Cell Stem Cell
Perspective

Assessing the Safety of Stem Cell Therapeutics

Chris E.P. Goldring,[1,13,*] Paul A. Duffy,[2,13] Nissim Benvenisty,[3,4,13,*] Peter W. Andrews,[5] Uri Ben-David,[3] Rowena Eakins,[1] Neil French,[1] Neil A. Hanley,[6] Lorna Kelly,[1] Neil R. Kitteringham,[1,*] Jens Kurth,[7] Deborah Ladenheim,[8] Hugh Laverty,[1] James McBlane,[9] Gopalan Narayanan,[9] Sara Patel,[10] Jens Reinhardt,[11] Annamaria Rossi,[12] Michaela Sharpe,[12] and B. Kevin Park[1]

[1]MRC Centre for Drug Safety Science, Division of Molecular & Clinical Pharmacology, The Institute of Translational Medicine, The University of Liverpool, Liverpool L69 3GE, UK
[2]AstraZeneca R&D Alderley Park, Safety Assessment UK, Mereside, Alderley Park, Macclesfield, Cheshire, SK10 4TG, UK
[3]Stem Cell Unit, Department of Genetics, Institute of Life Sciences, The Hebrew University of Jerusalem, Givat-Ram, Jerusalem 91904, Israel
[4]Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, CA 90048, USA
[5]Centre for Stem Cell Biology, Department of Biomedical Science, University of Sheffield, Western Bank, Sheffield S10 2TN, UK
[6]Endocrinology & Diabetes Group, Manchester Academic Health Science Centre, University of Manchester, AV Hill Building, Oxford Road, Manchester M13 9PT, UK
[7]Novartis Pharma AG, Forum 1, Novartis Campus, CH-4056 Basel, Switzerland
[8]Athersys, 3201 Carnegie Avenue, Cleveland, OH 44115-2634, USA
[9]Medicines and Healthcare Products Regulatory Agency (MHRA), 151 Buckingham Palace Road, Victoria, London SW1W 9SZ, UK
[10]ReNeuron Limited, 10 Nugent Road, Surrey Research Park, Guildford, Surrey GU2 7AF, UK
[11]Paul-Ehrlich Institut, Paul-Ehrlich-Straße 51-59, 63225 Langen, Germany
[12]Drug Safety Research and Development, Sandwich Laboratories, Pfizer Limited, Sandwich, Kent CT13 9NJ, UK
[13]These authors contributed equally to this work
*Correspondence: C.E.P.Goldring@liverpool.ac.uk (C.E.P.G.), nissimb@cc.huji.ac.il (N.B.), neilk@liv.ac.uk (N.R.K.)
DOI 10.1016/j.stem.2011.05.012

Unprecedented developments in stem cell research herald a new era of hope and expectation for novel therapies. However, they also present a major challenge for regulators since safety assessment criteria, designed for conventional agents, are largely inappropriate for cell-based therapies. This article aims to set out the safety issues pertaining to novel stem cell-derived treatments, to identify knowledge gaps that require further research, and to suggest a roadmap for developing safety assessment criteria. It is essential that regulators, pharmaceutical providers, and safety scientists work together to frame new safety guidelines, based on "acceptable risk," so that patients are adequately protected but the safety "bar" is not set so high that exciting new treatments are lost.

Immense expectation surrounds the area of stem cell therapeutics. Pressures are building to accelerate their development, from patients requiring effective therapy as well as companies requiring new products for dwindling pipelines and needing to diversify portfolios. This anticipation is independent of the source of stem cells (adult versus embryonic, or patient-derived autologous cells versus healthy donor adult or embryonic allogeneic cells). However, as with all new treatments, our knowledge about the safety of these medicinal products is still limited and needs to be expanded to assess their therapeutic safety more effectively. The purpose of this article (which arose from discussions at a workshop hosted by the MRC Center for Drug Safety Science in Liverpool) is to outline the major safety issues associated with stem cell therapeutics, to identify the gaps in our knowledge with respect to these issues, and to propose a set of recommendations designed to facilitate the development and clinical application of stem cell therapies from an industrial, clinical, and regulatory perspective. In 2008, the ISSCR published a detailed set of guidelines for the translation of stem cell research into clinical practice (Hyun et al., 2008). While there is some overlap in the issues addressed by both publications, the current article focuses specifically on the broader principles associated with the safe use of stem cell therapies and is intended to complement the ISSCR guidelines.

Current Status of Stem Cell Therapeutics and the Safety Challenge

Since they were first isolated by James Thomson (Thomson et al., 1998), the capacity of human embryonic stem cells (hESCs) for potentially unlimited self-renewal and differentiation has led to many attempts to exploit them in drug discovery, disease modeling, and regenerative medicine (Koay et al., 2007; Perin et al., 2008; Wong and Bernstein, 2010; Zaret and Grompe, 2008). Attempts are underway to differentiate hESCs into inter alia, hepatocytes (Baxter et al., 2010; Cai et al., 2007; Duan et al., 2007; Hay et al., 2008; Sullivan et al., 2010; Touboul et al., 2010; Vallier, 2011; Bone et al., 2011), cardiomyocytes (Kehat et al., 2001; Passier et al., 2005), neurones (Schuldiner and Benvenisty, 2003; Zhang et al., 2001), and intestinal tissue (Spence et al., 2011). Several pluripotent and multipotent stem cell-based therapeutics have entered clinical trials. Table 1 shows a summary of selected stem cell-based therapeutics approved for clinical trials by the United States Food and Drug Administration (FDA) or the UK Medicines and Healthcare Products Regulatory Agency (MHRA) to treat injuries to the central nervous system, myocardial infarction, and diabetes. Clearly, the explosive growth in interest in the use of induced pluripotent stem cells (iPSCs) opens up novel avenues of therapeutic development based on adult stem cells, thereby avoiding some of the ethical issues surrounding the use of human embryos to derive

Table 1. Selected Pluripotent and Multipotent Stem Cell-Based Therapeutics Currently Undergoing Clinical Trials in the US and UK

Condition	Intervention	Sponsor	Study Design	Sample size	Inclusion Criteria	Time Frame	Reference
Spinal cord injury (SCI)	GRNOPC1: oligodendrocyte progenitor cells	Geron Corp.	Non-randomized, single arm, uncontrolled	10	18–65 years, M+F. Neurologically complete, traumatic SCI. Single lesion	12 months	http://clinicaltrials.gov/ct2/show/NCT01217008?term=GRNOPC1&rank=1
Stable ischemic stroke (IS)	CTX0E03: neural stem cells	ReNeuron Ltd.	Non-randomized, single administration, ascending dose	12	M, > 60. Unilateral IS, > 1cm infarction. NIHSS minimum 6	2 year monitoring. 8 year follow-up trial	http://clinicaltrials.gov/ct2/show/NCT01151124?term=ctx0e03&rank=1
Acute myocardial infarction (AMI)	AMI MultiStem	Athersys Inc.	Non-randomized control and treatment groups. 3 dose escalation cohorts	28	18–80 years, M+F. 1st time diagnosis of ST-elevated AMI	Adverse events during 24 hr. Postacute events 30 days. 12 month follow-up	http://clinicaltrials.gov/ct2/show/NCT00677222?term=multistem&rank=3
Neuronal ceroid lipofuscinosis (Batten's disease, NCL)	Procedure HuCNS-SC: human neural stem cells	Stem Cells Inc.	Phase 1b. Single group assessment	6	6 months–6 yr M+F. CLN1 or CLN2 mutation. Clinical diagnosis of NCL	12 months	http://clinicaltrials.gov/ct2/show/study/NCT01238315?term=HuCNS-SC&rank=2
Stargardt's disease	Retinal pigment epithelial (RPE) derived from human embryonic stem cells	Advanced Cell Technology Inc. (ATC)	Nonrandomized, single administration	12	Not yet published	Not yet published	http://www.advancedcell.com/news-and-media/press-releases/advanced-cell-technology-receives-fda-clearance-for-the-first-clinical-trial-using-embryonic-stem-cel/
Dry age-related macular degeneration (AMD)	Retinal pigment epithelial (RPE) derived from human embryonic stem cells	Advanced Cell Technology Inc. (ATC)	Nonrandomized, single administration	12	Not yet published	Not yet published	http://www.actcblog.com/2011/01/act-receives-fda-clearance-for-clinical-trials-using-escs-to-treat-amd-afflicts-10-15-million-americans.html
Type 1 diabetes mellitus (DM)	PROCHYMAL: ex vivo adult mesenchymal stem cells	Osiris Therapeutics	Randomized placebo controlled, double blind. Phase 2	60	12-35 M+F. Type 1 DM, at least 1 DM-related autoantibody. Some beta-cell function	Not yet published	http://clinicaltrials.gov/ct2/show/NCT00690066

hESCs; however, translation of iPSC research into therapeutics is still at an early stage (for reviews on this subject see Nelson et al., 2010; Nishikawa et al., 2008; Vitale et al., 2011).

As well as iPSCs, other types of adult stem cells, such as mesenchymal stem cells (MSCs), have been shown to differentiate in vitro into cell lines displaying osteogenic, chondrogenic, or adipogenic characteristics (Prockop, 1997). Moreover, they have an immunomodulatory effect on their direct environment (Aggarwal and Pittenger, 2005), and they are able to secrete cytokines that are able to initiate intrinsic tissue regenerative processes (Caplan and Dennis, 2006). However, in contrast to iPSCs, MSCs are limited in their differentiation capacity. Nevertheless, due to their availability and potentially beneficial properties—through either autologous or allogenic donation—MSCs have been in the spotlight for regenerative medicine for various indications. As of May 5th, 2011, 168 studies have been registered at the U.S. NIH Clinical Trials registry (http://clinicaltrials.gov/ct2/results?term=mesenchymal+stem+cells) and 12 studies have been registered and uploaded onto the EU Clinical Trials Register (https://www.clinicaltrialsregister.eu/ctr-search/).

Clearly, stem cell-based therapies bring with them new safety challenges that cannot be addressed using standard analytical procedures developed for low-molecular-weight drugs or other biopharmaceuticals. A particular difficulty is the ability to monitor cell biodistribution, since once administered, the cells may be essentially indistinguishable from host cells. The ability to track the therapeutic cells is key to an objective assessment of risk with respect to inappropriate ectopic tissue formation or of tumorigencity. This is especially important where the cells are administered intravenously, rather than locally, since broad dissemination is likely to occur. The ability to determine the biodistribution of administered cells raises technical issues, as monitoring the fate of exogenous cells will require the development of novel technologies. Furthermore, the detection of misplaced cells may necessitate a mechanism for their removal, which again may not be technically feasible at present. Thus, there is a major need for technological advances in biomonitoring alongside the development of novel means for eliminating administered cells that become inappropriately located. Eliminating errant cells is likely to be a more challenging task and may involve incorporation of a "self-destruct" mechanism programmed into the cells to elicit apoptosis in response to a given stimulus.

A major concern with stem cell therapy is that of tumorigenic potential. The delivery of a cell with unlimited potential for renewal and the capacity to differentiate into any human cell type carries a burden of safety concern not associated with any other class of treatment. Whether these concerns are justified by solid research support is probably the most significant safety question that needs to be addressed at the current time. The finding that undifferentiated stem cells, introduced into immunocompromised animals, are capable of forming teratomas (tumors that are composed of a haphazard array of somatic cell types, sometimes arranged into tissues, and normally corresponding to all three germ layers) emphasizes the importance of addressing this issue. Furthermore, if the cells contain genetic abnormalities, these could potentially develop into teratocarcinomas (Ben-David and Benvenisty, 2011; Blum and Benvenisty, 2008), which are tumors composed of a teratoma element together with persisting undifferentiated stem cells. These would be expected to be highly malignant, like the corresponding testicular germ cell tumors that occur in young men.

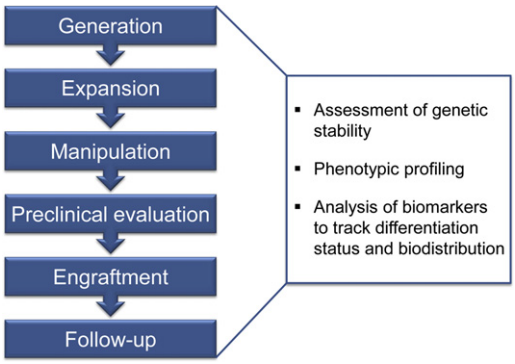

Figure 1. Workflow for Stem Cell-Derived Therapeutic Development
Genetic and phenotypic analysis must be a continuous process throughout product development, and differentiation status and biodistribution potential need to be tracked closely to ensure clinical effects are predictable and controllable.

Another evident safety issue that needs to be tackled by stem cell therapy providers is that of immunogenicity. Although there are reports of immune privilege of human embryonic stem cells (Drukker and Benvenisty, 2004), any foreign cell introduced into a patient will be subject to immune surveillance (Swijnenburg et al., 2008). While site of administration and multiple dosing may impact host-induced immunogenicity, a further significant difference between animal and human studies is that immunosuppression can be used in animal studies but may not be medically acceptable or necessary in trials in patients.

In addition to establishing the efficacy of stem cell therapies, the successful implementation of novel cell-based treatments will rely heavily on our ability to resolve these important safety issues, at both the preclinical and the clinical stages. Every step in the process of developing stem cell therapies requires rigorous scrutiny, from the origin of the cells used through expansion, manipulation, and preclinical evaluation to eventual engraftment in the host (Halme and Kessler, 2006; National Institutes of Health, 2006) (see Figure 1).

Importantly, the stem cell therapy field needs to interact at the level of therapy provider, safety scientist, and drug regulator in order to define the "acceptable risk" associated with a particular treatment and to set in place a framework for accurate assessment of that "risk." In our increasingly risk-averse society it is easy to err on the side of caution, but it should be acknowledged that if the safety "bar" is set unreasonably high then the enormous potential and promise of revolutionary medical treatments may never be realized.

The Safety Issues: Preclinical Assessment

In order to minimize patient risk, each stage of the cell therapy production should be assessed for potential safety concerns, before introduction to a human subject. This evaluation includes the manufacturing process itself, as well as the characterization and formal safety assessment of the finished product.

Manufacturing Consistency

A key area that must be addressed is the manufacturing process, i.e., the need for consistency of manufacture to ensure the

reproducible quality of the product. When preparing cells in vitro for transplant, it is essential to ensure that the culture is fully defined and characterized, as the consequences of poor definition may be far reaching. The importance of this issue from a regulatory perspective was underlined by the temporary hold placed by the FDA on Geron's first-in-human trial of an ESC-derived treatment for spinal cord injury (GRNOPC1) (Geron, 2009a). One of the concerns raised by the FDA—but subsequently allayed by the company—was surety that the manufactured cell product was fully characterized and that the mixtures of cells were predictable and free from contamination (Geron, 2009b). Clearly, for new treatments targeting clinical conditions of a less serious nature, the level of stringency of product quality may be set even higher to avoid the administration of undifferentiated cell contaminants.

Genetic Stability

Most, if not all, cell types acquire chromosomal aberrations during expansion in culture. As chromosomal aberrations are a hallmark of human cancer (Hanahan and Weinberg, 2011), it is very important to perform a detailed analysis of the genome prior to any cell-based treatment.

The inherent genetic instability of hESCs and iPSCs in culture has been demonstrated (Baker et al., 2007; Mayshar et al., 2010), and evidence for the instability of adult stem cells in culture is also beginning to emerge (Sareen et al., 2009; Ueyama et al., 2011). Consequently, not only gross karyotype but also detailed genetic profiling must be undertaken before engraftment into the host (Stephenson et al., 2010). As somatic cells within the body are often seen with copy-number variations (CNVs), any minor aberration that occurs in culture will not necessarily prevent its clinical use. The functional significance of specific aberrations that tend to occur in stem cell cultures will need to be assessed in safety preclinical trials. Acceptable degrees of genetic change must be established by a thorough examination of subcellular architecture, including chromosomes, small CNVs, and even point mutations (Gore et al., 2011; Laurent et al., 2011). Cell-surface markers and expression of transcription factors, as well as proliferation capacity and differentiation propensity, should also be evaluated, as these parameters have been suggested to change during the acquisition of genetic alterations (Blum and Benvenisty, 2009). Additionally, it is imperative to assess the heterogeneity of a culture, as the engraftment of undifferentiated or incorrectly differentiated cells may present a substantial tumorigenic or immunogenic risk to the recipient (Baker et al., 2007; Ben-David and Benvenisty, 2011; Fairchild, 2010). As the passage number of a stem cell lines increases, so too does the potential for chromosomal aberrations to arise (Hovatta et al., 2010; Maitra et al., 2005). Therefore, minimizing the culture time might be required in order to decrease the chance for in vitro genetic alterations.

Dosing and Pharmacokinetics

It is clear that conventional preclinical absorption, distribution, metabolism, excretion, and toxicity (ADMET) studies cannot be directly applied to cell-based products where there is a requirement to track differentiation and migration in vivo. How therefore can a dosing regimen be meaningfully calculated and pharmacokinetics/pharmacodynamics (PK/PD) be assessed? Such strategies are normally heavily reliant on risk-benefit analyses, but it presents a major challenge to make this analysis when the risks are poorly understood and the benefits are at present unknown.

Dosing regimens are conventionally based on in vivo dose response curves, but this method is difficult to translate to cell-based therapeutics. In determining an appropriate posology, it will be important to consider both evidence from dose-determining studies (i.e., it is necessary to consider how to derive a human equivalent dose; in many cases, animal models of disease are rodents— therefore, how will we determine how to scale up doses?) and rationale (comprising scientific and clinical logic). Dose selection considerations need to include both what is maximally feasible in the species chosen and the relevance to the intended human therapeutic dose. Data derived from tests with syngeneic cells can be useful to establish the dosing principles but are unlikely to contribute much to quantitative considerations, which are an essential part of determining initial human doses. It is also important to consider the route of administration of a product, i.e., whether it is administered systemically or locally. For example, MSCs are seen to home to sites of injury but a large proportion will accumulate in the lungs if administered systemically (Gao et al., 2001; Noort et al., 2002).

When selecting a relevant disease/injury model, it is important to understand both its attributes and limitations. When a well-developed animal model is available, evidence for robust proof-of-concept preclinical test results is valuable and informative, particularly if the targeted clinical indication requires administration of the stem cell-based product into a highly vulnerable anatomical site (Fink, 2009). A major issue for preclinical testing is the immunological relevance of testing human cells in an animal model. In certain circumstances, it may be possible to generate an analogous species-specific product, but this is not trivial and differences between the cells are likely to exist, which may limit the utility of this approach. Immunosuppression or immune-deficient animal models are likely to be employed, but this approach may mask immune-modulatory or immunotoxicological aspects of the cell-based therapy. Progress is being made with humanized mice but the clinical translatability of these studies is not yet clear.

Conventional toxicology and safety pharmacology studies rely on evaluating the effects of small molecules on normal physiological function (clinical observation, organ physiology, blood chemistry, and hematology) and histology of the organs. Often, the early studies supporting the first trials in humans are of short duration (up to 1 month). This timeframe is chosen based on known PK/PD and the location where the drug will eventually be eliminated from the system. For some stem cell therapies, a similar situation may be true and it would be possible to evaluate safety by adopting standard methods to determine effects on physiological function. However, for many cell-based therapies, the goal is to repair or replace damaged tissue specifically through engraftment. The potential lifetime exposure of a patient to a treatment the removal of which might not be feasible requires preclinical studies of significantly longer duration than are routine. Conventional histopathology is also problematic. Cell-based therapies will require new approaches in terms of cell localization and tracking and phenotype/genotype characterization. Furthermore, the potential chronology of tumor or teratoma formation will necessitate animal studies of substantially longer duration before starting trials in human subjects.

Biodistribution

The design of biodistribution studies conducted in animals must include a consideration of multiple factors: the methods applied to cellular detection and their sensitivity, the numbers of individual animals to be used, whether both sexes must be used, or whether a single sex can be considered adequate, whether a single species is adequate, and the appropriateness of the route of administration. Where systemic biodistribution of the product is likely, use of the intravenous route, in addition to the intended human route of administration, should be considered. In some instances, it will be feasible to conduct studies in animals with a condition resembling the human disease, either induced experimentally or using a strain with a genetic abnormality. However, in many instances it will be the case that human disease models do not exist: it is conceivable that distribution may be different between a diseased and nondiseased state and the relevance of any findings would need to be considered. Where the therapeutic use is in relation to surgery, it may be that small animals are not suitable and larger animals such as sheep or pigs may be required. Generally, the intended human cellular product should be used in these studies; however, doing so may require the use of immune-suppressed or immune-compromised animals. The immune system could in turn play a role in modulating cellular distribution in a patient, complicating the significance of any findings obtained in an immunodeficient animal model.

Biodistribution is a complex issue that relates to cell localization and migration as well as survival and differentiation status. At present, there is no single satisfactory method of tracking the fate of cells in vivo, and limitations of biodistribution assays arise in terms of sensitivity and limits of detection. In fact, is it important or even practically possible to track the fate of every cell over time? One method to do this is through the use of reporter probe imaging, as discussed by Sallam and Wu (2010). The use of model cells that have been modified to allow reporter-based tracking as a surrogate for the therapeutic product raises two issues. First, if the modified cell was being used only for the determination of distribution potential, has the addition of the reporter gene altered the function of the cell? (That is, how does it relate to the clinical product?) Second, if the clinical product itself contains the reporter construct, does the insertion of a transgene into a stem cell line mean that the stem cell product would have to meet the regulatory criteria for genetically modified organisms, as well as for cell-based therapies? Clearly, consensus needs to be reached regarding sensitivity of biodistribution assays and the characterization of extraneous phenotypes. The discovery of a small number of undifferentiated cells in the product, or a small number of cells migrating to ectopic locations, could lead to an assumption of risk that may not be functionally present and could halt the development of an otherwise efficacious product.

Attempts may be made to limit a risk of migration of stem cells from the target location, for example through the development of stem cell therapies using devices that are implanted in the target organ and constrain the stem cell product in this area. The encapsulation of the stem cell product would enable its future removal and prevent it from spreading in the patient's body; however, it is limited to the treatment of specific diseases, such as diabetes, in which the encapsulation would not diminish the functionality of the product and thus would not jeopardize its efficiency (Krishna et al., 2007).

The issues related to biodistribution should not be underestimated, but equally should not be considered insurmountable. There are emerging technologies in the fields of whole animal and tissue imaging, cellular biomarkers of phenotypic differentiation, and genetically modified tagging of cells that, if successful, will allow the monitoring of cell-based therapies. These approaches may offer further methods to address safety concerns related to biodistribution, and are further discussed in the safety assays section later in this article.

Immunogenicity and Immunotoxicity

Immunogenicity and immunotoxicity are potentially greater threats to recipients of cellular products than for conventional medicines, similar to transplantation therapies. Although the development of iPSCs holds promise for reducing this risk, the recent finding by Zhao and colleagues that in mouse iPSCs can induce a T cell-dependent immune response in syngeneic recipients (Zhao et al., 2011) shows that caution is warranted where these cells are used as the starting material. Immunotoxicity of the clinical product is difficult to assess as studies are typically run in immune-compromised or -suppressed animals. An alternative is to generate a species-specific homolog that may provide supportive data on the risk of immunotoxicity. A second issue is the potential that a human patient may generate an immune response to an administered product (immunogenicity). Okamura et al. (2007) attempted to reduce the risk of immunogenicity for their product by using in vitro assessments such as NK cell assays, serum cytotoxicity assays, mixed lymphocyte assays, FACS analysis of cell-surface expression, and cytokine assay. However, there needs to be some standardization of such assays and appropriate controls are required, particularly when looking for a negative result. Immunogenicity may be influenced by multiple factors including the site of administration (potential sites of immune privilege e.g., the eye), the maturation status of the cells, the number of doses, the immunological basis of the disease, and an aging immune system. Nonclinical studies with the clinical product may give rise to a xenogeneic response that may have little relevance to the clinical situation where there is a multidose arm.

The effect of a primed immune environment must be considered when multiple dosing is indicated; however, this concern may be more relevant for MSC-based products than for ES/iPS-derived cells. Moreover, the timeframe for immunogenicity assessment has to be of long duration, potentially up to the lifetime of a relevant animal model. The product must also be assessed in its final composition and in the absence of any manufacturing materials such as a scaffold, unless such material is part of the therapy. Due consideration must also be given to the possibility of secondary pharmacology, such as off-target effects resulting from the secretion of bioactive compounds from the graft. Similarly, safety pharmacology issues may result from physiological impairment of organ function due to migration of cells to an unwanted site.

Tumorigenicity

The capacity to form teratomas in immunocompromised animals is a characteristic of pluripotent stem cells (Ben-David and Benvenisty, 2011; Blum and Benvenisty, 2007). A donor-derived brain tumor following neural stem cell transplantation was also

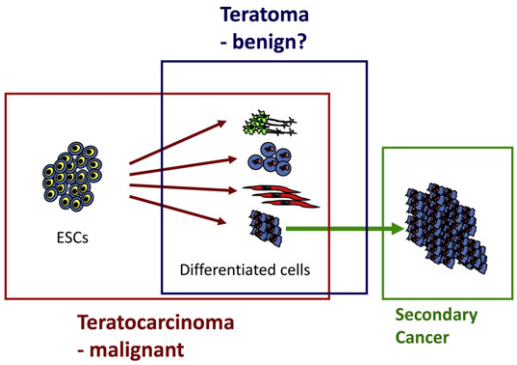

Figure 2. Three Classes of Tumors Could Be Envisaged Arising from ESCs and Their Derivatives

Initially, pluripotent stem cells produce "teratomas," which comprise a haphazard array of somatic cell types, sometimes arranged into tissues, and corresponding to all three germ layers. These tumors are typically regarded as benign. However, if undifferentiated stem cells are present in the tumor, then the tumor is regarded as a teratocarcinoma and would most likely be malignant. A third type of tumor that could arise would be one formed from the differentiated cells themselves. Such a "secondary" tumor would not have characteristics of teratomas or teratocarcinomas but would be most likely akin to tumors that arise from corresponding tissues in a person or animal.

reported in the literature (Amariglio et al., 2009). However, it is not yet possible to quantify the tumor risk associated with the introduction of stem cells and stem cell-derived products in vivo. Where that risk exists, the type of tumor must be considered, as well as how susceptible the tumor may be to therapeutic intervention. All these factors contribute to assessing acceptable risk, as tumorigenicity must be assessed on a case-by-case basis, dependent on the intended therapeutic indication and the recipient's prognosis.

Although most attention has focused on the dangers of teratoma or teratocarcinoma development compromising the use of pluripotent cell derivatives for regenerative medicine, it should be remembered that it is unlikely that undifferentiated cells will themselves be deliberately used for transplantation. Thus the risk posed by these cells is the potential for their contamination of the preparation of differentiated cells for transplantation. This risk will be ameliorated by developing appropriate purification protocols and the means for monitoring contamination. However, rather more insidious is the possibility that adult stem cells or stem cell-derived differentiated cells themselves may be tumorigenic, perhaps due to mutations acquired during culture of the parent stem cell. These issues are summarized in Figure 2.

The combination of interspecies differences in tumor development between rodents and humans (Anisimov et al., 2005) and the immunodeficient status of mice used for xenograft models compromises the translatability of some tumorigenic risks from animals to man. Ultimately, a collaborative effort between academia and industry may be the most fruitful approach to define markers of tumorigenicity. To this end, access to legacy data on primary hazard identification from studies that were halted during preclinical testing would greatly assist the risk:benefit analysis for stem cell-based therapies.

The Safety Issues: Clinical assessment

The seminal first-in-human safety trials of stem cell-derived products to treat spinal injury (Mayor, 2010) and ischemic stroke (Wise, 2010) have had a major impact in this field. Preclinical tests have been conducted on nude and SCID mice, which have severely compromised immune systems, but it is unclear how this model might compare to a human patient and how the condition of the patient (age, disease, nutrition, gender, medication, etc.) might affect the efficacy of the introduced cells. Once the cells have been successfully engrafted, the most appropriate method of assessing cell migration and biodistribution must be employed, and this may be, as discussed previously, through the employment of validated markers and new technologies such as imaging.

Obviously, the basic risks of the trial and the therapy must be assessed, both to patients and to donors (see earlier immune section), although the net clinical benefit of the product will evolve through its development life cycle. Due consideration must be given to timeframes for clinical evaluation and latency, based on data from animal models, as well as consideration for the disease status of the recipient. Nevertheless, there is a lack of data to support the long-term safety of stem cell transplantation, and also it is unclear exactly how clinically manageable cell-based adverse events may be. It will therefore be beneficial to establish a registry to centralize records of donors and recipients.

Finally, an option that should be considered as a fail-safe mechanism for halting therapy postadministration is through the deployment of a "suicide gene"—a genetic antidote that could be activated in vivo to ablate all grafted cells, or to select out all undifferentiated cells. This may take the form of an apoptosis-regulating gene that could be directed to a safe-harbor genomic location, as has recently been proposed in iPSC-based therapies (Papapetrou et al., 2011), although such genetic modification could potentially alter the characteristics of the cells. While iPSC-based therapies may be inherently "safer," since they can be derived from the same patient, the fact that they have been removed from the host, manipulated to dedifferentiate or transdifferentiate, often with genetic manipulation, as well as the important emerging evidence of the retention of the epigenetic status of their former cell type (Kim et al., 2010; Polo et al., 2010), brings the possibility that they also create adverse effects that would warrant a means for halting therapy.

Current Assays for Stem Cell Therapy Safety Assessment

A stem cell therapeutic may contain multiple cell types, depending on the source of the cells, the purification process, and the nature of the differentiation process employed. The most obvious safety risk of such differentiated cultures would be residual undifferentiated cells that might be tumorigenic (Anisimov et al., 2010; Ben-David and Benvenisty, 2011). Engraftment of undesired, fully differentiated cell types into an ectopic tissue might also have detrimental effects. The purity of the differentiated cells can be fully characterized by evaluating various markers of undifferentiated cells (such as TRA-1-60), markers of the specific cell type of interest, and markers of undesired cell types of the same/other lineages. The evaluation of such markers can be achieved using a quantitative polymerase chain reaction assay (qPCR), flow cytometry, and immunohistochemistry, and a combination of these methods might provide detailed knowledge of the purity of the culture (Adewumi et al., 2007;

Lavon et al., 2004; Noaksson et al., 2005). In addition, detailed clonogenic assays, such as soft agar colony formation assay (Hamburger, 1987) in the case of pluripotent stem cells and neurosphere formation assay (Reynolds and Weiss, 1992) in the case of neural stem cells, provide the most direct method to assess the existence of functional stem cells in a population. Knowledge of cell purity is crucial, since a mixed cell population might be beneficial for the pharmacodynamic effect (for example, mesenchymal stem cells have been suggested to support the engraftment of other cell types (Cristofanilli et al., 2011), but residual undifferentiated cells may contribute to the safety risk, as mentioned above).

Genetic changes in culture must also be evaluated in order to determine the safety of the therapy. Cells in culture, and stem cells in particular, accumulate chromosomal aberrations, especially at high passage numbers (Baker et al., 2007; Mayshar et al., 2010). These chromosomal abnormalities must be fully characterized and risk assessed before exposure to a patient. Analysis of karyotypic changes at passage numbers corresponding to those found in the product would help to assess safety. Furthermore, where transgenes are used in a product, the possibility of insertional mutagenesis, and therefore a cancer risk, must be studied. In addition, as recent studies have demonstrated that subkaryotypic changes, and even point mutations in coding regions, might arise in stem cell cultures (Gore et al., 2011; Hussein et al., 2011; Laurent et al., 2011), more accurate and expensive methods for the evaluation of the genomic integrity—such as array comparative genomic hybridization (aCGH) and single-nucleotide polymorphism array (SNP array)—might also be required.

With regard to animal testing of stem cell products, analysis is limited by the lifespan of the animal, compared with the lifespan of a human patient, and longer follow-up studies are likely to be required for early human stem cell trials as compared to conventional medicines. Such studies in animals would consist of histology, imaging and behavioral studies, and monitoring of the interaction of the product with surrounding tissues. Furthermore, testing of the product may be carried out at passage numbers beyond routine use, to ensure the product remains safe, particularly with regards to tumor formation and immunogenicity. Care should be taken with the use of passage numbers far in excess of the therapy product as these may carry altered genetic and phenotypic characteristics that are not clinically relevant.

Another potential safety issue is that of migration of cells from the graft. Cells can be tracked using several different methods, such as genetic labeling, immunohistochemistry, and bioluminescence techniques. An important issue to consider in this regard is the level of sensitivity, and methodological challenges can increase when a large animal model is required. GFP-labeled cells can be administered to an animal model and the migration to organs other than the intended target can be monitored using qPCR and histology (Xiong et al., 2010), although GFP labeling can potentially alter cellular characteristics. Alternatively, the cells can be incubated with a labeled perfluorocarbon nanoemulsion before exposure of animals (Hertlein et al., 2011), to act as a contrast agent for tracing them using nuclear magnetic resonance (NMR) of organs or magnetic resonance imaging (MRI) scans of either the whole animal or fixed slices of tissue. Cells can also be tracked using immunohistochemistry and bioluminescence techniques. Of course, administering human cells to an animal model makes analysis of biodistribution and migration very easy to monitor (Ellis et al., 2010). However, it becomes more complicated to analyze biodistribution of human cells in a human host. If the cells in the stem cell therapeutic are adequately characterized, the HLA type should be known and the host and graft cells can be discriminated based on immunological characteristics, assuming that imaging technology has sufficient resolution in human subjects.

Another relevant issue is that preclinical testing of products designated for closed compartments such as the brain and CNS has involved the maximum number of cells that can physically fit in the available tissue space, and clinical trial design has involved an extrapolation of this cell number based on the physiological difference between the preclinical species and humans (Redmond et al., 2007). Therefore, techniques to evaluate the safety of cell-based therapies at the critical stage of transition from preclinical to clinical trials ought to be developed and standardized.

Notwithstanding these important preclinical safety assessment issues, there are also concerns at the clinical trial phase surrounding lack of blinding or placebos, although for some disease areas, there may need to be new thinking due to the ethical issues associated with use of placebo cells. There are also possible issues regarding the effects of concomitant surgery and/or medication. These require further discussion and recommendations, which exceed the scope of this article.

Regulation of Stem Cell Therapeutics

The new era of stem cell-based therapies brings new challenges to drug regulators as well as to safety assessment scientists. In order to avoid regulatory inconsistencies, which could compromise the translation of novel therapies into clinical usage, it is essential that a dialog between regulators and therapy providers be initiated at an early stage. This approach would allow the identification of potential safety issues and define the expectation of regulators with respect to risk assessment.

An earlier review in 2006 attempted to apply the current FDA guidelines for biologics and cell-and-tissue products to emerging stem cell therapeutics (Halme and Kessler, 2006). As well as covering some of the issues discussed in this review, the authors made recommendations for reducing risk to the recipient from the graft itself, by screening for potentially harmful or problematic genetic conditions in the donor cells, and by adequate characterization of the product, particularly since clonal expansion in vitro may require the use of animal sera or animal feeder cells. In addition, Figure 3 shows a diagrammatic representation of an interim regulatory route map from the UK perspective.

Regulatory experience is rather limited, particularly where product failure is concerned. Preclinical failures are not brought to the attention of the regulatory agencies, and as such the regulators have only limited knowledge of the preclinical issues with product development. Data-sharing consortia may go some way to addressing these knowledge gaps. Clearly, proof-of-concept is more important at early stages of development than mechanistic data, but a mechanism is reassuring from a regulatory standpoint.

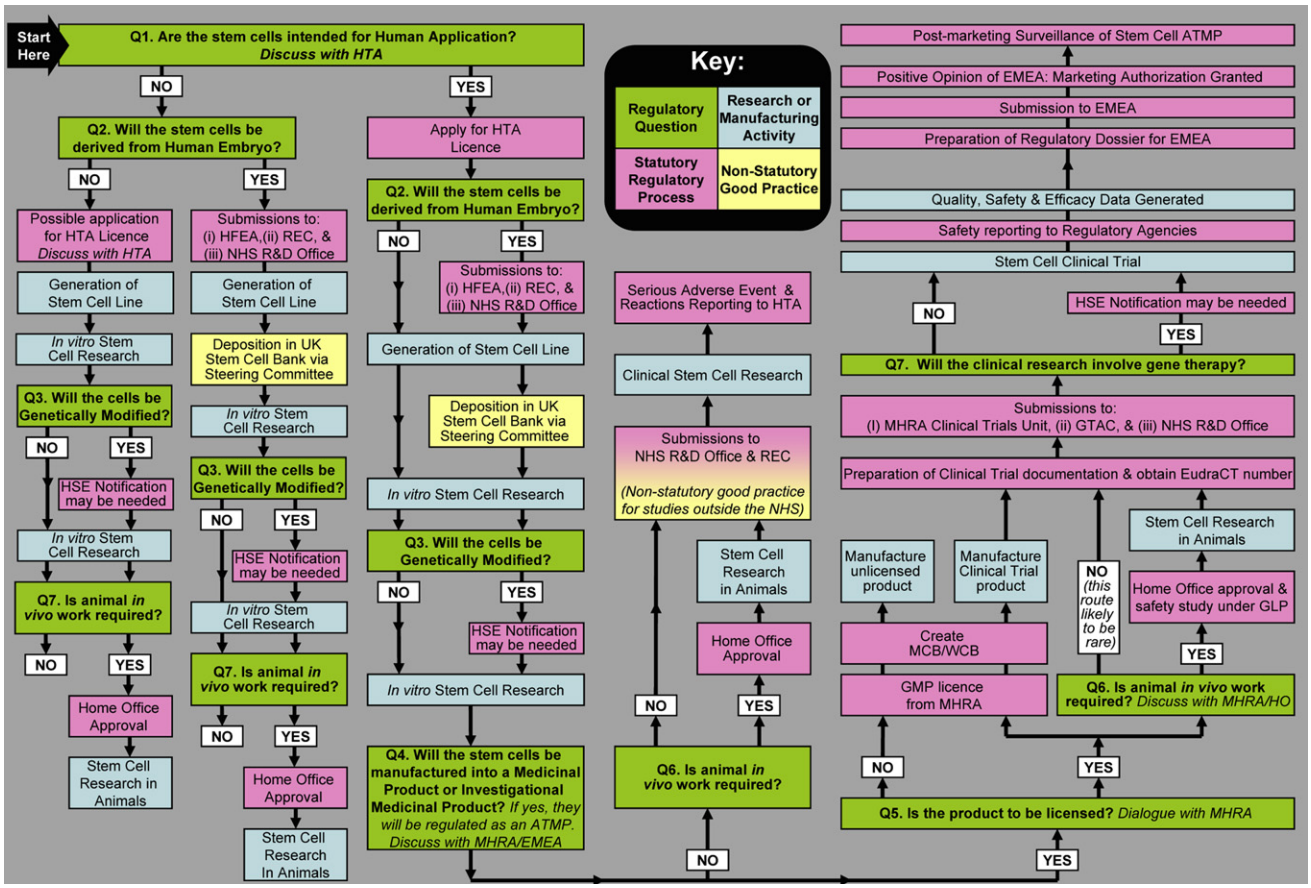

Figure 3. Illustrative UK Regulatory Route Map for Stem Cell Research and Manufacture

The UK Medicines and Healthcare Products Agency (MHRA) participated in the production of this regulatory route map for stem cell research and manufacture, which has been developed by the Department of Health (DoH) with the support of regulatory bodies and the Gene Therapy Advisory Committee (GTAC). This *interim* UK regulatory route map is intended to be a reference tool for those who wish to develop a program of stem cell research and manufacture ultimately leading to clinical application. A web tool to apply this in practice, using a decision-tree approach, is available at http://www.sc-toolkit.ac.uk/home.cfm. Other abbreviations: HTA, human tissue authority; NHS, National Health Service; GLP, Good Laboratory Practice; GMP, Good Manufacturing Practice; R&D, Research and Development; HFEA, The Human Fertilisation and Embryology Authority; REC, Research Ethics Committee; ATMP, Advanced Therapy Medicinal Products; EMEA, European Medicines Agency; EudraCT, a database of all clinical trials commencing in the European Union from May 1, 2004 onward. (See http://www.mhra.gov.uk/Howweregulate/Medicines/Medicinesregulatorynews/CON041337.)

It may be instructive in this context to outline the remit of The European Medicines Agency (EMA) Committee for Advanced Therapies (CAT), which was set up in 2008 to regulate new therapies such as cell-based therapies and is made up of European regulators, academics, clinicians, companies, and patient societies. As a multidisciplinary committee, the CAT is designed to cover a wide range of aspects of all advanced therapies, including stem-cell based therapies. Any new therapies are authorized centrally, but the decision as to whether a member state actually permits the use of the therapy is made at a national level (e.g., Germany, in which treatment with medicinal products containing embryonic stem cells is not permitted). Understanding and learning from this type of approach may be valuable in deciding how to address the issue of stem cell regulation.

Regulatory Safety Requirements for Stem Cell Therapeutics

For stem cell therapies, a regulatory approach based on conventional pharmaceutical products is not appropriate. Licensing decisions are made on a case-by-case basis, using a risk-benefit approach. Ultimately, in terms of safety, there is no distinction between small-molecule and cell-based therapies—they all need to meet acceptable standards of quality, safety, and efficacy before they can be widely used. Obviously, a stem cell product must be produced under Good Manufacturing Practice, with operations fully characterized and records and standard operating procedures in place. The product must also be adequately characterized. Knowledge of the purity of the product must be known, since extraneous phenotypes may either influence efficacy or contribute a significant safety risk. Any chromosomal abnormalities must be well characterized, in case there is a risk of contributing to tumor formation. While animal data should be supplied, where appropriate and informative, nonhuman primates should be used only if they are the best model.

The general consensus of regulatory agencies with regard to tracking biodistribution is to favor pragmatism as opposed to exhaustive analysis. For example, MRI and 3D imaging generates a huge amount of data, which can be difficult to interpret without good bioinformatics and systems analysis. Regulators

require that safety consideration is part of the manufacturing process as a whole. If the manufacturing process is changed to account for an issue with safety, the product must be proven to remain safe and efficacious.

Dose-escalation studies are difficult to carry out using cell-based therapeutics, but there must be a clear evidence-based rationale underlying choice of dose in clinical trials. To some extent, the strategy will depend on the product. The risks associated with the procedure to administer a cell-based therapeutic must be fully understood, i.e., the route of application, duration of exposure if this is not indefinite, and need for repeat applications. Furthermore, any device used for implantation needs to be approved for use in patients.

After characterization in animal studies and allometric scaling to human requirements, it is acknowledged that formal dose-escalation studies in humans might not always be feasible, although the bespoke nature of different cell-based therapies suggests that this may not always be the case. Lessons may be learned from the approaches used in the biologics/antibody world. If such studies are required, then they would be for the purposes of both tolerability and efficacy with the appropriate indices and biomarkers.

Regulatory Support

Communication is a key cornerstone of regulatory support. We particularly highlight the importance of establishing a dialog with the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), as well as improving communications with health authorities. In particular, improved collaborations between production and preclinical testing specialists, to ensure comparability of product or adequate testing where a product changes, may help streamline safety evaluations.

Increasingly, there are examples of industry and regulatory collaborations and consortia, such as the European Innovative Medicines Initiative (IMI), that adopt a precompetitive data-sharing approach to facilitate development of new safety evaluation methods. Nevertheless, only positive examples are taken forward into clinical development, meaning that the examples that fail are not publicized and information is not shared with others. Early interaction between regulatory agencies, therapy developers, and drug safety scientists is important in this evolving field, since clear regulatory guidelines help in planning product development. Precompetitive data-sharing approaches can involve the use of third-party organizations that act as a central and anonymous repository for storage and analysis of data, before sharing with the consortia members. Furthermore, discussions with regulators can assist companies working on individual development programs. As a corollary, companies that have been through the regulatory process before may have an insight into current regulatory requirements, and sharing of this type of information could also form an industry precompetitive approach.

Proposals

To expedite the advancement of the field of safety assessment of stem cell therapeutics, we would submit the following proposals:

- Collaborations between industry, academia, and regulatory authorities should be undertaken at every opportunity, using current centers of excellence, leading to the establishment of cross-institution expert cell-based therapy safety groups.
- Consortia that adopt a precompetitive data-sharing approach to facilitate development of new safety evaluation methods should be encouraged. Although data sharing can be challenging in practice, there are already emerging examples, such as through the IMI strategy described above.
- A centralized registry of donors and recipients should be established in order to best manage adverse events.
- Efforts need to be made to educate the public and media on the benefits and risks of stem cell-based therapies, and to explain issues rationally. This type of approach is key for articulating the notion of "acceptable risk" for a novel therapy.
- Research efforts that prioritize the following areas should be facilitated:
  1. Model systems. Research is required into the establishment of relevant animal models to improve preclinical testing. The development and inclusion of positive and negative control cell lines, for efficacy and/or toxicity, wherever possible, would add value to animal data.
  2. Safety and efficacy biomarkers. There is an immediate and pressing need to establish appropriate biomarkers for each stage of the development process. There is also a need for funding of research into markers of cell function, differentiation, and migration in vivo.
  3. Immunogenicity/Immunotoxicity. The risk of immunotoxicity is poorly characterized at present, largely due to a paucity of appropriate preclinical models, and further research is necessary to elucidate the interactions of grafted cells with the host immune system.
  4. Tumorogenicity. Tumorigenesis is also of great concern, and we suggest that research into the possibility of employing "stop methods" would be of great value as a means of ablating all grafted cells. In addition, marker panels for tumorigenic risk both pre- and postengraftment would be highly informative.

In summary, it is not clear whether the state of our understanding is sufficient to appraise the safety of these therapies in a comprehensive manner, and we therefore require further sensitive and robust approaches in bioanalysis to monitor them. At a broader level, it is also important to raise the question of whether we are setting a higher bar for the clinical implementation of stem cell-derived therapeutics than we currently apply for other types of cellular therapy. There is a danger that if perfection is a prerequisite for beginning, then we will never begin. Ultimately, while stem cell therapy is an area of rapid advancement, the science of stem cell safety assessment must also evolve, not to hinder progress, but to support, guide, and expedite patient treatment. The development of such technology is necessary to ensure that we can proceed with appropriate safeguards in place and allow that stem cell-based therapeutic approaches develop in a way that benefits society overall.

ACKNOWLEDGMENTS

C.E.P.G., R.E., N.F., N.A.H., L.K., N.R.K., H.L., and B.K.P. would like to thank the Medical Research Council (MRC); Frank Bonner, Ernie Harpur, and Rebecca Lumsden at Stem Cells for Safer Medicines; and The Association of British Pharmaceutical Companies (ABPI). C.E.P.G and R.E. thank Gillian Wallace and acknowledge the support of Life Technologies. N.H. acknowledges the support of The Wellcome Trust and The National Institute for Health Research. Peter Andrews wishes to thank the MRC and Yorkshire Cancer Research (YCR). An Industry-Academic-Regulator workshop that was held in Liverpool, UK, on Nov. 24, 2010, formed the initiative for the development of this manuscript.

REFERENCES

Aggarwal, S., and Pittenger, M.F. (2005). Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood *105*, 1815–1822.

Amariglio, N., Hirshberg, A., Scheithauer, B.W., Cohen, Y., Loewenthal, R., Trakhtenbrot, L., Paz, N., Koren-Michowitz, M., Waldman, D., Leider-Trejo, L., et al. (2009). Donor-derived brain tumor following neural stem cell transplantation in an ataxia telangiectasia patient. PLoS Med. *6*, e1000029.

Anisimov, V.N., Ukraintseva, S.V., and Yashin, A.I. (2005). Cancer in rodents: does it tell us about cancer in humans? Nat. Rev. Cancer *5*, 807–819.

Anisimov, S.V., Morizane, A., and Correia, A.S. (2010). Risks and mechanisms of oncological disease following stem cell transplantation. Stem Cell Rev. *6*, 411–424.

Baker, D.E., Harrison, N.J., Maltby, E., Smith, K., Moore, H.D., Shaw, P.J., Heath, P.R., Holden, H., and Andrews, P.W. (2007). Adaptation to culture of human embryonic stem cells and oncogenesis in vivo. Nat. Biotechnol. *25*, 207–215.

Baxter, M.A., Rowe, C., Alder, J., Harrison, S., Hanley, K.P., Park, B.K., Kitteringham, N.R., Goldring, C.E., and Hanley, N.A. (2010). Generating hepatic cell lineages from pluripotent stem cells for drug toxicity screening. Stem Cell Res. (Amst.) *5*, 4–22.

Ben-David, U., and Benvenisty, N. (2011). The tumorigenicity of human embryonic and induced pluripotent stem cells. Nat. Rev. Cancer *11*, 268–277.

Bone, H.K., Nelson, A.S., Goldring, C.E., Tosh, D., and Welham, M.J. (2011). A novel chemically directed route for the generation of definitive endoderm from human embryonic stem cells based on inhibition of GSK-3. J. Cell Sci., in press.

Blum, B., and Benvenisty, N. (2007). Clonal analysis of human embryonic stem cell differentiation into teratomas. Stem Cells *25*, 1924–1930.

Blum, B., and Benvenisty, N. (2008). The tumorigenicity of human embryonic stem cells. Adv. Cancer Res. *100*, 133–158.

Blum, B., and Benvenisty, N. (2009). The tumorigenicity of diploid and aneuploid human pluripotent stem cells. Cell Cycle *8*, 3822–3830.

Cai, J., Zhao, Y., Liu, Y., Ye, F., Song, Z., Qin, H., Meng, S., Chen, Y., Zhou, R., Song, X., et al. (2007). Directed differentiation of human embryonic stem cells into functional hepatic cells. Hepatology *45*, 1229–1239.

Caplan, A.I., and Dennis, J.E. (2006). Mesenchymal stem cells as trophic mediators. J. Cell. Biochem. *98*, 1076–1084.

Cristofanilli, M., Harris, V.K., Zigelbaum, A., Goossens, A.M., Lu, A., Rosenthal, H., and Sadiq, S.A. (2011). Mesenchymal stem cells enhance the engraftment and myelinating ability of allogeneic oligodendrocyte progenitors in dysmyelinated mice. Stem Cells Dev., in press. Published online March 12, 2011. 10.1089/scd.2010.0547.

Drukker, M., and Benvenisty, N. (2004). The immunogenicity of human embryonic stem-derived cells. Trends Biotechnol. *22*, 136–141.

Duan, Y., Catana, A., Meng, Y., Yamamoto, N., He, S., Gupta, S., Gambhir, S.S., and Zern, M.A. (2007). Differentiation and enrichment of hepatocyte-like cells from human embryonic stem cells in vitro and in vivo. Stem Cells *25*, 3058–3068.

Ellis, J., Baum, C., Benvenisty, N., Mostoslavsky, G., Okano, H., Stanford, W.L., Porteus, M., and Sadelain, M. (2010). Benefits of utilizing gene-modified iPSCs for clinical applications. Cell Stem Cell *7*, 429–430.

Fairchild, P.J. (2010). The challenge of immunogenicity in the quest for induced pluripotency. Nat. Rev. Immunol. *10*, 868–875.

Fink, D.W., Jr. (2009). FDA regulation of stem cell-based products. Science *324*, 1662–1663.

Gao, J., Dennis, J.E., Muzic, R.F., Lundberg, M., and Caplan, A.I. (2001). The dynamic in vivo distribution of bone marrow-derived mesenchymal stem cells after infusion. Cells Tissues Organs (Print) *169*, 12–20.

Geron (2009a). Geron Comments on FDA Hold on Spinal Cord Injury Trial (http://www.geron.com/media/pressview.aspx?id=1188).

Geron (2009b). Geron receives FDA clearance to begin world's first human clinical trial of embryonic stem cell-based therapy (http://www.geron.com/media/pressview.aspx?id=1148).

Gore, A., Li, Z., Fung, H.L., Young, J.E., Agarwal, S., Antosiewicz-Bourget, J., Canto, I., Giorgetti, A., Israel, M.A., Kiskinis, E., et al. (2011). Somatic coding mutations in human induced pluripotent stem cells. Nature *471*, 63–67.

Halme, D.G., and Kessler, D.A. (2006). FDA regulation of stem-cell-based therapies. N. Engl. J. Med. *355*, 1730–1735.

Hamburger, A.W. (1987). The human tumor clonogenic assay as a model system in cell biology. Int. J. Cell Cloning *5*, 89–107.

Hanahan, D., and Weinberg, R.A. (2011). Hallmarks of cancer: the next generation. Cell *144*, 646–674.

Hay, D.C., Fletcher, J., Payne, C., Terrace, J.D., Gallagher, R.C., Snoeys, J., Black, J.R., Wojtacha, D., Samuel, K., Hannoun, Z., et al. (2008). Highly efficient differentiation of hESCs to functional hepatic endoderm requires ActivinA and Wnt3a signaling. Proc. Natl. Acad. Sci U S A *105*, 12301–12306.

Hertlein, T., Sturm, V., Kircher, S., Basse-Lüsebrink, T., Haddad, D., Ohlsen, K., and Jakob, P. (2011). Visualization of abscess formation in a murine thigh infection model of Staphylococcus aureus by 19F-magnetic resonance imaging (MRI). PLoS ONE *6*, e18246.

Hovatta, O., Jaconi, M., Töhönen, V., Béna, F., Gimelli, S., Bosman, A., Holm, F., Wyder, S., Zdobnov, E.M., Irion, O., et al. (2010). A teratocarcinoma-like human embryonic stem cell (hESC) line and four hESC lines reveal potentially oncogenic genomic changes. PLoS ONE *5*, e10263.

Hussein, S.M., Batada, N.N., Vuoristo, S., Ching, R.W., Autio, R., Närvä, E., Ng, S., Sourour, M., Hämäläinen, R., Olsson, C., et al. (2011). Copy number variation and selection during reprogramming to pluripotency. Nature *471*, 58–62.

Hyun, I., Lindvall, O., Ahrlund-Richter, L., Cattaneo, E., Cavazzana-Calvo, M., Cossu, G., De Luca, M., Fox, I.J., Gerstle, C., Goldstein, R.A., et al. (2008). New ISSCR guidelines underscore major principles for responsible translational stem cell research. Cell Stem Cell *3*, 607–609.

International Stem Cell Initiative, Adewumi, O., Aflatoonian, B., Ahrlund-Richter, L., Amit, M., Andrews, P.W., Beighton, G., Bello, P.A., Benvenisty, N., Berry, L.S., et al. (2007). Characterization of human embryonic stem cell lines by the International Stem Cell Initiative. Nat. Biotechnol. *25*, 803–816.

Kehat, I., Kenyagin-Karsenti, D., Snir, M., Segev, H., Amit, M., Gepstein, A., Livne, E., Binah, O., Itskovitz-Eldor, J., and Gepstein, L. (2001). Human embryonic stem cells can differentiate into myocytes with structural and functional properties of cardiomyocytes. J. Clin. Invest. *108*, 407–414.

Kim, K., Doi, A., Wen, B., Ng, K., Zhao, R., Cahan, P., Kim, J., Aryee, M.J., Ji, H., Ehrlich, L.I., et al. (2010). Epigenetic memory in induced pluripotent stem cells. Nature *467*, 285–290.

Koay, E.J., Hoben, G.M., and Athanasiou, K.A. (2007). Tissue engineering with chondrogenically differentiated human embryonic stem cells. Stem Cells *25*, 2183–2190.

Krishna, K.A., Rao, G.V., and Rao, K.S. (2007). Stem cell-based therapy for the treatment of Type 1 diabetes mellitus. Regen. Med. *2*, 171–177.

Laurent, L.C., Ulitsky, I., Slavin, I., Tran, H., Schork, A., Morey, R., Lynch, C., Harness, J.V., Lee, S., Barrero, M.J., et al. (2011). Dynamic changes in the copy number of pluripotency and cell proliferation genes in human ESCs and iPSCs during reprogramming and time in culture. Cell Stem Cell *8*, 106–118.

Lavon, N., Yanuka, O., and Benvenisty, N. (2004). Differentiation and isolation of hepatic-like cells from human embryonic stem cells. Differentiation *72*, 230–238.

Maitra, A., Arking, D.E., Shivapurkar, N., Ikeda, M., Stastny, V., Kassauei, K., Sui, G., Cutler, D.J., Liu, Y., Brimble, S.N., et al. (2005). Genomic alterations in cultured human embryonic stem cells. Nat. Genet. *37*, 1099–1103.

Mayor, S. (2010). First patient enters trial to test safety of stem cells in spinal injury. BMJ *341*, c5724.

Mayshar, Y., Ben-David, U., Lavon, N., Biancotti, J.C., Yakir, B., Clark, A.T., Plath, K., Lowry, W.E., and Benvenisty, N. (2010). Identification and classification of chromosomal aberrations in human induced pluripotent stem cells. Cell Stem Cell *7*, 521–531.

National Institutes of Health. (2006). Regenerative Medicine. http://stemcellsnihgov/info/scireport/2006reporthtm.

Nelson, T.J., Martinez-Fernandez, A., Yamada, S., Ikeda, Y., Perez-Terzic, C., and Terzic, A. (2010). Induced pluripotent stem cells: advances to applications. Stem Cells Cloning *3*, 29–37.

Nishikawa, S., Goldstein, R.A., and Nierras, C.R. (2008). The promise of human induced pluripotent stem cells for research and therapy. Nat. Rev. Mol. Cell Biol. *9*, 725–729.

Noaksson, K., Zoric, N., Zeng, X., Rao, M.S., Hyllner, J., Semb, H., Kubista, M., and Sartipy, P. (2005). Monitoring differentiation of human embryonic stem cells using real-time PCR. Stem Cells *23*, 1460–1467.

Noort, W.A., Kruisselbrink, A.B., in't Anker, P.S., Kruger, M., van Bezooijen, R.L., de Paus, R.A., Heemskerk, M.H., Lowik, C.W., Falkenburg, J.H., Willemze, R., et al. (2002). Mesenchymal stem cells promote engraftment of human umbilical cord blood-derived CD34(+) cells in NOD/SCID mice. Exp. Hematol. *30*, 870–878.

Okamura, R.M., Lebkowski, J., Au, M., Priest, C.A., Denham, J., and Majumdar, A.S. (2007). Immunological properties of human embryonic stem cell-derived oligodendrocyte progenitor cells. J. Neuroimmunol. *192*, 134–144.

Papapetrou, E.P., Lee, G., Malani, N., Setty, M., Riviere, I., Tirunagari, L.M., Kadota, K., Roth, S.L., Giardina, P., Viale, A., et al. (2011). Genomic safe harbors permit high β-globin transgene expression in thalassemia induced pluripotent stem cells. Nat. Biotechnol. *29*, 73–78.

Passier, R., Oostwaard, D.W., Snapper, J., Kloots, J., Hassink, R.J., Kuijk, E., Roelen, B., de la Riviere, A.B., and Mummery, C. (2005). Increased cardiomyocyte differentiation from human embryonic stem cells in serum-free cultures. Stem Cells *23*, 772–780.

Perin, L., Giuliani, S., Sedrakyan, S., DA Sacco, S., and De Filippo, R.E. (2008). Stem cell and regenerative science applications in the development of bioengineering of renal tissue. Pediatr. Res. *63*, 467–471.

Polo, J.M., Liu, S., Figueroa, M.E., Kulalert, W., Eminli, S., Tan, K.Y., Apostolou, E., Stadtfeld, M., Li, Y., Shioda, T., et al. (2010). Cell type of origin influences the molecular and functional properties of mouse induced pluripotent stem cells. Nat. Biotechnol. *28*, 848–855.

Prockop, D.J. (1997). Marrow stromal cells as stem cells for nonhematopoietic tissues. Science *276*, 71–74.

Redmond, D.E., Jr., Bjugstad, K.B., Teng, Y.D., Ourednik, V., Ourednik, J., Wakeman, D.R., Parsons, X.H., Gonzalez, R., Blanchard, B.C., Kim, S.U., et al. (2007). Behavioral improvement in a primate Parkinson's model is associated with multiple homeostatic effects of human neural stem cells. Proc. Natl. Acad. Sci. USA *104*, 12175–12180.

Reynolds, B.A., and Weiss, S. (1992). Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. Science *255*, 1707–1710.

Sallam, K., and Wu, J.C. (2010). Embryonic stem cell biology: insights from molecular imaging. Methods Mol. Biol. *660*, 185–199.

Sareen, D., McMillan, E., Ebert, A.D., Shelley, B.C., Johnson, J.A., Meisner, L.F., and Svendsen, C.N. (2009). Chromosome 7 and 19 trisomy in cultured human neural progenitor cells. PLoS ONE *4*, e7630.

Schuldiner, M., and Benvenisty, N. (2003). Factors controlling human embryonic stem cell differentiation. Methods Enzymol. *365*, 446–461.

Spence, J.R., Mayhew, C.N., Rankin, S.A., Kuhar, M.F., Vallance, J.E., Tolle, K., Hoskins, E.E., Kalinichenko, V.V., Wells, S.I., Zorn, A.M., et al. (2011). Directed differentiation of human pluripotent stem cells into intestinal tissue in vitro. Nature *470*, 105–109.

Stephenson, E., Ogilvie, C.M., Patel, H., Cornwell, G., Jacquet, L., Kadeva, N., Braude, P., and Ilic, D. (2010). Safety paradigm: genetic evaluation of therapeutic grade human embryonic stem cells. J. R. Soc. Interface *7* (*Suppl 6*), S677–S688.

Sullivan, G.J., Hay, D.C., Park, I.H., Fletcher, J., Hannoun, Z., Payne, C.M., Dalgetty, D., Black, J.R., Ross, J.A., Samuel, K., et al. (2010). Generation of functional human hepatic endoderm from human induced pluripotent stem cells. Hepatology *51*, 329–335.

Swijnenburg, R.J., Schrepfer, S., Govaert, J.A., Cao, F., Ransohoff, K., Sheikh, A.Y., Haddad, M., Connolly, A.J., Davis, M.M., Robbins, R.C., and Wu, J.C. (2008). Immunosuppressive therapy mitigates immunological rejection of human embryonic stem cell xenografts. Proc. Natl. Acad. Sci. USA *105*, 12991–12996.

Thomson, J.A., Itskovitz-Eldor, J., Shapiro, S.S., Waknitz, M.A., Swiergiel, J.J., Marshall, V.S., and Jones, J.M. (1998). Embryonic stem cell lines derived from human blastocysts. Science *282*, 1145–1147.

Touboul, T., Hannan, N.R., Corbineau, S., Martinez, A., Martinet, C., Branchereau, S., Mainot, S., Strick-Marchand, H., Pedersen, R., Di Santo, J., et al. (2010). Generation of functional hepatocytes from human embryonic stem cells under chemically defined conditions that recapitulate liver development. Hepatology *51*, 1754–1765.

Ueyama, H., Horibe, T., Hinotsu, S., Tanaka, T., Inoue, T., Urushihara, H., Kitagawa, A., and Kawakami, K. (2011). Chromosomal variability of human mesenchymal stem cells cultured under hypoxic conditions. J Cell Mol. Med., in press. Published online March 21, 2011. 10.1111/j.1582-4934.2011.01303.x.

Vallier, L. (2011). Serum-free and feeder-free culture conditions for human embryonic stem cells. Methods Mol. Biol. *690*, 57–66.

Vitale, A.M., Wolvetang, E., and Mackay-Sim, A. (2011). Induced pluripotent stem cells: A new technology to study human diseases. Int. J. Biochem. Cell Biol. *43*, 843–846.

Wise, J. (2010). Stroke patients take part in "milestone" UK trial of stem cell therapy. BMJ *341*, c6574.

Wong, S.S., and Bernstein, H.S. (2010). Cardiac regeneration using human embryonic stem cells: producing cells for future therapy. Regen. Med. *5*, 763–775.

Xiong, Q., Hill, K.L., Li, Q., Suntharalingam, P., Mansoor, A., Wang, X., Jameel, M.N., Zhang, P., Swingen, C., Kaufman, D.S., et al. (2010). A Fibrin Patch-Based Enhanced Delivery of Human Embryonic Stem Cell-Derived Vascular Cell Transplantation in a Porcine Model of Postinfarction LV Remodeling. Stem Cells, in press. Published online December 23, 2010. 10.1002/stem.580.

Zaret, K.S., and Grompe, M. (2008). Generation and regeneration of cells of the liver and pancreas. Science *322*, 1490–1494.

Zhang, S.C., Wernig, M., Duncan, I.D., Brüstle, O., and Thomson, J.A. (2001). In vitro differentiation of transplantable neural precursors from human embryonic stem cells. Nat. Biotechnol. *19*, 1129–1133.

Zhao, T., Zhang, Z.N., Rong, Z., and Xu, Y. (2011). Immunogenicity of induced pluripotent stem cells. Nature, in press. Published online May 13, 2011. 10.1038/nature10135.