

Human Stem Cell Lines: The Role of Cell Banks in Assuring Quality for Research and Clinical Development in Cell Therapy

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Professor Glyn Stacey's background is in microbiology and cancer research, and he has established special interests and activities in the field of cell biology relating to genetic stability, safety issues and standardisation. From 1989-1998, he worked at the Centre for Applied Biology and Research, where he was involved in developing cell culture and cell banking procedures, and in establishing a cell biology unit. He joined NIBSC in 1998 where he has developed a broad remit on the appropriate development of cell biology activities relevant to the quality and safety of new biological medicines and therapies based on the use of human and animal cells. In addition, he has acted as an advisor to the UK Department of Health and the World Health Organization and was a member of the consultation group that drafted the UK Code of Practice for the Production of Human-derived Therapeutic Products (June 2002). Glyn is now in charge of the UK Stem Cell Bank, funded by the Medical Research Council and BBSRC to prepare banks of stem cell lines for use in research and the development of new therapies.

Bone marrow has been used as a source of stem cells for transplantation for many years and today it is finding an expanding range of clinical applications, including the treatment of auto-immune diseases and as a vehicle for gene therapy. Stem cells in peripheral and cord blood may be expanded to a limited degree *in vitro* but are at an early stage in terms of evaluation for therapy. The use of stem cell lines takes stem cell therapy into a new realm of possibilities, but there are some challenges regarding quality and safety in clinical applications and a number of these relate to the process of *in vitro* passage. As I will explain, particular concerns include: cell identity, stability of characteristics *in vitro*, reproducibility of differentiation protocols and microbiological status. Many of these challenges are in fact not new and have been addressed in various fields where cell lines are used and have received particular attention for the manufacture of biological medicines, including viral vaccines and recombinant therapeutic proteins. This article aims to identify some of the main issues that will need to be addressed in the development of therapies based on the use of stem cell therapies, and will outline the potentially valuable role that public service cell banks can play in promoting stem cell research and the quality and safety of new therapies.

GENERAL CONSIDERATIONS FOR THE QUALITY OF STEM CELL LINES USED IN RESEARCH AND DEVELOPMENT

The key features for gaining reliable and accurate data from cell lines are that they should be:

- ◆ Authentic (of the correct origin and not cross-contaminated or switched with another cell line)
- ◆ Pure (free of contamination by micro-organisms)
- ◆ Stable (the desired characteristics are sustained on extended passage *in vitro*)

Authenticity

The history of cell culture is punctuated with reports of instances of cell line cross-contamination where laboratory errors and poor cell culture practice seem to have been the major culprits (1). This process started a long time ago, with the discovery of widespread 'cross-contamination' of new cell lines with the HeLa tumour cell line (2). Other commonly used cell lines have been implicated in cross-contamination and even the originating laboratories have been shown to have provided cross-contaminated cell lines (3). Given the unique and important potential of stem cell lines, it is vitally important that

we do not ignore the lessons provided by early experiences in cell culture. Stem cell line banking centres have an important role to play through international collaboration to ensure that stocks of stem cell released for research are authentic.

Purity

The risk of cell contamination from donors with serious pathogens should be considered by all laboratory users of human tissues and cell. Persistent microbial infections can become established in cell lines, and some of these, whilst not being hazardous to laboratory workers, can alter the performance of cell lines and invalidate research work. Mycoplasma species are one of the most common known contaminants of cell lines. These can often go unnoticed by laboratory workers, but are well documented to have a broad range of genetic and phenotypic effects on their host cultures (4). Detection methods are readily available and routine screening for these organisms, along with destruction of contaminated cultures, is the best means of control in the long-term.

Other organisms, including viruses, may be introduced through cell culture media components (4,5) or by contamination with organisms from the environment or laboratory worker. The routine use of antibiotics may only suppress resistant contaminants and lead to recurring difficulties. Staff training in current best practice in cell culture (6) is vital to prevent contamination through appropriate laboratory procedures and careful selection of culture media.

Stability

A critical issue in the use of human stem cells is their ability to maintain undifferentiated characteristics on long-term passage *in vitro*, whilst also sustaining the capacity to undergo differentiation. Certain stem cell lines have shown the potential for genetic change *in vitro* and require careful qualification in extended passage experiments.

THE PRINCIPLE OF MASTER AND WORKING CELL BANKS

Experience in industry has established the importance of a cryopreserved 'master cell bank' from which to develop 'working cell banks' for all future uses. This tiered system is central to assuring long-term provision of good quality cells and should be a core tool for any stem cell line bank. These banks of homogenous vials of cells can be characterised, safety tested and made available for use over many decades, and the master-working bank system is a vital tool for reliable research and development of safe and standardised cell therapies.

Cryopreservation of human stem cells is an area that requires further investigation. Standard cryopreservation methods using dimethylsulphoxide (DMSO) have failed to work well for embryonic stem cell lines and DMSO is known to have broad ranging effects on cell biology. Vitrification methods have been evaluated that seem to offer immediate benefits, however, such methods will need considerable development and validation before they can be relied upon to ensure long-term viability of preserved cultures.

FURTHER CONSIDERATIONS FOR STEM CELL LINES USED IN THE DEVELOPMENT OF THERAPIES

Obviously, the issues raised above for research work are also basic requirements for the development of human stem cell lines for therapeutic applications. However, some particular areas demand more intensive consideration.

Adventitious Agents

In cell, tissue or organ transplantation, standard donor screening tests for key viral pathogens have been established to ensure a good record of safety for transplanted material (7). However, in the future, as the immunological barriers to stem cell transplantation are explored, the use of individual human stem cell lines for large numbers of patients may well expand. The use of a cell culture product in many patients will alter the risk benefit considerations and there will, potentially, be a broad range of contaminating agents that are not considered a hazard in R&D but which have significance when injected directly into patients. A risk assessment should be carried out for all critical reagents in direct contact with the cells in order to establish appropriate specifications for these reagents and identify any potential microbial risks, particularly in respect of materials of animal origin (8). In many cases, the careful selection and testing of media components will provide safe solutions (8), and there is already a formal European process for dealing with such risks (9).

Evaluating Risk from Source Tissue

A screening process for all donor cells and tissues is important to exclude the most likely and serious infections (7). In addition, a medical history for donors can be used to identify or exclude patient specific risk factors (for example, inherited disease or drug abuse). Microbiological screening data for donors should be scrutinised with regard to the sensitivity and specificity of the test methods used and the quality of laboratory testing (such as accreditation under an appropriate quality standard). As the range of cell therapy products derived using cell culture increases, there is a growing need for microbiological safety testing of an accredited standard. The cell banks servicing the research community and clinical development should be expected to assist workers in sourcing appropriate testing or to provide these tests as a service.

Stability and Tumourigenicity

The loss of ability of stem cell lines to differentiate following extended passage *in vitro* is a critical issue for stem cell therapy. Reliability will be promoted by master and working cell banks, but maximum population doubling levels (PDLs) may need to be established to provide limits for clinical use and this may vary between stem cell lines. The need for reliable differentiation for clinical therapy and in research means that careful validation of cell differentiation at extended passage is an important requirement for cell banking centres to address.

It is known that both haematopoietic stem cells and embryonic stem cell lines may undergo transformation or fusion *in vitro*

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and both of these events raise safety concerns regarding the potential tumorigenicity of stem cell products for therapy. Since the pluripotency of embryonic stem cell lines is characterised by their capacity to generate tumours in mice, it seems sensible to base their clinical use on differentiated cultures. It will also be necessary to have sensitive methods to test for the presence of residual undifferentiated and potentially tumourigenic cells.

For stem cell products from both embryonic and somatic sources, new markers for transformation events associated with tumourigenicity will be helpful. The presence of potentially transforming proteins is also an issue that may require further attention in risk assessment of cells for therapy.

NEED FOR GUIDELINES AND STANDARDS

Recently, guidelines on good cell culture practice have been developed by expert groups (10,11). For cell lines used for the manufacture of biological medicines, there are regulatory guidelines (12,13). However, the guidance required for the new therapies using stem cell lines is a combination of those already described, and guidelines for human tissues for transplantation. In some European countries, useful interim codes of practice are in use (14,15,16) whilst we await the European Human Tissues Directive.

It is also crucial to the success of any stem cell product that ethical and legal dimensions are addressed. This varies somewhat between different European countries (17). All stem cell workers should utilise ethical and consented sources of donor cells and familiarise themselves with the relevant legal requirements both locally and in collaborating countries.

Appropriate quality standards must be established for the process of stem cell line banking and for the UK Stem Cell Bank a standard has been established for clinical grade cell banks that requires banking in EU GMP grade facilities with all procedures subject to the UK Code of Practice for Tissue Banks (14) and inspected by the UK Medical and Healthcare products Regulatory Agency (MHRA). Whether stem cell lines are intended for clinical or research purposes, operation to an appropriate international quality standard is useful for any bank actively involved in distributing stem cell lines, both to demonstrate the quality of work and safety testing being carried out in preparing the cell banks and also to promote international standardisation. The quality system should not be self-serving, but is a vital part of the constant effort to

ensure that current best practice is implemented in the provision of stem cell cultures in order to ensure reliability and the safety of cells released for therapy.

CONCLUSION

There is a clear need for collaboration on an international basis between stem cell banking centres to establish and maintain ‘current best practice’ as a basis for high quality research and promotion of safe and efficacious stem cell therapies. It is vital that the stem cell community learns from the lessons provided by the past 50 years of cell culture to safeguard against the consequences of poor laboratory practice.

The maintenance and quality control of stem cell lines is a laborious task and it is important that public service cell banks are active in delivering high quality cell lines in the long-term. They will also have to keep pace with the rapidly developing scientific knowledge of stem cells, which will change the demands on cell characterisation and testing. A smooth and economical path from research bench to clinical trial can be greatly assisted by stem cell banks providing good quality and reliable cells for research programmes and also appropriately prepared stem cell lines for clinical use. ♦

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