Stem Cell Technology–Emerging Framework for Hazard Assessment and Biosafety Considerations

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Abstract

A vast spectrum of therapeutic interventions is based on stem cell technology, ranging from inert biomaterials to pluripotent, virally-modified cells. On the other hand, the scientific and regulatory community is concerned with the safety of stem cell-based products and therapies and the potential health risks associated with cell-based therapies. Scientific publications have revealed shared genetic similarities and cellular morphologic and phenotypic properties between stem cells obtained from embryonic and adult sources and cancer stem cells, thus creating considerable uncertainties about the long-term safety of stem cell-based products and therapies. Existing regulatory policy and guidelines are generally inadequate to address safety assessment and risk mitigation approaches to stem cell technology-derived products and therapies. Therefore, a need exists to develop core competencies within regulatory and standards-setting institutions to better understand the unique characteristics of stem cell-derived products and to perform safety assessments on a case-by-case basis, taking into account potential hazards and risk mitigation considerations. This article outlines an evidence-based risk mitigation framework that discusses two interrelated pathways in stem cell-based biotechnologies—stem cell-derived product development and stem cell-based therapies. The proposed conceptual framework integrates biohazard considerations for cell-based products with product and facility biosafety issues that are critical to research and product development activities. The stem cell-based clinical therapeutic pathway addresses the need for integrated Institutional Review Board and Institutional Biosafety Committee reviews as a way to develop core competencies to mitigate risks and ensure that institutional policy and safety considerations are adequately addressed in stem cell-based clinical therapy and product development activities.

Introduction

Development of stem cell-related research technologies shows considerable promise for disease diagnosis and therapeutics. According to industry estimates, stem cell-related therapy and product development are likely to be an $8.5 billion global market by 2016 (Young, 2009). The commercial potential is partly due to the fact that development of stem cell-based diagnostic and therapeutic products spans a wide range of pharmacological interventions involving acute and chronic disease conditions ranging from spinal chord injuries, Alzheimer's disease, Parkinson's disease, diabetes, cardiovascular diseases, to cancer. Adult stem cells such as the human Multipotent Mesenchymal Stromal Cells (MSC) are ideal candidates for clinical research and product development, as they have committed phenotypic properties for expansion and differentiation to specific cell and tissue lineages. As a result, MSC-derived cell lineages are ideal candidates for development of stem cell-based diagnostics and therapeutic product development. However, published literature indicates that human stem cells, including MSC and cancer stem cells, demonstrate shared genetic similarities, morphology, and phenotypic characteristics related to self-renewal and the capability for differentiation. This raises concern about the long-term biosafety of stem cell-based product applications in regenerative medicine (Lazennec & Jorgensen, 2008). Cell culture studies have reported the presence of cancer stem cells in several forms of tumors including myeloid leukemia, breast cancer, and glioblastoma (Pardal et al., 2003).

The National Institutes of Health (NIH) released a draft guideline for federal funding of human embryologic stem cell (hESC) research in response to the 2009 Executive Order removing several key restrictions on research funding for hESC lines (NIH, 2009). The NIH guidelines were not specific to research involving stem cells derived from other methods such as in vitro fertilization and somatic cell nuclear transfer.

NIH has published a safety assessment of human stem cells taking into account the derivation, expansion, manipulation, and characterization of human stem cell lines as well as preclinical toxicity and efficacy testing (NIH, 2006). The guiding rationale for the NIH report is that establishment of standardized practices and procedures for human stem cells enhances safety, uniformity, and reliability of stem cell preparations for therapeutic applications. However, current guidelines do not provide a technical framework to assess the potential for hazard and risk mitigation options for stem cell-based therapeutic interventions, which for the most part are in preliminary clinical and experimental stages. No clear regulatory guidelines for advanced product development exist since most of the stem cell-based technologies are in the...
early stages of product development/commercialization pipelines.

As far as we know, none of the published technical reports or currently available regulatory guidelines provides a technical or regulatory framework that considers the unique characteristics of stem cell-derived products and clinical investigations. Likewise, existing biohazard assessment approaches are less relevant to complex cell-based products and therapeutics development. This article reviews published findings on the potential hazards associated with human tissue-derived stem cells and outlines an evidence-based risk mitigation framework that takes into account two interrelated pathways in stem cell-based biotechnologies—stem cell-derived product development and stem cell-based therapies.

Assessment Methods and Results

Hazard Assessment

A conceptual framework for biohazard analysis of stem cell-derived products and therapy generally would have to rely on a conventional risk assessment paradigm consisting of the following:

(a) A hazard assessment component involving identification and characterization of key macromolecular, biochemical, cellular, and phenotypic functional categories that determine intrinsic hazards potentially attributable to these properties;

(b) Dose-response evaluation, which in the case of cellular, tissue-based systems such as stem cell-derived products involves cell dose and host site-dependent evidence such as tumorigenesis at the site of injection, oncogenic transformation in multiple-serial passages, or expression of oncogenes from in vitro and in vivo non-clinical studies;

(c) Exposure assessment which would predominantly involve therapeutic applications and mode of application whether used under homologous or non-homologous settings, and occupational exposure to extraneous pathogenic contaminants; and

(d) A risk mitigation strategy that formally integrates practices and processes on product development research at the facility level or therapeutic applications that address potentials for hazard and adverse exposures, and that ensures integrity, uniformity, and reliability of stem cell-based products and therapy.

The capability of stem cells for self-renewal and differentiation into more specialized cell lines is both their strength and a potential risk factor that needs to be addressed in safety and risk assessments. Table 1 summarizes the source of stem cells with varying capabilities for self-renewal and differentiation, providing key parameters for safety assessments based on biological source/origin of stem cells. Stem cells of embryonic or placental origin with potential for full-spectrum tissue differentiation pose the highest risk for carcinogenic transformation during sequential passage under in vitro conditions and induction of tumors in vivo at multiple sites in both experimental animal and human studies. Therefore, stem cell classification based on whether they are of embryonic, fetal, or adult origin constitutes a baseline criterion for biohazard assessment.

Table 1

<table>
<thead>
<tr>
<th>Origin</th>
<th>Stem Cell Source</th>
<th>Stem Cell Type</th>
<th>Cell Type Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Embryonic</td>
<td>Blastocyst (5-7 days)</td>
<td>Embryonic stem cells</td>
<td>All three germ layers</td>
</tr>
<tr>
<td></td>
<td>Gonadal ridge (6 weeks)</td>
<td>Embryonic germ cells</td>
<td>All three germ layers</td>
</tr>
<tr>
<td>Fetal</td>
<td>Abortus</td>
<td>Fetal stem cells</td>
<td>Neural crest stem cells</td>
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<td></td>
<td></td>
<td></td>
<td>Fetal hematopoietic stem cells</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Pancreatic islet progenitor</td>
</tr>
<tr>
<td>Infant</td>
<td>Umbilical cord blood</td>
<td>Umbilical cord blood stem cells</td>
<td>Hematopoietic stem cells</td>
</tr>
<tr>
<td></td>
<td>Wharton’s Jelly</td>
<td>Umbilical cord matrix stem cells</td>
<td>Hematopoietic stem cells</td>
</tr>
<tr>
<td>Adult</td>
<td>Germline</td>
<td>Spermatagonia, Oogonia</td>
<td>Bone marrow stem cells</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Peripheral blood</td>
</tr>
<tr>
<td></td>
<td>Somatic</td>
<td>Hemopoietic</td>
<td>Bone marrow stroma</td>
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<tr>
<td></td>
<td></td>
<td>Mesenchymal</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Others</td>
<td>Liver</td>
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<td></td>
<td></td>
<td></td>
<td>Epidermal</td>
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<td>Neuronal</td>
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<td>Eye</td>
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<td></td>
<td></td>
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<td>Gastrointestinal</td>
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<td></td>
<td></td>
<td></td>
<td>Pancreas</td>
</tr>
</tbody>
</table>

Based on Bongso & Lee (2005).
The risk of tumorigenesis is a primary concern with the use of embryonic stem cells (ESC) in regenerative medicine (Lazennec & Jorgensen, 2008; Perez-Caro et al., 2009; Rubio et al., 2005; Shiras et al., 2007). Experimental human studies with ESC grafts have shown that tumors can arise at multiple locations. ESC seems to share pluripotency-related cellular and biochemical properties, such as cellular plasticity and motility, with cancer stem cells involved in rapidly proliferating tumors. Hypoxia is postulated as a shared characteristic of rapidly proliferating tumors and ESC (Silvan et al., 2009). Genetic transcription factors associated with ESC pluripotency share common homology with cancer transcription factors linked to non-small lung cell carcinoma and pulmonary non-endocrine tumors (Sholl et al., 2009). Several published studies on experimental animals have unequivocally established ESC linkage to induction of tumors at multiple sites.

However, published studies from clinical and experimental animal models have yielded ambivalent results on adult tumor cells, such as the MSC, in tumor promotion and tumor inhibition.

According to the International Society for Cellular Therapy, for a stem cell line to be designated as MSC, it should meet the following criteria: Demonstrate (a) the property of adherence to plastic; (b) cell-surface antigenic phenotypes; and (c) the capacity to differentiate into three lineages—chondrocyte, osteoblast, and adipocyte (Dominici et al., 2006). MSC induces the release of growth factors such as stem cell factor (SCF), Interleukin-6 (IL-6), and granulocyte macrophage colony stimulating factor (GM-CSF), providing a growth effect on hematopoietic stem cells (Majumdar et al., 2000), as well as an inhibitory effect through stimulation of negative regulators such as a IL-8/CXCL8 and tumor necrosis factor alpha (TNF-α) at various points in the hematopoietic tissue growth and proliferation pathways (Liu & Hwang, 2005). Much of the evidence simultaneously linking MSC-induced promotion and inhibition of carcinogenesis is attributed to their induction of molecular signaling pathways in hematopoietic systems.

In vivo and in vitro studies have reported formation of tumors at the MSC injection site or its proximity. The cancer-promoting effects of MSC have been reported, both in terms of incidence and multiplicity of tumors, together with extensive necrosis and angiogenesis when compared with experimental animals injected only with tumor cells (Zhu et al., 2006). Studies have suggested that MSC nonspecifically target a wide range of organs including the site of active tumorigenesis, suggesting a complex interplay of stem cells simultaneously with tissue growth, development, and repair, and tumorigenesis. Human and experimental animal studies indicate the homing of MSC non-specifically to not only tumor-bearing organs but also to those without any signs and symptoms of cancer. MSC’s immunosuppressive properties may cause tumor promotion in experimental carcinogenesis. Tasso et al. (2009) reported induction of cancer at the injection site when MSC was used for immunosuppressant therapy, eventually leading to transformation of normal cells and creating a surrogate stromal tumor.

Initiation of carcinogenesis involves a multi-stage process linked to defects in the control of stem cell differentiation (Scott & Maercklein, 1985). Increased spontaneous transformation of MSC in cell cultures exposed to a low dose of UV radiation is interpreted as part of the multi-stage process in carcinogenesis. Investigations by Rubio et al. (2005) indicated spontaneous transformation of human adipose tissue-derived MSC populations when maintained for extended periods (4-5 months), resulting in altered phenotypes, lost contact inhibition, and growth on semi-solid agar, properties that are typical of neoplastic cells. These transformed MSC were shown to induce neoplasm, at the injected site, in experimental animals.

Pisati et al. (2007) reported the antitumor activity of human skin-derived stem cells in human brain tumor models, where direct implantation of stem cells into glioblastomas resulted in reduced tumor size and reduced tumor vessel density. Intravenous administration of MSC in an in vivo model of Kaposi’s sarcoma resulted in inhibition of tumor growth (Khakoo et al., 2006). Similarly, MSC-induced tumor growth-inhibiting activities are reported in experimental animal models such as Lewis lung carcinoma and B16 melanoma (Maestroni et al., 1999) in mice, and colon carcinogenesis (Ohlsson et al., 2003) and glioma cells (Nakamura et al., 2004) in rats and mice (Pisati et al., 2007).

Based on these results, carcinogenic risks associated with embryonic stem cells should be seen as a baseline hazard when used in therapeutic settings. In the case of adult stem cell sources, such as MSC, scientific investigations from both clinical and experimental animal models have yielded ambivalent results, with evidence for both promotion and inhibition of tumor formation in the recipient systems.

Existing Safety Guidelines and the Regulatory Environment: Figure 1 illustrates the stem cell therapy development pipeline driving both product and facility-related biosafety considerations. Current industry practices for stem cell therapy development are based on either therapies produced from stem cell preparations or therapies from products derived from stem cell lines. Therapies based on stem cell preparations directly employ select stem cell lines with minimal processing for clinical trials for safety and efficacy for targeted therapeutic applications. In contrast, therapies based on products derived from stem cells involve extended development of stem cell banking and characterization studies, production processes, and pilot scale manufacture for supply of stem cells. Biosafety considerations gain greater importance when larger volumes are generated via cell banking and pilot scale production processes.
Figure 1
Product and facility biosafety requirements are driven by stem cell product development pipelines.

Existing regulatory guidelines and industry best practices related to biosafety and the safety assessment of pharmaceuticals and biologics may not adequately address the unique requirements of complex live cells. For instance, current Office for Human Research Protections (OHRP) and FDA guidelines for clinical trials involving transfer of genes into humans are primarily for cellular and genetic manipulation and gene insertion-related biotechnologies and do not address the unique nature of stem cell-based technologies (45CFR46, 2005; 21CFR50, 2003a; 21CFR56, 2003b).

Existing regulatory policy and guidelines are generally inadequate to address safety assessment and risk mitigation approaches to stem cell technology-derived products and therapies. A need exists to develop core competencies within the regulatory and standards-setting institutions to better understand the unique characteristics of stem cell-derived products and to perform safety assessments taking into account potential hazards and ethical considerations. The U.S. Food and Drug Administration and the U.K. Department of Health, Medicines and Healthcare Products Regulatory Agency have embarked on a rather flexible regulatory pathway placing a two-tier review process for stem cell-based products with a more exhaustive review for uses in non-homologous settings compared to uses in homologous settings.

Stem cell guidelines emphasize the need for a comprehensive strategy that includes alternatives to culturing on feeder layers of animal cells for improved safety and mitigates the risk of unintended xenotransplantation of animal viruses into human stem cells (NIH, 2006). Scientific investigations are actively exploring alternatives to culture human stem cells without direct contact with the mouse feeder cells, albeit most of these are experimental efforts requiring optimization of culture conditions and scale-up for commercial development. Safety assessment of alternative stem cell culturing methodologies requires an extensive battery of stem cell characterization parameters aimed at establishing the purity of the stem cell population and relevant biological activity.

NIH Guidelines for research involving recombinant DNA molecules provides a comprehensive approach to the facility biosafety requirements including the Institutional Biosafety Committee oversight, Institutional Review Board (IRB) approval, and Recombinant DNA Advisory Committee (RAC) reviews prior to human cellular and tissue products-related clinical studies. Current regulations require a detailed assessment by the facility’s institutional biosafety officer (IBSO) to be used in the storage, processing, or pilot-scale manufacturing of human cellular and tissue-based products for clinical trials. Before a new study is initiated, the IBSO reviews the study protocols and suggests modifications as needed. A biannual biosafety inspection of the facility used for stem cell-related studies is required to verify compliance with all applicable regulatory guidelines, facility in-house policies, and standard operating procedures.

Nevertheless, existing guidelines on product safety and facility biosafety-related issues do not address the potential broad scope of stem cell technologies ranging from inert biomaterial and autologous cells, over to pluripotent, virally-modified cells with extensive applications both under homologous and non-homologous settings.

The FDA’s regulations for human cell and tissue-based products (HCT/P) require compliance with the current good tissue practice (CGTP), which governs the methods used in, and the facilities and controls used for, the manufacture of HCT/P, including record-keeping and establishment of a quality program (U.S. FDA, 2004). The overarching objective of the FDA regulation of HCT/P facilities involved in research, process development, manufacture, and testing-related activities is to improve
product safety, facility biosafety, and the protection of public health and the general environment.

The FDA regulatory pathway is a risk-based approach for stem cell-based technology and therapeutics, whereby high-risk products are required to comply with the more stringent requirements stipulated under the Public Health Service (PHS) Act, Section 351, whereas low-risk products are required to comply with Section 361 of the same Act (U.S. FDA, 2007). Human cell and tissue-based products, derived through minimal manipulation, are broadly defined as low-risk products and are required to comply with Section 351 that includes: (a) no clinical trial requirements; (b) no license requirement; and (c) focus on prevention of communicable diseases.

For regulatory purposes, stem cell-based therapeutics and product lines with more extensive manipulation so as to modify structure and the biological properties of products are treated like any other biological therapeutic products. The requirements include: (a) biological license application; (b) establishment of safety and efficacy; (c) Investigational New Drug Application; and (d) compliance with Good Manufacturing Practice (GMP). However, compliance with Good Tissue Practice (CGTP) is a requirement for both low- and high-risk HCT/P.

**Risk Mitigation Framework:** Existing risk assessment guidelines are generally driven by health and environmental hazards posed by chemicals, toxins, and biologic and infectious agents. The existing guidelines appear to be inadequate to address the unique and complex nature of live cell therapeutic solutions. The explosive growth in stem cell-based products for R&D, diagnostics, and therapies in recent years covers a broad spectrum ranging from pluripotent, virally-modified cells to inert biomaterials and autologous cells used in stem cell transplantation. Given the widening scope of therapeutic applications together with the increasing evidence from experimental animal and clinical studies of a vast divergence in the stem cell source-based genomic instability and cancer-inducing potential, a refined risk mitigation framework is needed that takes into account the unique nature and the varied hazard potential for different products and therapies derived from stem cells.

Figure 2 illustrates a proposed conceptual outline for risk mitigation for stem cell-derived products and therapies that takes into consideration the existing biosafety guidelines for facilities involved in cell-based products R&D, cell banking, manufacture, and testing activities, with evidence of the potential health risk associated

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**Figure 2**

Conceptual Framework for Evidence-based Risk Mitigation for Stem Cell Derived Products and Therapeutics Research and Development

- **Stem Cell-Derived Products Pipeline**
  - Evidence-Based safety and risk assessment
    - Cytogenetic
    - Biochemical
    - Molecular Markers
    - Cell surface
    - Functional markers

- **Pathogenic agent contaminants**
  - Biohazard assessment
    - OSHA blood-borne Pathogen Standard
    - CDC/NIH BMBL

- **Product development & Clinical applications**
  - Monitoring process
  - Testing requirements
  - Study protocols
  - Reporting requirements

- **Stem Cell Therapeutics**
  - IRB
  - ESCRO
  - IACUC
  - IBC
  - eGLP
eGTP
HCT/P
with certain sources of stem-cells used in medical therapy and product development projects. The evidence-based criteria development outlined here conceptually rests on conventional weight-of-evidence approaches to biohazard assessment of biological agents and toxins (Rao et al., 2004, 2006).

The proposed biohazard evidence-based risk mitigation framework takes into consideration safety and potential health hazards associated with stem cell-derived products and therapy, as well as facility operational processes likely to create occupational exposures to microbiological contamination during stem cell and feeder cell sorting and processing activities.

The first set of biohazard considerations driving risk mitigation stems from an assortment of cellular and biochemical evidence, such as: (a) potential hazards associated with the biological source or origination of stem cells; (b) type of tissue selected for harvesting cell lines; (c) inherent genomic instability in early-stage stem cell populations exhibiting a high propensity for totipotency and in several investigations reporting close similarity to tumor cell populations; (d) presence of biochemical and molecular markers closely linked to oncogenic potentials; and (e) cellular and phenotypic carcinogenic transformations more frequently observed in stem cells from embryonic and to some extent even adult stem cell sources during a serial passage. Determining the overall safety associated with a particular type of stem cell-based product or therapy under development on a case-by-case basis is critical. Research publications and technical monographs were reviewed to identify and categorize stem cell-specific hazard criteria with the potential to have a direct impact on a risk mitigation framework. Table 2 outlines a risk mitigation schema based on these hazard criteria applicable to both embryonic and adult stem cell technology-derived products and therapies. The hazard criteria listed in the table correspond to the conceptual framework illustrated in Figure 2.

### Table 2

*Risk Mitigation Schema for Embryonic and Adult Stem Cell Technologies-based Products and Therapeutics Research and Development*

<table>
<thead>
<tr>
<th>Hazard Criteria*</th>
<th>Safety and Biohazard Descriptors for a Risk Mitigation Schema</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source</td>
<td>Source based on origin: (1) Embryonic; (2) Fetal; (3) Umbilical cord; and (4) Adult</td>
</tr>
<tr>
<td>Application</td>
<td>Homologous, non-homologous</td>
</tr>
<tr>
<td>Tissue Type</td>
<td>(1) Totipotent embryonic stem cell, (2) Pluripotent fetal stem cell, (3) Umbilical cord stem cell, (4) Adult stem cell that includes: (a) Hematopoietic stem cells; (b) Multipotent mesenchymal stem cell; (c) Gut stem cell; (d) Liver stem cell; (e) Bone and cartilage stem cell; (f) Epidermal stem cell; (g) Neuronal stem cell; (h) Pancreatic stem cell; and (i) Eye stem cell</td>
</tr>
<tr>
<td>Activity Type</td>
<td>R&amp;D, Cell banking, pre-clinical experimental studies, cGMP production, clinical studies, post-therapeutic clinical monitoring and long-term safety studies</td>
</tr>
<tr>
<td>Cytogenetics/Genomic Instability</td>
<td>(1) Genetic instability: G-banding, FISH; (2) Source; (3) Introduction of oncogenes</td>
</tr>
<tr>
<td>Biochemical/Molecular Markers Linked to Carcinogenesis</td>
<td>Chemokines (CCL5, CXCL12); Secretion of interleukin-6 [IL-6]; TGF-β, MMP14</td>
</tr>
<tr>
<td>Molecular Markers</td>
<td>(1) Embryonic [SSEA, oct-4]; (2) Hematopoietic [CD14, CD11b, CD19, CD79a, CD34, CD45, HLA-DR, CD73*, CD90*, CD105*]; (3) Multipotent mesenchymal [STRO-1]; (4) Neural [p75 Neurotropin R, PSA-NCAM, Nestin]</td>
</tr>
<tr>
<td>Cell Surface Characteristics</td>
<td>(1) Capacity to convert to progenitor and highly differentiated cells; (2) Changes to culture microenvironment; (3) Adherence to plastic</td>
</tr>
<tr>
<td>Functional Areas</td>
<td>(1) Phenotype; (2) Proliferative and selective or non-selective migratory effects towards tumor site; (3) Immunosuppressive properties; (4) Tumoral angiogenesis</td>
</tr>
<tr>
<td>Risk of Adventitious Agents</td>
<td>(1) Blood borne pathogen (antigenic screening, co-cultivation with various indicators cells that allow contaminants to grow, PCR, or nucleic acid hybridization); (2) Tissue source to determine likelihood of carrying infectious agents (screen for included list); (3) Evidence of pathogenic agent, bacteria including <em>Mycoplasma</em>, yeast and fungi, transfer and survival in experimental animals (screen for included list); (4) Bacteria</td>
</tr>
<tr>
<td>Biosafety Level Requirement</td>
<td>Need for worker protection and engineering control</td>
</tr>
</tbody>
</table>

*Based on stem-cell specific hazard evaluation collected from a multitude of safety evaluations, cellular, biochemical, cytogenetic and molecular analysis.
Stem cell-specific tissue source/origin constitutes the principal hazard criteria for safety assessment. Experimental investigations with laboratory animal models and investigational therapeutics research involving human patients have identified a number of stem cell source-specific cellular, biochemical, and molecular markers linked to tumorigenesis. The literature is replete with tumorigenic transformation of stem cell lines of embryonic origin. However, a growing body of investigations has reported stem cell lines from adult sources, such as MSC, homing to tumor sites as non-specific, with the result that their anti-cancer effects may be highly variable with implicit safety-related considerations when used as a therapeutic agent. Therefore, investigators have suggested a thorough evaluation of the potential health risks associated with the use of MSC and related adult-tissue stem cell sources (Lazennec & Jorgensen, 2008). No risk assessment framework is currently available that considers the unique source-specific totipotent and pluripotent characteristics of stem cells to enable evidence-based risk analysis decisions in clinical therapy and product development projects together with facility and process-related biosafety consideration.

Toxicity data, in terms of the cell dose-dependent proliferation potential of undifferentiated adult stem cells including MSC, are obtained from animal models. The capacity of stem cell test candidates demonstrating a propensity for proliferation in animal models resulting in increased incidence and multiplicity of tumors in the transplanted host system is a reliable indicator of carcinogenic risks. Most embryonic stem cells under these test conditions have demonstrated tumor-induction potentials in transplanted host systems. Toxicity assessment would have to carefully consider the potential for undifferentiated stem cell proliferation compared to cellular growth leading to differentiation. Thus, the proliferative potential of stem cells in animal models appears to be a reliable hazard parameter for toxicity assessments.

The second hazard criteria for biosafety-related consideration in risk mitigation stems from key processes in cell-based laboratory or large-scale, cell-based product processing facilities causing potential risk of exposure to infectious agents (Table 2).

Preparation of stem cells from biological samples creates a potential risk of exposure to genetically manipulated cells that may contain genomic sequences of potentially pathogenic agents. The International Society of Analytical Cytology Biohazard Working Group published guidelines for sorting unfixed cells because of the potential for personnel exposure to droplets and aerosols containing biological agents present in the samples (Schmid et al., 1997). Use of a cell sorter is a common procedure during stem cell processing operations. An exposure scenario might involve the generation of aerosol and droplets during the cell sorting process. According to Schmid et al. (1997), cell sorters produce droplets in the range of 40 μm to 200 μm range and micro droplets in the 3 μm to 7 μm range during normal operations. Although droplets in the 80 μm and higher ranges will coalesce and not remain air borne, smaller droplets will remain aerosolized, particularly in the high air pressure cell sorter environment. Depending on the type and nature of stem cell formulations, generating a sizeable aerosol during the cell sorting process is possible.

Although the most likely route of exposure to pathogenic agents in a laboratory setting is through direct contact to cut skin or mucous surface membrane during an injury, intake via inhalation of aerosol particulates containing the infectious agents is well documented in occupational settings. For example, HIV-1 and Hepatitis B (HBV) virus are known to have caused infection among laboratory workers through aerosolization (Almeida et al., 1971). Accidental exposure to aerosol particulates generated by cell sorters and flow cytometry equipment, most commonly used in stem cell processing facilities, is considered a potential exposure risk. The International Society for Analytical Cytologists (ISAC) established biosafety guidelines for laboratories performing viable cell sorting experiments (Schmid et al., 2007).

Risks associated with adventitious agents such as blood borne pathogens and other opportunistic pathogenic agents is evaluated by a routine screening of tissue-engineered medical products to assess safety and risks associated with adventitious agents and their by-products (Table 2). These agents include bacteria, fungi, Mycoplasma, viruses, endotoxins, Transmissible Spongiform Encephalitis (TSE), and parasitic organisms. Blood borne pathogen standards are not limited to HIV and HBV; they also include the entire spectrum of adventitious agents in cell-based process and product development activities. For example, Cobo et al. (2007) reported microbial contamination in stem cell cultures by bacteria (including Mycoplasma), yeast, and fungi. This study, based on an analysis of 32 stem cell and feeder cell lines, revealed contamination of nearly 12 percent of the cell lines mainly with gram-positive cocci and Mycoplasma species, followed by gram-negative rods. Mycoplasma species alone accounted for a 4 percent microbial contamination rate in the tested stem cell and feeder cell samples (Cobo et al., 2007). As such, risk mitigation should integrate a test plan and reporting requirements for adventitious agents for stem cell-derived cell banking and production activities.

Finally, use of stem cell-based therapy at research facilities, be they academic medical centers or commercial clinical research hospitals, requires institutional oversight involving an Institutional Review Board (IRB) (Figure 2). The U.S. National Academy of Sciences Committee for human embryonic stem cell research for the first time in 2005 outlined guidelines for clinical research on advanced research and therapeutics development (NRC, 2005). This was followed by the publication of two amendments broadening the scope of the guidelines to include other sources such as pluripotent and...
multipotent stem cells (NRC, 2007; NRC, 2008).

Given the strong ethical and legal overtures related to the use of embryonic stem cells in clinical research, the National Academy of Sciences Committee recommended increased institutional oversight through an IRB process and establishment of an Embryonic Stem Cell Research Oversight (ESCRRO) committee to maintain institutional oversight on embryonic and adult stem cell-based clinical studies. The ESCRRO/IRB process outlined in these guidelines requires a careful assessment at the investigator and institutional levels of the applicable regulations, including safety considerations reviewed by the Institutional Biosafety Committee (IBC), of the proposed research (NRC, 2008).

Clinical studies are required to adhere to all relevant FDA regulations and compliance review requirements by the Institutional Animal Care and Use Committee (IACUC), if animals are to be used in the research. Current good tissue practices (CGTP), published by the FDA in 2009, address the safety of stem cell-derived product and therapy development in terms of donor screening to prevent the spread of communicable disease through stem cell and feeder cells in culturing and processing activities, and an audit system to track source-specific stem cells used by facilities in the manufacture of tissue-derived products (U.S. FDA, 2005; CFR, 2005).

NRC guidelines are unequivocal about the need for safety evaluations, particularly due to the potential risks posed by the introduction of retroviruses that carry the inducing genes to generate pluripotent cells from adult sources (NRC, 2008). Moreover, studies have indicated that inserting retroviruses leads to the induction of cancer and an increase in the tumor-causing potential of the derived pluripotent or multipotent cells with the capability to differentiate into a limited number of specialized cell types. IRB review of study protocol prior to ESCRRO approval should specifically examine supportive safety documentation, including informed consent documents, if applicable. The ESCRRO/IRB approval would have to take into account potential risks associated with the candidate product in the study protocol.

At the institutional level, the IBC should establish a separate subcommittee to review and evaluate on a case-by-case basis the laboratory protocols and procedures used in sorting and processing stem cells and feeder cells and the requirements of the containment process. Use of flow cytometry and cell sorters is a common procedure in cell-based product development facilities. Laboratory-acquired infections from handling test specimens and exposure to aerosols are reported among workers handling Hepatitis B and C, Mycobacterium tuberculosis, Brucella spp., and Epstein-Barr virus. Personnel in laboratories with centralized services for cell sorters and flow cytometry that handle large volumes from multiple sources are especially at risk of exposure to infectious pathogens. High-speed cell sorters generate aerosols, which in the presence of pathogenic contaminants pose biosafety hazards for workers in the facility. Although no documented reports specifically link workers contracting infections to aerosol exposure in cell sorter and flow cytometry work environments, a proactive initiative in the high-growth stem cell-derived product and therapy biotechnology sector is a relevant and appropriate measure to improve stem cell-based product and process-related biosafety practices overall.

Conclusions

As stem cell-based diagnostic and therapeutic product developments activities surge, a critical need exists to assess the safety of stem cell-based products and therapies and the potential for exposure of workers at facilities performing large-scale processing of automated cell sorting and flow cytometry. Published literature reveals shared genetic similarities and phenotypic properties between stem cells from embryonic and adult sources and cancer stem cells, introducing considerable uncertainties on the safety of stem cell-based products and therapies.

None of the existing guidelines for tissue-engineered medical product development provides a comprehensive regulatory framework to assess safety and potential long-term health risks for cell-based therapeutic products and their development activities. Existing guidelines on facility biosafety and safety of pharmaceutical products are inadequate for complex live cells. Therefore, a need exists to create distinctive competence in the field of risk mitigation for stem cell-based therapy and product development activities.

A systematic review of the vast published literature was performed to construct a conceptual framework for evidence-based risk mitigation for stem cell-based products and therapies. The conceptual framework attempts to integrate key biohazard criteria for cell-based products and establish facility-related biosafety practices. To a considerable measure, key biohazard drivers are: (a) hazards associated with the biological source/origin of stem cells; (b) tissue type selected for harvesting stem cell for cell banking and process development; (c) inherent genomic instability in early-stage stem cell populations and biochemical and molecular markers closely linked to oncogenic potentials; and (d) evidence of phenotypic carcinogenic transformations. Therefore, a need exists to determine on a case-by-case basis the overall safety of the stem cell line selected for therapy/product development.

Risk mitigation should clearly address clinical applications of stem cell-based products through an active IRB/ESCRRO process together with the relevant institutional biosafety committee oversight and review to ensure that institutional policy and safety considerations are adequately addressed in clinical research studies involving stem cells.

Finally, better understanding of stem cell biology is a
clear prerequisite to establish biosafety criteria for stem cells clinical/therapeutic applications. This requires a collaborative undertaking, involving public sector-private sector stakeholders, aimed at developing the distinctive competencies and knowledge base in risk mitigation. For example, application of stem cells in predictive toxicology could have a tremendous impact, for it offers a closer human phenotypic match compared to animal materials and a more reliable assessment of safety during early-stage toxicity screening, resulting in overall safety and efficacy of the conventional biologics and galvanizing the development of an entirely new class of stem cell-based therapeutics with proven safety and efficacy.

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References


Articles


OBA News

News from the NIH Office of Biotechnology Activities

March 1, 2010

Expert Panel Discussion on Dual Use Research for International Audiences Now Available on the OBA Web Site—Expert Panel Discussion on Dual Use Research for International Audiences Now Available on the OBA Web Site

A charge to the National Science Advisory Board for Biosecurity, and a key activity of the U.S. government, is to foster international dialogue on dual use life sciences research—or life sciences research that is conducted for legitimate scientific purposes but produces information, knowledge, or technology that may be misused to threaten human, animal or plant health and/or national security. Toward that end, on October 22, 2009, the NSABB hosted a moderated discussion, sponsored by NIH, with participants across the Americas to address the concepts of “dual use research” and “dual use research of concern,” how risks associated with dual use research of concern can be meaningfully assessed, and strategies for managing and addressing these risks.

The videocast of this event is now available for viewing in both English and Spanish at: http://oba.od.nih.gov/biosecurity/internationalwebcast.html. The program included presentations on the following topics:

• What is “dual use research” and “dual use research of concern”?
• How can risks associated with “dual use research of concern” be meaningfully assessed?
• What strategies for managing and/or addressing these risks have been proposed or implemented?
• What is being done to strengthen research governance in the Americas?
• Remote participants in South America, Central America, the Caribbean, and North America submitted questions and comments for the panel to consider and discuss. For more information about this event, contact Abby Rives at 301-594-1976 or by e-mail at rivesa@od.nih.gov.