10. ASSESSING HUMAN STEM CELL SAFETY

The isolation of human stem cells offers the promise of a remarkable array of novel therapeutics. Biologic therapies derived from such cells—through tissue regeneration and repair as well as through the targeted delivery of genetic material—are expected to be effective in the treatment of a wide range of medical conditions. Efforts to analyze and assess the safety of using human stem cells in the clinical setting are vitally important to this endeavor.

Transplanted human stem cells are dynamic biological entities that interact intimately with—and are influenced by—the physiology of the recipient. Before they are transplanted, cultured human stem cells are maintained under conditions that promote either the self-renewing expansion of undifferentiated progenitors or the acquisition of differentiated properties indicative of the phenotype the cells will assume. After incompletely differentiated human stem cells are transplanted, additional fine-tuning occurs as a consequence of instructions received from the cells' physiologic microenvironments within the recipient. The capabilities to self-renew and differentiate that are inherent to human stem cells point simultaneously to their perceived therapeutic potential and to the challenge of assessing their safety.

Assessing human stem cell safety requires the implementation of a comprehensive strategy. Each step in the human stem cell development process—beginning with identifying and evaluating suitable human stem cell sources—must be carefully scrutinized. Included in this global assessment are the derivation, expansion, manipulation, and characterization of human stem cell lines, as well as preclinical efficacy and toxicity testing in appropriate animal models. Being able to trace back from the cell population prepared for transplantation to the source of the founder human stem cells also allows each safety checkpoint to be connected, one to the other.

WEAVING A STEM CELL SAFETY NET

A diversity of opinion exists among researchers about the feasibility of initiating pilot clinical studies using human stem cells. Some are of the view that it is reasonable to expect within the next five years that human stem cells will be used in transplantation settings to replace dead or dying cells within organs such as the failing heart or that genetically modified human stem cells will be created for delivery of therapeutic genes. Others argue that a good deal more information about the basic biology of human stem cells needs to be accumulated before their therapeutic potential in humans can be assessed.

Clinical studies involving the transplantation of blood-restoring, or hematopoietic, stem cells have been under way for a number of years. Reconstituting the blood and immune systems through stem cell transplantation is an established practice for treating hematological malignancies such as leukemia and lymphoma. Transplanting hematopoietic stem cells resident in the bone marrow or isolated from cord blood or circulating peripheral blood is used to counter the destruction of certain bone marrow cells caused by high-intensity chemotherapeutic regimens used to battle various solid tumors. Moreover, clinical trials are being conducted to assess the safety and efficacy of using hematopoietic stem cell transplantation to treat various autoimmune conditions including multiple sclerosis, lupus, and rheumatoid arthritis.

Although precedents exist for the clinical use of human stem cells, there is considerable reluctance to proceed with clinical trials involving human stem cells derived from embryonic and fetal sources. This hesitancy extends to adult human stem cells of non-hematopoietic origin, even though, by contrast, their plasticity is generally considered to be lower than that of their embryo- and fetus-derived counterparts. For

human stem cells to advance to the stage of clinical investigation, a virtual safety net composed of a core set of safeguards is required (see Table 10.1. Safeguards for clinical applications of human stem cells, by source of cell).

Safety Assurance Begins with Adequate Donor Screening

Whether human stem cells are of embryonic, fetal, or adult origin, donor sources must be carefully screened. Routine testing should be done to guard against the inadvertent transmission of infectious diseases. Additionally, pedigree assessment and molecular genetic testing appear to be warranted. This is arguably the case when human stem cells intended for transplantation are derived from an allogeneic donor—that is, someone other that the recipient—and especially if the cells are obtained from a master cell bank that has been established using human embryonic stem or human embryonic germ cells.

The purpose of pedigree evaluation and/or genetic testing is to establish whether the human stem cells in question are suitable for use in the context of a particular clinical situation. For example, embryos derived from a donor with a family history of cardiovascular diseases may not be the best suited for the derivation of cardiac muscle cells intended to repair damaged heart tissue. Similarly, the use of molecular

Table 10.1. Safeguards for clinical applications of human stem cells, by source of cell*

Safeguard	Embryo	Fetus	Adult: Autologous (self)	Adult: Allogeneic (nonself)
Screen donors • Infectious-agent testing • Pedigree assessment • Molecular genetic testing	++	++	+	++
Use controlled, standardized practices and procedures for establishing stem cell lines	++	++	++	++
Develop alternatives to culturing on cell-feeder layer	++	++	NA	NA
Perform detailed characterization of tem cell lines • Morphology • Cell-surface antigens • Biochemical markers • Gene expression • Karyotype analysis • Biologic activity	++	++	++	++
Conduct preclinical animal testing • Proof of concept: disease models – Cell integration – Cell migration • Comprehensive toxicity • Proliferative potential	++ ++ ++	++ ++ ++	++ ++ +	++ ++ +
Monitor patient and do long-term follow-up	++	++	++	++

++= more important; +=less important; NA=not applicable.

genetic analysis could detect a mutation in the gene for alpha-synuclein. This gene is known to be responsible for the rare occurrence of early onset Parkinson's Disease. Detecting such a genetic abnormality in neuronal progenitor cells derived from an established embryonic germ cell line could block the use of those cells as a treatment for a number of neurodegenerative conditions, including Parkinson's Disease.

The number of genes known to be directly responsible for causing disease or anomalous physiologic function is relatively small. Advances in techniques for identifying, isolating, and analyzing genes, coupled with the wealth of information destined to become available as one outcome of the human genome sequencing projects, will raise this number. Considerably more will also be learned about how multiple gene products, each contributing an incremental quantity to the overall sum, predispose an individual to develop particular diseases. Clearly, it will eventually not be possible, or even necessary, to screen every source of human stem cells for the entire panoply of disease-associated genes. The screening of targeted genes will be conducted within the context of the relevant clinical population.

Using Controlled, Standardized Practices and Procedures for Establishing Cultured Human Stem Cell Lines Enhances Safety

To ensure the integrity, uniformity, and reliability of human stem cell preparations intended for clinical use, it is essential to demonstrate that rigorously controlled, standardized practices and procedures are being followed in establishing and maintaining human stem cell lines in culture.

Human stem cells from virtually every source other than blood-derived hematopoietic stem cells are maintained in tissue culture for some defined period of time. This is necessary to obtain a sufficient number of cells for use in clinical studies involving transplantation. Culturing human stem cells requires the use of formulated liquid media supplemented with growth factors and other chemical substances that promote cellular replication and govern the differentiation of the cultured human stem cells. Since human stem cells are a dynamic, biological entity, failure to standardize procedures for maintaining and expanding cells in culture could result in unintended alterations in the intrinsic properties of the cells. The initial seeding density of the cells, the frequency with which the culture medium is replenished, and the density cells are permitted to achieve before subdividing will all affect the characteristics of human stem cells maintained in culture. Altering the concentrations of supplemental growth factors and chemical substances, even switching from one supplier to another, may lead to changes in cell growth rate, expression of defining cell markers, and differentiation potential. Alterations in stem cell properties caused by the use of nonstandardized culture practices are likely to affect the behavior and effectiveness of the cells once transplanted.

One particular concern is how safe it is to use serum derived from cows as a supplement to culture media. Due to the outbreak of bovine spongiform encephalopathy (BSE) in cattle herds, primarily those raised in the United Kingdom, only serum produced from cows reared in countries certified to be free of BSE should be used. Consumption of beef contaminated with the agent responsible for causing BSE has lead to the limited emergence of new variant Creutzfeldt-Jakob disease (nvCJD) in humans. This disease results in the relentless destruction of brain tissue and is invariably fatal. Placing neural stem cells contaminated with the BSE infectious agent in a patient's nervous system to investigate cellular-replacement therapies for neurological disorders would be both irresponsible and devastating. Researchers are engaged in a vigorous effort to develop serum-free, chemically defined media that obviate risks associated with the use of bovine serum.

Alternatives to Culturing on a Feeder Layer of Animal Cells Improve Safety

An issue unique to the culturing of human embryonic stem and embryonic germ cells involves the use of mouse embryonic fibroblast feeder cells to keep the embryonic cells in a proliferating, undifferentiated condition. Human embryonic stem and embryonic germ cells are seeded directly onto a bed of irradiated mouse feeder cells. Transplanting into humans stem cell preparations derived from founder cells that have been in direct, intimate contact with nonhuman animal cells constitutes xenotransplantation—the use of organs, tissues, and cells derived from animals to treat human disease. The principal concern of xenotransplantation is the unintended transfer of animal viruses into humans.

Researchers are devoting considerable attention to developing culture conditions that do not use mouse feeder cells. In February of this year, scientists from

Geron Corporation, a biotech company focusing on the development of embryonic stem cell technology for treating disease, presented findings at a scientific conference demonstrating that human embryonic stem cells can be maintained without mouse feeder cells. Human embryonic stem cells seeded on a commercially available basement membrane matrix in media conditioned by feeder cells retain their proliferative potential and capacity to form all three embryonic germ layers (mesoderm, endoderm, and ectoderm). This suggests that human embryonic stem cells maintained in the absence of direct culture on a mouse feeder cell layer are comparable to human embryonic stem cells co-cultured with mouse feeder cells.

Detailed Characterization of Human Stem Cell Populations Reinforces the Safety Net

Detailed characterization of cell preparations intended for transplantation is critical to the development of human stem cells for clinical use. Identifying the cells that make up an human stem cell population intended for clinical study requires identifying cells exhibiting the desired phenotype within the preparation, as well as those that do not. This poses considerable challenges because human embryonic stem and embryonic germ cells have the capacity to give rise to all differentiated cell types, while adult human stem cells, though generally more restricted in their plasticity, are capable of generating all cell types that make up the tissue from which they were derived.

On the basis of the complex biological properties of human stem cells, including their potential to differentiate along multiple lineages and give rise to a variety of cell types, it is expected that the characterization of stem cell preparations will require a panel of orthogonal assessments. Parameters that will prove useful in establishing identity include 1) cell morphology (visual microscopic inspection of cells to assess their appearance), 2) expression of unique cell-surface antigens (as is the case for $CD34^+$ hematopoietic stem cells), 3) characterization of biochemical markers such as a tissue-specific enzymatic activity (e.g., enzymes that produce neurotransmitters for nerve cells), and 4) expression of genes that are unique to a particular cell type. Further, analysis of the nuclear chromosomal karyotype may be used to assess genetic stability of established human embryonic stem and embryonic germ cell lines maintained in culture for extended periods of time. Continued development and standardization of DNA microarray analysis (simultaneous screening for many genes) and proteomics (protein profiling) technologies will significantly enhance stem cell characterization.

Rigorous and quantitative identification of cell types within a heterogeneous population of differentiating human stem cells provides the means to gauge purity of a cellular preparation. In turn, this permits evaluation of the extent to which purity of a human stem cell preparation predicts efficacy after transplantation. It is not necessarily the case that homogenous populations composed of a single cell type will be more effective as a cell-replacement therapy than mixed populations of cells. It is conceivable that the reason differentiation of cultured stem cells obtained from the brain leads to formation of all the cell types found within the nervous system (namely, neurons, astrocytes, and oligodendrocytes) is that their coincidental presence is required to ensure maximum survival and functional capability. The interaction of various phenotypic cell types within a preparation of progenitor cells obtained after the controlled differentiation of cultured human embryonic stem cells is being actively investigated.

Once the purity profile has been established for a population of human stem cells generated using standardized procedures, deviations that occur outside what is expected due to normal biologic variation serve as a harbinger that significant, and possibly deleterious, changes may have occurred. Such alterations could reflect the introduction of genetic mutations as a consequence of culture conditions used to promote expansion and to induce differentiation of the progenitor cell population.

Before clinical studies involving human stem cell transplantation can be done, it is essential to demonstrate that human stem cell preparations possess relevant biological activity. The bioassay provides a quantitative measure of the potency of a cell preparation and ensures that cells destined for transplantation are not inert. Assays may be based on a biologic activity such as insulin release from pancreatic islet-like cells, glycogen storage by cells intended for regeneration of liver tissue, or synchronous contraction in the case of stem cell-derived cardiomyocytes to be used for repairing damaged heart muscle. When cells that have not acquired fully

differentiated functionality are to be transplanted, it may be appropriate to use surrogate markers that predict the acquisition of the intended biologic activity upon further differentiation. (For example, counting tyrosine hydroxylase-expressing neural progenitor cells in a mixed population of cells intended to provide dopaminergic neurons for treating Parkinson's Disease could predict the acquisition of relevant biologic activity after transplantation.)

Proof of Concept, Toxicity Testing, and Evaluation of Proliferative Potential in Animal Models Are Important to the Assessment of Human Stem Cell Safety

A critical element of the safety net is the transplantation of human stem cells into animals to demonstrate that the therapy does what it is supposed to do ("proof of concept") and to assess toxicity. Admittedly, animal models of human disease are imperfect because most human maladies do not spontaneously occur in animals. Chemical, surgical, and immunologic methods are used to damage neurons; induce diabetes; simulate heart attacks, stroke, and hypertension; or compromise organ function. In situations when focal genetic lesions are known to cause disease, the creation of transgenic mouse colonies in which the culpable gene is either eliminated or overexpressed results in disease models that are capable of faithfully reproducing human-disease-specific pathologies.

Human stem cells must be transplanted into animal models of human disease. Transplantation of neural stem cells should demonstrate measurable evidence of efficacy in models of neurodegenerative disease, such as Parkinson's Disease, Huntington's disease, and amyotrophic lateral sclerosis (ALS), Alzheimer's disease, as well as spinal cord injury and stroke. Improved liver function after transplantation of hepatocyte precursors should be observed in an animal model of hepatic failure. Normalization of blood insulin concentrations and amelioration of diabetic disease symptoms should result from the transplantation of pancreatic islet progenitors in a mouse model of diabetes. It is likely that in all cases, immunosuppression will be required due to immunologic incompatibility between humans and the animal model species (usually mouse or rat).

In addition to efficacy, evidence for anatomic and functional integration of transplanted human stem cells should be assessed. human stem cells destined for transplantation may be tagged with a marker, such as green fluorescent protein, that allows transplanted cells to be readily identified upon histological examination. A similar approach should be used to evaluate the migration of transplanted human stem cells from the site of injection into adjacent and more distant tissues. The migration of transplanted human stem cells to a nontarget site and subsequent differentiation into a tissue type that is inappropriate for that anatomic location could be problematic.

Questions about the use of embryonic compared with adult stem cells with respect to robustness and durability should be addressed in animal-transplantation models. Similarly, the issue of whether less-differentiated cells will be more effective than more-differentiated cells following transplantation should be investigated. Continued advancements in noninvasive imaging technologies, such as magnetic resonance imaging (MRI) and positron emission tomography (PET scanning), will allow these events to be observed in real time with reasonable resolution and without having to use large numbers of animals.

From the perspective of toxicology, the proliferative potential of undifferentiated human embryonic and embryonic germ cells evokes the greatest level of concern. A characteristic of human embryonic stem cells is their capacity to generate teratomas when transplanted into immunologically incompetent strains of mice. Undifferentiated embryonic stem cells are not considered as suitable for transplantation due to the risk of unregulated growth. The question that remains is, at what point during differentiation does this risk become insignificant, if ever? Identifying the stage at which the risk for tumor formation is minimized will depend on whether the process of stem cell differentiation occurs only in a forward direction or is reversible. Before clinical trials are begun in humans, the issue of unregulated growth potential and its relationship to stem cell differentiation must be evaluated. It is essential that careful toxicology studies are performed that are of the appropriate duration and that involve transplantation into immunocompromised animals of undifferentiated or partially differentiated embryonic stem cells, as well as adult stem cells.

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