

Newsletter

September 2006

Editorial

Dear Readers,

I hope this newsletter finds you well, as you plough the fertile grounds of research this Summer!

In this issue, in the “Chairman’s word”, Florian and the committee grapples with the organisation of the next ESACT meeting to maintain diversity while trying to keep focus for the increasing number of participants. From Singapore, BTI scores a coup with the formation of the CHO Consortium with industrial participants. There is a commentary on the Whistler conference in April. Following the ‘earthquakes’ created in stem cell research, there is an encouraging report in the Lancet that bone marrow stem cells may improve myocardial infarction in a double blinded study with placebo.

We feature the story of an antibody Phase 1 safety trial in the UK which had tragic consequences for the volunteers. A special issue of Cytotechnology on animal cell technology updates has been published in July. Amgen following the footsteps of Wyeth, also expands their manufacturing facilities in Ireland with their 2 best selling products.

We welcome new members to ESACT since Apr. ‘05 and round off with forthcoming meeting announcements.

Till Christmas, enjoy the summer!

Chief Editor, Steve Oh



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A Word from the Chairman

Dear members of the ESACT community,

Here we are in the middle of a phase now where urgencies of the next new meeting (in Dresden, as you remember) have not yet arrived, and the memories of the past event in Harrogate are still there but not in the forefront of our thinking anymore. I believe we are mostly concerned now to imagine what could we do in the lab to generate a new level of knowledge and scientific excitement that we would be able to share again eventually with our colleagues.

There were of course other meetings where some of us met again (Whistler, Canada, organized by our friends Konstantin Konstantinov and Jamie Piret).

With respect to the ESACT community and its future we have to think, also in response to the widening demand for the expertise our meetings provide, how we will handle the larger "crowds". In the executive board of ESACT this is a reoccurring theme, but it appears that it becomes more and more urgent with 800 to 900 people or more wishing to be part of the crowd. This of course, is also a question about what we consider to be the most important themes for the future. In my view, the largest part of our community is focused on processes and products. It may not be always the most exciting type of science that is behind these topics, but it surely is still very complex. I believe we can only do a good job if we do not loose contact with the huge diversity and depth of the general biological and medical sciences. Surely a lot of inspiration can be obtained from hearing from and talking to those people who represent the top in these respective fields. The executive board of ESACT has been challenged to come forward with a suggestion or position that would represent the two areas and how to integrate them into a vision for the decade ahead for ESACT.

Stay tuned... we are still working on it.

Best wishes.

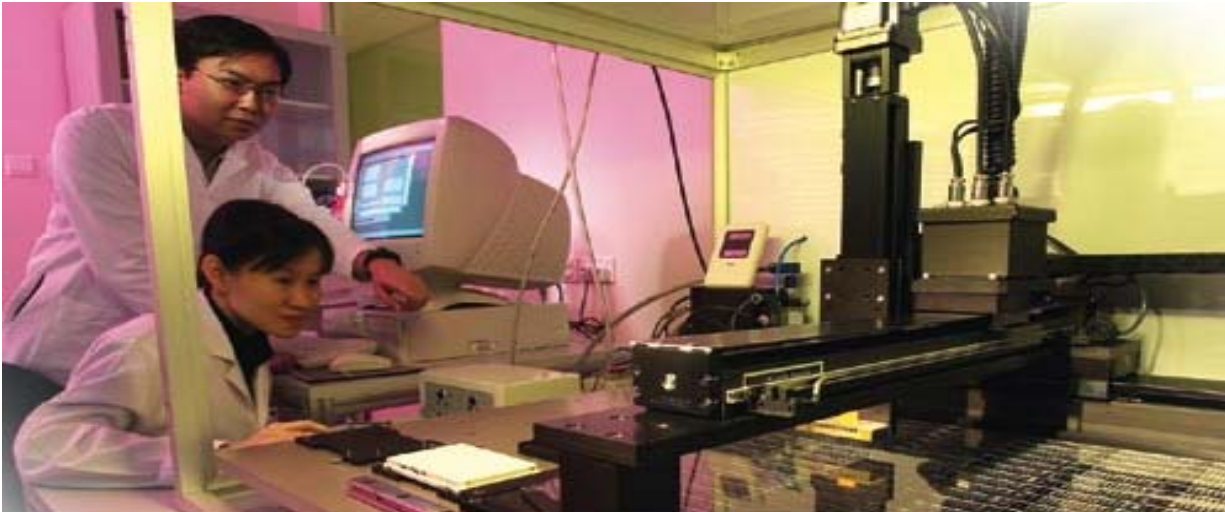
Florian



BTI forms CHO Consortium with industry

A*STAR Bioprocessing Technology Institute (BTI) and the University of Minnesota, USA has recently organized the formation of a consortium to further the development of genomic research tools for Chinese Hamster Ovary (CHO) cell lines.

The Consortium members comprise major pharmaceutical and biotechnology companies. Together, these companies will provide up to US\$2 million through the Society for Biological Engineering, USA to kick-start the collaborative research effort.



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Deciphering Dots and Spots

CHO cells are used to produce about 70 percent of all pharmaceutically important recombinant proteins. Research on CHO cells is thus considered especially significant for facilitating drug discovery and to increase production efficiencies of medicines for fighting certain cancers, controlling bleeding disorders and boosting blood cell production.

Scientists from BTI and the University of Minnesota will develop and use genomic tools to learn more about the biological and molecular make-up of CHO cells in order to gain insights to the cellular machinery, and to improve the capability of CHO cells to produce recombinant proteins.

The Bioprocessing Technology Institute (BTI) is a national institute funded by the Agency for Science, Technology and Research (A*STAR) through its Biomedical Research Council. BTI spearheads bioprocess science and engineering research by combining molecular biology, biochemistry, proteomic and genomic sciences, to understand how to enhance the productivity of cells, develop better cell culture, fermentation and separation processes to manufacture important molecules such as antibodies, recombinant DNA and proteins that target a myriad of diseases. Contact detail: BTI Bioprocessing Technology Institute, 20 Biopolis Way #06-01 Centros, Singapore 138668, admin@bti.a-star.edu.sg

Conference Report on Cell Culture Engineering X, Canada, April 2006

This conference was organized by Engineering Conferences International (ECI), the successor program to the United Engineering Foundation conferences program that was established in 1962 to provide an opportunity for the exploration of problems and issues of concern to engineers from many disciplines.

Summary of Oral Presentation

Listed below are brief summaries of some of oral presentations:

Session 1: Accelerating Cell Line Development and Clinical Candidate Optimization

Overall, the presentations suggested that there has not been a major breakthrough in this area. Most of the work utilized FACS to enrich cell population but the time needed for cell line development remains at about 3-6 months. The major bottleneck still appears to be the time needed for single cell cloning and subcloning.

Two industrial presenters (Amgen & Wyeth) showed data suggesting that gel microencapsulation drop (GMD) techniques allowed for the clonal population to be enriched for higher producers. The mechanism for this is still unknown. On the aspect of transient gene expression (TGE), a presentation from ImClone showed that with scale increase (30mL to 3L scale-up) there is a significant decrease in productivity (50% decrease) from TGE. Interestingly, it was mentioned that only on rare occasions is the protein in stable clones different from the TGE pools. It was also mentioned that for TGE, they preferred to use CHO or NSo because the GS system does not work very well in 293 cells.

Session 2: Physiology & Engineering of Production Cell Lines

Malcolm Kennard (Chromos) presented the use of the Artificial Chromosome Expression (ACE) system. The ACE system is based on pre-engineered artificial chromosomes with multiple recombination acceptor sites that allowed for the specific introduction of single or multiple gene copies and eliminates the need for random integration into native host chromosomes. They were able to generate single cell subclones in 6 months with stable expression for Mab titres of up to 1g/L.

Session 3: Proteomics & Genomics for Process Development

The collaborative efforts between University of Minnesota and BTI were presented by Prof. Wei Shou Hu in this session. In the same session, Martin Sinacore from Wyeth presented their efforts in creating a proteomics/genomics platform to examine CHO cell phenotypes. Currently, the platform consists of 3,500 CHO sequence (Affymetrix) and for the second generation platform, Wyeth is aiming to reach 15,000 sequences.

Session 4: Product Quality/Immunogenicity

Annie De Groot (EpiVax Inc.) presented their work in re-engineering proteins using pro-

proprietary 'immunoinformatics' so that they will have low *in vivo* immunogenicity while retaining their therapeutic properties.

Sigma Mostafa (Eli Lilly) presented work on product quality challenges. Not only are there glycosylation changes (e.g. non-human glycosylation) but also N-terminal cleavage, AA modification, pyruvateylation, alternative splicing, aggregates/isoforms. They found cleavage products tend to increase with culture time.

Session 5: Accelerating Process Development & Scale-Up

This session concentrated on the advances and challenges in accelerating process development and scale-up for clinical and commercial manufacturing of biopharmaceuticals. Most presenters (Amgen, Genentech, Biogen Idec) have a platform for the process development & scale-up whereby a SOP which has been proven to work is repeated only with minimal optimization steps (achieving ~5-10x increase in product titre).

First Stage: Explore different media usage in 2-5L bench top reactors

Second Stage: Develop Feed strategy and bioreactor environmental parameters

Third Stage: Consolidation runs in pilot-scale bioreactors

In addition, the need to define the threshold operating parameters of a process was stressed by the presenters. Eg. if seeding density is kept at $\pm 35\%$ product titre is maintained but beyond $\pm 45\%$ product titre decreases.

Session 6: Viral Vectors for Gene Therapy & Vaccination

Several modeling approaches were presented. Udo Reichl (Max Plank) reported on the modeling aspects of influenza A virus replication in mammalian cells while Antonio Roldao (IBET) presented a model of rotavirus VLPS production. Nedim Altaras (Merck) presented an approach that used PERC6 cells as an alternative for adenovirus production. High passage number cells have higher viral production capability (~3x virus/cell).

Session 7: Case Studies in Cell Culture process Development & Technology Transfer

In the workshop, there was a debate on how to carry out technology transfer efficiently. Most of the participants agree that with bigger biopharma, there is a need to have dedicated process development (PD) and Manufacturing sciences (MS) personnel. The PD is responsible for developing the process while the MS is dedicated for tech transfer.

Session 8: High-Throughput Screening Systems for Mammalian Cell Bioprocessing

A few presentations focused on the development of micro-bioreactors that will allow for high throughput runs for process optimization. Alice Chuck (Amgen) presented some preliminary set-up experience with SimCell micro-bioreactors but even the set-up seems to be plagued with problems (interface issues with incubator, robotics). Francesc Godia (Universitat Autònoma de Barcelona) presented the group's newly developed minibioreactor platform. This is also still in the prototype stage.

Judy Chou (Wyeth) presented an interesting high-throughput assay for cell line & cell

culture development. They directly linked protein A onto 96-well plates for quick purification of MAb. This format allowed sufficient quantity to be purified for quantity and quality assessments. They can screen up to 1500 clones in a day. More importantly, they showed that the well method has similar binding capability to columns.

Session 9: Downstream Processing, Challenges & Prospects

Philip Lester (Genentech) stressed that while industry has managed to reach high yields of grams per L, the high cell mass from the production process can impact harvest and initial filtration operations and may also deliver high levels of host cell and process related impurities to the downstream process.

Danny Wong

Application of bone marrow stem cells in myocardial infarction leads to a reduction of the infarct

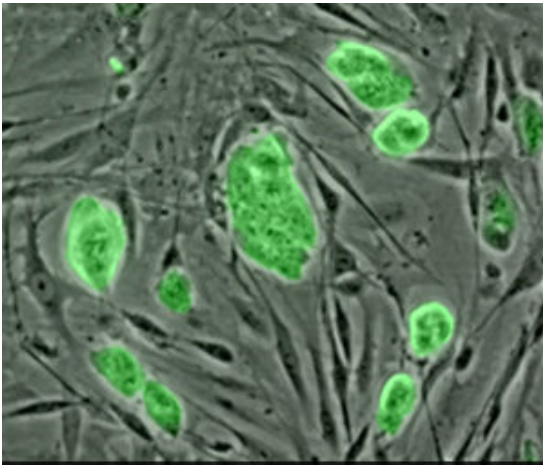
Doctors at the Catholic University of Leuven, connected with the University Hospital - Gasthuisberg, the Stem Cell Institute Leuven (SCIL), and the Flanders Interuniversity Institute for Biotechnology (VIB), are publishing a major breakthrough in the treatment of patients with acute myocardial infarction. Their research shows that the administration of a patient's own stem cells has a significant positive effect on the heart's recovery: in the patients studied, the size of the infarct was clearly reduced. The use of stem cells appears to be safe, and to date no side effects have occurred that can be attributed to the stem cells. This study is a world-first – its exciting results are being published in the prominent medical journal *The Lancet*.

In an acute myocardial infarction, the flow of blood from a blood vessel in the heart is blocked, whereby the cardiac muscle receives insufficient oxygen and heart tissue dies. In many cases, the supply of blood in the deadened portion of the heart can be restored via the so-called balloon technique. But the heart suffers permanent damage, primarily to the left ventricle.

Researchers in Leuven have tested the administration of bone marrow stem cells on patients stricken with acute myocardial infarction. In the 67 patients of the study, the supply of blood in the heart was restored optimally via the balloon technique. Then, within 24 hours, some patients received an injection of stem cells from their own bone marrow and some received an aqueous (placebo) solution (the patients in each group were selected by drawing lots). Such a double-blind, placebo-controlled study has never before been conducted.

Collaboration among the cardiology, hematology, radiology and nuclear medicine services yielded an unparalleled study in which state-of-the-art technology was used to investigate changes in the left ventricle, blood supply and heart metabolism.

Improvement in the global functioning of the left ventricle was comparable in both the control group (injected with the placebo) and the group that received the stem cells. But a clear global improvement in function was found in the sub-group of patients who had



been afflicted with the most serious infarctions. Moreover, the reduction of the size of infarct was significantly greater in all patients in the 'stem cell group' and correlates with a better preserved regional left ventricle function. It is still much too early to conclude that every patient with a myocardial infarction should be treated with stem cells. Indeed, there is still a long road to travel in the development of a medication, and no risks must be taken along the way.

One of the major scientific merits of this study is that it has investigated – in a rigorously controlled manner – the possible role as well as the

limitations of the administration of stem cells. The findings are thus an important driving force for further targeted clinical and pre-clinical research. This study is the initial impetus for VIB and the Catholic University of Leuven and the newly established SCIL to quickly combine fundamental research on stem cells with clinical applications for the benefit of patients.

Monoclonal Antibody Drug Trial in London causes Serious Consequences for Six Volunteers

The doctors who treated six British men in a trial of a drug in March which went horribly wrong, say they believe the men suffered an immune system overreaction known as a cytokine storm. The men were testing an experimental drug in a phase 1 safety trial; the drug was designed to treat chronic inflammatory conditions such as rheumatoid arthritis, multiple sclerosis and some forms of leukemia. The drug is a monoclonal antibody, an immune system protein engineered to target immune system cells known as T-cells that directly stimulate the immune system. In a healthy person the T-cells dampen the function of other parts of the immune system by preventing the body from attacking itself and when this safeguard fails, it can lead to autoimmune diseases. GN1412 was designed to deal with such a response.

Although all the men have since recovered it has not been without certain ramifications regarding their long term health; one already has the early signs of lymphatic cancer. Ryan Wilson age 21 was the most seriously injured volunteer and had to have parts of his fingers and toes amputated because he developed gangrene. Of eight healthy men, six were given the drug TGN1412 and two a placebo; within a short space of time the six men who received the drug developed headaches, shivers, nausea, diarrhoea and lower back pain; within twelve hours one of the patients became severely ill and some of the men became very disturbed, their blood pressures all plunged and their organs began to fail.

All were fortunately transferred to an intensive care unit where they received intensive cardiopulmonary treatment, including dialysis, high-dose Medrol, a corticosteroid used to reduce inflammation and an anti-interleukin-2 receptor antibody. Two of the patients developed cardiovascular shock and acute respiratory distress syndrome, which required

eight to 16 days of organ support. Dr. Ganesh Suntharalingam and colleagues at Northwick Park Hospital in northwest London who treated the men have written a report of the incident and along with comments from other medical experts, and hope the information provided will contribute to the debate over identifying potentially dangerous drugs and stop such an event from happening again. The report is published in the New England Journal of Medicine.

The doctors say the incident appears to have been an unforeseeable consequence of taking the drug, but believe the case may help doctors understand what happens when the immune system overreacts in such a way, which could occur again in future clinical trials. The ensuing two weeks were pretty dramatic as the previously healthy men developed symptoms that resembled sepsis, a very serious infection. They experienced difficulty breathing and in the fight to keep the men alive the patients were given transfusions of blood products after their blood started to clot abnormally and tissue started to die and peeled off at the ends of their fingers.

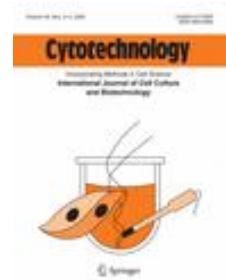
It eventually took the doctors two weeks to stabilize the patients and to lead them to recovery, but some remained in hospital for months. Blood tests showed they had a cytokine storm, a sudden over-release of immune system inflammatory chemicals and such an over-response can be a killer. This had not happened in the animal trials; possibly say some experts because lab animals are often kept in super-clean environments and their immune systems have never been challenged.

Researchers say regulatory authorities, who tested TGN1412 from the same batch as the infused drug, found no errors in its manufacture, formulation, or administration and found no contamination. They advise that scientists running clinical trials should be aware of the possible dangers and equip themselves to handle such cases. The volunteers were each paid about US\$ 3,500 for taking part in the trial. Four of the men have already received compensation payments of about US\$25,000 from TeGenero. The others are waiting for the results of medical tests before making specific compensation claims. Lead report author Dr. Ganesh Suntharalingam says the patients were given the drug at 10-minute intervals, and perhaps there needs to be more time between doses. He says the men were lucky that the trial took place at a fully equipped medical centre, and were able to get to intensive care very quickly when they needed to. Other experts are now questioning the medical ethics of phase 1 trials where high risks are taken for money and believe more animal experiments with experimental drugs need to be done before drugs are tried on patients. Others are concerned that the fiasco will be detrimental to the use of such new drugs which have the potential to benefit many people.

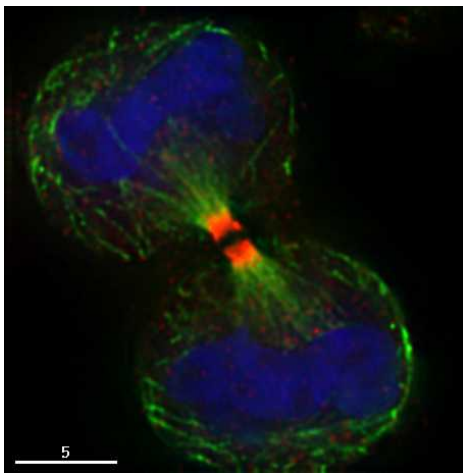
TGN1412 is made by German biotechnology firm TeGenero AG and the trial was run by U.S. drug research company Parexel International on behalf of the company and has since filed for insolvency.

Special issue of Cytotechnology

A special issue of Cytotechnology on “Animal Cell Technology – Past, Present, and Future “ has been edited and was published in July. The objectives of this issue are to provide an update on recent achievements and future developments in animal cell technology. The following topics are covered:-



1. O.-W. Merten. Introduction to animal cell culture technology – Past, present and future.
2. A. Nienow. Reactor engineering in large scale animal culture.
3. J.P. Carvell and J.E. Dowd. On-line measurements and control of viable cell density in cell culture manufacturing processes using radio-frequency impedance.
4. J. Keenan et al. The role of recombinant proteins in the development of serum-free media.
5. M. Butler. Optimisation of cellular metabolism and glycosylation of recombinant proteins produced by mammalian cell systems.
6. N. Arden and M.J. Betenbaugh. Regulating apoptosis in mammalian cell cultures.
7. A. Oumard et al. Recommended method for chromosome exploitation: RMCE-based cassette-exchange systems in animal cell biotechnology.
8. T. May et al. Current status of transcriptional regulation systems.
9. K.F. Wlaschin, G. Seth, W.-S. Hu. Toward genomic cell culture engineering.
10. J.N. Warnock et al. Cell culture processes for the production of viral vectors for gene therapy purposes.
11. S. Diekmann et al. Present and future developments in hepatic tissue engineering for liver support systems. State of the art and future developments of hepatic cell culture techniques for the use in liver support systems.
12. S.K.W. Oh and A.B.H. Choo. Human embryonic stem cell technology: large scale cell amplification and differentiation.



HeLa cell about to undergo abscission
(www.gla.ac.uk/ibls/BMB/gwg/memtraff.html)

Amgen expands manufacturing in Ireland

Amgen, the world's biggest biotechnology company, said Tuesday that it would build a manufacturing plant in Ireland to supply its growing European customer base.

The plant, to be built near the southwestern city of Cork and to open in 2009, would employ 1,100 people and cost more than \$1 billion, Amgen and the Irish government said.

Amgen also plans to expand its existing U.S. and British research facilities and build a new development center in Uxbridge, England.

The facility in Ireland will produce drugs like Aranesp and Epogen. The drugs, which are used to treat anemia, are among the world's best-selling biotechnology drugs and had sales of \$5.1 billion in 2004. Revenue from Aranesp jumped 38 percent in the third quarter to \$840 million.

"This is not a transfer of capacity, its organic growth and the need to serve a growing number of patients," Fabrizio Bonanni, Amgen's senior vice president for manufacturing, said by telephone. "As we grow in Europe in terms of patients served we have to establish a presence and that has to include our ability to manufacture here."

Several European countries had competed for the investment won by Ireland, which has the fastest-growing economy in Europe and has specialized in attracting foreign pharmaceutical companies.

"Investments of this scale speak volumes about Ireland's ability to compete and win the most advanced and innovative business from the biggest biotechnology company in the world," said Ireland's minister for employment, trade and enterprise, Michael Martin.

Amgen received undisclosed of grants from the Irish government as part of the deal. But company officials stressed that they picked Ireland primarily for other reasons, notably Ireland's concentration of drug companies and its corporate tax rate of 12.5 percent - about a third the rate of many European rivals.



Cork— site of Amgen's newest manufacturing plant

New ESACT members since April 2005

Surname	First Name	Institution
Ansel	Emilie	GlaxoSmithKline Biologicals
Baumann	Sebastian	GlaxoSmithKline
Berberof	Magali	GlaxoSmithKline Biologicals
Bollati	Mariela	German Research Center of Biotechnology
Caloz	Pierre	Laboratoires Serono
Christensen	Klaus	F. Hoffmann La Roche
Coco Martin	José	Animal Sciences Group, Wageningen UR
Daugherty	Peter	Upstate Biotech
De Firesta	Françoise	GlaxoSmithKline Biologicals
de Waard	Rick	DMV International
Dillingham	Matthew	Serologicals Ltd
Dumas	Patrick	GlaxoSmithKline Biologicals
Gallili	Gilad	Abic, Biological Laboratories Teva
Garnas	Annika	L.A.B. Sweden AB
Garnier	Alain	Laval University
Geserick	Christoph	CNIO Spanish National Cancer Center
Gorenflo	Volker	Aventis-Pasteur
Hamilton	Bruce	GlaxoSmithKline
Hunt	Ian	Novartis Institute of Biomedical Research
Johnsen	Laust Bruun	Novo Nordisk
Jonniaux	Jean-luc	Crucell
Kapat	Arnab	Reliance Life Sciences Pty Ltd
Knott	Isabelle	GlaxoSmithKline Biologicals
Kotsopoulou	Ekaterini	GlaxoSmithKline
Long	Joanne	Vertex Pharmaceuticals
Luo	Shun	JRH Biosciences, Inc.
McCall	Martin	Acyte Biotech Pty Ltd
McNorton	Sandra	JRH Biosciences
Mygind	Ann Merete	Novo Nordisk
Oh	Duk Jae	Sejong University
Onadipe	Adekunle	Pfizer Global Research & Development
Oppenheim	Sheldon	Millennium Pharmaceuticals
Perani	Angelo	Ludwig Institute for Cancer Research
Radcliffe	Pippa	Oxford BioMedica
Repping	Oscar	Intervet International BV
Rhodes	Malcolm	Serologicals
Singh	mallika	Human Genome Sciences, Inc
Sobczyk	Andre	Vivalis
van de Griend	Rene J.	Biocult bv
van der Velden-de Groot	Tiny	Netherlands Vaccine Institute
Wiedemann	Philipp	Boehringer Ingelheim Pharma GmbH
Yeow	May Ling	Pall Europe Limited

Forthcoming Meetings

Cell and Molecular Biology of TRP Channels
Sep 7 - 8, 2006
Bath, United Kingdom

European BioTechnology Workshop
Sep 17 - 19, 2006
Karthause Ittingen, Warth near Zurich, Switzerland

Cell Culture Scale-up
September 18 – 20, 2006
Hyatt Westlake Plaza - Thousand Oaks, CA

19th Annual and International Meeting of the Japanese Association for Animal Cell
Technology JAACT
September 25-28, 2006
Kyoto Japan.

Cancer Genomics and Emerging Technologies Conference
Oct 2 - 4, 2006
Boston Marriott Cambridge, Cambridge, MA

Manchester Stem Cell Meeting
Oct 25, 2006
Manchester, UK

Immunocytochemistry, In Situ Hybridization & Live Cell Imaging
Oct 23 - Nov 5, 2006
Cold Spring Harbor Laboratory, Cold Spring Harbor, New York USA

59th Annual Symposium on Cancer Research. "Stem Cells in Cancer and Regenerative
Medicine"
Oct 27 - 29, 2006
Houston, Texas

Biotechnología Habana 2006
Nov 13 - 16, 2006
Havana, Cuba

Neurodegenerative Disease: Biology and Therapeutics
Nov 30 - 3, 2006
Cold Spring Harbor, New York, USA

Stem Cells 2006
Dec 14 - 17, 2006
Cancun, Mexico

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