multi-epitope display. While current production process (using IC-BEVS) is well established, ADDomer purification process still entails technologies that are difficult to scale-up and/or non-GMP compliant. Therefore, the aim of this study is to develop a scalable, GMP compliant purification process.

**Methods/Approach:**
Cell lysis and lysate clarification steps were optimized, with different surfactant agents being studied. Capture and polishing approaches based on ionic exchange, hydrophobic interaction and multimodal chromatography, as well as tangential flow filtration (TFF) were evaluated. Membranes of different chemistries (PES, PVDF, regenerated cellulose and cellulose acetate) were tested for sterile filtration.

**Results:**
An integrated process of cell concentration, lysis and lysate clarification was implemented, using TFF in a hollow fiber device, with the surfactant agent Deviron C-16 allowing better lysis efficiency. As capture step, anion exchange chromatography yielded the highest ADDomer purity (66 %, assessed by HPLC), which was further increased to 81 % by fine-tuning buffer conductivity and pH for binding, washing and elution. TFF membranes with pore sizes of 300, 500 and 1000 kDa were evaluated for polishing and concentration; the 1000 kDa membrane enabled high purity (97 %) and ADDomer recovery (> 85 %) in shorter processing time. Finally, the performance of sterile filtration was similar irrespectively of membrane used. Overall, this purification process yielded 45 mg of ADDomer per L of culture, with purity of 97 %.

**Conclusion:**
The innovative downstream process implemented here is both scalable and GMP compliant, holding the potential for application in an industrial setting.

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# Reaching Cell Culture Process Productivity Edge and Pushing it Further

Mr Bassem Ben Yahia[1], **Mr Antoine PIEDNOIR**[1], Mr Thomas Dahomais[1], Ms Laetitia Morel[1], Mr Neil Kearney[1], Mr Arnaldo de Souza[1], Mr Wolfgang Paul[1]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
This study focuses on addressing the manufacturing process challenges associated with monoclonal antibody production and present innovative ideas to overcomes them in order to transfer cost efficient processes.

**Impact/Novelty:**
Our study will demonstrate how media optimization, process modelling, and other tricks can overcome misincorporation, cell culture pH and clone selection challenges in order to push process productivity further.

**Introduction:**
Many process tricks to optimize volumetric productivity of cell culture processes have been developed which led to significant productivity increase. Nevertheless, as the high productivity of such processes are pushing the cells to their “limits”, the risk of reaching a productivity plateau is increased. In this presentation, we will share challenges observed for some cell culture processes where volumetric productivity of producing clones were pushed to their potential limits. Three examples of challenges will be shared i.e. (a) a first example focusing on misincorporation issue, (b) a second example focusing on cell culture pH challenges and (c) a third example focusing on simple cell culture modelling for clone selection for intensified processes.

**Methods/Approach:**
Three CHO clones producing three mAbs (mAb1, mAb2, mAb3) in Fed-Batch processes with intensified N-1 step are studied in Ambr250, 2L and 80L bioreactors.

**Results:**
In the first study (mAb1) (a), in order to reduce the level of misincorporation, multiple strategies were successfully tested, including media optimization and supplementation of metabolites, and led to significant productivity increase with reduced misincorporation level. In the second study (mAb2) (b), multiple strategies, including media optimization, were studied in order to improve cell culture pH during production leading to more than 20% productivity increase. In the last study (mAb3) (c), a simple modeling methodology using logistic equations was developed and tested in order to select the best clone leading to the highest productivity with intensified process.

**Conclusion:**
The process improvements developed over the years in the cell culture fields could lead to some process challenges. The strategies to overcome each challenge, using for instance media optimization and process modelling have lead to more than 20% productivity increase. Those strategies, included within a cell culture process toolbox, allow to develop more robust processes and to push further the cell culture process productivity “limit”.

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# Data-driven feed strategy optimisation and scale-up of mAb-expressing CHO cell line bioprocess.

**Mr Jeremie Barbau**[1], Ms Milla Neffling[1], Dr Christopher Schultz[2], Ms Bethany Kerr[2], Dr William Morgan-Evans[2], Ms Samantha Colbeck[2], Ms Ji Young Anderson-Czajkowski[1], Dr Michael Hartlep[3], Dr Wolfgang Kuennecke[3], Dr Graziella Piras[1]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
**Transitioning from development to manufacture** – understand and characterise CHO mAb-producing bioprocess with insight into specific key nutrient consumption and metabolite accumulation that enables data-driven optimisation, scale-up, and control.

**Impact/Novelty:**
A case study of previously unattainable approaches to optimise bioprocess feeding strategy to specifically address cells' needs – and implementing on-line monitoring and control of glucose to ensure bioprocess operational feasibility.

**Introduction:**
In today's bioprocessing, accelerated timelines and reduced costs are important, while maintaining product quality despite process modifications. To achieve this, at-line and on-line technologies are necessary for informing bioprocess parameters and cell-feeding strategies. The goal was to optimize monoclonal antibody production and CHO cell health through data-driven decision-making, including the selection of appropriate commercial cell culture media and feeds. At-line spent media analysis and on-line glucose/lactate monitoring are used for feeding strategy optimization.

**Methods/Approach:**
CHO GS mAb-producing cell line was used in a DOE and scale-up in Sartorius Ambr15, to Ambr250, and further to 10L stir-tank bioreactors. A cell culture media panel from Thermo Fisher Scientific was tested, and a CD feed was fed bi-daily, with key nutrients, amino acids, added separately. Three strategies for glucose feeding were tested.
908 Devices at-line and on-line analytics were used to enabled the feed-strategy optimisation.

**Results:**
Overall reduction in CD feed volume resulted in improved lactate and ammonia profiles, and thus improved cell viability (60%+) at the end of the cell culture, allowing a better environment that will protect the product from degradation and other negative impact on quality attributes. Bioprocess scaled-up was achieved with no significant changes in the key component consumption across the bioprocess scales. Target titer was maintained, and at the final stage, adding a single amino acid increased the titer by 6%. In addition to optimisation of feed volume and composition, and feeding regime, glucose feeding was optimised from daily bolus to continuous feeding. No differences in product quality were observed.

**Conclusion:**
We show accelerated process development through data-driven decision-making, achieving cost-reduction whilst improving process health by using at-line and on-line technology to characterise and control bioprocess.

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# PAT-Enhanced Perfusion Process by In-line Raman Probe and On-line Nanoplasmonic Sensor.

**Mr Maxime Hervé**[1,3], Ms. Michaela Dölle[1,3], Dr. Meeri Mäkinen[1,3], Dr. Thuy Tran[2,3], Prof. Véronique Chotteau[1,3]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:**This research addresses the critical need for advanced process analytical technologies (PAT) in bioprocess development, using on-line nanoplasmonic sensor and in-line time-gated Raman probes for perfusion processes.

**Impact/Novelty:** A novel sensor by on-line nanoplasmonic sensing for the quantification of antibodies is integrated on-line in a perfusion process, as well as an in-line time-gated Raman probe, where time-gating is applied to remove the fluorescence interference. These real-time sensors improve the monitoring in perfusion processes, offering as well the possibility of feed-back control.

**Introduction:**Perfusion processes are becoming increasingly popular in the biopharmaceutical industry for protein manufacturing. However, this technology still faces several challenges, including appropriate on-line process monitoring. We propose to address these issues through the use of an in-line time-gated Raman probe and a nanoplasmonic sensor.

**Methods/Approach:**We cultivate CHO-M cells at a 1.5L scale on perfusion mode with an Alternating Tangential Flow (ATF) system as cell-separation device. A time-gated Raman Probe is mounted in the bioreactor, enabling real-time analysis of metabolites and IgG glycosylation, based on partial least square modelling. The IgG concentration is measured in the harvest line where the on-line nanoplasmonic flow cell sensor is implemented.

**Results:**The results demonstrate the successful implementation of in-line time-gated Raman probes and on-line nanoplasmonic sensors to monitor the perfusion process. Real-time data acquisition significantly enhance the reliability and reproducibility of complex processes.

**Conclusion:**This study concludes that the incorporation of real-time monitoring sensors in perfusion process is a convenient solution to continuously monitor the process parameters. They represent key elements to set-up more robust and potentially cheaper perfusion process development. These technologies pave the way for processes empowered by feed-back control automation.

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# Case study: Improvement of powder media robustness for CHO perfusion cell culture

**Mrs Nandita Vishwanathan**[1], Mr Thomas Vuillemin, Dr Martin Jordan, Mr Raphael Guillot, Ms Prudence Bourlier, Ms Sandrine Richard, Dr Jean-Marc Bielser, Arnaud Perilleux, Jonathan Souquet
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
Media component stability is a critical material attribute for ensuring process robustness and reproducibility for biomanufacturing. Hence, it is relevant to the theme of transitioning to biomanufacturing.

**Impact/Novelty:**
Critical material attributes of complex cell culture media are often overlooked in the process control strategy, leading to unexplained process variability during routine manufacturing. The novelty is in the fact that there are not many such reports available in literature, especially for continuous manufacturing.

**Introduction:**
Biopharma is at the cusp of adopting integrated continuous processes with the hope of achieving higher efficiency, quality and consistency. High density perfusion cell culture is employed for bioproduction in continuous manufacturing of biologics. Powders for high performing perfusion media are richer in amino acids with lower proportion of inert salts, leading to new challenges for maintaining lot -to-lot consistency and stability. Our standard cell growth based stability test surprisingly did not meet acceptance criteria for our perfusion media powder despite formulation being close to our platform cell expansion medium.

**Methods/Approach:**
Our first approach was to accelerate powder aging artificially to replicate powder degradation during storage. Using our model we were able to replicate the effect observed during the cell growth test with regard to appearance and performance. Mass spectroscopic analysis was employed to detect majority of components and discover a few expected degradation products.

**Results:**
The reduced performance of the medium was correlated to the reduced concentration (i.e., stability) of one highly reactive medium component. In the ‘stressed’ conditions, the component was reduced to 20% of its target concentration. The aging of the powder had an impact on the cell growth and titer, but only limited impact on product quality in our screening model. While tested in a perfusion process, the impact on ‘growth phase’ and ‘production phase’ were significantly different (same media is used for both growth and production).

**Conclusion:**
Temperature and humidity are critical factors for the stability of complex media powders. From the results of this investigation, we were able to develop a new formulation that has potentially lesser sensitivity to degradation during storage by adding back certain components of our cell expansion medium which were removed, replacing certain raw materials and improving handling and storage of powders.

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# Achieving High Productivities Through Bioreactor Optimization at Lower Peak Cell Densities

**Dr Luis Rodriguez**[1], Ociel Ferreyra[1], Ericka Agredano[1], Joshua Yokoo[1], Kristi Wong[1], Catherine Nguyen[1], Isabelle Jones[1], Raul Tholley[1]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
Scaling up a perfusion process.

**Impact/Novelty:**
A perfusion process can achieve high productivities at suboptimal peak cell densities.

**Introduction:**
A renewed interest in perfusion cell culture technologies for continuous production of therapeutic recombinant proteins has led to the development of cell culture media that can support high cell densities. Perfusion is also being driven by the biopharmaceutical industry moving towards more complex protein forms including bispecific and fusion proteins, while requiring a need for flexibility in the production process and intensified manufacturing processes. However, implementing a perfusion process instead of a traditional fed-batch process requires the development of the process prior to scaling up to a production bioreactor.

**Methods/Approach:**
A CHO perfusion media was evaluated with perfusion mimic models and an alternating tangential flow filtration perfusion capable tabletop bioreactor in both an N-1 perfusion process and production of a recombinant antibody. Cell culture mini bioreactor centrifuge tubes and an Ambr15 micro bioreactor system were utilized to maximize cell densities and attain initial bioreactor parameters, respectively. The Ambr15 parameters were optimized on a 0.25L perfusion capable tabletop bioreactor to achieve improved cell-specific productivity (qP), volumetric productivity (VP), and cell specific perfusion rate (CSPR).

**Results:**
We have demonstrated the optimization of a perfusion process in a tabletop perfusion capable bioreactor, utilizing small scale perfusion mimic models to assess the cell culture medium. We assayed peak cell densities in an N-1 perfusion process, while demonstrating how productivity is related to various metabolites, glucose, and aeration during a continuous steady-state culture. Finally, we achieved high productivities in a continuous steady-state culture without maintaining peak cell densities.

**Conclusion:**
Our results demonstrate that success of a continuous steady state perfusion culture can be achieved through the optimization of bioreactor parameters without achieving peak cell densities.

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# Advancements in Microcarrier based stem cell culture process and effortless transition to 10L single-use bioreactors

**Ms Namitha Haridas**[1]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Scientific Topic 4: Transitioning from development to manufacture – How do science and innovation translate into highly efficient biomanufacturing.**

**Impact/Novelty:**
To smoothly implement complex cell culture processes at a higher scale, it is imperative for the bioreactor to ensure accurate control of crucial process parameters, including oxygen supply, perfusion rate, and pressure. This study exemplifies the successful transition from 2D to 3D process from development to production scale, with a focus on adeptly controlling these vital parameters.

**Introduction:**
Stem cells isolated in Sartorius' Process Development lab were used for 2D and 3D scale process development. Process optimization was conducted in a prototype Single-use (SU) UniVessel 10L bioreactor using Sartorius' cell culture media and microcarriers.

**Methods:**
Vessel integrity was confirmed by performing a pressure check with the BioStat® B control tower prior to culture initiation. Samples from the SU vessels were retrieved daily to assess cell growth, viability, and nutrient metabolite profiles. A 50% media exchange was performed on day 3or4, and cells were harvested from microcarriers on day 6.

**Results:**
The process utilized in the 10L SU prototype vessel was characterized by its scalability in terms of growth performance. With the enhanced gassing parameters used, the culture was maintained for 6 days, and the growth profile obtained was comparable to results achieved in other Sartorius bioreactors. Viability of > 90% was obtained daily and the stemness nature of cells after culture was confirmed by using the iQue screener plus and a stem cell-specific antibody marker panel for analysis.

**Conclusion:**
The results presented in this study suggest that upon commercialization, the 10L represents an ideal production bioreactor for manufacture of intermediate-scale products and will serve as an excellent n-1 vessel for production of cells in larger vessels.

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# Delivering CMC Initiatives using Design for Manufacturing, Modern Design of Experiments, Prediction, and Machine Learning

**Dr. Tiffany Rau**[1,2], Dr. Philip Ramsey[3]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**

Addresses design for manufacturability, CMC, challenges of new modalities, data analysis, predictive modelling, and scale-down models.

**Impact/Novelty:**

New techniques in data analysis and experimental design are needed to address small highly interactive data sets such as SVEM in addition, efficient Space Filling Designs are needed.

**Introduction:**
The bioprocessing space is extensive and diverse but whether you are developing a monoclonal antibody, a cell therapy, a gene therapy, a vaccine, a protein, mRNA, or something that cannot even be imagined today…. we have similar opportunities and challenges in the areas of development, manufacturing, and regulatory compliance (CMC). It is critical that the discoveries and learnings that are made early in the process are leveraged throughout the development program.

**Methods/Approach:**
For US FDA regulated products, the development must include process characterization before filing a Biologic Licensing Application (BLA) with the agency and leveraging data obtained throughout the process not only de-risks the scale-up and further development but also supports the process characterization activity. Characterization, optimization, and long-term control of these processes requires several things such as benchtop experiments, Design of Experiments, scale down models and scale-up models, analytical development, and the development of accurate and validated predictive models. Process Development and later characterization is critical but often the learnings from the work are not always incorporated into an effective predictive model for multiple stakeholders. Another challenge is the data sets are often small especially in Cell Therapies and thus new and novel methods must be used to analyze the data and then generate predictive models; we introduce a new machine learning method for predictive modelling with an emphasize on small data referred to as *self-validating ensemble modelling* (SVEM).

**Results:**
In this presentation, we propose by using highly efficient space filling experimental designs combined with predictive modelling methods from machine learning, cost effective and reliable predictive models can be developed. The models subsequently may be used for scale-down modelling, process characterization, optimization, and identification of critical process parameters for manufacturing control strategies.

**Conclusion:**
A case study will be presented showing how predictive modelling can support CMC initiatives.

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# Reduction of LMW species during upstream process

**Mr Thomas Vuillemin**[1], Dr Martin Jordan[1], Dr Nandita Vishwanathan[1], Dr Jean-Marc Bielser[1], Mr Arnaud Perilleux[1], Dr Jonathan Souquet[1]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
Presentation of a design of experiments (DoE) that explores the main effects and interactions of various factors in order to reduce low molecular weights.

**Impact/Novelty:**
A single experiment leveraging robotic scale (Ambr250) was used to define a second-generation process to reduce LMW species from the upstream process.

**Introduction:**
Low Molecular Weight (LMW) species are byproducts of bioprocess mainly produced during upstream process and are hard to purify. They consist of partial protein of interest. For mAb, they are generated either by proteolytic digestion or by reducing the disulfide bond and present various variants. It is crucial to prevent the appearance of LMW as they impact the safety of the drug. On this poster, we proposed to investigate the medium and feed composition as well as process parameters to reduce the amounts of LMW induced by disulfide bond reduction.

**Methods/Approach:**
A design of experiment was performed using 4 different factors including process parameters, media component concentration and media component form (pure or complex). Up to three levels of each factor were evaluated. The experiment was conducted in ambr250. The process was locked after the DoE and the stability of the new formulation was also assessed in 3.5L bioreactors. All experiments were done in fed-batch mode. Cultures were sampled to count cells and to quantify metabolites, amino acids, mAb titer and LMW each day.

**Results:**
A LMW decrease was observed with the modulation of the level of the media component form.

**Conclusion:**
A one-step DoE can be applied to fine-tune product quality for fedbatch processes. Such approaches are highly valuable to improve processes within tight timelines.

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# Adeno-associated Virus Production in Suspension Cell Culture Using Bioprocess Control Systems and BioBLU® Single-Use Bioreactors

**Mr David Solbach**[1], Mr Jorge Escobar[2]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:**
Transitioning of viral vector production from research to production requires sufficient cell numbers. Here we present a cultivation method that allows process scale-up in a reproducible manner.

**Impact/Novelty:**
Establishing large-scale AAV production processes requires scalable systems and precise parameter control. We demonstrate the feasibility of a controllable and scalable production platform based on suspension cultures and stirred-tank bioreactors.

**Introduction:**
Mammalian cells represent one of the most important manufacturing platforms for vaccine and gene therapy developers. Especially, HEK293 cells are an attractive host for numerous biotherapeutic platforms. They are easy to transfect and can produce large amounts of recombinant proteins or virus particles. However, a major limitation of this adherent cell line is its tendency to clump when converted to suspension format. This limitation can be overcome by suspension-adapted HEK293 cells, such as the Expi293F cell line.

**Method/Approach:**
The bench scale BioBLU Single-Use Bioreactors regulated by Eppendorf bioprocess controllers were utilized to establish an Expi293F cell culture fit for the production of adeno-associated virus (AAV) capsids. Such cell culture-produced AAV vectors are an integral tool for both vaccine production and gene therapy development. First, the growth properties of the Expi293F cell line were evaluated in a batch culture system by employing the precise process parameter control properties of the bioprocess controllers. Subsequently, an AAV capsid production platform was established, using a helper-free AAV system.

**Results:**
Batch culture under precise parameter control resulted in a peak density of $13 \times 10^6$ viable cells/mL culture volume.
A robust AAV capsid titer of around $10^{12}$ capsids/mL was reached, which is in line with typical industrial yields and much more efficient than uncontrolled shake flask culture.

**Conclusion:**
This study underscores a suspension culture-based viral vector production platform in stirred-tank bioreactors as a more advantageous alternative to attachment culture approaches.

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# Influence of animal origin free (AOF) peptone supplementation on CHO productivity and glycosylation profiles

**Maren Grün**[1], Ashwin Gurunathan[2], PhD Anna-Barbara Hachmann[3], Bilal Mughal[2], Saranya Chitturi[2], Meagan Haynes[2], Alexander Fox[3], Megan Pajak-Lee[3], Stacy Holdread[2], PhD James Brooks[2]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
The use of peptones as either media supplement or feed for Chinese Hamster Ovary (CHO) cell-based protein production processes can increase product titer and improve product quality attributes.

**Impact/Novelty:**
Whilst peptones are commonly used in microbial fermentation processes, this poster showcases their versatility and ability to also improve CHO-based protein production.

**Introduction:**
The current demand in the bioproduction industry is to produce high quality monoclonal antibodies (mAb) both in terms of high titer and desirable protein quality profiles. CHO cell lines have been the workhorse of the pharmaceutical industry to produce them. Desired attributes can be achieved by cell line engineering, process optimization or media supplementation and feed optimization. Whilst cell line engineering and process optimization is a time-consuming process, media, supplements and feeding optimization provides a quick solution to achieve high titer and alter product quality attributes. Here, we investigated the impact of peptone (protein hydrolysates) supplementation on mAb production and quality attributes.

**Methods/Approach:**
Different mAb producing CHO cell lines were cultivated using respective chemically defined media and feeds, whilst adding distinct animal origin free (AOF) peptones as either supplement or feed. During this, cell growth, productivity, glycosylation profiles and charge variants were measured and analysed.

**Results:**
The obtained data presented here strongly demonstrate that peptones can be used to improve cell growth and mAb titer. Furthermore, they also can modulate glycosylation profiles and positively impact charge variants.

**Conclusion:**
The poster describes the impact that AOF peptones have on viable cell density, viability, mAb titer and glycosylation profiles. We also see the various ways a peptone maybe be used in conjunction to a chemically defined feed and the benefits it offers in terms of boosting mAb titer. Thus, targeted supplementation of CHO cell cultures with peptone supplements can improve productivity and glycosylation profiles.

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# Seed Train Intensification and Production Process Automation for Pilot-Scale Fed-Batch and Perfusion Processes

**Mr Jan Ott**[1], Vivian Ott[1], Christopher Brau[2], Sandra Beyer[3], Prof. Dr. Dieter Eibl[1], Prof. Dr. Regine Eibl[1]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
Approaches for scaling highly efficient intensified mAb production processes with CHO cells from lab to pilot scale are presented with glucose and cell bleed automation.

**Impact/Novelty:**
High cell density cryovials and the 10:1 turndown ratio of the DynaDrive Single-Use Bioreactor allow for direct inoculation of a 50 L Bioreactor from a single vial.

**Introduction:**
Many approaches are currently aimed at intensifying biopharmaceutical production processes. Three areas are targeted in the upstream process: 1. the cryopreservation, 2. the seed train, and 3. the production process.

**Methods/Approach:**
An established ultra-high cell density cell bank and the 10:1 turndown ratio of the DynaDrive Single-Use Bioreactor (Thermo Scientific) were used to eliminate the seed train prior to the 50 L production bioreactor. For this purpose, the 50 L bioreactor was inoculated with a 5 mL cryovial containing 260x$10^6$ cells/mL at 5 L starting volume. To demonstrate the scalability, the procedure was also tested in an ambr vessel (Sartorius). Additionally, 50-day perfusion processes, which were fully automated in cell bleeding and glucose addition, were performed in the 50 L DynaDrive and a 2 L glass bioreactor.

**Results:**
Scaling approaches yielded comparable results across lab and pilot scale. The cryovial inoculation of the 50 L DynaDrive could reduce the time for the seed train by 60% while achieving the same IgG titres as in the standard fed-batch experiments. In the perfusion experiments, more than 1 g/L/d IgG could be produced while sustaining >100 $mm^3$/mL viable cell volume.

**Conclusion:**
Intensified cell banks and modern single-use bioreactors enable seed train bioreactors to be omitted. In addition, highly productive long-term perfusion processes can further increase the efficiency of production processes and are amicable to labour and risk saving automation approaches.

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# Development of a high-titer Bi-paratopic tetravalent diabody-Fc-Fab: IcoCell Line, Design for Manufacturability and Modern DOE

**Dr. Eva-maria Tombak**[1], Evaliisa Sejev[1], Jelizaveta Geimanen[1], Katrin Orro[1], Andres Männik[1], Mihhail Kurašin[1], Kristo Kuus[1], Kiira Gildemann[1], Nele Tamberg[1], Evelin Väljaots[1], Shane Miersch[2], Sachdev Sidhu[2]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:**
Addresses methods to streamline development and tech transfer to manufacturing, design for manufacturability, high-throughput screening, complex “molecules” that do not fit historic IgG1 platforms, data analysis, Modern DOE.

**Impact/Novelty:**
Co-location of process development and manufacturing teams along with new cell lines, high-throughput PD and modern DOE and machine learning allows non-platform processes to be developed, transferred and scaled-up faster.

**Introduction:**
Many organizations have built platforms around IgG1 but the platforms often become irrelevant when moving to “off platform” molecules. One needs to allow for additional time to deliver the process and also entertain the possibility of lower titers and challenges in purification for non-IgG1 and non-IgG4 molecule types.

**Methods/Approach:** (heading must be in bold)
It is critical to build flexibility during cell line development and process development to account for opportunities which one may not be realized if a platform process is only used An additional way of increasing the likelihood of success for complex molecules is co-locating development and manufacturing allowing for collaboration and decreases the complexity of transitioning a molecule to manufacturing which is not platform and requires modifications in feeding strategies, gassing strategies and purification methods for example.

**Results:**
An example of a “difficult to express” molecule Bi-paratopic tetravalent diabody-Fc-Fab (198 kDa) where over 4 g/l was obtained using the IcoCell CHO cell line with minimum process development will be presented in a streamlined fashion and how Modern DoE was leveraged for data analysis.

**Conclusion:**
It will be described how early in the upstream process development the purification team was engaged to deliver a purification strategy that not only met the needs of the process but was also designed to be implemented into a future manufacturing environment. Non-platform molecules are more challenging than platform IgG molecules, but they offer many advantages i.e. right fit for the product and thus the development and commercialization must fit both.

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# Chicken Feather Follicle Cells as a source of fat for cultivated meat

**Mr Hélder Bandarra-Tavares**[1,2,3], Prof. Pedro Fonte[3], Dr. Ana Fernandes-Platzgummer[1,2]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
Finding suitable cells to produce cultivated fat is necessary to manufacture attractive cultivated products. The expansion and adipogenic differentiation of cells derived from chicken feathers were assessed and optimized.

**Impact/Novelty:**
Chicken feathers can be sourced in a minimally invasive way, unlike other cell sources with adipogenic potential, like the bone marrow. Their differentiation and proliferation capacity *in vitro* were demonstrated.

**Introduction:**
Globally, more land is dedicated to the production of animal-based food than any other application. Global meat production and consumption is increasing, and forecasts show that it will more than double by 2050. Consequently, there has been a growing interest in cultivated meat. However, the best cell sources and are still under debate. Their choice must account for their availability, isolation process, proliferation, and differentiation potential, with myogenic and adipogenic lineages being fundamental to achieve meat-like properties.

**Methods/Approach:**
Chicken Feather Follicle Cells (cFFC) were isolated from pin feathers through enzymatic digestion with collagenase IV and expanded with DMEM supplemented with 10% and 20% fetal bovine serum (FBS) combined with three coating substrates (no coating, gelatin and laminin). Afterwards, cFFC were characterized with flow cytometry and PCR and differentiated towards the adipogenic line.

**Results:**
DMEM+20%FBS with gelatin coating was the best culture condition, reaching 13.9 total population doublings over 6 passages. cFFC tested positive for the surface markers CD73, CD44, CD90, CD29, CD105, CD56, CD31 and CD45. Most importantly, cFFC presented the ability to commit towards the adipogenic lineage, forming lipid vacuoles within the cells.

**Conclusion:**
cFFC's proliferation and adipogenic differentiation potential makes them suitable candidates for manufacturing cultivated fat for cultivated meat products. Future studies will focus on cFFC expansion and differentiation under dynamic conditions with appropriate microcarriers. Their organoleptic and nutritional properties will be optimized for incorporation into final products.

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# Development of a scalable platform for the production of bovine fat for cultivated meat

**Mr Hélder Bandarra-Tavares**[1,2,3], Mr. Pedro Agostinho[1,2], Prof. Pedro Fonte[3], Dr. Ana Fernandes-Platzgummer[1,2]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
Cultivated meat is a promising alternative to conventional meat, and achieving large-scale production is crucial. The expansion of bovine mesenchymal stromal cells (bMSC) under dynamic conditions was assessed and optimized.

**Impact/Novelty:**
bMSC from the umbilical cord, a minimally invasive source, were isolated with no impact on animal welfare. Their adipogenic differentiation potential makes them prime options for cultivated fat production.

**Introduction:**
There has been an increasing interest in cellular agriculture, which aims to overcome most issues associated with livestock meat consumption, such as its environmental impact, risk of foodborne diseases, and animal slaughtering. Different approaches have been attempted to produce cultivated meat, but high production costs and scalability limitations have hampered this technology.

**Methods/Approach:**
bMSC were isolated from the umbilical cord Wharton's Jelly (bMSC(WJ)) using explant culture and enzymatic digestion with collagenase IV. Afterwards, cells were characterized according to the expression of key MSC markers (CD105, CD90, CD29, CD44), absence of endothelial and hematopoietic markers (CD31, CD45), and differentiation potential. Finally, bMSC(WJ) proliferation was optimized under static and dynamic conditions, using dissolvable microcarriers in the latter to facilitate cell harvesting.

**Results:**
bMSC(WJ) were successfully cultured throughout multiple passages with culture medium supplemented with 20% fetal bovine serum. Culture conditions were optimized by adding growth factors (FGF, IGF, VEGF, HGF, PDGF) previously described to improve MSC proliferation. Supplementation with FGF-2 was found to be the optimal condition for bMSC(WJ) growth and was used under dynamic conditions, achieving a cell fold increase of 13.8 and a doubling time of 1.2 days. Adipogenic differentiation was successfully performed in static conditions.

**Conclusion:**
bMSC(WJ) were successfully expanded in dynamic culture. Coupled with their adipogenic differentiation potential, this paves the way for the development of a platform to produce cultivated fat from a sustainable source without compromising animal welfare.

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# Scale-up or Scale-down? - Biomanufacturing Process Intensification by Converting Fed-batch Into High-density Perfusion Processes

**Ms Caroline Noack**[1], Mr. Lukas Hostettler[1], Mr Frederic Houioua[1]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:**
Innovative process design principles, such as intensification of upstream processes using high-density perfusion, can significantly benefit the manufacturing of therapeutic proteins. A glimpse into the potential of perfusion process intensification.

**Impact/Novelty:**
The presented study aims to contribute to an understanding of how converting existing fed-batch production processes into high-density perfusion can be leveraged to improve manufacturing agility and sustainability.

**Introduction:**
Innovative process design principles, such as intensification of upstream processes using high-density perfusion, can significantly benefit the manufacturing of therapeutic proteins. The presented study aims to contribute to a better understanding of how converting existing fed-batch production processes into high-density perfusion can be leveraged to improve manufacturing agility, cost, and sustainability.

**Methods/Approach:**
Several CHO cell lines expressing different therapeutic protein formats were generated using Novartis' proprietary cell line development technology and fed-batch process platform. To further optimize these production processes and improve general manufacturing agility, cost, and sustainability, a conversion into high-density perfusion including scale-up to manufacturing scale was performed.

**Results:**
A direct comparison of process performance between fed-batch and high-density perfusion biomanufacturing processes was conducted. The results of the intensified process indicate a significant multiple increase in product yield, independent of the produced target protein.

**Conclusion:**
In this study, we show that successful intensification of biomanufacturing processes – by converting the operational mode from fed-batch to high-density perfusion – can be an efficient tool to significantly increase therapeutic protein yields.

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# Continuous Production of Influenza VLPs Using IC-BEVS: a Multi-Stage Bioreactor Approach

**Dr Ricardo Correia**[1,2], Ms Taja Zotler[3], Mr Miguel Graça[1,2], Dr Bárbara Fernandes[1,2], Dr Gorben Pijlman[3], Dr António Roldão[1,2]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:** As the biotechnology field advances towards implementation of intensified bioprocesses, continuous operations like the one herein described arise as leading modalities for efficient biomanufacturing.

**Impact/Novelty:** This work showcases an efficient continuous multi-stage bioreactor process using the insect cell-baculovirus expression vector system (IC-BEVS), expanding the applicability of this expression system for process intensification approaches.

**Introduction:** The lytic nature of IC-BEVS limits its transition from traditional batch-based to intensified continuous bioprocessing. Cascaded bioreactor setups have mitigated the limitations of viral systems for continuous operation but have not yet been efficiently applied to IC-BEVS.

**Methods/Approach:** A continuous multi-stage bioreactor process was established to produce influenza hemagglutinin-displaying virus like particles (HA-VLPs) using IC-BEVS. Two process designs and three residence times (RT) (18, 36, and 54 hours) were tested. Design 1 comprised neutral pH-adapted insect High Five cells and a recombinant baculovirus (rBAC) generated via Bac-to-Bac technology, which previously allowed 3-fold higher productivity. Design 2 comprised insect Sf9 cells and a rBAC generated with flashBAC™ technology.

**Results:** Processes were efficiently operated in continuous mode for 14 (design 1) and 20 (design 2) days; cell growth kinetics and viability varied across RT. rBAC titers were consistent in design 2 ($10^8$-$10^9$ pfu/mL), unlike in design 1. Higher RT resulted in increased expression of HA-VLPs. Production of HA-VLPs was consistent during operation using design 2 (34 ± 14 HA titer/mL); in design 1, a decreasing trend in HA-VLPs production was noted. The presence of particles resembling HA-VLPs was confirmed by transmission electron microscopy throughout both continuous operations.

**Conclusion:** This work shows that process design was key for the successful continuous HA-VLPs production using IC-BEVS, and paves the way for establishing continuous, integrated setups using this expression system.

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# Massive hiPSC-derived Cardiomyocyte Production for Regenerative Medicine: combining WNT Activation and Oxygen Modulation in Bioreactors

**Miss Lara Inocêncio**[1,2], Mr Pedro Vicente[1,2], Miss Beatriz Gamelas[1,2], Mr. Asier Ullate-Agote[3], Mr Olalla Iglesias García[3], Mr Patxi Martin-Uriz[3], Mr Felipe Prósper[3], Mr Manuel M Mazo Vega[3], Prof. Paula Alves[1,2], Dr Margarida Serra[1,2]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
We developed a scalable bioprocess to expand hiPSC-CMs with high purity, functionality, and high therapeutic potential for cardiac regenerative medicine.

**Impact/Novelty:**
We demonstrate for the first time the importance of combining sustained Wnt activation with dissolved oxygen control to establish a robust bioprocess for massive expansion of hiPSC-CMs in scalable STBs.

**Introduction:**
The use of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) has shown potential to improve cardiac regeneration after myocardial infarction. However, producing enough cardiomyocytes (> 1 billion) is extremely laborious, hampering the clinical use of this technology. Here, we developed a bioreactor-based protocol that combines continuous WnT activation and oxygen control to create a scalable and reliable process, while improving hiPSC-CMs' expansion and quality attributes.

**Methods/Approach:**
hiPSC-CMs were cultured as 3D aggregates in 200mL stirred-tank bioreactors (STBs). hiPSC-CM expansion was induced by CHIR99021 addition through perfusion, and aiming at bioprocess intensification we modulated the dissolved oxygen (DO) concentration. We then scaled-up the bioprocess 10x in single-use 2L STBs, keeping power input per volume constant. The produced hiPSC-CMs phenotype was characterized by flow cytometry, immunofluorescence microscopy, RNA-seq analysis and calcium imaging.
**Results:**
When DO was controlled at mild-hypoxic levels (10%$O_2$), a reduction of 30% in reactive oxygen species production together with a 70% increase in the concentration of proliferative cells (Ki67+ cells) was observed when compared to the hiPSC-CMs cultured in STBs operated under atmospheric normoxia (21%$O_2$). After 11 days of culture, a higher hiPSC-CM expansion factor was attained in STB-10%$O_2$ (10.3) relative to STB-21%$O_2$ (4.4) and static culture (3.6). STB cultures also present a higher percentage of cells positive for cardiac markers (>98%), cTnT and α-actinin, when compared to the static cultures (~90%). hiPSC-CM aggregates showed spontaneous beating, expressed cardiac markers, and were able to mature in culture, revealing improved gene expression, sarcomere alignment, and cardiac calcium transient. Scale-up was successfully performed, confirming the feasibility of the optimized bioprocess to produce clinically relevant numbers of hiPSC-CMs.
**Conclusion:**
In this work we rationally developed a robust STB-based bioprocess to produce billions of hiPSC-CMs, showing the importance of controlling DO at low levels to boost hiPSC-CMs' expansion and their quality attributes.

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# Optimizing Mammalian Cell Culture Strategies: Validation of a Model-Based Platform for Medium and Feeding Design

**Dr Ivan Paredes**[1,3], Mr. Temístocles Molina[1,3], Mr. Aarón Canales[2,3], Ms. Francisca Pizarro[2,3], Mr. Bastian Herrera[2,3], Ms Isidora Prat[1,3], Dr J. Cristian Salgado[2,3], Dr. Ziomara Gerdtzen[1,3]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
A methodology is implemented to determine cell culture requirements, optimize media composition, and refine feeding strategies, for enhancing productivity in bioreactors using a model-based platform.

**Impact/Novelty:**
By integrating cell and product data into a cell-line-specific dynamic model, we can predict culture requirements, to design a customized media and feeding strategy for enhancing productivity.

**Introduction:**
The continuous growth of the biopharmaceutical industry emphasizes the need to enhance production strategies, prioritizing effectiveness and efficiency in bioprocess design. Our efforts center around optimizing bioproduct production through the assessment of culture requirements, and the employment of a cell line specific model-based platform to determine a culture media composition and feeding strategy that improves productivity in the production system of interest.

**Methods/Approach:**
Batch cultures were performed for the selected cell lines in 500 mL bioreactors, monitoring biomass, nutrient consumption, and production to determine cellular requirements for growth and product synthesis. Subsequently, a specific model was fitted for each cell line and applied for the identification of a media composition and feeding strategy to optimize biomass and/or product yield. This methodology was applied to CHO-tPA, PK15, and Hi5 cell lines, and a similar methodology was applied to hMSC cells.

**Results:**
In the case of PK15, the optimized fed-batch culture exhibited a 69% increase in biomass compared to batch and a 35% increase compared to non-optimized fed-batch cultures. Meanwhile, for CHO-tPA, the optimized approach resulted in a 46% boost in biomass compared to batch and a 24% increase compared to non-optimized cultures. Additionally, for hMSC a 52% reduction in media requirement was achieved.

**Conclusion:**
Results show that implementing a cell-line-specific feeding strategy through a model-based platform, leads to a significant increase in bioproduct yield and process efficiency.

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# Advancing Manufacture of hiPSC-Hepatocytes with improved functionality: a nature-inspired bioprocess

**Dr Margarida Serra**[1,2], Dr Joana Almeida[1,2], Mr Pedro Vicente[1,2], Ms Inês Crespo[1,2], Dr Nikolaus Virgolini[1,2], Dr Inês Isidro[1,2], Dr Juan R. Rodriguez-Madoz[3], Dr Felipe Prosper[3], Dr Pedro Baptista[4], Prof Paula Alves[1,2]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
Theme 4 - Transitioning from development to manufacture – How do science and innovation translate into highly efficient biomanufacturing?
This work aims to use the scientific knowledge of liver development biology to develop novel and efficient bioprocesses (nature-inspired bioprocesses) to produce hiPSC-derived hepatocytes with high quality.

**Impact/Novelty:**
We show the importance of modulating key environmental factors (oxygen and microbiome) to improve hepatocyte differentiation and cells' quality attributes and identify the cell maturation stage that ensures efficient engraftment.

Introduction
Hepatocytes differentiated from human induced pluripotent stem cells (hiPSC-Hep) provide unprecedented opportunities for hepatic cell-based therapies. However, the hybrid fetal-adult phenotype of these cells, the lack of reproducible protocols for their scalable production and the challenges regarding their engraftment ability are hampering a faster clinical application. In this work, we designed a nature-inspired strategy and combined advanced manufacturing platforms with omics technologies and process analytical tools to better recapitulate and monitor the microenvironment of physiological liver development during hiPSC-Hep bioprocessing.

Methods/Approach
hiPSC-Hep were cultured as 3D cell aggregates in stirred-tank bioreactors (STB) operated in perfusion and equipped with a capacitance probe. The hepatic differentiation/maturation steps were optimized by controlling the dissolved oxygen and by culturing hiPSC-Hep with the secretome of human intestinal microbiota, recapitulating what happens in liver development.

Results
When dissolved oxygen was controlled at physiologic levels, higher hiPSC-Hep production ($2\times10^6$ cell/mL) and differentiation efficiencies (> 80% $Albumin^+$ cells) were obtained when compared to uncontrolled condition ($0.6\times10^6$ cell/mL, <45% $Albumin^+$ cells). The generated hiPSC-Hep showed synthesis of key hepatic proteins (albumin, alpha 1 antitrypsin), urea and bile acids secretion and drug metabolization capacity, CYP450 activity and glycogen storage. Our results also show a good correlation between the cell permittivity measured online and the aggregate biovolume measured by offline methods, demonstrating the potential of dielectric spectroscopy to monitor hiPSC expansion/differentiation in STB.
hiPSC-Hep treated with microbiome secretome showed improved expression of critical hallmarks of human hepatocytes. Finally, we used transcriptomic analysis (RNA-Seq) to confirm that hiPSC-Hep maturation levels modulate the "machinery" that mediates cell engraftment and identify the cell

maturation stage that ensures efficient cell' engraftment in vitro and in vivo, with preservation of hepatic functionality.

Conclusion

We show that a rational modulation of key environmental factors (dissolved oxygen and gut microbiome) is critical to improve hPSC-Hepatocytes bioprocessing and cells' quality attributes.

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# Single-Use Bioreactors designed according to traditional bioreactor principles facilitate transition from development to production scale.

**Dr Alicja Zimmer**[1], Mr. Dai Quach[2], Mr. Jorg Bolder[1], Dr. Sufia Karim[2]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Impact/Novelty:** Single-Use Bioreactors designed according to traditional bioreactor principles allow for a rapid and smooth scaling-up of cell culturing processes, but also facilitate continuous process development by accurate scaling-down.

**Introduction:** Scaling both up and down of bioprocesses is vital for bioprocess development. Poor process outcomes are often a result of scaling-up mammalian cell culture processes in large scale Single-Use bioreactors using different (Single-Use) bioreactor designs. Bioreactors used for production can have entirely different designs from smaller scale bioreactors resulting in a challenging scaling-up processes. Differences in geometric ratios (e.g. impeller diameter : vessel diameter or volume height : vessel diameter) can lead to sub-optimal results due to changes in mixing times, mass transfer coefficients, or shear stress levels. The current study aims to investigate the performance of Single-Use Bioreactors designed according to traditional bioreactor principles on the scaling-up of cell culture processes.

**Methods:** *Chinese Hamster Ovary* cell cultivations were carried out in stirred tank bioreactors with identical geometric ratios at different working volumes (2 L, 50 L and 250 L). Process parameters were adjusted according to proven scaling strategies (e.g. tip speed, VVM, etc.). Criteria used for the evaluation of scale-up success were viable cell density, viability, nutrient and metabolite titers, and monoclonal antibody titers.

**Results:** Results of the SUB cultivations at 50 L and 250 L working volume show all evaluated criteria to be comparable to the 2 L glass autoclavable stirred tank bioreactor.

**Conclusion:** This study demonstrates that Single-Use Bioreactors designed according to traditional bioreactor principles facilitate the scaling-up of mammalian cell culture processes, thereby leading to acceleration of development of vital medical therapies worldwide.

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# Structural identifiability and parameter estimation of dynamic metabolic models used for cell culture media optimization

Aaron Canales[1,3], Francisca Pizarro[1,3], Bastian Herrera[1,3], Ivan Paredes[2,3], Isidora Prat[2,3], Temístocles Molina[2,3], Dr. J. Cristian Salgado[1,3], **Dr. Ziomara P. Gerdtzen**[2,3]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
Utilization of mathematical modelling tools for formulating metabolic models of mammalian cells with the aim of optimizing the manufacturing of cellular products through the design of culture media and feeding strategies.

**Impact/Novelty:**
Identifiability analysis of metabolic models allows the incorporation of improvements to their structure, facilitating parameter estimation processes through Bayesian and frequentist methods.

**Introduction:**
The cell-derived products market's growth and the pressing need to establish competitive local manufacturing processes, emphasize the importance of achieving high productivity and reducing manufacturing costs. This interdisciplinary effort contributes to a project with the goal of creating a platform that optimizes production of cell-derived products through the design of culture media and feeding strategies for mammalian cells. A crucial step in the development of this platform involves studying a generalized mathematical model describing mammalian cell metabolism to determine the feasibility of inferring unique values for their unknown parameters.

**Approach:**
This work analyses the structural identifiability of the model using a probabilistic algorithm with a differential geometry approach, recognizing improvement opportunities, and subsequently addressing the parameter estimation problem. The model is predictive, dynamic, and phenomenological, describing cellular metabolite mass balances through differential equations, incorporating also specific metabolic characteristics of the studied cell lines.

**Results:**
The identifiability analysis of the model with its initial mathematical structure shows that it is not possible to infer all its parameters from observations. Therefore, structural improvements are integrated into the model, reducing unknown parameters, and facilitating the current parameter estimation process.

**Conclusions:**
The initial model's structure for optimizing animal cell culture media was significantly improved. Future efforts will explore alternative strategies for solving the inverse problem using frequentist and Bayesian approaches, aiming to enhance computational efficiency and reducing computing time.

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# Advancing Biomanufacturing Excellence: Harnessing Model Predictive Control for Optimal Process Efficiency and High-Quality Product Outputs

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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
Model Predictive Control (MPC) can enhance biomanufacturing through the integration of predictive models to support cost-efficient, scalable processes, aligning with ongoing efforts in automation and quality by design process characterization.

**Impact/Novelty:**
MPC, integrated with cell data and dynamic modelling, offers a ground-breaking approach to biomanufacturing. Customized feeding strategies, guided by dynamic models, enhance precision and adaptability, marking a significant advancement in process optimization.

**Introduction**:
The biopharmaceutical market's growth demands efficient production. A suitable substrate feeding strategy impacts efficiency, quality, and reproducibility. Proper design and control of this are vital for success. MPC excels in fed-batch process control, adapting to nonlinear dynamics, and considering multiple variables and constraints for optimized production and process robustness.

**Methods/Approach:**
Feeding rates are determined by employing MPC, assessing differences between model predictions and reference values. Constrained optimization guides the process to determine the optimal feeding rate. MPC's performance was compared with classical control using criteria such as performance metrics, the percentage of time within a 20% range of the setpoint, and adherence to operational constraints.

**Results:**
The strategy obtained with P control violates pump operational constraints. Through MPC, the feeding strategy considers constraints on pump limits and feeding time instances for cell culture. MPC outperforms classical control, reducing errors by up to 65% based on the ISE performance criterion Additionally, it extends the duration of controlled variables within the setpoint range.

**Conclusion:**
MPC, along with a generalized dynamic model, holds high potential to enhance the development of biopharmaceutical production processes by increasing process robustness from the design stage. MPC outperforms classical control, reducing errors and improving the stability of controlled variables, offering a promising avenue for improved bioprocessing.

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# Lot To Lot Consistency of AOF Peptones and Application of Key Driver Identification Service (KDI)

Mr Ashwin Gurunathan[1], Neelanjan Sengupta[1], Juliet Macharia[1], Nathan Cordell[1], Stacy Holdread[1], **James Brooks**[1]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:**
Transitioning from development to manufacture-The key driven identification service will use statistical models to identify components which can help make the process consistent as well enhance cell culture performance.

**Impact/Novelty:**
This poster addresses the question around lot-to-lot consistency when using peptones in a biologic process and the benefits of key driven identification service in improving cell culture performance.

**Introduction:**
Peptones (or protein hydrolysates) consist of water-soluble products including peptide fragments and various other nutritional components derived from the partial hydrolysis of proteins from plants, yeast, or animal tissues. Peptones offer several benefits such as nutritional buffering, protection from toxic byproducts, potentially delaying apoptosis and improving monoclonal antibody titers. With peptones having a rich nutrition profile, they can be a quick and cost-effective solution to optimize media and feeds for higher cell growth and titers. One of the major questions surrounding peptones is regarding lot-to-lot consistency and its performance analytically and biologically across several lots.

**Methods/Approach:**
Chinese hamster Ovary (CHO) cell line expressing a monoclonal antibody was cultured in simple fed batch mode in cell culture media with several lots of key animal origin free peptones. Cell culture performance was assessed using viable cell density, viability, antibody titer, charge variants, glycan profile and aggregation.

**Results:**
The data generated shows the consistency between several lots of animal origin free components. The data also shows how the KDI service can help in identifying key drivers and achieving consistent biological performance.

**Conclusion:**
Overall, we have shown the consistency between several lots of key Animal Origin Free peptones by comparing analytical and biological metrics such as viable cell density, viability, titer, charge variants, glycan profile, and aggregation. We have also shown the impact of the key driver identification service to help enhance bioproduction outcome and achieve consistent performance.

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# Accelerating CHO Bulk Harvest Testing: A Complete Set of Rapid Methods for Adventitious Agent Detection

**Dr Anna Woodward**[1], Ms Melisa Wilson[2], Dr Manjula Aysola[2], Dr Pamela Hamill[1], Dr Alison Armstrong[1]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
Intensified processes and continuous manufacturing necessitate state-of-the-art QC testing methods to prevent bottlenecks in processing and ensure that maximum efficiencies can be achieved.

**Impact/Novelty:**
This is an innovative technology to enable virus detection at speed, without compromising on detection capabilities or sensitivity compared to current methods.

**Introduction:**
Adventitious agent testing is often a rate-limiting step in downstream processing. Traditionally, most biosafety testing at the bulk harvest stage is performed using time-consuming culture-based methods, which rely on amplification of the contaminating agent. Alternative methods that offer a more rapid turnaround are needed to relieve this bottleneck and address the increasing pressures on manufacturing speed.

**Methods/Approach:**
In line with current regulatory guidance, we have developed an advanced multiplex and degenerate PCR technology for adventitious virus detection. The assay has been designed to detect virus families that are relevant for CHO cell processes, including emerging viruses and those that may infect human cells. Full validation of the assay has been performed to enable GMP-compliant testing.

**Results:**
Our innovative Blazar® CHO AOF assay facilitates a rapid testing strategy for CHO-based bulk harvest samples, which combines a very broad virus detection capability with high sensitivity. This new technology provides an assay that is a suitable replacement for in vitro testing for adventitious viruses, with the advantage of significantly reduced timelines and sample volume requirements without compromising on virus detection capabilities.

**Conclusion:**
By combining the Blazar® CHO AOF assay with other validated assays for detection of bacteria and fungi, mycoplasma, viruses and retrovirus-like particles, a full set of results for CHO bulk harvest testing can be obtained in less than two weeks.

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# Impact of ICH Q5A revision on quality control testing and virus safety strategies

**Dr Anna Woodward**[1], Dr Manjula Aysola[2], Mr Steven McDade[1], Dr Alison Armstrong[1]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
To ensure product safety, any strategy for process monitoring and control should include consideration of the fundamental principles of viral safety, as outlined in the ICH Q5A document.
**Impact/Novelty:**
In light of finalisation of the revised ICH Q5A guideline in November 2023, this presentation highlights key regulatory changes to raise awareness amongst developers and manufacturers of future regulatory expectations.

**Introduction:**
All biologics require extensive quality control testing and prevention measures throughout their manufacturing process to ensure freedom from adventitious contaminants. The ICH Q5A guidance document, which outlines global regulatory expectations for mitigating the risk of viral contamination, was first published in the 1990s. It advocates a three-part approach to viral safety, by applying the principles of prevent, detect and remove.

**Methods/Approach:**
ICH Q5A was recently revised to reflect recent scientific developments such as new therapeutic modalities, advanced manufacturing and novel detection technologies. We have reviewed the revised version and summarised the key changes that developers and manufacturers may wish to consider in relation to their viral safety strategy.

**Results:**
This presentation will consider the impact of the revised guideline on quality control of biologics manufacturing, with particular reference to new technologies such as Next Generation Sequencing and PCR-based methods. These technologies offer increased breadth of detection, improved sensitivity and faster timelines. The requirements for implementing such technologies to improve viral safety strategies will be presented, including validation approaches. Opportunities for replacement of *in vivo* and *in vitro* assays with molecular methods will also be discussed. Furthermore, the section of ICH Q5A that covers viral clearance has been expanded, particularly with respect to viral vectors and the application of previous data to products manufactured using platform processes. Implications for the design of viral clearance studies will be outlined.

**Conclusion:**
The ICH Q5A revision is now broader in scope and encompasses some of the newer modalities such as viral vectors and virus-like particles. It enables developers to realize the benefits of molecular methods, thus strengthening the ability to detect emerging virus threats as well as furthering ethical and sustainability goals. The revised document also provides opportunities to apply prior knowledge when designing viral clearance studies.

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# Novel vector system for one-step generation of diverse vector assemblies, enhancing titres of multispecific antibodies

**Dr Stephen Jaffé**[1], Miss Changhavi Ragurajan[1], Dr Alice Seleiro[1], Miss Tejal Bhure[1], Dr Michael Anbar[1], Dr Bernie Sweeney[1]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

At the core of any production process lies the need to design and construct vectors for high level expression of the target molecule. Current industry standard processes can be lengthy, requiring multistep cloning procedures. This can extend timelines, adding to project costs and delaying the ability of drug developers to generate their molecule and proceed to clinical trials.

As the biologics sector continues to develop, there is an increasing focus on the development of advanced multi-chain, multi-specific antibodies. However, the optimal vector design for these molecules isn't known with highly diverse molecules requiring diverse vector solutions. The ability to rapidly modify the promoters and chain order of these molecules independently for optimal chain expression stoichiometry and subsequent product titre and quality is highly desirable.

Lonza have developed a novel, robust, vector construction system. The system provides one-step generation of vectors for the expression of single to four chain molecules, allowing rapid generation or modification of chain order and promoter variants.

We hereby demonstrate a portion of the ongoing R&D optimisation of this system at multiple key stages in the production process with high throughput, automated plasmid generation of diverse panels of chain order and promoter permutations for the expression of multispecific antibodies. We demonstrate the impact that diverse libraries of plasmids have on stable CHO pools generated with GS piggyBac® transposase technology, enhancing yield for correctly assembled constructs over 2-fold compared to classical vector assembly designs.

Key points

- Industry needs
- Multispecific antibodies
- Vector Assembly
- Rapid modifications of chain order
- Integration of novel genetic elements
- Easily manipulate the expression of different chains to optimise stoichiometry

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# Cell culture-based bioprocess development and simplification for Influenza A virus

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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:** GMP implementation is a hurdle on the way from academic research to clinical trials. For mitigation, we develop simple, cost-effective and high yielding bioprocesses for viral based therapeutics.

**Impact/Novelty:** Here we aim to establish a high yielding fed-batch process while minimizing process complexity using influenza A as a model virus.

**Introduction:** Annual epidemics and the threat of a serious pandemic make Influenza A virus (IAV) one of the most important human pathogens. In response, innovative antivirals and vaccine approaches are under continuous further development which need to take the step into clinical trials. Recent advances in large-scale cell culture techniques have made cell culture-based production a promising strategy for IAV. Here, simple fed-batch processes could have great potential for process intensification and have so far been given little consideration.

**Methods/Approach:** In this study, we tested different infection strategies, including time of infection, media dilution (MD) and feed supplements prior infection. Infection experiments were performed in shake flasks, with Influenza A/Puerto Rico/8/34 (H1N1) and MDCK suspension cells. Virus production was evaluated by cell specific virus yield (CSVY) and volumetric productivity (VP) based on total virions and active virus titer.

**Results:** Higher cell densities resulted in higher virus yields. However, infection at the end of exponential growth phase, i.e. at peak cell concentration, decreased virus productivity, which could be overcome by a media dilution step prior infection. To allow productive infection in the exponential growth at higher cell densities, various feeding strategies are now being investigated.

**Conclusion:** For further process intensification, supplementation of feeds and/ or dilution of inhibitors prior infection might be beneficial. Future work includes the media screening of commercial fed-batch media, following development of a fed-batch process for IAV production to achieve even higher yields.

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# Vision, Planning, and Implementation of a Fully Automated, Bench-Scale Bioreactor Platform

**Dr Frank Ritacco**[1], John Czukkerman[1], Kyle Doce[1], Evelyn Schleicher[1], Brandon Veres[1], Dr. Hanne Bak[1], Dr. Shawn Lawrence[1]
[1]Regeneron Pharmaceuticals, Tarrytown, United States

Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
Accelerating development and tech transfer of bioprocesses, while incorporating quality by design, process monitoring and control by integrating multiple technologies into a fully automated, bench-scale bioreactor laboratory.

**Impact/Novelty:**
Commercially available automated microscale systems have limitations (sampling frequency, PAT, number of ports, volume of material generated for downstream development,) which are addressed by implementation of bench-scale automation (2-15L).

**Introduction:**
[*Anonymous*]'s protein-based therapeutics are produced in fed-batch bioreactors using Chinese Hamster Ovary (CHO) cells. Development and optimization of bioreactor processes requires many precisely timed daily operations to ensure product quality, optimize productivity, and enable understanding of culture conditions. These operations include inoculation, nutrient additions, and sampling, which often occur during weekends or outside normal working hours. Development experiments require multiple bioreactors running in parallel, further increasing the number of manual activities as well as the risk of human error.

**Methods/Approach:**
To increase operational efficiency, improve work-life balance, and minimize operator error, we have initiated full automation of bench scale bioreactor operations. This poster details our rationale and approach, including identification of automatable unit operations and workflows, factors and criteria evaluated during strategic planning and equipment selection, and experimental efforts to confirm comparability between automated and manual operations.

**Results:**
We have successfully begun implementing complete automation of bench-scale bioreactor operations, including inoculation, sampling, nutrient addition, sample processing, retain collection and storage. Identification and evaluation of automatable equipment platforms and workflows for all critical unit operations have resulted in the establishment of a new benchtop layout, incorporating automation-capable bioreactors, autosamplers, retain collectors, and a workcell including a liquid handler, robotic arm, vial labeler, centrifuge and refrigerator.

**Conclusion:**

[*Anonymous*]'s newly designed upstream "Bench of the Future" enables a fully automated bench-scale bioreactor workflow, providing substantial improvements in work-life balance, operational efficiency, consistency, and reduced risk of operator error.

# Poster Presentations

# Innovate or Die - Technology Innovation

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# Innovate or Die: A Business Methodology for Biomanufacturing Innovation Strategy and Deployment

**Mr Martijn Wapenaar**[1]
[1]Johnson & Johnson, Leiden, Netherlands

Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:**
Innovation within commercial biomanufacturing is essential for business to stay competitive and keep delivering the products of the highest quality to patients in need.

**Impact/Novelty:**
A specific business method for technology innovation at a major biopharmaceutical company.

**Introduction:**
To drive operational excellence on safety, quality and reliability, concepts like handsfree bioreactor control or automated process control will be of great value to the future of biopharmaceutical manufacturing. Instead of standard ways of checking product quality from samples taken at the end of a batch, integrated quality control provides real time product release and offers options to make process adjustments if parameters are about to go out of control. This handsfree automated process control means the quality of processes is controlled in real time with the potential to minimize/obsolete quality investigations. Sampling would simply become a non-value-added activity which liberates operational personnel to work on other tasks, like setting up the next process to drive increased output on the same footprint.

**Methods/Approach:**
Fully reaching handsfree process control requires a variety of technologies to be deployed. In a multinational organization, a well-considered governance model needs to be in place and maintained for successful technology deployment. Our organization established a team in early 2023 to aid in the strategy and deployment of innovations. The goal of this team is to enable a future of biopharmaceutical manufacturing where the best process can be ensured.

**Results:**
The proposed presentation/poster will discuss the learnings of the new team and their business method for technology innovation.

**Conclusion:**
Technology innovation within commercial biotherapeutic manufacturing is a complex process that needs guidance and acceptance from the wider organization to be successful. The methodology presented is one possible way to achieve this.

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# Dielectric spectroscopy as PAT tool to monitor specific growth rate and increase monoclonal antibodies production

**Miss Adèle Schini**[1], Dr. Bruno Ebel[2], Dr. Emmanuel Guedon[2], Dr. Laurent Jourdainne[1]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
Dielectric spectroscopy can be used to understand and estimate a metabolic and physiologic parameter that influences antibody production. Its control throughout the bioprocess opens to an increased productivity.

**Impact/Novelty:**
Current in situ monitoring methods for cell culture don't provide access to metabolic parameters. Using dielectric spectroscopy, this work studies the link between the specific growth rate (μ) and the antibody production.

**Introduction:**
In the biopharmaceutical industry, the use of mammalian cells to produce therapeutic proteins becomes widespread. Monitoring of these cultures via different process analytical technologies (PAT) is essential to ensure a good quality product while respecting good manufacturing practice regulations. PAT especially spectroscopic methods, provide real-time measurements of culture physiological state. Dielectric spectroscopy can be used to monitor the viable cell concentration (VCC) after processing raw data through mathematical models. Therefore, it's possible to estimate a functional parameter, μ, directly influenced by the glucose availability in the medium, and consequently to improve the production of secreted antibodies.

**Methods/Approach:**
CHO cell cultures are performed in bioreactors in fed-batch mode and monitored with a capacitance probe to generate spectroscopic data which are then processed through a mathematical model to estimate VCC and μ. Samples are taken daily to measure glucose and antibody offline concentrations.

**Results:**
The addition of boluses when the offline glucose concentration fell below a threshold, enabled the specific growth rate to be maintained as positive values, thereby increasing biomass and antibody productions.

**Conclusion:**
VCC monitoring using dielectric spectroscopy enables the estimation of μ that isn't generally accessible. As this key physiologic parameter is linked to antibody production, it may be possible to associate also an in-situ solution for glucose monitoring (ex: Raman spectroscopy) to automate feeding for enhanced production.

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# Hollow fiber-based cell retention: Recent advancements to reduce product retention in perfusion cell culture

**Dr Patrick Romann**[1], Dr Jean-Marc Bielser[2], Prof. Dr. Thomas K. Villiger[3], Prof. Dr. Andrew L. Zydney[4], Mr. Philip Giller[3], Dr Antony Sibilia[1]
[1]Levitronix GmbH, Zürich, Switzerland, [2]Merck Serono SA, Corsier-sur-Vevey, Switzerland, [3]University of Applied Sciences and Arts Northwestern Switzerland, Muttenz, Switzerland, [4]Pennsylvania State University, Pennsylvania, USA

Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:** This study demonstrates how the combination of process understanding with latest technology allows to tackle one of the most critical bottlenecks in perfusion cell culture, the product sieving challenge.

**Impact/Novelty:** Controlling Starling recirculation, either by optimizing operating parameters or by applying co-current filtrate flow, greatly improves product sieving, thereby addressing one of the most critical bottlenecks in perfusion cell culture.

**Introduction:** Continuous manufacturing of complex therapeutic modalities has gained momentum due to product quality, manufacturing flexibility, and cost considerations. A such, perfusion cell culture processes, with their cell retention capability, represent great potential for process intensification. Even though hollow fiber-based cell retention has emerged to the preferred technology within the industry, filter fouling remains a major bottleneck in current manufacturing scenarios and scale-up. Huge potential to improve performance lies within the identification of optimal operating parameters and evaluation of innovative technology to better control the filtration process.

**Methods/Approach:** Extensive pressure characterization studies in hollow fiber modules at lab-scale as well as manufacturing scale were performed to understand the relationship between pressure profiles and operating parameters. Gained knowledge was subsequently leveraged for optimization of operating parameters of existing processes. Additionally, novel hollow-fiber based perfusion operation modes were developed and subsequently verified in cell culture perfusion processes.

**Results:** Recirculation, caused by the axial pressure drop within the fiber module, was identified as major factor to consider when optimizing tangential flow filtration (TFF) in perfusion cell culture. In unidirectional and alternating TFF systems, minimizing the Starling flow was therefore the suggested strategy to maintain high product sieving. Controlling the Starling recirculation in a novel cell retention system, by applying a co-current filtrate flow, resulted in even higher filtration performance with minimal product retention in steady-state perfusion cell cultures.

**Conclusion:** Overall, this study highlights the importance of Starling flow in hollow fiber filtration and suggests strategies to either minimize, or control Starling recirculation with the aim to address bottlenecks in cell retention devices for perfusion cell culture.

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# Tailoring an intensified fed-batch process to large scale manufacturing needs

**Dr Christian Kaisermayer**[1], Dominik Schieman[2], Nuno Buxo Carinhas[2], Kristina Kliche[1]
[1]Novartis, Langkampfen, Austria, [2]Novartis, Basel, Switzerland

Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected (Innovate or die)**
This work describes process intensification as a relevant emerging technology to improve facility utilization and reduce manufacturing cost.

**Impact/Novelty:**
Process intensification is gradually adopted in the biotech industry to boost the productivity of traditional fed-batch culture. Commercial utilization typically requires to maintain product quality and puts restrictions on media preparation volumes, equipment residence time and performance of the cell retention unit.

**Methods/Approach:**
This work describes the development of an intensified fed-batch process using perfusion in seed culture to inoculate the production bioreactor at up to 10x increased seeding VCD. Several strategies were combined to accommodate large scale equipment restrictions e.g. inoculating several production bioreactors from the same seed train, minimizing media preparation volumes by using concentrates in the perfusion process and optimizing the feed strategy to allow unrestricted cell growth while optimizing also the performance of the cell retention unit.

**Results and conclusion:**
A consistent run rate of production bioreactors was enabled using a rolling inoculum strategy in the seed train. To minimize media hold volumes, a 2x concentrate was prepared and used for direct dilution with WFI in seed bioreactors without negatively affecting cell growth. The perfusion rate in seed bioreactors was adapted based on the residual nutrient concentration supporting unrestricted cell growth. Limiting components were identified via spent media analysis. The adapted perfusion rate allowed for repeated use of the filtration unit for cell retention during the rolling inoculum. Intensified fed-batch processes inoculated from the optimized seed train delivered a significantly increased yield of recombinant protein, while simultaneously reducing the process time required in the production reactor compared with traditional fed-batch. Key quality parameters of the recombinant protein like aggregates, fragments, charge variants and glycosylation were comparable to the established fed-batch process.

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# Unlocking Process Insights: High-Throughput Miniaturized Cell Culture System for Data-Rich Process Modelling

**Dr Vishwanathgouda Maralingannavar**[1], Ms Alexandra Gheorghe[1], Dr Sandeep Ranpura[1], Dr Colin Jaques[1], Dr Deniz Demirhan[1]
[1]Lonza Biologics Plc, Slough, United Kingdom

Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
In this study we discuss an innovative sub-mL scale miniaturised cell culture system for continuous culture systems and its selected use cases.

**Impact/Novelty:**
Generating necessary volume of data for precise modelling is a precursor for realising the potential of big data to revolutionize cell culture bioprocessing. This work is a step towards it.

**Introduction:**
As the field of bioprocessing continues to evolve, the demand for efficient and data-driven methodologies has become increasingly vital. High-throughput miniaturized cell culture systems have emerged as powerful tools in the pursuit of generating abundant data for precise process modelling. This work aims to explore one of such systems that we have developed on accelerating data acquisition and enhancing our understanding continuous culture systems.

**Methods/Approach:**
In this work, a panel of CHOK1SV GS-KO® clones expressing either mAbs or bispecific antibodies were used. Different medium compositions were achieved by fortifying a medium component at various concentrations. For clone screening application, 12 representative clones were used. Cell culture performance with the bioreactor were compared with respect to growth profile and extracellular metabolites.

**Results:**
This study provides an overview of the capabilities of the system, offering insights into its application in clone and medium screening and process optimisation. Miniaturized cell culture platform enabled the simultaneous cultivation of numerous cell cultures in sub-mL scale volumes, providing a rapid and cost-effective means to explore a wide range of experimental conditions. Cell concentrations reaching ~$50\times10^6$ cells/mL were achieved. Growth and extracellular metabolite profiles were compared with lab-scale bioreactors and a comparable performance within 25% was found. There are inherent trade-offs of the system when compared to lab-scale bioreactors and we discuss strategies to reduce such differences.

**Conclusion:**
In summary, this work highlights the significance of high-throughput miniaturized cell culture systems in generating extensive/comprehensive datasets for process modelling.

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# Automation of perfusion process for high cell densities: Maintaining constant cell specific perfusion rate (CSPR)

**Mr Niklas Welzenbach**[1], Mr Lisa Wolowczyk[1], Ms Mona Bausch[1], Mr Luis Ayala[1], Dr Jochen Sieck[1]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:**
Continuous processes offer flexibility, smaller scale and higher space-time yields. However, perfusion bioreactors require more operator interventions. Process automation can help to simplify and intensify these processes.

**Impact/Novelty:**
In a dynamic perfusion process the perfusion rate needs to be adapted with increasing cell densities to maintain a constant CSPR. This can be automated using a biomass probe.

**Introduction:**
Process automation in perfusion bioreactor processes offers several advantages. It enhances productivity by streamlining operations and optimizing resource utilization. Media demand and logistics are considered roadblocks for adoption of perfusion processes. Automation of perfusion process control based on cell density or biomass as a proxy can help reduce media demand and enhance process efficiency.

**Methods/Approach:**
This approach required the generation of a $CSPR_{stat}$ operation in a respective software in which the perfusion rate could be controlled. The goal of this operation was the automated translation of the online biomass signal into pump rates to maintain a constant CSPR of 18 pL/cell/day. Daily recalibration of the biomass signal via an offline VCD measurement helped to fine-tune control and adapt to changes in cell morphology.

**Results:**
A proof-of-concept automated $CSPR_{stat}$ operation could be shown. With process optimization CSPR could be well controlled ranging close to the desired setpoint in a second run. By automation of the continuously increasing perfusion rates, cell densities up to 400e6 cells/mL could be reached. Those high cell densities led to technical limitations regarding dissolved oxygen (DO) control, blocking of the cell retention device, and maxing out the biomass signal, that must be considered to improve the process further.

**Conclusion:**
A $CSPR_{stat}$ operation was developed automatically adjusting perfusion rate according to the biomass signal. Constant CSPR profiles confirmed the functionality of the operation. Some technical challenges were encountered during the operation, primarily related to the extremely high cell densities achieved via $CSPR_{stat}$ operation.

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# Assessment of cloning efficiency and error rates of various single-cell dispensing technologies

**Ms Olga Rimkevich**[1], Mr. Sven Loebrich[1]
[1]Mural Oncology, Waltham, United States

Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Introduction:**
In recent years, the advent of new single-cell dispensing technologies has significantly shortened the cell line development workflow for novel clinical assets. However, each novel technology has pros and cons in terms of ease-of-use, cost of implementation, efficiency of seeding and cloning, and – most importantly – error rates. Here, we assess the error rates of different established and emerging technologies by direct side-by-side comparison, including i) impedance, ii) microfluidics, iii) nanodroplet, and iv) gravity-based deposition. We contrast these findings to conventional limiting dilution cloning (LDC).

**Methods/Approach:**
We generated stably transfected CHO clonal cell lines expressing red or green fluorescent proteins (RFP, GFP, respectively) for high-sensitivity detection. Then RFP and GFP expressing clones were mixed at equal ratio and single-cell dispensed into 96-well plates for each of the four different dispensing technologies. After 14 days of incubation, plates were imaged on a high-resolution automated microscopic whole well imager using both red and green fluorescence channels. Outgrowth (cloning efficiency) and the rate of undetected errors from each technology was determined across two different CHO expression system backgrounds.

**Results:**
We made several key observations: i) We found differences in outgrowth rates across all evaluated technologies, with the best results being comparable to those from limiting dilution cloning. ii) All tested dispensing modalities show superior outcomes with regards to cloning error rates when compared to limiting dilution cloning. iii) We observe meaningful differences in the error rates between impedance, microfluidics, nanodroplet and gravity-based deposition technologies.

**Conclusion:**
Taken together, we outline a rigorous comparative approach for the assessment, qualification, and selection of different single cell cloning technologies in different CHO genetic backgrounds.

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# Process Intensification Strategies to Improve Process Economics in Biologics Development and Manufacturing

Mr Madhava Ram Paranandi[1], **Mr Jagadeesh Kanagala**[1]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to theme selected:**
Process intensification strategies discussed in this abstract are novel and unique to the biopharmaceutical manufacturing in general.

**Impact/Novelty:**
The strategies described herein, have the potential to reduce the overall cost of biologics manufacturing.

**Introduction:**
With the advent of various biotherapeutic modalities, the emphasis has shifted to manufacturing these complex biologicals in a cost-effective manner. Continuous manufacturing has made rapid strides in this area as a good alternative. However, companies that have been manufacturing using the traditional fed-batch technology, would have to invest in new facilities altogether. Process intensification strategies have proven to provide good alternative options in such cases. We have successfully demonstrated a couple of such options at development scale.

**Methods/Approach:**
For seed intensification, perfusion was implemented at N-1 stage using ATF technology. A high cell density of 40-50x$10^6$ cells/ml was generated in 5 days. Fed-batch cultures were initiated at 10X – 20X concentrations of the standard fed-batch, resulting in same antibody titer in less duration, thus enhancing the facility capacity. In a separate production intensification, ATF was used differently, where, instead of a 0.2µm filter, a 30kDa filter was used for perfusion. Unlike traditional perfusion, the protein was retained inside the bioreactor along with cells. Due to very high cell densities achieved, the titer productivity increased significantly.

**Results:**
The concentrated fed-batch option resulted in a 260% increase in titer productivity in the same duration as a fed-batch culture. The perfusion rate had to be optimized to achieve the high titers. The key is to maintain a high viability even at the very high cell densities to ensure a higher specific productivity.

**Conclusion:**
The high titer produced due to the concentrated fed-batch option resulted in a significant reduction of cost of goods for the product. This has been selected for further scale-up.

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# Intensified production of recombinant vesicular stomatitis virus-based vectors by tangential flow depth filtration

**Mr Sven Göbel**[1], Mr. Lars Pelz[1], Ms. Rachel Legmann[2], Dr Jennifer Altomonte[3], Prof. Amine Kamen[4], Prof. Udo Reichl[1], Dr. Yvonne Genzel[1]
[1]Max-Planck Institute, Magdeburg, Germany, [2]Repligen, Waltham, United States, [3]Department of Internal Medicine II, Klinikum rechts der Isar, Technische Universität München, Munich, Germany, [4]Department of Bioengineering, McGill University, Montréal, Canada

Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
Production, harvesting and clarification using scalable integrated closed single-use perfusion systems (tangential flow depth filtration) allows reduction of unit operations and enables intensification and integration of recombinant viral vector-based vaccine manufacturing.

**Impact/Novelty:**
In a proof-of-concept study using tangential flow depth filtration (TFDF) perfusion, we evaluated production, continuous virus harvesting and clarification of vesicular stomatitis virus vectors (rVSV). Space-time yields improved up to 460%.

**Introduction:**
In recent decades, recombinant virus vector-based therapies have been under intense investigation to combat, both, infectious diseases and cancer. Compared to other approaches, viral vector-based therapies rely on the infection of cells, thereby inducing robust immune responses elucidating both humoral and cellular immunity. To address the unprecedented demand and to cope with the high dose input required, batch-based manufacturing strategies need to be intensified.

**Methods/Approach:**
In this study, we evaluated the applicability of a TFDF perfusion system as a novel cell retention device for both perfusion cultivation and continuous harvesting with clarification in a single unit operation at the 3 L scale. The intensified production of two different rVSV-based vectors, one which induces classical cytopathic effects and one that mediates cell fusion reactions, were compared to optimized batch processes.

**Results:**
Using HEK293-SF cells and a recombinant vesicular stomatitis virus (rVSV) vector expressing a green fluorescent protein, perfusion cultivations resulted in a maximum viable cell concentration (VCC) of $11.3 \times 10^6$ cells/mL and infectious virus titers of up to $7.1 \times 10^{10}$ $TCID_{50}$/mL in the permeate. Although the cell-specific virus yield decreased relative to a batch process established as a control, an increased space-time yield was obtained. Using suspension BHK-21 cells and a fusogenic oncolytic hybrid of rVSV and Newcastle disease virus (rVSV-NDV), the TFDF unit allowed us to achieve high VCC (16.4-$20.6 \times 10^6$ cells/mL), continuous vector harvesting and clarification. Compared to an optimized batch process, 11-fold higher infectious virus titers were obtained in the permeate (maximum $7.5 \times 10^9$ $TCID_{50}$/mL).

**Conclusion:**
Overall, the TFDF module showed very good performance as a perfusion system for the tested rVSV-based vectors and cell lines. Continuous virus harvesting with subsequent clarification through the TFDF module in one step can simplified process operation and helped to develop an integrated, scalable (up to 2000 L), sustainable and economical process for future vaccine manufacturing.

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# Automated Cell Line Development: Identifying Top Candidate Clones More Efficiently

**Dr Michael Hoffman**[1], Zach Goldblatt[1], Jason Vitko[2], Thomas King[2], Tiffany Mclamarrah[2], Jen Tedstone[2], Hope Divello[1], Steve McLellan[1], Victor Cairns[2], Jack Scarcelli[2], Christine DeMaria[1]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
In an effort to develop a more efficient CLD workflow, a multifunctional CLD automation platform was implemented, utilizing integrated robotic cell culture handling technologies with software-based data handling applications.

**Impact/Novelty:**
Generation of high producing cell lines in manual CLD workflows is an intensive process and demands new methods that accurately identify lead candidate clones in as expedited timeline as possible.

**Introduction:**
A combinatorial CLD automated approach was used to screen top candidate clones from 3 unique uncloned pool populations, as compared to a manual control process. In total, over 10,000 clones were generated, screened, and expanded.

**Methods/Approach:**
To accomplish this, an automated CLD platform was implemented using a Phenomex Beacon, as well as a robotic liquid handling platform integrated with both incubation and cell imaging functionalities for storage/incubation, imaging, screening, hit picking, feeding, and passaging. This automation platform was paired with a data handling network to enable complete system integration and more efficient screening of candidate clones. A 24-deep well plate fed-batch screen was implemented in the workflow to enable early selection of candidate clones based on growth properties and productivity. A fed-batch screen was performed on top candidates to access clone performance and make final selections.

**Results:**
Results demonstrated that, compared to the historic manual CLD workflow, the new automated workflow identified clones faster more efficiently, thereby enabling faster project timelines. Clones generated in the automated workflow were shown to exhibit better attributes associated with growth and productivity, contributing to improved outcome metrics and a more predictable and successful CLD process.

**Conclusion:**
This work validated the implementation and utilization of laboratory robotics and high throughput CLD automation and represent a critical effort to continuously improve the CLD ways of working and shorten our fast-to-clinic timelines.

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# Pilot Scale Bioreactor Design for Dynamic Perfusion with Tangential Flow Filtration

**Dr Nicholas Guros**[1], Alex Gadberry[1], Elise Woodall[1], Michael Mollet[1]
[1]AstraZeneca, Gaithersburg, United States

Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:** Dynamic perfusion processing presents as the most feasible platform to meet industry demand on biologicals manufacturing – we must innovate or die.

**Impact/Novelty:** This work demonstrates the successful scale-up of a bench-scale dynamic perfusion process to a 500 L pilot process leveraging hollow fiber tangential flow filtration (TFF) to continuously separate mAbs from cells.

**Introduction:** There is an increasingly urgent need to expand the manufacturing capacity of biopharmaceuticals. The cost and timeframe of constructing new commercial (> 10 kL) fed-batch facilities ($400 – 800 M and five years respectively) warrants the development of an alternative manufacturing strategy to the historical fed-batch standard.

**Methods/Approach:** A bioreactor was designed to enable 500 L dynamic perfusion. Bioreactor components including single-user container holder, agitator, and sparger were engineered to maintain high cell density CHO culture in combination with a TFF hollow fiber skid to continuously separate mAb from culture. Bioreactor components, operating parameters, and automation schemes were designed to satisfy the delicate balance of $O_2$ for cellular respiration, air sparge for $CO_2$ removal, foam generation, condensate in the exhaust stream, and shear forces on the cells through the TFF recirculation loop.

**Results:** The bioreactor supported a peak cell density of almost 120 M cells/mL, and yielded comparable $pCO_2$, metabolite, and cell health attributes as the bench-scale dynamic perfusion platform. Gas entrainment into the TFF skid was mitigated while managing foam generation through excellent automation control. Exhaust filter health was mitigated by a condenser skid and exhaust blower.

**Conclusion:** This work demonstrates the next iteration of bioreactor engineering for a single-use, high cell density, TFF dynamic perfusion process that is potentially scalable to the commercial scale (2 – 6 kL), on track to be a less expensive and more efficient alternative to presently available fed-batch manufacturing platforms.

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# Promises and pitfalls of process intensification implementation

**Dr Patrick Mayrhofer**[1]
[1]Novartis, Schaftenau, Austria

Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
Challenges and solutions for implementing innovative upstream cell culture bioprocesses.

**Impact/Novelty:**
Today, a multitude of novel technologies and concepts are available that have been shown to significantly improve the performance and productivity of bioprocesses.

**Introduction:**
Two key strategies to intensify existing or novel bioprocesses are the use of perfusion technologies and shortened, high-density main-stage cultivations.

**Methods/Approach:** (heading must be in bold)
In this presentation, various options for process intensification are highlighted and extended by practical experience.

**Results:**
Identified challenges and risks concerning implementation of these technologies or transfer between scales and sites are discussed.

**Conclusion:**
The target audience is a group of experts interested in process intensification strategies in general or specifically preparing for ongoing intensification projects.

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# The Future of Making Cell Culture Media

**Ms. Kaitlynn Bayne**[1], Mr. Tom Fletcher[1], Mr. Bryan Tamashiro[1], Miss Brooke West[1]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected**
Game changing innovation for automated and consistent media hydration.

**Impact/Novelty**
Media preparation is often overlooked as an area for optimization in manufacturing processes. This novel approach to hydration offers an automated system that hydrates media quickly and consistently.

**Introduction**
The biopharmaceutical industry continues to face pressure to deliver new products to patients as quickly as possible. To accomplish this, manufacturers need to develop and then optimize cost-effective production processes, all without comprising quality or safety. While many technologies have been developed to increase efficiency and cost savings at different stages of the manufacturing process, one area of production has been neglected - media preparation. As a supporting process, this has yet to be targeted as an opportunity for optimization, despite being labor intensive, time consuming, and a known source of variation. To improve the traditional approach to media hydration, an automated single-use hydration system has been developed. It is a single-unit operation process that integrates hydration and filtration, and allows manufacturing personnel to focus on other critical process steps.

**Methods/Approach**
Media was produced both in the automated hydration system and in a traditional manufacturing facility. The time to make the media was measured and the quality of the media produced was evaluated via amino acid and vitamin concentrations as well as pH and osmolality readings.

**Results**
The automated hydration system produces equivalent media in a more consistent and shorter time (100 L in an average of 17 minutes), which is 2-fold to 5-fold faster when compared to current media manufacturing methods.

**Conclusion**
The new automated hydration system provides flexibility and efficiency to the media preparation process, while producing equivalent results consistently.

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# Advancing Cell Culture Media Preparation in CDMOs

**Ms. Kaitlynn Bayne**[1], Mr. Chris Cline[2], Mr. Mark Patton[2], Mr. Scotty Bailey[2], Miss Sharyn Farnsworth[2]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected**
Using a novel approach to hydrating cell culture media in a Contract Development and Manufacturing Organization (CDMO) platform process.

**Impact/Novelty**
A new automated cell culture media hydration system used by a CDMO for their platform process, which is an option they can now offer to their customers.

**Introduction**
CDMOs help develop and optimize manufacturing processes for their clients. When it comes to cell culture media, CDMOs often do not have the ability to optimize much of the media preparation activities at large scale. Media preparation has historically been labor intensive, time consuming, and a known source of variation. There is a new, automated single-use hydration system available that has the ability to optimize this previously untouched step of the manufacturing process. It is a single-unit operation process that integrates hydration and filtration, and allows for manufacturing personnel to focus on other critical process steps. This gives CDMOs the flexibility to offer more speed and consistency in the media preparation process for their clients.

**Methods/Approach**
Eight bioreactor runs were performed to compare media hydrated in the new automated hydration system, media hydrated using the CDMO's traditional method, and liquid media purchased directly from the supplier. Time to prepare the media was compared as well as cell growth and productivity data, including cell counts, viability, titer, and nutrient and metabolite levels.

**Results**
The CDMO's platform media was hydrated 6-fold faster in the automated system compared to traditional hydration in a mixer. Data concludes that cells behaved similarly across all conditions.

**Conclusion**
The media made in the new automated hydration system was made in a fraction of the time and was equivalent to that made by the CDMO's traditional manufacturing methods.

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# Raman-based Lactate Feeding Impact on Culture Characteristics and Product Quality in Mammalian Cell Cultures

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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Innovate or Die:**
This work utilizes a novel Raman-based analytical device that requires no chemometric modelling for implementation and acquisition of real-time, in-line glucose, lactate, and biomass data.

**Impact/Novelty:**
This is the first instance of using Raman-based feedback control for lactate feeding in fed-batch mammalian cell cultures to assess impacts on culture performance and product quality.

**Introduction:**
The pharmaceutical industry is increasingly motivated to improve process control, understanding, and repeatability using process analytical technology (PAT) methods. More specifically, Raman-based methods have shown promise as a way to monitor critical performance parameters including glucose and lactate concentrations in real time. Recently, this has translated to the implementation of Raman-based glucose feeding strategies to modulate productivity and product quality in Chinese hamster ovary (CHO) cell cultures. This work implements Raman-based lactate feeding strategies as a means of modulating product quality and improving culture conditions, primarily though decreased ammonia accumulation.

**Methods/Approach:**
The NISTCHO cell line was cultured in EX-CELL Advanced media as a fed-batch culture with EX-CELL Feed 1 added at 5% v/v every other day. Lactate concentration was continuously monitored throughout the culture. In the first few days of the culture, lactate accumulates as a waste product from the cells; however, there is a subsequent lactate consumption phase. During this consumption phase, sodium lactate is fed to maintain the lactate concentration at a desired set-point, avoiding the complete depletion of lactate in the culture.

**Results:**
Preliminary results demonstrate that lactate feeding is an effective way to decrease ammonia accumulation at the end of mammalian fed-batch culture. Critical quality attributes including glycosylation and charge variants will be analyzed to assess the impact of a continuous lactate feed on antibody product quality.

**Conclusion:**
Raman-based analytical devices have the ability to increase process control through feeding strategies of critical substrates, in this case lactate, for improved culture performance and tunable product quality attributes. It is important to implement these techniques to further understand the connections between substrate metabolism and culture productivity and product quality.

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# Off-Gas based Soft-Sensor for Real-Time Monitoring of Biomass and Metabolism in CHO Continuous Fermentation Processes

**Mr Tobias Wallocha**[1], Dr. Oliver Popp[1]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:** “Innovate or Die – Technology Innovation”.
The poster illustrates that implementing off-gas analysis in a simple, robust manner facilitates better bioprocess understanding and real-time biomass monitoring and control. It takes PAT control to the next level.

**Impact/Novelty:**
Our work shows that off-gas analysis can be essential for meeting modern PAT demands in nowadays bioprocesses, providing detailed information for novel soft sensor methods and enabling real-time process control.

**Introduction:**
Chinese hamster ovary (CHO) cells are widely used in biotechnological companies and academic working groups as main working horses for production of recombinant therapeutic glycoproteins such as monoclonal antibodies. The assessment of viable cell density (VCD) or the viable cell volume (VCV) are important and mandatory critical process parameters (CPP) for the determination of active biomass in cell cultivation processing steps. We highlight for the first time the advantageous usage of VCV instead of VCD in combination with an oxygen-uptake-rate (OUR) based soft-sensor for real-time biomass prediction in continuous single-use (SUB) fermentation systems.

**Methods/Approach:**
We exemplarily show for a continuous CHO process that the developed real-time biomass soft-sensor can be used for the prediction of underestimated CPPs like distinct cell metabolic phases and dependent bioprocess variables such as the cell-specific pyruvate consumption/production rate qPyr, which enables in-depth process monitoring or even metabolic augmented process feedback control strategies.

**Results:**
Our proof-of-concept data set, consisting of 14 similar SUB continuous fermentation processes including 14 different monoclonal antibody (mAB) producing cell lines, shows significantly higher model accuracy when VCV instead of VCD was used as biomass depiction. This strengthens our strong belief in a paradigm change regarding nowadays biomass description in modern bioprocesses.

**Conclusion:**
In summary, the application of an easy to implement off-gas analyzer can deliver meaningful bioprocess key parameters in real-time to further monitor and optimize state of the art bioprocesses in bioreactors from research to manufacturing.

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# Modular approaches to integrated and continuous biomanufacturing improve supply chain agility

**John Raven**[1], Graeme Burton[1], Robyn Hoare[1], Sam Whitwam[1], Richard Lugg[1], Charlie Heise[1], Terri Dover[1], Tibor Nagy[1], Michelle Lyons[1], Benjamin Hastings[1], Caitlin Morter[1], Chathumi Maduwage[1], Fay Saunders[1], Leon Pybus[1]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Innovate or Die – Technology Innovation**
Market growth, demand uncertainty, new modalities, cost of goods (CoGs) reduction, and improving speed, quality, agility, and sustainability challenge biomanufacturing. Integrated and continuous biomanufacturing (ICB) can address these challenges.

**Impact/Novelty:**
We describe ICB-based methods to maximise unit operation, manufacturing process, and facility output productivity. Step-by-step (per unit operation) or end-to-end approaches maximise impact and supply chain agility.

**Introduction:**
Current biopharmaceutical production for mammalian cell lines involves building biomanufacturing facilities with fixed capacity, focusing on fed-batch bioreactor production with independent, and batch downstream unit operations. ICB offers inherent advantages of reduced capital and operating costs; increased productivity and reduced facility size through process intensification; facilitation of multiproduct facilities; improved flexibility to changing demand; increased process sustainability through a reduction in process mass intensity (PMI); and improved product quality consistency.

**Methods/Approach:**
A number of ICB based case studies are presented which either intensify the seed train, N-stage production bioreactor and/or downstream unit operations on a unit-by-unit operation or an end-to-end basis. These studies inform the design of next-generation manufacturing facilities (small scale single-use and large-scale stainless steel) that maximise supply chain agility.

**Results:**
Next-generation manufacturing facilities can deliver <1 kg to >100's/kg of product with smaller footprints (50-70% smaller; single-use; continuous harvest allows run flexibility to demand), sustainability (less water and $CO_2$, energy savings), lower CapEx investment (<50%), rapid deployment (<2 years), increased space time yield (2-3x through high density perfusion culture), low CoGs (>30%; improved), and improved product quality (reduced time of product in process; enabling manufacture of next generation products).

**Conclusion:**
Current and future multiple modality pipelines require flexible and modular manufacturing options for clinical and commercial supply: scale-up (20 kL stainless steel); scale-out (2 kL single-use); and scale-on (c500L single-use continuous manufacturing). ICB based approaches can be applied holistically by unit-by-unit operation (scale-up/scale-on) or end-to-end (continuous manufacturing) to maximise supply chain agility.

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# Major increase of the expression level of a hard-to-express bispecific biparatopic BEAT® antibody

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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Innovate or Die – Technology Innovation**
A combination of improvements in cell line development and upstream process optimization is leading to a major improvement in the production of multispecific antibodies of the BEAT® format.

**Impact/Novelty:**
Achieving expression levels of more than 10 g/L for a multispecific antibody is a significant target in our industry. Following a systematic optimization workflow, a hard-to-express bispecific biparatopic antibody (= trispecific mAb) was expressed above 10 g/L (product) in a scalable fed-batch process. Such developments will be useful to enable industrial expression of new challenging molecules and formats entering CMC.

**Introduction:**

Multispecific format antibodies have a great therapeutic potential. However, they are also known to be a source of challenges in CMC, notably in terms of expression level. A proprietary bispecific biparatopic antibody was found difficult-to-express using the initial platform process. As the initial cell population did not respond to process development, a change of clone was performed using an improved vector design, followed by process development with the aim of boosting the expression levels.

**Methods/Approach:**
Clones were evaluated in fed-batch process varying multiple parameters, including feeding strategies, temperature shift, different gassing and stirring strategies. In addition, the use of an N-1 intensified process was evaluated.

**Results:**
Among the tested parameters, a temperature shift at peak cell density was identified as a key factor for titer optimization. From the initial 1 g/L titer, the optimized fed-batch titer increased to 4.5 g/L. Development of a N-1 intensified process increased the titer up to 11 g/L.

**Conclusion:**
A platform approach is a valuable tool to move quickly preclinical projects into clinical Phase I studies. However, most multispecific antibody formats are difficult-to-express molecules. N-1 intensified process led to a major increase of 1100 % in titer compared to the initial platform fed-batch titer.

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# Implementing multi-modal non-destructive online monitoring to qualify 3D tissues

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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:** Innovate or die _This study describes the implementation of two non-destructive technologies applied to monitoring 3D culture at a large scale for tissue engineering.

**Impact/Novelty:** Suggest innovative analytical instruments for the emerging 3D culture and regenerative medicine field.

**Introduction:**
Tissue engineering (TE) and regenerative medicine (RM) have experienced significant growth in the last decade, integrating diverse technologies to address challenges. Despite advancements in 3D biofabrication, strategies for tissue maturation in clinical settings remain nascent, posing urgent concerns. Notably, current imaging modalities for tissue characterisation are constrained in scale and depth. Recent endeavours aim to implement real-time monitoring on tissue cultivation vessels to track tissue evolution by applying bioprocess principles and tools for enhanced and informed fabrication.

**Methods/Approach:**
Two technologies, Raman spectroscopy and Magnetic Resonance Imaging (MRI), were chosen for their capabilities. Conjunctive tissue models of 10 cm3 were monitored using Raman spectroscopy over 2 to 3 weeks. The Kaiser-Endress Rxn2 IOT Raman analyser, equipped with a Raman bIO-LAB 220 immersed probe or a non-contact PhAt probe, facilitated online acquisition of both medium and tissue composition. MRI, at high-field (7-Tesla) and low-field (0.3-Tesla), provided insights into internal morphology and nutritive flow path, demanding specific sequences and data treatment for a 3D qualification of flow path and intensity distribution.

**Results:**
Raman models described lactic acid release by conjunctive tissues, revealing distinct metabolisms in 3D culture cells versus 2D adherent cells, with a precise 0.1g/L prediction. Online monitoring of lactate release identified various growth patterns. Additionally, Raman models inside a 3D Gelatin-based matrix, developed with the PhAt probe, enabled biomass cell counting with a precision of 0.7 x $10^6$ cells/ml. MRI analyses validated CFD (Computational Fluid Dynamic) simulated flow paths and unveiled internal tissue morphological shapes previously inaccessible without tissue destruction.

**Conclusion:**
Both technologies yielded valuable information on cell growth, tissue metabolism, and morphological evolution without sample interference. These analytical tools advance tissue characterisation and qualification before clinical application and implantation, marking progress in the field.

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# Process analytical toolkit for optimizing continuous bioprocess modelling for manufacturing

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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
In this study, we present an overview of possible process analytical toolkit for optimizing continuous bioprocess modelling for manufacturing purposes.

**Impact/Novelty:**
Automating, analysing and storing diverse input and output dataset for continuous bioprocess modelling can direct us towards lights-out manufacturing.

**Introduction:**
Setting up automated smart factories will require whole process operations and control decisions to be executed without human intervention. To foray into this domain, it starts with adopting technologies for constant monitoring of process and media composition. Here we aim to employ Raman, Capacitance and orthogonal technologies to gather dataset for optimizing continuous bioprocessing which can be transferred to manufacturing.

**Methods/Approach:**
In this work, we have employed bispecific antibody producing CHOK1SV GS-KO® clone in a lab scale reactor connected to ATF cell retention device. Samples were analysed offline for titer using orthogonal techniques. Continuous feed addition was controlled by Raman. The cell number was controlled in N-1 bioreactor with capacitance probe. Mass spectroscopy and ProteinA HPLC were used as orthogonal methods.

**Results:**
This study provides an overview of toolkit that could be deployed for process optimization and modelling. Cell concentrations reached expected peak cell density of $20X10^6$ cells/mL. Comparable metabolite profiles and product quality were observed. The titre uplift was over 30% with PAT controlled intensified fed-batch process compared to low inoculum fed-batch processes. The comparability of orthogonal techniques to ProteinA HPLC for titer measurement was successfully demonstrated to be over 85%.

**Conclusion:**
In summary, this work highlights potential toolkit that can be deployed for process optimization and a step towards lights-out manufacturing.

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# Dielectric spectroscopy as PAT tool to monitor the viable cell concentration (VCC) of CHO cells

**Miss Adèle Schini**[1], Dr. Bruno Ebel[2], Dr Karine Elise Voltz[1], Dr. Emmanuel Guedon[2], Dr. Laurent Jourdainne[1]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
Dielectric spectroscopy can be used to understand and estimate a metabolic and physiologic parameter that influences antibody production. Its control throughout the bioprocess opens to an increased productivity.

**Impact/Novelty:**
The goal of this project is to develop a novel method for converting dielectric spectroscopy permittivity measurements into precise qualitative and quantitative insights on cell physiology.

**Introduction:**
In the biopharmaceutical industry, widespread adoption of mammalian cells for therapeutic protein production requires continuous monitoring through Process Analytical Technologies (PAT). Spectroscopic methods, particularly dielectric spectroscopy, enable real-time measurement of cell culture physiological states, including monitoring VCC and cell radius by processing raw data through mathematical models, ensuring product quality and compliance with manufacturing regulations.

**Methods/Approach:**
CHO cell cultures are performed in bioreactors in batch mode and monitored with a capacitance probe to generate spectroscopic data which are then processed through mathematical models to estimate VCC and radius. Samples are taken daily to estimate the offline reference values of these two parameters.

**Results:**
Uncertainties related to different cell-specific parameters of the mathematical equations such as internal conductivity (σi) and the membrane capacitance (Cm) indicated that they are critical to optimize model precision. The most accurate optimization method found involves in-process adjustments of Cm and σi in the model equations with samplings from the bioreactor.

**Conclusion:**
This combination of offline and in situ data improved the estimation accuracy of the VCC by 69%. The availability of biomass parameters monitored in real-time opens the possibility of developing process control strategies based on permittivity variations. The full extent of applications of this technology is still being determined and it is promising that other modelling strategies will provide more information on cell physiology in culture.

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# High-throughput Biacore™ assays for screening and characterization of bispecific antibodies

**Dr Johanna Rodhe**[1], Bahareh Motaghi Moghaddam Shahri[1], Gustav Johnson[1], Narasimha Murthy Bandaru[1], Linnéa Nygren Babol[1], Albin Larsson[1], Malin Kruukka[1], Dr Prajakta Khalkar[1], Dr Ann Lövgren[1], Daniel Ivansson[1]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected: Innovate or Die – Technology Innovation**
Bispecific antibodies (bsAbs) are analytically challenging due to the complicated product-specific impurity profile. We demonstrate novel high-throughput assays to support clone screening during cell line development (CLD).

**Impact/Novelty:**
We demonstrate novel assay opportunities to assess purity and amount of correct product during CLD for bsAbs.

**Introduction:**
Producing bispecific and multispecific antibodies is typically more challenging than standard mAb production. Therefore, screening of many clones during CLD may be required.
Protein A (ProtA)-based methods frequently fail to quantitate the correct form. Methods such as ELISA or mass spectroscopy are labor-intensive and/or not sufficiently sensitive. We report the development of surface plasmon resonance (SPR) assays implemented on the Biacore-8K+ platform, which can be used for high-throughput quantitation of the desired bsAb and to generate purity estimates concerning product-related impurities.

**Methods/Approach:**
Antigens and protein-based affinity binders directed to different antibody domains are utilized to develop assays for the characterization of bsAbs using Biacore™ SPR technology. We evaluated assays based on either single interaction or dual interactions (sandwich assays).

**Results:**
By combining a Biacore sandwich assay with a ProtA assay, we generated three data points: (i) concentration of the correct bsAb format, (ii) apparent concentration of product-related species binding the first antigen, and (iii) apparent concentration of product-related species containing an Fc domain and hence binding to ProtA. By comparing the two apparent concentrations with the correct form, we estimated the purity and profile of the product-related impurities.

**Conclusion:**
We show how the Biacore 8K+ platform offers opportunities for early characterization and selection of bsAb-producer clones as both the level and purity of the correct bispecific form can be assessed at high throughput.

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# Overcoming Challenges in a Novel Coagulation Factor Production: Fed-batch versus Perfusion

**Dr Iván Martínez-Monge**[1]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected**
This case showcases a strategic commitment to innovation, exemplified by our successful application of novel technologies in challenging coagulation factor production.

**Impact/Novelty**
An already established production process for a novel, life-saving coagulation factor for anti-coagulated patients was compared to alternative processes to improve output, product quality and Cost of Goods.

**Introduction**
Millions of people rely on synthetic anticoagulants to prevent stroke and thrombosis. The lack of a counteracting agent puts patients with severe bleeding or emergency surgery at high risk. VMX-C001 was developed to induce clotting despite the presence of anticoagulants. The product is produced in a perfusion process using CHO cells that is challenged by molecule complexity and the vitamin K dependent gamma-carboxylation activation, resulting in a low ratio of correctly expressed pre-activated protein. Various strategies are explored to enhance productivity and facilitate VMX-C001's market entry, addressing a critical medical need.

**Methods/Approach**
Different strategies were investigated: (1) optimization of the perfusion process, (2) metabolomics study to identify potential metabolites enhancing activity, and (3) exploring alternative production modes such as fed-batch and intensified fed-batch. Process performance was determined by both titer and activity, while product quality was evaluated through SE-UHPLC, RP-HPLC, and activated FXa assays.

**Results**
The perfusion process yielded three successful 100L GMP batches. Inability to boost productivity risked the drug's market entry due to production costs. Metabolic analysis identified pathways enhancing protein activity but yielded no improvements. Fed-batch and intensified fed-batch were investigated as viable alternatives to perfusion, providing higher titer, activity, and a favorable product quality profile.

**Conclusions**
Due to the inherent instability of coagulation factors, perfusion processes are the preferred choice for production, although perfusion processes are costly and challenging to scale up. Successful fed-batch and intensified fed-batch processes have been developed for coagulation factor production, achieving a substantial reduction in cost-of-goods.

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# Technical approach of a concept for the closed-loop recycling of a small-scale plastic-based bioreactor

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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
Single-use plastic-based bioreactors offer many advantages for biotherapeutic development. However, with sustainability playing an increasingly important role, there is a need to explore plastic circularity options.

**Impact/Novelty:**
We investigated the technical feasibility of a closed-loop recycling concept for the main construction material of a small-scale bioreactor and tested these recycled bioreactors in application.

**Introduction:**
To accelerate biotherapeutic development while decreasing time and cost, small-scale single-use multi-parallel bioreactors enable rapid identification of production clones, media formulations, and optimal process parameters to maximize yield and quality. Despite these well-known advantages, the use of plastics raises concerns both in terms of social perception and end-of-life aspects, requiring actions to increase circularity.

**Methods/Approach:**
This study for closed-loop recycling included logistics, such as collection, autoclaving, and shipment of the bioreactors. Recycling was accomplished through disconnecting, sorting, and re-granulating polycarbonate, which was then used for injection and assembly of new bioreactors. In the last step, cytotoxicity and cell culture tests were performed to confirm the full functionality of the recycled bioreactors.

**Results:**
Virgin bioreactors were autoclaved and successfully recycled into polycarbonate granules, which were then injected using 100% recycled content. After injection and assembly, a color change in the recycled bioreactors was ascertained, which did not occur when using virgin material. Nevertheless, final application tests using an industrial-relevant antibody-producing cell line in a standard fed-batch process indicated no impact of the recycling process on biocompatibility, cell growth, or productivity.

**Conclusion:**
This novel study demonstrates the technical feasibility of such a closed-loop recycling concept. However, several challenges remain to enable implementation at a commercial scale. The impact of biomass, possibly biocide, and biotoxins on the recycling process and quality remains unknown. Recycling logistics are also an issue for higher quantities than demonstrated here. Finally, the holistic environmental impact of such a recycling process needs to be assessed.

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# Transposon system for stable non-coding RNA overexpression in CHO production cells

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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:**
For genome integration of transgenes coding for therapeutic proteins, transposase-mediated integration outperforms random integration. However, such integration systems are currently not available for integration of non-coding RNAs as engineering targets.

**Impact/Novelty:**
As targets for cell engineering, microRNAs require integration into the host genome for stable overexpression. We present a novel and efficient transposon system for stable microRNA overexpression in CHO cells.

**Introduction:**
Genomic integration methods as random integration and transposase-mediated integration play crucial roles in genetic engineering. Recent advancements highlighted the superiority of transposon systems compared to random integration. While transposon systems gained interest for the production of monoclonal antibodies (mAb), so far stable overexpression of microRNAs as cell engineering tool is exclusively performed by random integration.

**Methods/Approach:**
Classical restriction cloning including overhang primers for PCR generated transposon plasmids, which were transfected with transposase plasmids into CHO-K1-mAb1 cells using a chemical transfection reagent, followed by antibiotic selection. Cell growth and transgene (EGFP) expression were measured via flow cytometry. Copy number, microRNA expression and target regulation were quantified by qPCR. Antibody titer and fucosylation were measured via HPLC and MS, respectively.

**Results:**
Transposase-mediated integration via hyperactive *PiggyBac* (hypPB) and hyperactive *Sleeping Beauty* transposon systems were compared to random integration of transgenes into the CHO genome. Transfection with both transposase systems resulted in cell pools with approximately 90% EGFP-positive cells, while random integration rendered only 60% of EGFP-positive cells after selection. Additionally, cultivation over 40 days indicated stable expression of transgenes for at least a month with transposase-mediated integration.
Based on slightly better performance, hypPB-transposon system was selected to establish a stable microRNA-transposon-system. First, recognition sites for the transposase were cloned into a miRNA expression plasmid, followed by integration of 1, 2 and 4 copies of different miRNAs into the expression cassette. Next, stable miRNA-expressing cell pools were generated using the new miRNA-transposon-system and compared to random integration. In addition to analysis of copy number, superior performance was analyzed for miRNA overexpression, target gene regulation, and functional effect (mAb fucosylation) of the novel miRNA-transposon-system.

**Conclusion:**
The use of transposon systems can surpass conventional random integration. This novel technique can facilitate high-speed and high-performance integration for non-coding RNAs into CHO host or production clones for efficient cell engineering.

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# Inline viscometer as process analytical technology to measure real-time protein concentration in TFF operation

**Dr Patrick Romann**[1], Ms. Zsofia Bencze[2], Mr. Philipp Campos[1], Mr. Antony Sibilia[1], Dr. Daniel Steinert[1], Dr. Thomas Nussbaumer[1]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:** The study presents an innovative technology to measure viscosity, which is available as low-cost and single-use device that can be leveraged to improve bioprocess monitoring.

**Impact/Novelty:** A novel viscometer with a measurement principle based on magnetic levitation was successfully applied to quantify protein concentration in tangential flow filtration (TFF) operation.

**Introduction:** TFF is typically performed to formulate biologic therapeutics to high protein concentrations and includes ultrafiltration (UF) as well as diafiltration (DF) steps. During the process, protein concentrations are typically measured by at-line analytics to determine if target protein concentrations are reached to initiate the next processing step. Strongly increasing viscosities at target protein concentrations greater than 100 g/L pose however challenges to the sample measurement process with the risk of inaccurate concentration determination.

**Methods/Approach:** A novel single-use inline viscometer, based on magnetic levitation, was developed to expand the currently available inline process analytical technology (PAT) toolbox. The inline viscometer can be readily integrated into the feed stream of the TFF device. The performance of the inline viscometer was investigated to measure protein concentration in a TFF process consisting of a UF and DF step.

**Results:** A reliable correlation between protein concentration and viscosity could be established by reference analytics. Subsequently, real-time monitoring of protein concentration via the viscometer during the TFF operation enabled accurate process control during the UF and DF steps, without the need to sample the process. At the same time, implementation of the viscometer had no impact on product quality.

**Conclusion:** Overall, this study affirms the effectiveness of a novel viscometer in monitoring protein concentration during pharmaceutical processes, making it an ideal inline PAT tool. The real-time viscosity measurement capability has the potential to increase the control of TFF operations and improve process performance.

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# A novel hybrid bioprocess strategy addressing key challenges of advanced biomanufacturing

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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
Traditionally, bioprocesses are strictly separated into continuous and discontinuous operations, which have distinct limitations. Novel hybrid process strategies can overcome these limitations by synergistically combining benefits of both process formats.

**Impact/Novelty:**
The new continuous fed-batch strategy aims to combine both standard processes. Thereby, specific limitation of the individual processes such as low productivity (fed-batch) or high media consumption (perfusion) are overcome.

**Introduction:**
Biotherapeutic products are commonly produced by either steady-state perfusion or fed-batch (FB). Each process shows distinct advantages and disadvantages in areas such as plant utilisation, achievable cell densities and media consumption. The goal of this work was to show feasibility of a novel hybrid process strategy to combine advantages of both process formats, while eliminating the respective limitations.

**Methods/Approach:**
The novel continuous fed-batch (cFB) strategy involves a short-term FB with subsequent rapid and complete media exchange and cell bleed, followed by the next FB-cycle. The concept was firstly tested in small-scale, improving the FB-cycle duration and reinoculation cell density. Subsequently, beneficial process parameters were scaled-up to a 5 L benchtop system incorporating a fluidized-bed-centrifuge (FBC) as scalable media exchange system.

**Results:**
The novel cFB showed continuous characteristics as well as increased productivity (+217%) at benchtop-scale compared to the FB. In contrast to a perfusion process, lower media consumption (-50%) and decreased monitoring effort could be reported. Further, compared to filter-based perfusion, the FBC media exchange could bypass distinct effects like product sieving or filter fouling. Furthermore, product quality remained comparable to the perfusion process.

**Conclusion:**
To summarize, the novel developed hybrid process strategy addresses the main challenges of current bioproduction, namely either low productivity resulting from FB operations or high media consumption rates and filter-dependent issues that are common issues of perfusion cultivations. Therefore, the developed continuous fed-batch represents a new and innovative approach for future process intensification efforts.

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# CHO upstream platform development: Lower cost-of-goods and improved quality

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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
Next generation bioprocesses are challenged with providing a high-quality product with a reduced cost-of-goods to further democratise biopharmaceutical supply.

**Impact/Novelty:**
We describe two parallel innovation streams, upstream process development and feed media development, which converge to produce improved product quality attributes, at lower cost, in a fed batch process.

**Introduction:**
The work presented set out to achieve two main objectives. Firstly, the improvement of both product quality and process attributes (e.g., robustness and scalability), through modification of upstream process running conditions (e.g., bioreactor setpoints and feeding regimes). Secondly, lowering of cost of goods through development of a new production feed media.

**Methods/Approach:**
Upstream process development took an iterative, DoE led approach with multiple cell lines and products in an automated microbioreactor system, followed by fine-tuning and scale-up verification at bench scale. Novel lower cost feeds were incorporated into the process during scale-up.

**Results:**
The feeding aspects of the process development work is centred on responsiveness to nutrient requirement in the culture, and as such is designed to cater for the variable needs of different cell lines. The resultant upstream process delivered equivalent productivity but with a favourable shift in charge profile from acidic to main peak species. Furthermore, the process was characterised by lower demands for gassing input for oxygen and pH control, minimal base requirement for pH control, reduced foaming and lower levels of lactate, osmolality and $pCO_2$. The introduced new feeds are estimated to yield a reduction in feed costs of greater than 50 %.

**Conclusion:**
The approach outlined was successful in driving significant quality and cost advantages over the predecessor process and demonstrates the significant gains which can be made within the confines of an existing bioproduction platform.

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# End-to-end model-based continuous bioprocessing with central data management

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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:**
The critical role of a software platform in continuous bioprocessing, enabling cross-device model-based control and data management for optimized efficiency, adaptability and precision.

**Impact/Novelty:**
Interlinking all units and vendor-independent devices throughout the entire bioprocessing continuum, coupled with automated sampling, data management, direct incorporation of results, visualisation and model-based process control within one tool.

**Introduction:**
Continuous bioprocessing has emerged as a promising approach in biomanufacturing, offering increased efficiency and product consistency. This work explores the comprehensive integration of model-based control throughout the entire bioprocessing continuum, from upstream processing to downstream purification, with a central data management and control system orchestrating the entire operation. The aim is to showcase an end-to-end solution that maximizes process control, optimization and data-driven decision-making.

**Methods/Approach:**
All units and devices from bioreactors, pumps, scales, sensors, tangential flow filtration (TFF) to simulated moving bed (SMB) chromatography are interlinked. Central data management, facilitated by our software, acts as a centralized hub, consolidating data from various sources into a unified platform. Automated sampling with subsequent data processing and direct incorporation of the results can be applied for cross-device process control. This system is laying the foundation for process model development, advanced real-time monitoring and the implementation for early anomaly detection, proactive decision-making and flexible control strategies.

**Results:**
We have developed a platform for a fully integrated end-to-end continuous process with a digital representation of the complete process chain. Illustrated by case studies and practical applications, the comprehensive model-based continuous bioprocessing with central data management, demonstrates how ensuring real-time availability of all data in a suitable format and connecting it with mathematical models, facilitates steering the process in the correct direction. This showcases improved process efficiency and increased adaptability to dynamic manufacturing environments.

**Conclusion:**
The integration of our software into continuous biomanufacturing processes presents a promising avenue for a new era of precision and efficiency and has the potential to revolutionize the biomanufacturing landscape. By providing a holistic solution addressing challenges across the entire bioprocessing chain, this approach lays the groundwork for enhanced productivity, reduced costs and accelerated development timelines.

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# A novel cell retention strategy introducing scalable sorting of viable cells by fluidized bed centrifugation

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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
Biotherapeutic manufacturing processes are limited by the loss of cell viability and accumulation of apoptotic cells. Selective cell retention could reduce these challenges by extending cultivation time and process intensification.

**Impact/Novelty:**
The novel method enables sorting of the complete bioreactor cell culture to enrich viable cells during biopharmaceutical production. Based on this, an intensified cultivation concept with enhanced productivity was developed.

**Introduction:**
Fluidized bed centrifugation (FBC) enables the separation of viable and non-viable cells based on small differences in cell density, shape, and size. This method, also known as cell elutriation, has so far only been able to process small quantities of cells for research and biomedical applications. To apply cell elutriation in biopharmaceutical production a scalable and simple sorting method was developed using single-use based FBC systems.

**Methods/Approach:**
The sorting method was optimized in a small-scale FBC considering the viability of the cell culture to adapt the FBC process parameters. In a subsequent proof-of-concept study, the parameters were transferred to a technical-scale FBC system and used to sort 2 L of a low viable CHO cell broth expressing an antibody. Furthermore, its application as a selective cell retention strategy was tested in a hybrid fed-batch cultivation.

**Results:**
Most viable cells were retained in the FBC chambers and returned to the bioreactor, while most non-viable cells and cell debris were removed with the leached culture medium. Using the upscale setup, the complete broth with low viable cells was processed in a single step of less than 12 minutes, achieving a significant increase in cell viability from initially 51% to 81%. In a related study, sorted cell cultures showed higher cumulated product titers and, interestingly, higher cell specific productivities.

**Conclusion:**
Selective cell retention using FBC is a promising strategy to remove non-viable cells and thus to enrich viable cells in the production bioreactor, enabling intensified biomanufacturing.

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# Scale-down model of the future: Ambr250 ® 's Innovation sparks a bioprocess revolution

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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:** This study assesses the Ambr250® (automated microscale bioreactor system) as an alternative to 2L glass bioreactors.

**Impact/Novelty:** Our findings suggest that the Ambr250® holds promise as an advanced and reliable alternative for scale-down modeling in bioreactor applications.

**Introduction:** Efficient bioreactor scale-down models (SDM) expedite bioprocess optimization, simulating large-scale processes cost-effectively. They enable parameter optimization on a smaller scale before scaling up, reducing risks and resource expenditure. The Ambr250® stands out due to its advantages, such as scalability, precise control, and diminished resource demands. This study explores its potential to redefine the landscape of SDM development, laying the groundwork for more streamlined and resource-conscious bioprocess advancements.

**Methods/Approach:** Satellite and non-satellite cell culture production runs were performed with Chinese Hamster Ovary (CHO) cell lines expressing monoclonal antibodies. For satellite runs, aliquots from N-1 pre-culture and media were used from the manufacturing site, to run the Ambr250® system in parallel to the manufacturing campaign (2000L stainless-steel bioreactor). For the non-satellite runs, Ambr250® bioreactors were inoculated after expanding the cells separately with the same type of media used in manufacturing scale. The impact of process and engineering parameters on cell culture performance and product quality attributes were investigated in a design of experiment (DoE) approach. Samples from all process runs were collected, treated, and analyzed using high-throughput methods to generate product quality data. The results of the Ambr250® runs were compared to 2L (current validated SDM) and 2000L manufacturing bioreactor runs to evaluate predictability of control conditions and process parameter changes.

**Results:** The similar performance of Ambr250® to our validated model implies a seamless transition, offering the prospect of enhancing efficiency and predictability in the development of bioprocesses.
**Conclusion:** The Ambr250® emerges as a strong candidate, signaling a shift toward innovation in the generation of scale-down models for bioreactors.

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# Nanoparticle-based vaccines for COVID-19: Click chemistry functionalization of HIV-1 virus-like particles and extracellular vesicles

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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:**
COVID-19 human sera recognition of two selected B-cell epitopes from the SARS-CoV-2 Spike protein demonstrating the potential of click chemistry in nanoparticle-based vaccine development.

**Impact/Novelty:**
The potential of click chemistry for functionalizing extracellular vesicles is demonstrated, paving the way for its use in vaccine development or drug delivery approaches.

**Introduction:**
The COVID-19 pandemic highlighted the need for rapid vaccine development methods. Here a click chemistry reaction has been optimized to covalently attach linear B-cell epitopes from the SARS-CoV-2 Spike protein to HIV-1 Gag virus-like particles (VLPs) and extracellular vesicles (EVs). These nanoparticles, increasingly explored for vaccination and drug delivery, can be easily and quickly functionalized through this optimized approach, achieving a high epitope density, showing the potential of click chemistry in nanoparticle-based vaccine development.

**Methods/Approach:**
Cy5, used as reporter molecule, allowed to measure functionalization using nanoparticle tracking analysis and fluorimetry. Nanoparticle functionalization was characterized by super-resolution fluorescent microscopy (SRFM). Dot-blot with COVID-19 human sera validated the Spike protein epitopes selected using bioinformatic tools and bibliographic research.

**Results:**
After the proof of concept of the functionalization of VLPs and coproduced EVs, an optimization process based on reaction kinetics and design of experiments, allowed to determine the optimal reaction parameters and reagent concentrations, achieving 923.28 Cy5 molecules covalently linked per nanoparticle. SRFM analysis revealed remarkable differences in functionalization between VLPs and EVs. Due to this, two EV stocks, produced by mock transfection and cell growth, were functionalized achieving 3618.63 and 6498.75 Cy5 molecules covalently liked per nanoparticle. The different nanoparticle stocks were functionalized with Spike protein epitopes to perform an immunoassay using COVID-19 human sera with a high antibody recognition.

**Conclusion:**
This work has optimized a click chemistry method for rapidly functionalizing EVs with Spike protein epitopes. By achieving high epitope density, this approach highlights its potential to accelerate the early stages of vaccine development.

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# Evaluation of cell growth and AAV production in a fixed-bed bioreactor to streamline process development

**Anne MacIntyre**[1], Nathan Hazi[1], Andrew Laskowski[1], Michelle Olson Peabody[1]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:** The iCELLis Nano bioreactor is an effective tool for process development of adherent cell cultures. The studies performed provide insight into optimizing AAV processes using this technology.

**Impact/Novelty:** We evaluated how select process parameters affected cell attachment, cell growth and distribution, and recombinant adeno-associated virus serotype 5 (rAAV5) production in the iCELLis Nano bioreactor.

**Introduction:** The demand for viral vector-based gene therapies continues to grow as FDA approvals accelerate and the number of potential indications increase. To remain competitive, companies must streamline viral vector development timelines using advanced systems and efficient processes. For adherent cell culture processes, the iCELLis Nano bioreactor is an automated, single-use, fixed-bed bioreactor that facilitates excellent cell growth and volumetric productivity and offers high flexibility for gene therapy development.

**Methods/Approach:** We performed a series of growth and rAAV5 production cell culture runs using a HEK293 cell line. We evaluated the impact of linear speed and presence of Pluronic F-68 on cell growth and distribution. We also evaluated the impact of linear speed, cell density, and total DNA concentration during transient transfection on rAAV production.

**Results:** Pluronic F-68 exhibited no impact on cell growth, while linear speed was shown to influence cell growth and overall cell distribution. At the time of transfection, the cell density and total DNA concentration displayed an effect on rAAV production yield, while linear speed did not.

**Conclusion:** The results highlight areas of process robustness when using the iCELLis Nano bioreactor. They also suggest focus areas for process development when evaluating cell growth and rAAV production.

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# Advanced Shaken BioReactor (ASBR): Novel small-scale mammalian continuous cultivation platform

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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance/Impact/Novelty:** We present a novel, simple and inexpensive, yet predictive, scale-down system for perfusion processes.

**Introduction:** Intensified processes often involve perfusion processes and therefore there is an increasing need to scale these down.

**Methods/Approach:** A 20 mL scale-down system was implemented using orbitally-shaken centrifuge tubes with automated feed, harvest and pH control through Python and serial communication. The system combines an incubator, a medium pump, and a harvest pump. Sterile centrifuge tubes with a top filter for gas exchange are pierced with a needle for medium addition and a suction tip for level-controlled harvest.

Due to the difficulty of implementing effective cell retention and oxygenation in small scale, we scaled-down perfusion cultures to chemostat cultures. In order to enable sampling without perturbing the dilution rate, Exponentially increasing In-flow and Discontinuous Out-flow (EIDO) was implemented[1]: the working volume is reset daily through harvesting but increases exponentially over the day with medium additions every 2nd hour. To keep the dilution rate constant, additions are matched to the current working volume[1].

**Results:** ASBR is compared to ambr™ chemostat and classical bench top chemostat cultures and finally to a bench top ATF perfusion setup, illustrating the predictability of the scale-down system. The power of using chemostats to scale down perfusion is shown: when the chemostat dilution rate is equal to the perfusion bleed rate (same growth rates) the cells experience the same chemical conditions (concentration of substrates and products e.g., titre) in the two systems at steady state[1].

**Conclusion:** ASBR delivers prediction of perfusion: physiology, titre, VCD and quality; with 1) Reduced handling, 2) Minimal consumables, 3) Simplicity and therefore less failures, 4) Low cost and 5) Works equally well with 1 tank or 6 tanks.

1. Heitmann et al. 2024. Scale-down of CHO cell perfusion cultures to small-scale chemostat cultures. Biotechnology Progress (In Prep)

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# microRNA technology for optimized antibody galactosylation

**Dr René Handrick**[1], Florian Klingler[1], Patrick Schlossbauer[1], Lukas Naumann[2], Prof. Friedemann Hesse[1], Prof. Christian Neusüß[2], Prof. Kerstin Otte[1]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:**
Engineering of glycan structures has become an essential tool to modulate biological properties of monoclonal antibodies (mAbs) and adapt them to therapeutic needs. Recent genetic engineering approaches based on knock-out and overexpression are often unable to fine-tune glycosylation modulation. Therefore, novel approaches addressing/altering glycosylation pathways have are needed in cell line development.

**Impact/Novelty:**
We demonstrate the potential of small noncoding microRNA (miRNA) technology to modulate glycosylation in Chinese hamster ovary (CHO) cells establishing miRNAs as engineering tools for fine tuning of galactosylation.

**Introduction:**
Galactosylation is considered a critical quality attribute (CQA), as the glycan pattern modulates Immune response. Since N-linked terminal galactose residues can affect serum half-life, antibody-dependent cellular cytotoxicity (ADCC), complement-dependent cytolysis (CDC) and antiviral activity it has to be controlled to ensure therapeutic efficacy.

**Methods/Approach:**
The potential of miRNAs to alter galactosylation in CHO production cells was investigated by transient and pcDNA6.2-GW/EmGFP-miR-based stable transfection. Successful modulation of glycans was monitored by mass spectrometry. Molecular response of galactosylation pathways was analyzed by qPCR. Influence on growth/viability was investigated by quantitative flow cytometry (Calcein-AM staining) and trypan blue exclusion. Antibody concentration was determined by protein A high-performance liquid chromatography.

**Results:**
We identified, characterized and validated miRNAs as engineering tools for decreased (mmu-miR-7646-5p, mmu-miR-7243-3p, mmu-miR-1668, mmu-let-7c-1-3p, mmu-miR-7665-3p, mmu-miR-6403) or increased (mmu-miR-452-5p, mmu-miR-193b-3p) degree of galactosylation. Underlying regulation of gene expression of the galactosylation pathway was confirmed at mRNA level corresponding with respectively altered galactosylation profile. While downregulation of β-galactosyltransferases B4GALT1, B4GALT2 gene expression by mmu-miR-7243-3p, mmu-miR-1668, mmu-let-7c-1-3p, mmu-miR-7665-3p, mmu-miR-6403 resulted in decreased galactosylation, mmu-miR-452-5p and mmu-miR-193b-3p, which increased the amount of galactosylated glycoforms, increased expression of β-galactosyltransferases, precursor synthesis genes GALM, UGP2 and of a nucleotide sugar transporter SLC35A2. Finally, a 10 days batch cultivation of stable miRNA overexpressing pools revealed similar or even increased volumetric product concentrations compared to the mock control.

**Conclusion:**
Stable plasmid-based overexpression of miRNAs represents a versatile tool for engineering cell phenotypes inducing favorable galactosylation-patterns for biopharmaceutical production.

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# Fluorescence sensor development for online monitoring of critical process parameters in Tumour-infiltrating lymphocyte cultivations

**Ms Sandra Dienemann**[1], Dr. Ole Jacob Wohlenberg[1], Dr. Nicola Goddard[2], Dr. Lucia Teixeira[2], Dr. Mariana Werner Sunderland[2], Dr. John Churchwell[3], Dr. Vincenco Di Cerbo[3], Dr. Patrick Statham[3], Dr. Dörte Solle[1], Prof. Dr. Sascha Beutel[1]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:** The project aims to develop a smart manufacturing platform for gene and cell therapy manufacturing to improve efficacy, reduce costs, and minimise batch failures by implementing new sensor technologies.

**Impact/Novelty:** The first-of-its-kind single-use fluorescence sensor will enable non-invasive online monitoring of critical process parameters (CPPs) during the co-cultivation of Tumour-infiltrating lymphocytes (TIL) and dendritic cells, and subsequent cell expansion.

**Introduction:** TILs represent the next generation in autologous immunotherapies. They have the capability to surpass the constraints of current systems, such as Chimeric Antigen Receptor (CAR) T-cells utilised in haematological malignancies and can be employed for the treatment of solid tumours. The manufacturing process of complex personalised cell therapies faces a lack of fast, adaptive, controllable and scalable cultivation platforms. To address this bottleneck, the consortium aims to develop a new manufacturing platform for autologous immunotherapies by implementing online process analytical technologies (PAT) and adaptive process control.

**Methods/Approach:** The consortium emphasizes real-time monitoring of intrinsic fluorophores like proteins, nicotinamide adenine dinucleotide (NADH), or flavins using two-dimensional fluorescence measurements. Fluorescent process biomarkers are identified using offline measurements and their relevance to the process is confirmed by metabolomics analysis.

**Results:** Relevant wavelength combinations for a co-cultivation process of TILs and dendritic cells were identified using offline reference 2D fluorescence measurements. This information provides the basis for the development of a sensor prototype to monitor the cultivation process online in different scales.

**Conclusion:** The implementation of a fluorescence sensor for simultaneous monitoring of several metabolites during the co-cultivation of TILs and dendritic cells is a valuable tool to gain process knowledge as basis for process control. It is a promising approach in advancing the manufacturing of complex autologous gene and cell therapies and reducing manufacturing risks.

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# A 3D-printed microfluidic system for automated transient transfection of suspension cells at high-cell density

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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
Automated transient transfection is an essential step towards continuous, reproducible transfection which could make the production of biopharmaceuticals more flexible and faster compared to stable clones.

**Impact/Novelty:**
Our innovative 3D-printed microfluidic transfection system is easily operated, offers reproducible and improved results compared to manual methods, and can be used for continuous transient transfection.

**Introduction:**
Manual transient transfection often exhibits large fluctuations and cannot be carried out at high-throughputs – which is essential for the use in clinical or industrial purposes. However, the demand for patient-specific biopharmaceuticals is increasing and high-throughput screening is necessary. To close this gap, we have developed a 3D-printed microfluidic transfection system that can be integrated into cell culture processes and offers the ability for continuous transient transfection through automation and parallelization.

**Methods/Approach:**
The microfluidic system with integrated micromixers and connectors was developed using CAD software and printed via high-resolution 3D printer using a biocompatible, heat-stable material. The system was validated for transient transfection of CHO-K1 and HEK293E suspension cells at high-cell density ($20*10^6$ cells/mL) using linear polyethylenimine (PEI, 25 kDa) as transfection reagent and an EGFP plasmid. The reproducibility was also demonstrated by dynamic light scattering measurements.

**Results:**
By using the microfluidic system, we were able to achieve automated, reproducible transient transfection of the used cell lines with high cell viability and transfection efficiencies between 90-99%. Due to the integrated micromixer, the system produces stable and uniform PEI-DNA polyplexes, which can be modified based on the flow rates.

**Conclusion:**
By connecting the microfluidic system to a cell culture process, this facilitates the automated, continuous transient transfection of suspension cells which can be employed to produce recombinant proteins "on demand". The microfluidic system enables homogeneous polyplex formation and mixing of the cell suspension, resulting in significantly more reproducible results compared to manual methods.

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# Ambient intelligence for the quantification of cell culture operations

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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:**
This study focuses on the working process of culturing therapeutic cells and provides insight into techniques for analysing them in a quantitative manner.

**Impact/Novelty:**
A system was developed to quantitatively evaluate similarities from video data during cell culture operations and demonstrated that data-driven methods are effective in analysing and standardizing cell culture operations.

**Introduction:**
Cell-based therapies, emerging as a promising solution for challenging diseases, have increased the demand for large-scale and reliable cell production. Unfortunately, current cell culture processes are heavily reliant on manual labor, trailing behind other industries in technological advancement. The recent progress in image processing technology has enabled the quantification of human activities, a previously daunting task. In healthcare, this application of image-based measurements to professional procedures and patient activities is termed "ambient intelligence." This concept is a crucial component of data transformation (DX), which is vital for the development of intelligent facility management based on data. Research indicates that DX is efficient in improving processes that are dependent on manual labor.

**Methods/Approach:**
We utilized "ambient intelligence" in cell culture to quantitatively evaluate operator skills and enhance training. By analysing video data of operators at clean benches with a skeleton estimation algorithm, we tracked cell-passaging actions. A unique algorithm summarized actions, enabling comparison of operational characteristics and their training implications.

**Results:**
Our findings reveal that video analysis for data-driven operation profiling is an effective way to standardize operational skills. It helps both operators and trainers by ensuring consistent operational practices and improving training methods.

**Conclusion:**
Our results point to ambient intelligence-assisted cell manufacturing as a cutting-edge method for standardizing complex cell culture tasks and boosting the efficiency and safety of cell production.

**Reference:**
Haque A, Milstein A, Fei-Fei L.: Nature, 585(7824), 193-202 (2020)

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# Incorporation of a click-chemistry-reactive tetrazine amino acid for the production of site-specific antibody-drug conjugates

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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:** The presented technology allows the production of site-specific protein conjugates such as ADCs without heterogenous by-products.

**Impact/Novelty:**
Highly homologous, site-specific protein conjugation for e.g. ADCs overcomes existing challenges of heterogenous and uncontrolled traditional production methods.

**Introduction:**
Antibody-drug conjugates (ADCs) combine directed cancer therapy with conventional chemotherapy by coupling a small cytotoxic molecule via a short linker unit to a monoclonal antibody. All ADCs currently on the market rely on side-chain functionalization of surface-exposed amino acids (e.g. lysine or cysteine), resulting in heterogenous mixtures of protein conjugates. Here, we investigated the incorporation of the tetrazine-bearing non-canonical amino acid urea-Tet-Lys (uTL) into recombinant proteins using an orthogonal pyrrolysyl-tRNA/pyrrolysyl-tRNA synthetase pair for amber suppression in Chinese hamster ovary (CHO) cells. Bio-orthogonal click chemistry reactions of the tetrazine moiety enable the production of site-specific protein conjugates.

**Methods/Approach:**
As a proof-of-concept, uTL was incorporated in an mCherry-EGFP reporter fusion protein bearing a TAG stop codon between the two fluorophores after transient transfection of reporter and tRNA/tRNA-synthetase plasmids in CHO-K1 cells. To investigate the potential of producing a therapeutic ADC, a plasmid encoding trastuzumab with an amber stop codon in the heavy chain (trastuzumab-uTL) was expressed in ExpiCHO-S cells, purified and conjugated with a PEG12 molecule via click-chemistry. Product homogeneity and conjugation efficiency were analysed by protein electrophoresis and mass spectrometry.

**Results:**
uTL was incorporated in the mCherry-TAG-EGFP reporter protein with an efficiency of 24 % as assessed by flow cytometry. Tetrazine conjugation to a click chemistry reactive fluorophore was shown by protein electrophoresis. Analytical quantities of trastuzumab-uTL with two defined tetrazine conjugation sites in the heavy chain were purified. After conjugation of a functionalized PEG12 molecule to the antibody via click-chemistry, one single mass was identified for the heavy chain by mass spectrometry indicating that the click chemistry reaction generated a homogeneous protein conjugate.

**Conclusion:**
The results are a promising step towards overcoming the heterogeneous conjugate mixtures resulting from lysine and cysteine conjugation, which would facilitate the development of next generation site-specifically conjugated ADCs.

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# Disrupting Cell Therapy Storage and Distribution with Hypothermic Preservation of Adipose-Derived Mesenchymal Stromal Cells (MSC)

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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
Hypothermic storage in alginate beads as an alternative to cryopreservation is an emerging technology with the potential to improve the MSC production process, fast-tracking the development of new cell-based products.

**Impact/Novelty:**
MSC encapsulation for 12 days at 10-20°C retaining viability and function; hematopoietic support assay as a functional assay for MSC; human platelet lysate as an alternative to fetal bovine serum.

**Introduction:**
Cell and gene therapies (CGT) have reached new therapeutic targets but have noticeably high prices. Solutions to reduce production costs might be found in CGT storage and transportation since they typically involve cryopreservation, which is a heavily burdened process. Encapsulation at hypothermic temperatures (e.g., 2-8 °C) could be a feasible alternative.

**Methods/Approach:**
Adipose tissue-derived mesenchymal stromal cells (MSC(AT)) expanded using fetal bovine serum (FBS)- (MSC-FBS) or human platelet lysate (HPL)-supplemented mediums (MSC-HPL) were encapsulated in alginate beads and kept in at a temperature between 10 and 20 °C, to mimic possible temperature oscillations during transportation, for 30 min, 5 days, and 12 days. After bead release, cell recovery and viability were determined to assess encapsulation performance. MSC identity was verified by flow cytometry, and a set of assays was performed to evaluate functionality.

**Results:**
MSC(AT) were able to survive encapsulated for a standard transportation period of 5 days, with recovery values of 56 ± 5% for MSC-FBS and 77 ± 6% for MSC-HPL (which is a negligible drop compared to earlier timepoints). Importantly, MSC function did not suffer from encapsulation, with recovered cells showing robust differentiation potential, expression of immunomodulatory molecules, and hematopoietic support capacity. MSC(AT) encapsulation was proven possible for a remarkable 12-day period.

**Conclusion:**
There is currently no solution to completely replace cryopreservation in CGT logistics and supply chain, although encapsulation has shown potential to act as a serious competitor. Improving initial encapsulation efficiency will contribute to further enhance encapsulation potential.

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# New Era of Real-time Bioprocess Monitoring and Control –Raman-based Direct Measurements with De Novo Model

**Dr Stephen Driscoll**[1], Dr Chris Brown[1], Mr Colin Gavin[1], Dr Milla Neffling[1], Dr Graziella Piras[1], Ms Alison Siegmann[1], Mr Morgan Siegmann[1]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected**
Innovate or Die – The Raman-based technology presented here enables understanding and control of mammalian bioprocesses without the significant investments and long timelines of traditional black-box chemometric model development.

**Impact/Novelty**
Our innovative Raman-platform is run as simply as a pH-meter, while still offering access to high-quality, full-spectrum Raman data. It employs physics-based *de novo* model for real-time monitoring and control.

**Introduction**
Raman technology is of interest to bioprocess parameter and product characterization. The appeal of simple optical *in-situ* probes for multi-parameter measurements is obvious, but the expense associated with robust multivariate model development often hinders implementation. Considerable expertise is required in the nuances of the spectroscopic modality, the interplay of chemistry, physics and environmental factors, not to mention the multivariate model development and validation. We have developed a Raman platform built to scale from PD to GMP implementation for bioprocess monitoring and control. This system utilises a *de novo* data-analytic approach that enables generalization across a wide range of cell lines, media systems, and process scales.

**Approach**
Traditional chemometric multivariate models are trained empirically with offline reference values and bioprocess Raman measurements, but these approaches are per-process models, which are prone to overfitting and are not generalisable. The *de novo* model presented here is derived from measurements of individual biochemical species, a mathematical model of the optical spectrometer itself, and the fundamental physics of the Raman measurement. Given the time-variant aspect of several of these attributes, it is updated continuously in real-time. The platform incorporates direct feed-control capability with built-in controller output, and has other built-in features for cGMP support.

**Results**
Results from the validation of the *de novo* model across many different cell lines, scales, and widely varying media systems will be reviewed. We will also present the results of spiking experiments across a range of fresh and spent media, which demonstrate consistency with theoretical, analytical figures of merit of the platform.

**Conclusion**
Although convenient for their ‘black box’ properties, empirical models can be impractical to deploy in complex bioprocesses robustly. Our Raman-based platform with the *de novo* approach is run as simply as a pH-meter, while still offering access to high-quality, full-spectrum Raman data for bioprocess development.

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# De Novo Approaches for Bioprocess Parameter Estimation using Raman Spectroscopy

**Dr Stephen Driscoll**[1], Dr Chris Brown[1], Mr Colin Gavin[1], Dr Milla Neffling[1], Dr Graziella Piras[1], Ms Alison Siegmann[1], Mr Morgan Siegmann[1]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected**
Innovate or Die – the current black-box, per-process, Raman solutions are incompatible with complex bioprocesses development approaches and timelines. We discuss a solution to this problem that employs a dynamic *de novo* model.

**Impact/Novelty**
The proposed modelling approach offers a scalable framework that overcomes many of the challenges involved with the implementation of Raman for bioprocess monitoring using traditional black-box chemometrics.

**Introduction**
Traditional chemometric models, such as partial least squares (PLS), struggle to accommodate the unique characteristics of bioprocesses. These processes inherently defy the assumptions of standard multivariate modelling approaches, exhibiting highly heteroscedastic measurement error structure, non-stationarity, and extreme time-domain correlations. In contrast to empirical models, like PLS, we propose a continuously updated *de novo* model. This novel approach embraces the probabilistic nature of bioprocesses, calculating a "linear best estimator" with each new measurement sample. By explicitly adapting to the intrinsic properties of bioprocesses, our model aims to sidestep the limitations of traditional chemometric approaches. We anticipate that this innovative framework will yield more accurate estimations of bioprocess parameters, paving the way for enhanced process optimization and control.

**Approach**
Here, we propose the use of a *de novo* model to construct a posterior "linear best estimator" dynamically in real-time. The model is comprised of both static and dynamic terms. For example, a formulary prior, which contains a density estimate of spectrally dominant components commonly found in bioprocesses, and a system-filter function, which contains information specific to each device, are used statically, while terms such as the error covariance matrix at time *t* are updated dynamically. Under this framework, the proposed model does not require any empirical bioprocess training data.

**Results**
Results from the validation of the proposed *de novo* model across a diverse set of cell lines, scales, and media will be presented. This includes an evaluation of the method's analytical figures of merit and a comparison of experimentally obtained sensitivity and selectivity to the *a priori* estimates provided by the model.

**Conclusion**
The proposed *de novo* modelling approach overcomes the impracticalities of implementing traditional black-box empirical methods, enabling a new era of bioprocess development and control with the simplicity of a pH meter.

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# "Maximisation" of IgG Galactosylation: Model-led Cell Engineering Strategy for Quality Assurance in CHO mAb Products

**Dr Aoife Harbison**[1], Dr. Mina Ghahremanzamaneh[1], Mr. Itzcoatl Gómez-Aquino[1], Dr. Alfonso Blanco[2], Dr. Sara Carillo[3], Dr. Jonathan Bones[1,3], Dr. Ioscani Jiménez Del Val[1]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
We have developed a simple and robust CHO host cell engineering technology that curbs glycan variability throughout bioprocess development and scale up.
**Impact/Novelty:**
By identifying mechanistic and metabolic bottlenecks in mAb glycosylation, we were able to develop an easily implementable but novel strategy for galactosylation control and have published an international patent application.

**Introduction:**
For commercial monoclonal antibody (mAb) products, N-linked glycosylation is a critical quality attribute, where increased β4-galactosylation enhances mAb Complement Dependent Cytotoxicity (CDC) and Antibody-Dependent Cellular Cytotoxicity (ADCC) effector functions. However, glycan heterogeneity is often encountered throughout bioprocess development and must be addressed in order to progress through to clinical trials.
**Methods/Approach:**
Using metabolic modelling with CHOmpact, two significant bottlenecks were identified in the N-galactosylation pathway: (i) Diversion of nucleotide sugar donor supply towards cellular O-galactosylation and (ii) Limited accessibility/availability of the β4-galactosyltransferase enzyme in Golgi. To alleviate these bottlenecks, we established a two-step cell-engineering strategy, "GalMAX", where, respectively, (i) the β3-galactosyltransferase chaperone (COSMC) is knocked out using nuclease based tools (CRISPR Cas9 or TALENs) and (ii) β4-galactosyltransferase is ectopically overexpressed to drive product N-galactosylation. Cell pool enrichment was achieved using lectin-aided fluorescence cell sorting (LA-FCS).
**Results:**
Proof of concept studies were carried out on two CHO cell lines, VRC01 and DP12. The GalMAX variants of these cell lines were cultured in batch and fed-batch modes for characterisation. No negative impacts were observed in cell growth, metabolism, and mAb titre by the cell-engineering events. LC-MS glycoprofiling was performed on the mAb product. In batch mode, both DP12-GalMAX and VRC01-GalMAX cells yielded an increase of galactosylation by 40% and 44% respectively, compared with the parental cells. Similarly, in fed batch mode, galactosylation increased by 44% (DP12) and 50% (VRC01).

**Conclusions:**
By increasing β4-galacostylation to >90%, the GalMAX strategy has great promise for both quality assurance and potentially improved oncolytic activity of mAb products. We envision GalMAX as a robust "plug-and-play" technology to enhance the performance of biopharmaceutical host cell platforms.

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# Bispecific antibodies production by mRNA transfected CHO cells

Mr. Thornwit Chavalparit[1], Dr. Marianne Gillard[1], Associate Prof. Timothy Mercer[1,3], Prof. Esteban Marcellin[1,2], **Dr Veronica Martínez**[1]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:**
mRNA transfection of CHO cells for production of therapeutic proteins is an innovative technology that allow fast protein production, it is useful to optimize peptides transcripts ratios for optimal product.
**Impact/Novelty:**

To our knowledge, this is the first time of using mRNA transfection of CHO cells to produce a bispecific antibody. This novel technology allows faster production of bispecific antibodies.

**Introduction:**

Bispecific antibodies (BsAbs) are the next generation therapeutic proteins with expected sales exceeding US$ 20 USD by 2028. BsAbs combine the functionality of two monoclonal antibodies in a single drug, resulting in a more robust and tailored response. Because BsAbs are artificial proteins, Chinese Hamster Ovary (CHO) cells expression yields are low, and the correct assembly of the antibody fragments is a challenge. Therefore, there is a need to improve CHO cells manufactured BsAbs quantity and quality. mRNA transfection offers the advance of fast and high yield production of antibodies by CHO cells, as well as the potential to test the conditions to generate the correct BsAbs assembly.
**Methods/Approach:**

Four mRNA molecules will be co-transfected into CHO cells by lipofection, one molecule for each BsAbs fragment. Different ratios of the four BsAbs fragment mRNAs will be tested in the transfection, and the product quantity and quality will be measured.

**Results:**

We expect to produce correctly assembled BsAbs by mRNA transfection in CHO cells. We also expect to find the optimal ratio of the different fragments mRNAs that when transfected into CHO cells generate more of the desired asymmetric heterodimeric BsAbs.

**Conclusion:**

mRNA transfection of CHO cells offers an alternative to traditional cell line development methods for fast production of complex therapeutic proteins. Allowing the optimization of the conditions necessary to produce an optimal product in a time saving manner.

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# Implementation of Novel Single-use Technique for Clearance of Associated Host Cell Proteins

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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to theme selected:**
Implementation of the single use membrane adsorber with novel ligand chemistry aids in controlling complex associated host cell proteins generated from mammalian cell culture systems .

**Impact/Novelty:**
The case study provides a single use method for reducing associated host cell proteins and low molecular weight impurities with a novel ligand chemistry.

**Introduction:**
Controlling complex host cell proteins during the purification may require several stages with different modes of chromatography. Protein associated host cell proteins co-elute with target protein and therefore removal is challenging. Therefore, it becomes necessary to develop a combined strategy with a novel purification step which can control these host cell proteins along with other product related impurities. In this study a novel purification step is developed for removal of associated host cell protein and low molecular weight impurities for purification process without affinity as capture step.

**Methods/Approach:**
A non-Fc Fusion Protein was captured from the harvest by ion exchange chromatography. Different single use membrane adsorbers were used. Parameters like load factor, residence time and pH were studied to optimize the clearance for impurities. Further scale-up was performed to establish scalability.

**Results**:
Anion exchange membrane adsorber with a hybrid ligand chemistry was observed to significantly reduce co- eluting host cell proteins and fragments in a single unit operation. Reduction % of>95% was achieved for both the impurities. During scale up, correlation was observed between fragment % in load and clearance of host cell proteins. Reduction of fragment % in the load was observed to increase clearance of host cell proteins.

**Conclusion:**
The membrane adsorber with the hybrid ligand chemistry can be used as a platform process to control complex host cell proteins and size related impurities in a single unit operation thus increasing process economics and efficiency of purification.

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# High-throughput media optimization strategy for CHO Cell lines

**Dr. Jaeho Shin**[1], Dr. Swapnil Chaudhari[1], Dr. Ing Ali Safari[1], Dr. Dirk Müller[1]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Novelty:** Usage of robotics for media variant preparation is in the early phase. Manufacturers can accelerate media optimization using statistical design of experiments and multiplexed cell cultivations (e.g., microtiter plates).

**Introduction**: As more biologics are developed, shortening molecule development timelines can help improve process efficiency. Optimizing the composition of cell culture media is critical to maximizing yield and attaining key product quality attributes. But it can be challenging — media can contain more than 50 different compounds and modifying both basal media and feeds and performing multiple iterations is often required.

**Methods:** For a given optimization target (e.g., product titer or glycans), initial data is collected from screening runs with media variants from a library. Cell performance in the scaled-down target process is assessed experimentally using a high-throughput approach in deep-well plates. Based on spent media analytics and a computational workflow, sensitive compounds are identified and used for predicting a set of targeted follow-up experiments for further optimization, e.g., at the Ambr® 15 scale. Once the desired optimization target is reached, scale up testing is performed and optimized formulations media can be provided in liquid or powder format.

**Results:** High throughput media screening allowed reproducible titer improvement in DG-44 (by 150%), CHO-S (79%), and CHO-K1 (40%) cell lines. Media library collected during the study includes >200 media and continues to accumulate.
**Conclusion:** The integrated platform provides a structured media optimization workflow harnessing high-throughput cultivation media variant preparation and screening, adaptive spent media analytics and a data analytics pipeline to support focused media optimization. The platform can be applied to optimizing both basal and feed media compositions. Its modular nature also allows for easy integration of upgrades and extensions to individual modules and provides an approach extensible beyond CHO cells.

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# Faster cell line development by using targeted integration combined with a new automation device setting

**Dr Anke Mayer-Bartschmid**[1]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:**
Technology innovation in Cell line development (CLD) helps to select in a shorter timeframe more predictable clones.

## Impact:
A combination of a targeted integration expression system, new cell sorting and high throughput (HT) bioprocessing devices allows for faster supply of drugs to the clinics.

## Introduction:
CLD for biologics is historically seen as the time critical step on the way to first in human for protein-based drugs. Monoclonality assurance and the selection of high titer clones with favorable product quality is time and resource intensive. Newer approaches to overcome these limitations are the use of Targeted (TI) or Transposase -mediated integration. Still, this needs screening efforts to select a stable, monoclonal production cell.

## Approach:
A random screening approach with a TI capable landing pad led to a good and stable producing master clone. The gene of interest can be flipped in as a multicopy. By using the Cytena UP.SIGHT$^{TM}$ single cell printer non-exchanged clones were sorted out by their fluorescence signal. A small number of clones (96) were expanded into a 96well based automated fed-batch system with automated feeding and titer measurement capabilities. Samples are generated for a HT ddPCR analysis to check for correct copy number and integration.

## Results:
The isolated RMCE capable clone showed a reasonably good titer by integrating 2 copies of a monoclonal antibody combined with a stable expression. By using our automation platform, we could isolate in a fast and efficient manner isogenic, monoclonal clones. The final clone could be selected without further large scale bioreactor check and stability assessment. The overall timeline until final clone can be shorten to 3 months.

## Conclusion:
New HT technologies allow for selection of production cell lines with reproducible bio profile, defined and well characterized genetic integration in a fast and reliable way.

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# 2nd gen UP.SIGHT : Generation of monoclonal cell lines, from cloning to clone selection

**Lucia Gordillo-Perez**, Ms. Julia Scherzinger[1], Mr. Daniel Türk[1], Mr. Harrison Garner[1], Dr. Fernando Aprile Garcia[1]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
We present a novel single cell dispenser and imager for cell line development (CLD).

**Impact/Novelty:**
The 2nd gen UP.SIGHT covers cell line development processes from single cell cloning to selection of high producing clones with comprehensive documentation for IND/BLA submissions on a single instrument.)

**Introduction:**
There is constant pressure to reduce timelines in mammalian cell line development (CLD) for biotherapeutic protein production. Demonstration of clonal derivation of the generated cell lines is key for health authorities' approval. To meet these regulatory and process-oriented demands, single-cell dispensers and plate imagers are vital to any CLD laboratory. Here we present the 2nd gen UP.SIGHT, that combines single cell dispensing and plate imaging capabilities.

**Methods/Approach:**
Single cell dispensing experiments were performed to determine single-cell dispensing efficiency (SCDE) and probability of clonal derivation (p(clonal)). Cell growth and monoclonal antibody titer quantification were determined using the 2nd gen UP.SIGHT's imaging capabilities. Data was analysed using the C.STUDIO 2.0 software.

**Results**
The instrument's SCDE resulted in 97.81% and (p(clonal)) was higher than 99.99% . Furthermore, we show that the cloning efficiency can be up to 80% when working with optimized medium conditions. Importantly, we also demonstrate that growth assessment via well bottom imaging or cell counting and titer measurement of monoclonal antibody-like molecules can be performed on the 2nd gen UP.SIGHT. Data analysis in C.STUDIO 2.0 guides the user to select the best clones and provides comprehensive data logs and reports for IND/BLA submissions.

**Conclusion:**
2nd gen UP.SIGHT enables fast and efficient CLD workflows, covering all the steps from single-cell dispensing with assurance of clonality, colony tracking and titer measurement. This ultimately results in better documentation for improved quality of the final biological product.

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# Self-Organizing Murine Cardiac Organoids Towards Heart-On-Chip and Modeling of Congenital Defects

**Dr Sebastien Sart**[1]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:** We selected theme 5. Here we developed a new normal and pathological model of heart development. The heart-on-chip opens the way towards live-monitoring and regulation of heart functions.

**Impact/Novelty:** The mouse cardioids recapitulate the features of normal and pathogenic developing hearts. The heart-on-chip platform pave the way for curing congenital heart diseases.

**Introduction:** Congenital heart diseases are one of the most prevalent type of birth defects, which require better understanding. However, it is challenging to characterize the cardiac organogenesis *in utero* or to maintain embryos *ex vivo*. Thus, there is a need for novel *in vitro* model of heart development that enables quantitative analysis of normal and pathological development with immediate comparability *in vivo*.

**Methods/Approach:** We developed a novel protocol to induce differentiation into heart tissue using murine embryonic stem cells (ESCs). Time evolution of cardiac development was analyzed at the transcriptional level. The cellular diversity, structural organization and beating were investigated by quantitative imaging. *Greb1l*$^{+/tm1a}$ ESCs were used to model crisscross hearts. A perfused droplet microfluidic platform was developed to generate cardioids on chip.

**Results:** The aggregates started to express markers of first and second heart fields, followed by cardiomyocytes, epicardium and endothelium, just as during embryonic organogenesis. The cardioids displayed myofibrils and heart cavities, while beating at regular frequency. The downregulation of *Greb1l* restrained cardioid formation, through abrogation of beating, ribosome biogenesis, depletion of cardiac precursors, just as we found by *in vivo* labeling. Functional cardioid generation was successfully translated within droplets on chip.

**Conclusion:** Mouse cardioids display the main features of normal and pathological heart development. The microscale generation of cardioids on chip paves the way towards combinatorial drug screening on chip and the development of novel therapies of congenital heart diseases.

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# Scale-down of a continuous centrifuge for the harvest of mammalian cell culture

**Mrs Michaela Dölle**[1,2], Mr Maxime Hervé[1,2], Prof. Veronique Chotteau[1,2]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:** Continuous stack centrifugation is a classic method to clarify mammalian cell cultures. Down-scaling offers significant advantages, enhancing process development, increasing robustness, and accelerating process approval, thereby reducing time.
**Impact/Novelty:** A novel scaled-down continuous centrifuge, derived from a commercially available disc-stack centrifuge for mammalian cell clarification, enables early-stage scalable harvesting and clarification in process development.

**Introduction:** The initial stage of downstream processing often involves centrifugal harvesting, with non-continuous centrifugation at a small scale while continuous centrifugation is applied in large-scale processes. The prediction of the non-continuous centrifugation for the large-scale continuous centrifugation is limited, posing a challenge for manufacturing scale-up.

**Methods/Approach:** Our method involves adapting the continuous centrifugation process, typically designed for larger-scale applications, to a more compact and efficient configuration suitable for small-scale operations. By systematically modifying key parameters, including rotor size, speed, and operational conditions, we aim to enhance the performance and scalability of the centrifugation process in the context of mammalian cell clarification.

**Results:** Preliminary results indicate promising cell recovery and process efficiency, underscoring the potential of this approach for advancing cell clarification methodologies in biomanufacturing.

**Conclusion:** Our study demonstrates that the prototype of a small-scale continuous centrifuge holds substantial potential to enable the scalability of manufacturing processes from the early stages of development. This has significant implications for resource, cost, and time savings, paving the way for sustainable advancements in process development.

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# Accelerating cell line development through efficient single-cell isolation with DISPENCELL

**Mrs Marie Vingerhoets**[1], Dr. Audrey Berger[1], Dr. Arnaud Gelb[1], Mr. Loïc Pierson[1], Dr. Georges Muller[1], Dr. David Bonzon[1]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected**
Single-cell dispenser DISPENCELL represents an affordable and automated solution for cell line development processes. Its impedance-based technology constitutes an innovative and robust tool for single-cell identification.

**Impact/Novelty**
DISPENCELL-S3 provides an efficient solution for single-cell cloning. We successfully challenged the DISPENCELL workflow using samples with high cell debris concentration. Moreover, DISPENCELL-S4 allows an enhanced and automated user experience.

**Introduction**
Single-cell isolation is a critical step of cell line development and is also of great interest in precision medicine. It therefore requires reliable, fast and robust tools to perform single-cell assays or to obtain monoclonal cell lines. In this research, we highlight optimized process performances when using a cutting edge impedance-based spectroscopy technology called DISPENCELL.

**Methods/Approach**
The performances of an automated CHO single-cell isolation workflow using DISPENCELL-S3 and DISPENCELL-S4 were compared to the classical limiting dilution workflow. To improve the process, samples with a high concentration of debris were treated with Debris Removal Solution (Miltenyi Biotec).

**Results**
DISPENCELL-S3 workflow proved to be effective for isolation of CHO single cells. A 3.2 fold increase in the number of colonies was observed compared to limiting dilution. The in-line impedance signal detection facilitated the determination of wells containing single cells, thus accelerating the identification of monoclonal colonies. The debris removal procedure was efficient and allowed the withdrawal of up to 93% of debris.This procedure did not affect monoclonal outgrowth performances. The DISPENCELL-S4 new design improved process control, while maintaining high monoclonal outgrowth performances.

**Conclusion**
DISPENCELL workflow provides an efficient and simple method to accelerate single-cell isolation. This workflow can be further adapted for samples containing a high concentration of debris. DISPENCELL-S4 new design streamlines the cell line development process while maintaining high monoclonal outgrowth performances and facilitating integration into a GMP-friendly environment.

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# Omics to support mathematical modelling of CHO Cell processes

**Dr Meeri Mäkinen**[1,2], Dr Kevin Colin[1,2], Ms Markella Zacharouli[1,2], Prof Hakan Hjalmarsson[2,3], Prof. Veronique Chotteau[1,2]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to the theme selected:**
This study aims at using omics in mechanistic modelling of CHO cell process for better a better understanding of the process and as support for feed-back control.

**Introduction:**
Understanding the effect of the process parameters, such as feeding the amino acids is important for the process. The integration of information from omics into mechanistic models can be very valuable but represent an important challenge due to the nature of omics data, large amount of complex information, often without linear correlation with the metadata.

**Methods/Approach:**
Omics information was used in this work to identify the metabolic pathway and to support the mechanistic models. Two applications using CHO cells were performed, a perfusion process with variation of the perfusion rate including support of transcriptomics and a fed-batch process with variation of the feeding including support of LC-MS-MS metabolomics. Pipeline works to decipher the information were established for both cases and the data were then used in a mechanistic modelling approach, where the important reactions were first identified and then the kinetics.

**Results:**
The respective pipelines to treat the transcriptomics or the metabolomics were successfully established. In particular novel tools were developed to address the qualitative data generated by the LC-MS-MS on one hand. On the other hand, the information of genome-scale model was successfully used for the identification of a relevant reduced reaction network thanks to the transcriptomics information. This was then successfully used for the development of kinetic models.

**Conclusion:**
The presented workflow provides tools to capture relevant biological mechanisms of the process from transcriptomics or from metabolomics for perfusion or fed-batch processes.

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# Unlocking the future of Process Analytical Technologies for adaptive process control through revolutionary dielectric spectroscopy

**Dr Fernanda Masri**[1], Dr Lindsay Fraser[1]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:**
[The system] can revolutionise the development of therapeutic modalities from early development to production for real-time adaptive process control, with potential to provide a step-change in our bioprocess understanding.

**Impact/Novelty:** (30 words)
[The system] combines the limitless potential of dielectric spectroscopy and powerful algorithms that leverage Machine Learning to offer smarter PAT solutions by sensing intrinsic properties of single cells without labels.

**Introduction:**
[The system] is set to impact the life-science sector by streamlining cell line development (CLD) through fast identification of lead clones and detecting changes in cell phenotypes, addressing critical demand for real-time PAT, the key to unlocking continuous development and manufacturing.

**Methods:**
[The system] is a scalable technology that measures cellular physiology based on intrinsic single-cell properties. Using a wide range of frequencies, it builds a picture of the cells in high dimensional space, investigating both cell surface and intracellular features concurrently. This unique “digital fingerprint” is used to infer information about the cell population and predict behaviour in CLD and biomanufacturing events.
To gain value from the multi-dimensional information rich data sets that the instrument generates, our propriety data analytics software leverages multivariate statistics to help the user infer insight for decision making and control making the technology accessible and not reliant on expert users.
**Results:**
Case studies demonstrate the value of [The system] for bioprocessing of mammalian cell cultures using *Chinese Hamster Ovary* for production of monoclonal antibodies. The technology, capable of streamlining CLD, helps identify lead clones and cell lines based on productivity and stability. Relevant parallels are investigated for viral vector production.
Moreover, we explore how early detection of changes in cell phenotype can help identify cell populations, health and critical parameters that inform manufacturing decisions for cell therapies.
**Conclusion:**
[The system] is the brain underlying the variants planned within the product platform, making it suitable to measure off-line, in-line and real-time. This flexibility unlocks application potential in standard quality control (QC) programmes paving the road to become a staple QC assay for cell therapies. Simply, a solution to address key needs for smarter, faster, less labour intensive and more automated processing, enabling control and understanding based on cells rather than relying on proxy data.

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# Intensified clarification and capture strategy by synchronization of fluidized bed centrifugation and membrane-based multi-column chromatography

**Mr Fabian Schmitz**[1,2], Dr. Martin Saballus[1], Dr. Thomas Kruse[1], Prof. Mirjana Minceva[2], Dr. Markus Kampmann[1]
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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
The work addressed mAb process intensification using an advanced control strategy for continuous mAb processing by coupling a bioreactor with continuous harvest and capture technology.

**Impact/Novelty:**
The novel control strategy enabled continuous mAb processing by combining and coupling fluidized bed centrifugation (FBC) with multi-column chromatography (MCC) steps into one streamlined downstream process.

**Introduction:**
Ongoing improvements in upstream processing have shifted the bottleneck in mAb production to downstream processing. Intensification of downstream unit operations offers a high potential to overcome the current throughput limitations. As the first step of a downstream process, FBC is a promising technology for cell broth clarification. FBC promises mild process conditions compared to established clarification processes, offering a high mAb recovery and a low release of process-related impurities. However, FBC is a cyclic process resulting in a semi-continuous, variable harvest flow stream. This makes integrating an intensified capture step, such as MCC, for optimized membrane utilization and high productivity challenging.

**Methods/Approach:**
A 50 L bioreactor was harvested with one streamlined clarification and capture process. An FBC with integrated filtration was performed to clarify the cell broth and to remove cell debris. The clarified harvest stream was supplied to a surge tank and subsequently to an MCC unit for the capture step. A novel control strategy was used to adjust the loading flow rate of the MCC unit according to the variable FBC harvest stream. In addition, membrane adsorbers were used for the MCC to handle the high flow rates during the process.

**Results:**
The advanced control strategy enabled a robust and uninterrupted clarification and capture process. Overall, a high mAb yield was obtained with simultaneously high removals of HCP and DNA.

**Conclusion:**
By synchronizing both process steps, the advantages of FBC and MCC were combined to obtain high productivity and lower buffer consumption compared to batch process steps.

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# Benefits of animal origin free peptones on mammalian cell-based vaccine manufacturing

**James Brooks**[1], Wen-Yang Tsai[1], Ashwin Gurunathan[1], Matt Rosecrans[1], Aaron Robinson[1], Anna-Barbara Hachmann[2], Stacy Holdread[1], James Brooks[1]
[1]Thermo Fisher Scientific, Hunt Valley, United States, [2]Thermo Fisher Scientific, Grand Island, United States

Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme:**
Innovate or die – technology innovation driving the understanding and control of bioprocesses – Use of animal origin free (AOF) peptones allows vaccine manufacturers to eliminate animal serum, improve production, and reduce costs.

**Impact:**
Our work demonstrates the benefit of using AOF peptones in cell culture process during viral-vector based vaccine manufacturing.

**Introduction:**
Animal serum has been the most widely used supplement in cell culture and vaccine manufacturing for decades. However, the cost, potential adventitious pathogens, and insecure supply continues to drive up the costs and may not be a viable option for cost sensitive vaccine manufacturers. Peptones (or protein hydrolysates) are derived from the partial hydrolysis of proteins from plants, yeast, or animal tissues and can be safer and more reliable alternative to supplementation with serum. One potential use for peptones can be during viral vector vaccine production.

**Methods:**
Previously, we demonstrated that chemically defined (CD) feed, Gibco EfficientFeed C+ 2X, can boost wildtype adenovirus production in suspension HEK293 cells in Gibco Dynamis media. In the present study, we evaluated the effect of various animal-origin free (AOF) peptones—soy, yeast, cotton etc.—as feeds on AdV production in an Ambr15 micro-bioreactor. Viral titers were quantified by focus forming assay.

**Results:**
Addition of 2g/L or 6g/L peptones on 0 and 2 day post infection (dpi), resulted in peak AdV titers on 4 or 5 dpi. While peak viral titers in several peptone conditions (soy, yeast, and cotton) matched the titers in CD condition, two peptone blend conditions showed approximately 40% titer increase compared to CD feed control. In adherent Vero process, we also demonstrated that AOF peptones and the blends with reduced serum (2%) can support comparable cell growth when compared to respective 10% serum condition. Interestingly, higher vesicular stomatitis virus production titers were observed in conditions supplemented with AOF peptones or their blends in 2% reduced serum conditions.

**Conclusion:**
Overall, these results demonstrate that AOF peptones can be used to improve HEK293 and Vero based vaccine manufacturing processes.

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# Simple and High-Throughput Analytics for Proteins and Cells in Bioprocessing

**Dr Eligio Iannetti**[1]
[1]Beckman Coulter Life Sciences, Amsterdam, Netherlands

Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected**
Overcoming challenges in large biologics development involves advanced analytics for process control. We developed assays focusing on Key Process Parameters (IgG titer and cell viability) and Critical Quality Attributes (protein aggregation), simplifying assay complexity and significantly reducing assay duration.

**Impact/Novelty**
Novel integrated applications allows to quantify IgG titer and protein aggregation using Fluorescence Polarization (FP) assays, while cell count and viability from label-free cell images using Artificial Intelligence (Ai) (Convolutional Neural Networks). These innovative methods are simple, rapid, cost effective, readily automatable and can now be fully integrated in one workstation.

**Introduction**
Ensuring suitable bio-analytics are in place is critical in preventing bottlenecks in high-throughput and automation bioprocesses.
We present here innovative and integrated analytical technologies for rapid & high-throughput molecule and clone quantification and characterization with a focus on CHO antibody Cell Line Development and Process Development.

**Methods/Approach**
Here we present ValitaCell (now part of Beckman Coulter Life Sciences) innovative automated and now integrated analytical solutions:

- IgG quantification straight in crude samples - FP based assay
- Protein aggregation quantification in purified samples - FP based assay
- Label-free CHO cell count & viability - Ai based

**Results**
High correlation was observed between IgG titer measured with Valita Titer assay and industry standard technologies (HPLC and BLI). High correlation observed between Valita Aggregation Pure and HPLC-SEC. High correlation observed between CellAi viability measurements and industry standard technologies (staining-based like trypan blue).

**Conclusion**
Valita Titer plates enable rapid & high-throughput IgG quantification directly in crude samples. Valita Aggregation Pure plates enable rapid & high-throughput protein aggregate detection and quantification in pure sample solutions. CellAi enables cell count and viability measurements directly from label-free images of cells. These assays can be easily automated and fully integrated in one workstation.

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# Rapid and In-depth Proteomic Analysis of Chinese Hamster Ovary Cells Bioprocesses

**Miss Darina Stoyanova**[1], Dr Karl Burgess[1], Dr Mark Rendall[2], Dr Jeff Keen[2], Dr Leon Pybus[2], Mr Luke Johnston[1], Ms Lisa Imrie[1], Dr David Mentlak[3]
[1]University Of Edinburgh, Edinburgh, United Kingdom, [2]Fujifilm Diosynth Biotechnologies, Billingham, United Kingdom, [3]University of York, York, United Kingdom

Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
Established high throughput proteomic interrogation of Chinese hamster ovary cell molecular pathways allows decreased analysis time, increased culture sample coverage and depth of understanding

**Impact/Novelty:**
Characterisation of stress-induced cell death pathways in Chinese hamster ovary cells, utilising subcellular high throughput proteomics

**Introduction:**
Chinese hamster ovary cells (CHO) are the main platform for monoclonal antibody production in the growing biopharmaceutical market. Compared to any other production platform, CHO cells, being mammalian cells, have the major benefit of providing appropriate protein folding and glycosylation patterns. However, further knowledge on the effect of protein burden and optimisations aimed at improving the viability and culture longevity for higher protein production are required. Programmed cell death – apoptosis, is the predominant form of response occurring from biochemical stresses in bioreactor culturing. Cell longevity, yield and product quality are thus negatively affected. However, apoptosis is operated through multinodal pathways, not yet comprehensively understood.

**Methods/Approach:**
Proteomics - the investigation of the whole set of proteins and their associated changes in any cell, tissue or organism, allows global investigation of CHO cell bioprocesses and identification of pathways or markers of desired phenotype - targets for cell process improvement. Combining subcellular fractionation with novel data-independent acquisition strategy, allows for more sensitive and reproducible analysis of multiplex data for understanding the molecular reactions governing time and mechanisms of sensing, activation, and execution of cell death of industrially relevant CHO cell lines, on the basis of stresses caused by bioreactor culturing. Introducing a short liquid chromatography gradient allows increased high throughput, given the multiplied sample number due to the fractionation.
**Results:**
Investigation of different monoclonal antibody producing cell lines, with different productivities, allows the identification of key stress-induced pathways connected with increased protein burden. Additional comparison of bioreactor-adapted producer cell line and non-adapted non-producer cell line reveals the mechanisms through which cells adapt to the stress caused by change in the environment and the cell death mechanisms occurring when culturing non-adapted line in bioreactor conditions.
**Conclusion:**
Elucidating cellular responses to stress and cell death allows identifying key markers for targeting to increase cell longevity and alleviate stress. Cellular engineering strategies could be further considered once potential targets are decided.

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# Novel application of next generation sequencing to characterise recombinant cell lines and determine genetic stability

**Dr. Pamela Hamill**[1], Dr Leyla Diaz[2], Dr Danielle Hickman[2], Dr Alexei Slesarev[2]
[1]Merck , Glasgow, United Kingdom, [2]Merck, Rockville, USA

Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
Novel methods to analyse recombinant cell lines and establish their stability that increase the quality of data and speed of analysis will assist in accelerating development of novel biologics.

**Impact/Novelty:**
This novel single methodology can replace multiple different methods currently used to assess stability of recombinant cell lines and provides improved resolution and faster completion of analysis.

**Introduction:**
Innovation in technologies to generate more controllable or targeted integration of expression constructs is a priority for the biologics industry, particularly as more complex biotherapeutic formats are developed, since this can give rise to greater cell line stability and consistent protein expression. As cell line development evolves, however, advanced supporting technologies to analyse clones and final cell lines selected for the production of biotherapeutics are needed. Thorough characterisation of recombinant cell lines is essential to ensure suitability for manufacturing and to satisfy regulatory expectations, however analysis required to confirm the integrity of the expression cassette, its insertion location, copy number and stability over time relies on multiple older technologies which are performed separately and may lack sufficient resolution.

**Methods/Approach:**
The advent of advanced sequencing techniques and sophisticated data analysis capabilities has enabled the incorporation of next generation sequencing (NGS) based technology for quality control testing of biologics. Here, we describe the application of short read NGS technology and development of custom data analysis methodology to achieve whole genome sequencing enabling comprehensive and accurate analysis of expression cassettes in recombinant CHO cell lines.

**Results:**
The fully validated, GMP-compliant methodology generates high resolution sequence coverage of the CHO genome and inserted regions enabling accurate integration site analysis and determination of protein encoding and flanking regions to identify possible insertions, deletions or substitutions which could impact protein expression levels or quality. In the same sequencing reaction, this new method allows highly accurate determination of gene copy number.

**Conclusion:**
This novel NGS based approach for genetic stability determination of recombinant CHO cell lines results in streamlined analysis with greater accuracy and higher resolution results. Since no insert specific assay design is required and the method fulfils the most recent regulatory expectations, this approach helps accelerate biologics manufacturing.

# Poster Presentations

# Other Topics

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# Antiviral Activity of Influenza A Defective Interfering Particles against Multiple Viruses In Vitro

**Ms Patricia Opitz**[1], Mr Lars Pelz[2], Ms Elena Piagnani[2], Mr Patrick Marsall[2], Ms Nancy Wynserski[2], Mr Marc Dominique Hein[1], Mr Pavel Marichal-Gallardo[2], Dr Sascha Young Kupke[2], Prof Udo Reichl[1,2]
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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected**

With this contribution, we want to shed a light on use of Influenza A defective interfering particles (IAV DIPs) against a range of interferon (IFN)-sensitive viruses.

**Impact/Novelty**

In this study, we observed that IAV DIPs suppress the replication of respiratory syncytial virus (RSV), yellow fever virus (YFV), and Zika virus (ZIKV).

**Introduction**

There is an urgent need for novel potent antiviral agents against existing and emerging viruses. IAV DIPs cannot only suppress replication and spreading of IAV, but also interfere with the replication of unrelated viruses by triggering the innate immune system. To test the potential regarding the latter, we conducted co-infection experiments in human lung cells for a broader range of viruses.

**Methods/Approach**

In A549 cells, we studied the well-known IAV DIP “DI244”, which is characterized by a large deletion in genome segment 1. In addition, we used “OP7”, a DIP with multiple point mutations in segment 7. In co-infection experiments with RSV, YFV or ZIKV we monitored virus titres and quantified gene expression using real-time RT-qPCR.

**Results**

Co-infection with IAV DIPs effectively inhibited replication and spread of all three viruses in A549 cells. In addition, it induced an enhanced type I and type III interferon (IFN) response, shedding light on mechanism underlying the antiviral activity of IAV DIPs. Using Vero cells, which are deficient in IFN production, co-infections did not lead to any suppression of viral titres. The absence of antiviral activity in the presence of the JAK1/2 inhibitor ruxolitinib indicated a dependence on the JAK/STAT signalling pathway for the observed antiviral effect.t.

**Conclusion:**
In summary, this study affirmed the potential of IAV DIPs for use as broad-spectrum antiviral including infections caused by diverse IFN-sensitive viruses.

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# Tiny Trouble?: Long-Term Effects of Microplastics on Murine Macrophages

**Ms Johanna Fritsche**[1], Dr. Valérie Jérôme[1], Prof. Dr. Ruth Freitag[1]
[1]University Of Bayreuth, Bayreuth, Germany

Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Expanding Insights into Cellular Effects of Microplastic Particles Using a Long-Term Cultivation Approach**

The impact of microplastic pollution on aquatic, terrestrial, and human life is a central subject in today's research. *In vitro* experiments play a pivotal role in acquiring valuable insights into these questions.

## Novelty

We used long-term cultivation of cells with microplastic particles (MPP) to expand the current knowledge regarding effects of MPP on cells *in vitro*.

## Introduction

MPP (particles < 5 mm) pollution is a rising threat in our modern world, eliciting concerns regarding human health. However, most available *in vitro* studies regarding cellular effects of MPP focus on acute toxicity endpoints, lacking long-term exposure or post-exposure analysis.

## Approach

Murine macrophages (ImKCs) were used to evaluate the effect of pristine and artificial-weathered polystyrene MPP (2 µm) on immune cells. Cells were cultivated with MPP for 24 h (short-term exposure) or three weeks (long-term exposure). Multiple cellular responses were analyzed, including metabolic activity, upregulation of genes, and ROS production. Macrophage activation was analyzed by the expression of activation-specific surface markers and the release of a pro-inflammatory signal.

## Results

Artificial-weathered MPP lead to a lower metabolic activity than pristine, seen in both short-term and long-term exposure. Regarding macrophage activation, both particle types showed similar short-term effects. After prolonged exposure, the interaction between cells and artificial-weathered MPP resulted in a distinct reduction of pro-inflammatory markers, accompanied by a concurrent increase in anti-inflammatory markers. In contrast, pristine MPP did not impact pro-inflammatory surface markers.

## Conclusion

Our study highlights the importance of long-term analysis of MPP effects, emphasizing the value of using both pristine and artificial-weathered particles. Through a multi-parametric analysis, we obtained comprehensive insights into MPP effects. Our findings demonstrate the critical need for incorporating artificial-weathered particles in *in vitro* studies due to their closer resemblance to environmental MPP.

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# An Economic and Controlled Bioprocess for Manufacturing Red Blood Cells from Stem Cells

**Prof. Robert Thomas**[1,2], Dr Katie Glen[1,2], Dr Polina Vickhreva[1], Dr Mela Twist[1], Mrs Laura Erdos[1], Dr Vincent Ho[3,6], Dr Kenneth Church[4], Dr Janice Moser[4], Dr Michael Mansour[5], Mr Doug McConnell[1]
[1]Safi Biotherapeutics Inc, Boston and Loughborough, USA and UK, [2]Loughborough University, Loughborough, UK, [3]4DBio3 Centre for Biotechnology, USU, Bethesda, USA, [4]Sciperio Inc, Orlando, USA, [5]Massachusetts General Hospital, Boston, USA, [6]Department of Radiology and Radiological Sciences, USU, Bethesda, USA

Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:** Cross theme aspects (product specific approaches to process monitoring, intensification, and innovative bioprocess design)

**Impact/Novelty:** The system is novel in terms of intensification of a cell therapy in a 1L stirred tank, and has the impact of improving economics for high dose cell therapies
**Introduction:** Donor transfusion issues include variable cell quality/number, pathogens, transfusion reactions and donor shortages. A stem cell manufactured equivalent could replace donor cells, but requires surmounting multiple technical challenges to achieve an economic and controlled manufacture method.

**Methods/Approach:** To address these challenges we have developed a uniquely efficient manufacturing system with multiple innovative steps: a 1L stirred tank bioreactor with ultrafiltration perfusion loop is applied to deliver concentrated feeds, gas exchange membranes are used to improve mass transfer. Continual cell monitoring is achieved via an impedance probe, and periodic analysis of offline cell and medium quality attributes specific to the product.

**Results:** A culture density of ~1E8 cells/mL is achieved yielding 1E11 total cells/L; proliferation is sufficient to produce hundreds of units of packed red cells from a single mobilised peripheral blood donation. This is achieved with minimal exchange of expensive cell culture medium and additives. Cells enucleate, have normal biconcave morphology, and express typical levels of surface markers such as CD235a and Band3. Manufactured red cells contain approximately 34pg haemaglobin per cell, have a normal O2 p50, and deformability over a 0-30pa range is similar to native reticulocytes. Cells can be downstream processed via bulk filtration and centrifugal elutriation to remove the majority of nucleated or low erythroid marker expressing cells.

**Conclusion:** We have demonstrated it is feasible to produce red cells from stem cells in scalable stirred tank bioreactor systems at a level that will enable meaningful clinical dosing. The manufacture system is designed to enable further cost and scale optimisation that will support economically sustainable supply.

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# Cationic flocculants assisted clarification

**Ms Anna-Carina Frank**[1], Mr Daniel Komuczki[1], Ms Clara Hofmann[2], Ms Natalie Deiringer[2], Ms Felicitas Guth[2], Ms Elisabeth Grünstein[2], Mr Rainer Hahn[1], Mr Peter Satzer[1]
[1]Boku - University Of Natural Resources And Life Sciences, Vienna, Austria, [2]BASF SE, Ludwigshafen am Rhein, Germany

Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected**
"Other Topic" was selected. No relevance to the other themes available.

**Introduction:**
Intensification of cell culture-based unit operations such as fed-batch processes of monoclonal antibodies using Chinese hamster ovary cells leads to higher cell densities and high product titers. The high concentration of biomass significantly increases the burden on clarification steps such as depth filtration. In particular, depth filtration becomes less economically and technically feasible as substantially more filter area is required.

**Methods/Approach:**
It is known that specific cationic flocculants facilitate the separation of cells and the removal of host cell derived impurities. Although they have been extensively used before, a study on the correlation between these cationic flocculants and their removal potential of industrially relevant critical host cell proteins (HCP) has not yet been conducted.

**Results:**
We therefore established fingerprints of various flocculants on their impurity removal potential by analysis of residual HCP profiles using proteomics.

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# Exploring NutriStor as a novel Chemically Defined Solution for Short-Term Cold Storage of Cells

**Mrs Mira Genser Nir**[1], Ms Anat Vishlitsky[1], Ms Meital Gury Ben-Ari[1], Mr David Fiorentini[1]
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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
The poster focuses on the preservation of animal cells in the field of bio-preservation. These subject holds significance for any research, application, or process that involves utilizing cells as a tool or product.

**Impact/Novelty:**
The poster represents a novel product for cells preservation and represents an advanced approach in preserving of therapeutic cells, providing a valuable tool for the advancement of cell-based therapies.

**Introduction:**
Cryopreservation plays a pivotal role in preserving cells for various cell-based applications. However, the freeze-thaw cycles involved in the process can negatively impact both cell viability and yield. Additionally, most of the cryopreservation solutions incorporate irritant, cell permeable Cryoprotective Agents (CPAs), potentially affecting cellular performance and functionality. For Advanced Therapy (AT) applications, it is advantageous to avoid freezing and the exposure of cells to these irritant CPAs. Moreover, the short-term cold storage of cells can facilitate the stable shipment of cell-based products for therapeutic applications.

**Methods/Approach:**
This study introduces NutriStor, a unique Animal Component-Free (ACF), Protein-Free (PF), salt-based bio-preservation solution as an alternative to the traditional freezing methods. NutriStor enables the preservation of Human Mesenchymal Stem Cells (hMSCs) at 2-8°C for several days. The objective is to assess the effectiveness of NutriStor in maintaining high cell viability and yield, while preserving the identity and characteristics of hMSCs, including their multi-lineage differentiation potential, self-renewal capacity, immunophenotyping profile, proliferation capacity, and genomic stability. hMSCs from various sources undergo NutriStor cold storage, followed by assessment of cell's quality, quantity, identity, and performance.

**Results:**
The results demonstrate that NutriStor effectively preserved hMSCs during short-term storage (several days), exhibiting high cell viability and yield. The cells maintain essential characteristics, including multi-lineage differentiation potential, self-renewal capacity, immunophenotyping profile, proliferation capacity, and genomic stability.

**Conclusion:**
To summary, NutriStor offers a viable alternative to conventional freezing methods for short-term preservation of hMSCs. The solution ensures high cell viability and yield while preserving cell identity and characteristics, essential for ensuring the quality of cells and achieving optimal clinical outcomes. These findings support the use of NutriStor for stable shipment and short-term storage of cell-based products in therapeutic applications, providing a valuable tool for the advancement of cell-based therapies.

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# Catching the perfect sweetness: Critical parameters in CLD and USP to gain best-fitting protein N-glycosylation

**Dr Lena Tholen**[1]
[1]Fyonibio Gmbh, Berlin, Germany

Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Impact/Novelty:**
Production of complex N-glycosylated proteins is becoming increasingly important as more complex biopharmaceutical proteins come into focus. Due to highly individual requirements, customized cell hosts and USP approaches are required.

**Relevance to Theme Selected:**
The topic has generally high relevance for improving protein production systems for complex glycoproteins.

**Introduction:**
N-glycosylation is one of the most common modifications of biopharmaceuticals, affecting important pharmaceutical properties such as pharmacokinetics and half-life. The desired N-glycosylation is highly dependent on the biopharmaceutical characteristics and requirements and can be crucial for the final product function as well as an important critical quality attribute.

**Methods/Approach:**
The data presented will show a holistic approach to getting the best N-glycan properties out of a biopharmaceutical production process, based on both host cell selection and improvement as well bioprocess optimization. For process optimization an AMBR®15-based optimization approach will be combined with N-glycan profiling using RapiFluor® labelling and the in-house developed software GlycoFiler® for parallelized and optimized data evaluation.

**Results:**
The first part of the presentation will take a closer look at cell engineering and host cell selection strategies and how these approaches can lead to improved and maximized product sialylation as well as product afucosylation. A link to the role of individual N-glycan structures will be made to explain the importance of individual adaptations. In addition to the host cell level, a second topic will be discussed to show the impact of bioprocess conditions on complex N-glycan structures and how upstream process development can pave the way for perfectly matched N-glycosylation.

**Conclusion:**
The topic will highlight what tools are available and applicable to achieve the desired critical quality attributes and what might be the best way forward depending on the desired N-glycan profile.

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# Bioprocess Intensification with Peptide-Based Cell Culture Media Optimization Using Tyrosine and/or Cystine (Di-)Peptides

**Dr Stephan Brinkmann**[1], Ms Christina Jost[1], Ms Tamara Heinze[1], Mr Tomislav Trescec[1], Dr Anne Benedikt[1], Dr Martin Schilling[1]
[1]Evonik Operations GmbH, Darmstadt, Germany

Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:** The use of (di-)peptides for the optimization of cell culture media is emerging, e.g., to increase cell and process productivity and at the same time greatly reduce manufacturing costs.

**Impact/Novelty:** The application of tyrosine and/or cystine (di-)peptides in bioprocesses is an elegant solution to further optimize cell culture media resulting in an overall improved cell and process productivity.

**Introduction:** Over the past decade, cell culture media (CCM) optimization has been a key strategy for obtaining high yields and improving productivity, while ensuring product quality in biopharmaceutical production. However, further bioprocess intensification is limited by undesired CCM chemistry such as the formation of reactive oxygen species (ROS) that negatively impact cellular metabolism and final protein quality (Chevallier et. al., 2019).

**Methods/Approach:** Here, we tested stability and reactivity in model systems to better understand the roles L-tyrosine and L-cystine peptides play in CCM chemistry. We assessed their effects on CCM stabilization and their impact on the bioprocess.

**Results:** Compared to free Tyr, Tyr-dipeptides reduce the light-induced colorization of Trp-containing model solutions and CCM more strongly due to their higher solubility, as the natural, photoprotective ROS scavenging ability of the Tyr residue is preserved. Additionally, ROS formation and cell viability was studied as a function of Cu(II) ion concentration and increasing concentrations of free L-cysteine or L-cystine peptides. The viability strongly decreased with increasing L-cysteine concentration in the presence of Cu(II) ions whereas no such effect was observed in the presence of peptides.

**Conclusion:** Tyr-dipeptides can be used as light stabilizers while Cys-peptides can be applied in CCM to reduce/prevent ROS formation catalyzed by free L-cysteine. Their application in bioprocesses resulted in improved cell culture performance. Therefore, besides their nutritional function, these (di-)peptides are options to control/stabilize CCM chemistry to further improve CCM formulations and enable more efficient bioprocessing.

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# First ever animal-free, cell line-derived human S9 fraction for in vitro drug metabolisation

**Dr Beat Thalmann**[1]
[1]Scinora GmbH, Rafz, Switzerland

Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
In novel approach methodologies (NAMs), animal-derived *in vitro* metabolisation agents are used. To increase the sustainability, cell line-derived alternatives need to be established.

**Impact/Novelty:**
Hepatoma cell line-derived S9 metabolisation reagents increase the availability of human alternatives as well as the sustainability in toxicological risk assessment and are not available in market yet.

**Introduction:**
In a process to replace the animal liver-derived S9 fraction for in vitro metabolising agents, we have established proprietary hepatoma suspension cell lines grown in an optimised chemically defined media system and used these cells to produce the first animal-free human S9 fraction. Instead of killing animals or using donated liver for this purpose, the current process is efficiently able to produce animal-free alternatives on a larger scale.

**Methods/Approach:**
The presented process combines the advantages of suspension cell culture (high cell density, easy handling, high productivity) with a programmable conversion to improved liver-specific properties.

**Results:**
The hepatoma suspension cell lines used in the laboratory-scale process achieve high cell densities (>$5 \cdot 10^6$ cells/ml). The current process enables the weekly production of a human S9 fraction derived from cell lines equivalent to about one third of an average human liver. A 100-liter bioreactor is sufficient to replace a commercial batch consisting of the livers of four individuals. This process would increase the interest and likelihood of the product being included in the OECD/DIN guidelines, where the rat liver-derived S9 fraction is still standard (e.g. OECD TG471: bacterial reverse mutation test).

**Conclusion:**
Due to the increasing relevance for humans in human-based research, an improved and upscaled production process would reduce the risk of potential ethical implications due to the increased procurement of human livers as well as the parallel increase in demand for human liver S9 fraction. This increases the human relevance in parallel and improves the sustainability of *in vitro* metabolisation in toxicological evaluation.

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# Getting Fat: Adipogenesis of Pluripotent Stem Cells in Livestock Species for Cultivated Meat

**Miss Sarah Ho**[1], Dr Joe Mee[1], Dr Ana Almeida[1], Dr Pritika Singh[1], Miss Marta Esquiva Diaz[1], Miss Niamh Hyland[1]
[1]Roslin Technologies, Edinburgh, United Kingdom

Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Theme: Others topics**
Using animal pluripotent stem cells (PSCs), including induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs) to produce cultivated meat.

**Impact/Novelty:**
Fat contribute to the flavour, aroma, and texture of meat. Hence the generation of adipocytes, or fat cells, from pluripotent stem cells holds significant importance for cultivated meat applications.

**Introduction**
The use of PSCs in cultivated meat manufacturing can potentially reduce the environmental impact of traditional meat production as it requires significantly less land and water, and contributes to lower greenhouse gas emission.

PSCs have the capacity to self renew, expand indefinitely and can differentiate in to adipocytes and myocytes (muscle cells), which are the predominant cell types of interest for cultivated meat.

Adipocytes generated from PSCs offer several advantages for cultivated meat applications. They can allow the control of lipid profiles that can be optimised for taste, nutrition, texture and other enhanced sensory qualities relevant to human health and consumer perception. Once a suitable adipogenic profile and characterisation has been established, high density bioreactors can be used for manufacturing at an industrial scale.

**Methods/Approach**
Successful induction of adipogenesis has been demonstrated using a defined 2D or 3D differentiation protocol using Porcine and Ovine PSCs.

**Results**
We have developed a robust defined directed differentiation protocol to generate adipocytes from Ovine, Porcine and Bovine PSCs. The end stage adipocytes display oil droplets that are stained by Biotracker488 Green Lipid Droplet dye and Oil Red O. In addition, the adipocytes express increased levels of classic adipocyte markers such as PPARγ, CEBPα and FABP4 when compared to undifferentiated ESCs/PSCs.

**Conclusion**
Roslin Technologies has successfully demonstrated the differentiation of Porcine, Ovine and bovine PSCs into adipocytes using a defined protocol that is amenable to scale up for the cultivated meat industry.

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# Upskilling the cell therapy manufacturing workforce

Flora J Keumurian[1], Paul W. Barone[1], John Duguid[2], Caleb Neufeld[1], Betsy Skrip[1], Stacy Springs[1], Humberto Vega Mercado[3], Krystyn Van Vliet[4], Jacqueline Wolfrum[1], Mingyu Yang[1]
[1]MIT, Cambridge, United States, [2]Vericel, Cambridge, United States, [3]Bristol-Myers Squibb, Princeton, United States, [4]Cornell University, New York, United States

Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
With over 2,000 cell therapies in the pipeline, there is a growing need for a well-trained workforce. We developed a scalable training programme to support the increased workforce.

**Impact/Novelty:**
An academic-industry collaboration produced a blended-learning course to teach cell therapy manufacturing.

**Introduction:**
There are currently few training programmes to help individuals transition from traditional biotechnology fields to cell therapy manufacturing. An online resource that teaches important fundamental concepts coupled with virtual laboratory experiences that allow for experiments to be delivered in a time-efficient manner (with no consumables or equipment required) would accelerate the training of the workforce.

**Methods/Approach:**
We created an online course on the fundamentals of cell therapy manufacturing as well as authentic virtual modules that teach biotechnology laboratory skills in an industry-style setting. The online course covered manufacturing process requirements and analytical challenges, as well as the stringent regulatory requirements that ensure manufacturers produce safe and efficacious cell therapy products. The virtual laboratory modules covered cell culture, genetic modification, and aseptic processing. We highlight the key features of the online course (e.g., snippets of video lectures, examples of text and images, footage of laboratory equipment in action, interviews with subject matter experts) and virtual modules.

**Results:**
During five offerings of the online course, 12,600 learners enrolled, out of which 1,037 went on to earn verified certificates. Data taken from entrance and exit surveys show that 40% of respondents indicated that the course would help transition them into the cell therapy industry and broaden their career opportunities.

**Conclusion:**
We believe this course can serve as a model for how academia and industry can collaborate to create innovative, scalable training programmes to meet the demands of the modern biotechnology workforce.

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# A 3D-printed microfluidic cultivation system for parallel monitoring of perfused 3D cell cultures

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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:**
3D cell cultures offer opportunities to reduce the use of animals in drug testing and pre-clinical research, thereby increasing throughput, lowering costs, and addressing ethical concerns.

**Impact/Novelty:**
Three-dimensional microfluidic culture systems provide a promising option towards approaching physiological conditions *in vitro*. The system presented enables parallel cultivation, media supply and microscopic live-cell imaging of 3D cell cultures.

**Introduction:**
An increasing use of 3D cell models in drug discovery, disease modelling, and tissue regeneration demands appropriate culture systems. Besides high-throughput, easy monitoring of the cells is a crucial requirement for such systems. The 3D-printed microfluidic perfusion system offers flexible solutions for parallel cultivation of separate 3D cell cultures.

**Methods/Approach:**
The cultivation device was designed using SolidWorks®. Main parts were fabricated using high-resolution 3D printers. An integrated polycarbonate bottom sheet allows good microscopic examination, and a 3D-printed adapter ensures compatibility with standard imaging systems. Perfusion of the device (inside standard $CO_2$ incubators) is achieved by a media reservoir connected to a peristaltic pump. Following successful tests with hydrogel-embedded L-929 cells, the system has been optimized to house 3D cell cultures of cells critical to the blood-brain-barrier for molecular assessments of their ischemia-induced alterations in the ongoing study.

**Results:**
The presented system ensures precise, reproducible positioning of the cell cultures and sufficient gas exchange and nutrient supply to the cells. Thus, appropriate cell growth was successfully monitored over three days, and live/dead staining using live-cell imaging showed comparable results between hydrogel-embedded cells in 96-well plates and in the microfluidic cultivation device.

**Conclusion:**
The presented system allows for easy microscopic online monitoring of the cultured cells and significantly reduces the required volumes of hydrogel and cell suspension. Due to parallelization and automation of perfusion, it offers great potential to be used as a customizable platform for 3D cell culture applications for mechanistic studies and drug screening.

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# CRISPRing up CHO: Unveiling the blueprint for a dual-guide mediated genome-wide CRISPR/Cas9 screen in CHO

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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
This work covers genetic engineering and large-scale genomics in Chinese Hamster Ovary (CHO) cells and hence, does not readily fit into the larger themes given.

**Impact/Novelty:**
In this study, we lay the groundwork for a true genome-wide exploration in CHO cells, offering a gateway into understanding biopharmaceutical industry's fundamental organism.

**Introduction:**
The ease of CRISPR programmability facilitates the rapid creation of large libraries for comprehensive genetic screens, leading to valuable insights into genotype-phenotype interactions and potential gene targets for further research. Hence, by leveraging the CRISPR toolbox, we identify, verify, and establish essential components for executing a genome wide screen in CHO cells.

**Methods/Approach:**
Our study exploits the efficiency and flexibility of CRISPR by using a paired guide RNA (pgRNA) - mediated deletion strategy for complete knockout of selected genes-of-interest. Our approach involves co-transfection of carefully curated pgRNA sequences—spanning deletion sizes from 12 to 106 kbps—with a Cas9-encoding plasmid into CHO-K1 cells, followed by qualitative and quantitative assessments for validation.

**Results:**
Genomic DNA analysis consistently revealed Cas9-induced deletions at the targeted sites through deletion PCR. Furthermore, mRNA expression surveyed by qRT-PCR also showed a reduction in gene expression, even at a pool level. Thus, our varied design decisions for the pgRNA expression cassette successfully led to precise deletions, affirming the effectiveness of our approach.

**Conclusion:**
This robust workflow collectively enables the strategic application of CRISPR-Cas9-mediated editing within CHO cells, underpinned by a comprehensive suite of methodologies encompassing design, transfection, and detailed analysis. Thus, this work establishes the blueprint for genome-wide deletion approaches to understand the functionality of both coding and non-coding genomic regions in contributing to essentiality, productivity and product quality in CHO cells under bioprocessing conditions.

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# Trial for cell preparation for Cellular food

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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

**Relevance to Theme Selected:**
Examination of the cells that form the basis in cellular foods, which have been the focus of much attention in recent years.

**Impact/Novelty:** (
The efficient isolation of primary myoblasts derived from livestock and the culture conditions to maintain the differentiation potential of the cells were investigated.

**Introduction:** (
In recent years, Cellular Foods including cultured meats have been attracting attention as alternative proteins. However, various issues remain in their production. Preparation and quality of the seed cells are one of the issues. In particular, differentiation potency of myoblast into myocyte/myotube, which is considered important to make up texture of food, is the biggest challenges.
Primary cells are one of the options for the seed cells for cultured meat. In general, primary cells have the highest differentiation potency in just after establishment and gradually decline. In this study, we investigated ways to maintain the differentiation potency of these cells.

**Methods/Approach:**
We isolated the primary cells derived from edible beef, cultured in 20% FBS, 2ng/ml b-FGF, F10.
(1) In order to increase the percentage of myoblast at the time of establishment, the seeding conditions were examined using non-coating or collagen-coated dish. After establishment, the cells were induced differentiation into myocyte/myotube and evaluated the efficiency of differentiation.
(2) In order to keep differentiation potency, the culture conditions for the myoblast are important. We applied serum-free CHO-SFM-A medium into the primary cell culture. After several passage, the cells were induced differentiation into myocyte/myotube. After several days, the cells were evaluated the efficiency of differentiation.

**Results:**
(1) The ratio of myoblast differentiation increased by re-seeding the supernatant into another collagen-coated dish 24 hours after seeding on the non-coating dish. However, the differentiation potency decreased several passages.
(2) CHO-SFM-A media affected the growth of primary cells. After induction, high percentage of myocyte marker-expressing cells and cell-fusion were observed in CHO-SFM-A-added cell culture. but, the percentage was low and cell fusion was not observed in the cell culture without CHO-SFM-A addition.
**Conclusion:**
These results could contribute the method for maintaining the differentiation potential of primary cells.

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# Development of an edible and food-grade microcarrier for cultured meat production

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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to the theme selected:** Development of suitable microcarrier for the culture of anchorage-dependent animal cells is crucial for large-scale manufacturing in the cultured meat industry.

**Impact/Novelty:** Despite the strong efforts that have been made in recent years, limited edible biomaterials have been developed for food applications. Addressing this gap is fundamental for cultured meat development.

**Introduction:** Within the field of cultivated meat, cultivated fat is a new disruptive technology that aims to create a new source of animal fat, addressing the environmental, ethical, and economic drawbacks generated from traditional livestock farming. However, the most used cell types for this purpose require an adhesion material to be cultured. A new edible, cost-effective and animal-free microcarrier for the expansion and fattening of duck primary cells for cultivated fat production has been developed to address this issue.

**Methods/Approach:** A set of screening experiments was carried out to find the best material candidates for the culture of the cells of interest. Next, a spray-out protocol was implemented and optimized to produce micro spherical beads that were further chemically functionalized using the previously selected biomaterials based on their physicochemical properties.

**Results:** The results indicate that a polysaccharide matrix combined with vegetal protein provided a suitable structure for cell attachment, expansion, and fattening, reaching cell yields comparable with the commercially available microcarrier Cytodex-1. Furthermore, cell culture on designed microcarriers were successfully achieved in a lab-scale bioreactor.

**Conclusion:** An edible and animal-free microcarrier has been designed and optimized, sustaining primary duck cells expansion and differentiation. This technology provides an innovative solution to an unresolved challenge, opening new horizons in the field of cultured meat industry.

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# Process intensification opportunities in early phase development and large-scale production

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Poster Session 1, Cromdale Hall & Lennox Suite, June 24, 2024, 2:15 PM - 3:45 PM

**Relevance to Theme Selected:**
Process intensification in early phase development can significantly improve cost effectiveness. Although, the process needs to be robust and deliver a product with the required quality attributes.

**Impact/Novelty:**
Capabilities of a typical platform vs an intensified process are compared, thereby the great potential of applying process intensification, N-1 perfusion, and continuous capture, in early development is demonstrated.

**Introduction:**
The main benefit of a platform in biomanufacturing is to reduce time to clinic. Saving development time might translate to earlier availability of life saving therapies for patients and increases competitiveness, as resources can be allocated to other pipeline projects. Although a platform process allows for fast product development, efforts to increase process efficiency and enabling large-scale production at low cost of good are not yet well established in biopharmaceutical industry.

**Methods/Approach:**
Process development based on a traditional platform approach is compared to an intensified process. The upstream process was intensified by applying perfusion technology, while for downstream a continuous multi column approach for the capture step was considered with the focus to increase yields and reduce costs of goods. Transferability and scalability up to pilot was investigated and finally key product quality attributes were assessed.

**Results:**
Initial upstream development by applying a platform approach resulted in relatively low biomass (i.e. IVCD, integral of viable cell density). In a straightforward approach pre-stage perfusion (N-1) was introduced to increase seeding cell densities and to obtain an overall higher cell integral at the production bioreactor stage, resulting in a titer improvement of greater than 100%. Most importantly, only moderate, and acceptable changes in key product quality attributes were observed.
With regards to downstream, the introduction of a continuous capture step resulted in a significant reduction of process time, buffer consumption and costs.

**Conclusion:**
We demonstrated that applying process intensification already in early phase development and large-scale production should be considered as a standard tool in biopharmaceutical industry. By intensification of only two unit-operations the overall process yields were shown to be strongly increased while significantly reducing costs.

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# Convergence of Population Growth, Affluence, and Sustainable Meat Production: Insights from the Cultivated Meat Industry

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Poster Session 2 & Networking, Cromdale Hall & Lennox Suite, June 24, 2024, 7:30 PM - 9:00 PM

The global population is on an upward trajectory, expected to surpass 10 billion by 2050, accompanied by rising affluence in developing regions. This dual phenomenon poses a formidable challenge in meeting the escalating demand for meat through conventional means. The burgeoning cultivated meat industry presents a promising solution to this sustainability conundrum.

The legacy bioprocessing industry laid a solid foundation for the assessment and production of scaleable, efficacious, and safe biologic therapeutics. While certain paradigms align, distinctive themes emerge, particularly in building bioprocesses to attain critical quality attributes influencing flavor and nutritional composition. The raw materials within cell culture media play a pivotal role, influencing many aspects of cultivated meat production. This study delves into the design specifications, sources, and characteristics of critical nutrients contained in media that impact the performance, cost, composition, safety, and mission of cultivated meat. The imminent production of cultivated meat necessitates a paradigm shift in cell culture media production, impacting the global supply chain of raw materials pivotal in both food production and the biopharmaceutical industry.

This work encapsulates some of the complex landscape of cultivated meat production, highlighting the industry's potential to reconcile the demands of a burgeoning global population with the imperative of sustainable and efficient meat production.

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# GS Effex, a new Lonza host cell line for the production of antibodies with Enhanced ADCC activity

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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

**Relevance to Theme Selected:**
The continual R&D-driven evolution of the GS® toolbox ensures that it can rise to the challenge of increasingly diverse demands in biotherapeutic production.

**Impact/Novelty:**
Lonza has developed a new, high-performing, host cell line that generates specific product quality attributes of great benefit in the development of immunotherapeutics for the oncology and autoimmune therapy areas.

**Introduction:**
Lonza's GS Gene Expression System® is a platform widely used for the expression and manufacturing of biologics. Using state-of-the-art cell engineering technologies, Lonza has developed a new high-performing host cell line, GS Effex®. Derived from the industry-known GS Xceed® CHOK1SV GS-KO cell line, GS Effex® lacks expression of the α1,6-fucosyltransferase (encoded by the FUT8 gene). This novel platform enables the production of afucosylated antibodies with enhanced antibody-dependent cellular cytotoxicity (ADCC).

**Methods/Approach:**
GS Effex® cell line was generated by engineering GS Xceed® using innovative gene editing technology. The cell line performance attributes were evaluated by generating stable pools expressing different mAbs and isolating clones expressing the antibodies.

**Results:**

- Gene editing events are observed in the FUT8 gene. LCA shows the absence of fucose in proteins. The growth of GS Effex® host cell line is comparable to GS Xceed®.
- GS Effex® derived mAb-expressing pools show comparable growth to GS Xceed® in Ambr® 15 and comparable titers. Afucosylated antibody without G0F glycan form produced in GS Effex® hosts shows enhanced ADCC activity than GS Xceed®.
- GS Effex® mAb-expressing derived clones show comparable growth to GS Xceed® and comparable titers.

**Conclusion:**
Compatible with Lonza's platform processes and GS® toolbox, the GS Effex® cell line enables immunotherapeutic developers to produce molecules with increased potency, in a proven system, without compromising on critical attributes such as cell growth, titer and product quality.

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# Study of the role of chronokine X35 in the spinal cord and peripheral nerve injuries

**Mr Rubén Guerrero-Yagüe**[1,2,3], Dr Sergi Verdés[1,2,3], Dr Maria Puigdomenech[1,4,5], Dr Mireia Herrando-Grabulosa[1,4,5], Dr Esther Udina[1,4,5], Dr Miguel Chillón[1,2,3,6], Dr Rubèn López-Vales[1,4,5], Dr Assumpció Bosch[1,2,3,5]
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**Relevance to Theme Selected:** Chronokine X35, a pleiotropic protein, shows neuroprotective, remyelinating, antioxidant, and anti-inflammatory properties, offering a promising therapeutic avenue for spinal cord and peripheral nerve injuries, pathologies that lack effective curative treatments.

**Impact/Novelty:** Advances in molecular biology and neural networks are shedding light on the complexity of spinal cord and peripheral nerve injuries, highlighting the urgent need for innovative treatments.

**Introduction:** Following the initial mechanical insult of a traumatic spinal cord injury (SCI), a secondary injury cascade occurs resulting in ischemia, excitotoxicity, microglia activation, inflammation, and oxidative stress. To a lesser degree, these processes also underlie some traumatic peripheral nerve injuries (PNI).

**Methods/Approach:** This study investigates the neuroprotective and neuroregenerative potential of the chronokine X35 to counteract these detrimental effects, proposing a synergistic approach using AAV gene therapy to address the mechanisms activated after SCI or PNIs.

**Results:** Immunohistochemical assays revealed chronokine X35 protein expression in key neural regions such as the spinal cord, dorsal root ganglia and brain. mRNA analysis demonstrated a significant decrease in X35 expression for at least 28 days post-SCI in the spinal cord. After PNI, isoform-specific downregulation was observed in different affected tissues.
On the other hand, both X35 isoforms exhibited complete protection against glutamate-induced motoneuron death in rat spinal cord cultures, indicating their potential as therapeutic agents. Furthermore, overexpression of X35 using self-complementary AAVs produced by HEK293 to overexpress the chronokine X35 appears to increase the rate of slow fibre regeneration after sciatic crush in adult mice.

**Conclusion:** Chronokine X35's neuroprotective effects suggest therapeutic potential through X35 via recombinant protein or gene therapy for SCI and PNI. Further research is needed for clinical applications to offer new perspectives in the treatment of these lesions.

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# Mechanistic modelling of bioreactors: development of a digital twin

**Dr. Andrej Pohar**[1], Tina Šmuc[1]
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**Relevance to Theme Selected: Modelling**
A mathematical model was developed by incorporating chemical kinetics, cell metabolism, transport phenomena, hydrodynamics, and pH and dissolved oxygen process control.

**Impact/Novelty:**
A comprehensive bioreactor model that incorporates all the governing phenomena and is a precise replication of the realistic system is not yet available in the literature.

**Introduction:**
The bioreactor is a complex multiphase system, and its operation is governed by fundamental physical, chemical, and biochemical phenomena, fluid dynamics and process control. Successful bioreactor operation requires a detailed understanding of these processes, and resolving the mathematical description requires the development of a mechanistic model. The developed model is a “digital twin” of the realistic system, and can be used for testing operational scenarios, parametric sensitivity, troubleshooting, and for scale-up and transfer to other facilities with different process equipment.

**Methods/Approach:**
Computational fluid dynamics provide the hydrodynamic conditions such as the specific power input and homogenisation time. Mass balance equations are used for describing the headspace, bubbles, and cultivation medium. This includes gases, gas dissolution reactions, cells, metabolites, pH and dissolved oxygen, under various operational conditions such as fed-batch or perfusion.

**Results:**
The results of the modelling provide predictions of cell growth and viability, metabolite profiles, pH and dissolved oxygen profile, gas concentrations in the headspace and bubbles, and on-demand oxygen and $CO_2$ flows. The process can be further simulated for pre-stage stirred tank or rocking motion bioreactors, the production of small-scale models, efficient scale-up to the manufacturing scale, and real-time monitoring.

**Conclusion:**
The developed model provides insights into the operation of bioreactors and the complex interdependencies of the underlying phenomena. A mechanistic understanding allows for efficient planning of development activities and for a robust process in production, with less failed batches and resolved unexpected events.

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# Mesenchymal stem cell encapsulation in alginate beads using microfluidics

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Poster Session 3, Cromdale Hall & Lennox Suite, June 25, 2024, 2:30 PM - 4:00 PM

## Background and Novelty

Mesenchymal stem cells (MSCs) have attracted a lot of attention in the field of regenerative medicine owing to their ability to recruit progenitor cells, secrete immunomodulatory molecules, and in some cases differentiate [1]. However, MSCs have shown limited effects *in vivo,* mainly due to their low survival post-transplantation. Several strategies aiming to increase their viability post-transplant have been developed, amongst them, encapsulation of cells in hydrogels. Indeed, hydrogels such as alginate are biocompatible and can provide a supportive and protective microenvironment. Cell encapsulation in hydrogels can be achieved by using a microfluidic system. MSC spheroid can also be formed using this technology.

## Experimental Approach

A microfluidics system was used to produce alginate beads. The outer organic phase was a fluorinated oil containing surfactants (Fluosurf). The inner aqueous phase was Alginate (+additives). MSCs retrieved from the apical papilla of human teeth (SCAP)[2] were used. Several parameters were studied (phase composition and gelation time) to obtain beads containing an even distribution of cells with high viability. Then, spheroids were obtained in double emulsion droplets (DE) by adding a second chip downstream [3]. The DE of alginate allowed the formation of spheroid after 150min.

## Results and Discussion

The most promising method to obtain viable cell-containing beads was to allow in-chip cross-linking of the alginate (containing Ca-EDTA) [4]. Indeed, by adding acetic acid to the oil phase, the interaction between the acid and alginate occurred inside the chip. The collected beads were immediately released from the oil after production, which improved cell survival.
Spheroids production still need to be optimized with this method, but it allowed the formation of small size spheroids encapsulated in alginate.
With this optimized method, we plan on offering a reliable platform to encapsulate cells in microbeads as a suspension or as spheroids. By doing so, we aim to improve the efficacy of MSCs.

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