CTGTAC Meeting # 45

Cellular Therapies Derived from Human Embryonic Stem Cells – Considerations for Pre-Clinical Safety Testing and Patient Monitoring

April 10, 2008

BRIEFING DOCUMENT

INTRODUCTION

This meeting is being convened to provide the Food and Drug Administration (FDA) with insight and perspectives regarding safety concerns confronting development of cellular therapies derived from human embryonic stem cells (hESCs). No specific products will be discussed for regulatory review purposes. Instead, invited experts and manufacturers who are developing cellular therapies derived from hESCs will present information on some of the issues concerning development of these types of products. Members of the committee will be requested to consider this information and provide a response to FDA questions. Discussion will be limited to characterization of hESC products, appropriate animal models for preclinical testing, and suitable monitoring for clinical studies.

BACKGROUND

Properties of Human Embryonic Stem Cells

There is considerable interest in development of cellular therapy products derived from human embryonic stem cells (hESCs), primarily due to their ability to self renew and proliferate while maintaining pluripotency and their capacity to differentiate in culture. These properties allow production of large numbers of undifferentiated hESCs that can then be induced to differentiate along specific cell lineages under carefully controlled manufacturing conditions. Due to the potential for hESCs to contribute to the repair or replacement of damaged or diseased cells and tissues, it is anticipated that differentiated cells derived from hESCs will be proposed as investigational cell therapy products for multiple clinical uses.

Human ESCs have an intrinsic capacity to generate teratomas (1, 2), which may contain differentiated cells originating from all three embryonic tissue types, endoderm, mesoderm, and ectoderm. This characteristic provides evidence of pluripotency but also raises a potential safety concern. When administered to animals in sufficient numbers, hESCs give rise to teratomas comprised of either differentiated or undifferentiated cell types, depending on the microenvironment at the site of administration(3-7).
It is conceivable that cell therapy products derived from hESCs will be heterogeneous in their composition and consist of cells that have differentiated to variable degrees. Residual undifferentiated hESCs and partially differentiated cells will retain the capacity to proliferate and differentiate further. A related potential safety concern is the ability of cells to migrate from their target site of administration and possibly undergo differentiation that is inappropriate to a non-target location.
The goal of the meeting is to obtain expert advice regarding product characterization, preclinical testing, and design of clinical studies sufficient to ensure patient safety in the first clinical trials of hESC-derived cell therapy products. FDA has considerable experience in the evaluation of investigational cell therapy products, and has published several relevant guidance documents to facilitate safe progress in this field (8, 9). However, the use of cellular products derived from hESCs present unique challenges worthy of further consideration.

PRODUCT CONSIDERATIONS

As with any investigational cell therapy product, detailed and comprehensive characterization of hESC source cultures and derivative cellular products is critical to ensuring the safety of a cellular therapy product derived from hESCs. An important approach to safe clinical use of this type of product will be to adopt manufacturing practices that minimize the number of undifferentiated hESCs present in the final formulated preparation. Reduction or elimination of undifferentiated hESCs from the final cellular product may be desirable or even necessary to reduce the potential for teratoma formation and diminish the possibility for inappropriate differentiation. Appropriate analytical methods will be needed to evaluate the products. The sensitivity, specificity, robustness, accuracy, and precision of assays used to characterize hESC-derived cellular products must be sufficient to provide a reasonable assurance of safety

when administered to humans. Assays used as process controls and for lot release should include tests capable of detecting unacceptable levels of undifferentiated hESCs or other cellular impurities in hESC-derived cellular products that may form tumors, differentiate inappropriately, or present other safety concerns.

To achieve these goals, several parameters should be tested to optimize the ability of analytical test results to predict the behavior of the cellular product *in vivo* reliably. Current methods for characterization of source hESCs and derivative cellular products include detection of stage-specific markers by flow cytometry, analysis of gene expression by RT-PCR, and analysis of protein expression by Western blot; analytical technology is evolving rapidly that will complement or replace these methods. The eventual choice of parameters that will be used to develop in-process and release tests will depend on the results of these characterization studies. Presently, there is no consensus in the field regarding the number, nature, or optimal methods for analyzing markers best suited to predict safety and efficacy of hESC-derived cellular products. Accordingly, it will be important to evaluate characteristics predictive not only of clinical effectiveness, but also of potential adverse outcomes. In the latter case, the question of analytical sensitivity is particularly important. The desired goal is to use the product characterization data in tandem with the animal data to determine a safe first-in-human starting dose level accurately and to optimize both safety and potential benefit of early phase and subsequent trials.

PRECLINICAL CONSIDERATIONS

According to Title 21 of the Code of Federal Regulations (CFR) Part 312.23 (a)(8), the sponsor of a clinical trial should provide *"…adequate information about the pharmacological and toxicological studies…on the basis of which the sponsor has concluded that it is reasonably safe to conduct the proposed clinical investigations. The kind, duration, and scope of animal and other tests required vary with the duration and nature of the proposed clinical investigations."* The design and conduct of the preclinical studies are thus critical to the regulatory decisions made in allowing the administration of a cellular therapy into humans.

To evaluate the safety of a hESC-derived cellular product *in vivo* adequately, comprehensive preclinical studies to identify and understand potential toxicities need to be conducted before entering clinical trials. Based on the biological properties of these cells, both the potential for tumorigenicity and the potential for inappropriate differentiation at a non-target location are significant safety concerns. It is therefore important to consider carefully the biological relevance of the animal species and animal models used to assess the *in vivo* safety of the hESC-derived cellular product. Selection of the most appropriate animal species and models is a major unresolved issue that revolves around the issue of immune tolerance to hESC-derived cellular products. Based on the biology of the hESC-derived cellular product, as well as the disease/injury of clinical focus, the route of administration, and other factors, more than one animal species may be needed to provide a comprehensive *in vivo* characterization and safety profile of these products.

In addition to the species used, the safety assessment of many cellular therapies has also made use of animal models of disease/injury that mimic some aspect of the pathophysiology of the proposed patient population. Such models help provide insight regarding dose/activity and dose/toxicity relationships. Thus, the applicability of such models in the context of possible species restrictions due to the biology and immunologic tolerance of the animals to hESC-derived cellular products should be addressed. These factors will affect cell fate. Thus cell survival, migration/trafficking, differentiation/mRNA or protein expression profile, integration (anatomical/functional), and proliferation also may need to be considered when selecting appropriate preclinical models prior to first administration into humans.

Animal Models for Preclinical Testing of hESC-derived Cellular Products

When conducting preclinical testing in an animal model, the impact of the immunosuppressive regimen or the immune deficient state of the animal model on the engraftment of the implanted hESC-derived cellular product needs to be assessed. Each hESC-derived cellular product may have unique patterns of proliferation and expression of antigens and will likely contain various ratios of differentiated and non-differentiated cells which can affect the biological actions of the administered product. These factors may affect what happens to the cells after administration and thus affect the safety and biological activity of the investigational hESC-derived cellular product in host animals. In order to allow for reasonable extrapolation of data generated in animals to humans, it is important to assess the engraftment potential of the cells in animal models. Animal models should be sufficiently sensitive to predict whether unacceptable levels of undesirable cells could be present in the final preparation, especially with regard to tumorigenic potential.

Immunological Tolerance to Cells of Human Origin

The criteria for selection of the host animal(s) in order to support engraftment of the hESC-derived cellular product need to be considered carefully. Ideally the animal model should be immunologically tolerant to cells of human origin. The effects of humoral and cellular immunity on hESCs implanted into mice are important considerations that have been investigated. For example, Drukker *et al.* (10) compared undifferentiated hESCs in immunocompetent (BALB/c, C57Bl, SJL, and CB6F1) and immunodeficient (NOD/SCID, C57BL/6J-Lyst^{bg}, Balb-nude, and CBA/CaHN Btk^{xid}) mice by implantation of 1 x 10^6 undifferentiated hESCs in kidney capsules. Over the course of one month, all implanted immunocompetent mice failed to develop teratomas, while the immunodeficient mice differed in their ability to reject hESCs. C57BL/6J-Lyst^{bg} (NK-deficient) and CBA/CaHN Btk^{xid} (B cell-deficient) mice failed to develop teratomas. In contrast, NOD/SCID mice (B and T cell-deficient) developed tumors. These results suggest that T cells play an important role in xenorejection of implanted hESCs. Tian *et al.* (11) compared teratoma formation following intramuscular implantation of hESCs in NOD/SCID (B and T cell-deficient) and SCID/Beige (SCID/Bg) mice (B, T, and NK cell-deficient). All implanted mice developed teratomas, but the tumors formed at a

faster rate in the SCID/Bg mice. This study suggests that NK cells may play a role in xenorejection of implanted hESCs.

Tian *et al.* (11) also studied the relationship between the immune status of the animal, engraftment potential of hESCs, and teratoma formation. The hESCs were allowed to differentiate on mouse bone marrow (BM) stromal cells for 7-24 days. The pre-differentiated hESCs cells were given 2 or 4 x 10^6 cells/mouse by intravenous or intra-medullary infusion in NOD-SCID mice (B and T cell-deficient) or in NOD-SCID mice that were pretreated with anti-ASGM1 to also delete NK cells. Mice were followed for 3 to 6 months. Although no teratomas were observed in any animal, NOD-SCID mice treated with anti-ASGM1antibody showed 3- to 10-fold better cell engraftment at 3 months post BM implantation than mice that were injected intravenously. These results suggest that NK cells play a role in xenorejection of hESC-derived cells and that antibody-mediated suppression of NK cells may enhance engraftment *in vivo*.

Erdo *et al.* (12) performed direct comparisons of allogeneic (mice) and xenogeneic (rats) administration of mouse embryonic stem cells (mESCs). The mESCs were implanted intracerebrally in mice or rats immunosuppressed with Cyclosporine A. A tumor incidence of 75-100% was observed in mice receiving 500 or more mESCs. In contrast, no tumors were detected in rats receiving 8 x 10^5 mESCs.

To address tumorigenic assay sensitivity, Lawrenz *et al.* (13) developed a spiking assay in an immunodeficient mouse model that could detect low numbers of mESCs present within a large dose of human fibroblasts. The mESCs were implanted into Balb/c nude mice using two different approaches. One method used subcutaneous injection of mixtures of cells embedded in Matrigel® containing 2 x 10^6 human fibroblasts spiked with different numbers of mESCs. The second method used kidney capsule implantation of mixtures containing 10^6 human fibroblasts and different numbers of mESCs. Both methods could detect teratoma formation in cell mixtures containing as few as two mESCs. No tumors were observed in immunocompetent mice.

These studies show increased sensitivity for detection of tumorigenic cells following administration of allogeneic compared to xenogeneic ESCs. In the allogeneic situation, immunosuppression is important for sensitive detection of tumorigenic cells. Thus, the immune status of the host animal, whether due to administration of exogenous immunosuppressive agents or the use of genetically immune-deficient animals, may be a major determinant of long-term *in vivo* outcome. Each model provides distinct advantages and limitations. Therefore the criteria for the selection of host animal(s) that support engraftment of the hESC-derived cellular product need to be adequate and justified with regard to proposed clinical trials.

Selecting Cell Dose Levels and Starting Cell Populations

Cell dose is an important consideration when designing preclinical animal studies, especially for cellular products that may consist of partially differentiated cells, fully differentiated cells, and residual undifferentiated hESCs. General recommendations for

preclinical study designs for cellular therapy products include several dose levels that bracket and exceed the anticipated clinical dose range, based on a predefined parameter, such as body weight or organ weight/size/volume. However, for hESC-derived cellular products, the potential contribution of the heterogeneous population of cells in the final clinical product to adverse findings, such as tumor formation and/or inappropriate differentiation, is an important issue that may affect selection of the cell doses used in animals.

The importance of reducing the numbers of undifferentiated hESCs in the final cellular product has been investigated in a rat model. Brederlau *et al.* (3) investigated the effect of pre-differentiation of hESCs on teratoma formation. The hESCs were differentiated in culture for various times, followed by administration of 10^5 hESCs into 6-OHDA lesioned striata of (female) hemi-Parkinsonian Sprague-Dawley rats. The animals were immunosuppressed transiently with cyclosporine A, beginning at one day prior to cell administration and continuing for two weeks. The rats were followed for 13 weeks. The incidence of teratoma formation correlated inversely with the pre-differentiation culture time. The incidence of teratoma formation was 100, 25, and 0% for hESC pre-differentiated for 16, 20, and 23 days, respectively. Notably, 82% mice implanted with 16-day pre-differentiated hESC were lost due to teratoma formation between 6 to 11 weeks post implantation mice, while only 25% mice implanted with 20-day pre-differentiated hESCs were lost due to teratoma formation between 12 to 13 weeks post implantation. Implanted cells that underwent longer *in vitro* pre-differentiation had increased numbers of β-III-tubulin (marker of progenitor cells) and tyrosine hydroxylase (marker of dopaminergic neurons) positive cells, with a corresponding decrease in Oct4 (marker of undifferentiated cells) positive cells in the brains of rats examined at 2 weeks post implantation**.** Overall, the data suggest that prolonged *in vitro* pre-differentiation of hESCs can reduce the incidence of teratomas *in vivo*. Thus, the relative composition of the cellular product in the host animal(s) is likely a major contributing factor to the formation of tumors; therefore the criteria for the selection and adequate characterization of cell dose need to be examined.

Selecting Site of Cell Administration

The anatomic location of the implantation site in animals is another important consideration. The local environmental niche of the host animal will affect cell survival and subsequent differentiation, and thus could 1) diminish or enhance the desired biological response or 2) result in misleading conclusions regarding the safety and effectiveness of a cellular product if cell fate is compromised. Cell fate could also be influenced differently by implantation into a normal microenvironment vs. a site of disease or injury. In addition, undesirable proliferation or differentiation that occurs in some anatomical sites may be more deleterious than for others; for example spinal cord or brain vs. peritoneal cavity.

Impact of the Host Microenvironment

The physiological environment and the anatomical location where cellular products are administered may exert a significant influence on safety. Shih *et al.* (14) investigated whether engraftment of human fetal tissues in severe combined immunodeficient (SCID) mice could provide a physiologically relevant microenvironment for hESCs to differentiate. Human fetal tissues from thymus, pancreas, and lung were engrafted under the kidney capsules of SCID mice. Three months later, 5×10^3 hESCs (two different lines) were implanted into the engrafted fetal tissues. The fetal tissues were harvested 2-3 months post cell implantation. Tumors were observed in all human thymus and lung grafts implanted with hESCs derived from the two different lines. Depending on the cell line administered, from 42-50% of the human pancreatic grafts had tumors. These tumors displayed an aggressive growth pattern, with histological characteristics of primitive, undifferentiated teratocarcinomas rather than non-malignant, differentiated teratomas. Tumor formation was dose dependent in the spleen and lung grafts at 8-12 weeks post cell implantation, with 0%, 25-35%, or 100% teratoma formation at doses of 50, 500, or 5000 cells, respectively. In contrast, approximately one million hESCs given in various anatomical sites in NOD-SCID mice were necessary for tumor development. These results suggest that the physiological environment and the anatomical location may exert a significant influence on tumor formation. Therefore the site of cell implantation in the animal host(s) that will be biologically relevant to the clinical situation needs to be considered with regard to the proposed clinical trail.

Determining Study Duration

The duration of preclinical studies should be adequate to assess the potential for tumorigenicity and other long-term consequences associated with administration of hESC-derived cellular products in humans. Optimally, animal studies should be extended for the lifespan of the animal, which will vary with the species, strain, disease/injury condition, and/or immune status. Based on these parameters however, study durations can potentially vary to a great extent, thus the question of extrapolation from resulting animal safety data to the clinical circumstance remains. Given the considerations presented in this document and the questions regarding relevant animal species/models that still remain, discussion regarding 1) the limitation of study duration intervals in animal studies and 2) the translation of animal study results to the safety profile of the hESC-derived cellular product in humans is warranted.

Safety Assessment

As with any investigational cell therapy product, it is important to understand the conditions under which undesirable events can occur, and to determine how these safety concerns can best be evaluated by *in vitro* and *in vivo* preclinical studies before clinical use. As expressed throughout this document, the tumorigenic potential of hESC-derived cellular products is a significant safety concern. In addition to the tumorigenicity question, the potential for other adverse events exists. Thus it is important to have the tools to assess endpoints of toxicity, such as ectopic tissue expression, inappropriate differentiation, and undesired phenotype expression.

CLINICAL CONSIDERATIONS

The decision to initiate clinical trials of hESC-derived cellular products requires consideration of factors that are common to trials of cell therapy products in general: the characteristics of the cellular product; the results of comprehensive pharmacology/toxicology studies in relevant animal models; the nature and severity of the targeted illness; the age, gender, and other demographic characteristics of the intended patient population; and the proposed anatomical site(s) of administration. As with all cellular therapies there is also need to consider the effects of concomitant medications and treatments on both the patient and the cellular product. The possibilities of immune rejection or other unanticipated immunological responses must be addressed as well. Early clinical trials of novel cell therapies should be designed to take all these factors into account, with enrollment criteria permitting maximum possible benefit to patients, given the potential risks.

In addition to these general principles that are applicable to all cellular therapies, there are special safety concerns for hESC-derived cellular products that need to be considered carefully in designing clinical trials.
It is expected that the administered hESC products may consist of cell populations comprised of fully differentiated cells; partially differentiated progenitor cells; and, possibly, low levels of undifferentiated hESCs. Subsets of this heterogeneous cell population may have the potential for functional integration, as well as de-differentiation, migration, further differentiation, proliferation, and tumor formation. Clinical trials must be capable of monitoring and detecting those events which may pose safety concerns. It is important to recognize that many of these potential adverse events may occur over protracted periods of time. Early manifestations of potential adverse events, including formation of teratomas or other tumors, may not be detectable with current non-invasive technology, which will include imaging as well as possible use of blood-borne markers. These considerations will be important in determining key design parameters for clinical trials of hESC-derived cellular products: duration of patient follow-up; selection of procedures for safety monitoring (e.g., conventional X-ray, ultrasound, CT and MRI scanning, PET scanning, testing of immune responses to the cellular product, and other clinical and laboratory modalities).

Early-phase clinical trials of all cell therapies expose subjects to potential risks that differ substantially from those associated with phase 1 drug trials. Accordingly, there are generally significant differences between the two product classes in early trial design. Cellular products cannot be subjected to terminal sterilization, and their pharmacological disposition is unpredictable; for some products, unchecked proliferation, as opposed to exponential decay for conventional drug, is a real possibility. Many indications under consideration are serious and/or life-threatening, but the life expectancy of the study population may be measurable in years or decades. The anatomical sites of administration (e.g., intracranial, intraspinal, intracardiac) proposed for many cellular products, including hESC-derived products, may pose additional risks arising from the surgical

procedures, the vulnerabilities of the sites themselves, and subsequent accessibility of the sites in the event of medical necessity, including removal of the product.

For cellular products, a reasonable balance between risk and benefit will be likely only in patients with the targeted disease. Given the additional safety concerns for hESCs, the risk-to-benefit evaluation is brought into even sharper focus.

Early-phase clinical trials of hESC-derived cellular products will have to be designed carefully in order to ensure the safety of enrolled subjects, who will undoubtedly be patients with the targeted disease. Given the potential risks of hESC-derived cellular products, data supporting a reasonable possibility of efficacy may need to be particularly strong, and design parameters may need to allow for detection of clinical benefit. As for all cell therapies, such expectations of potential therapeutic action are generally based on pre-clinical demonstrations of proof-of-concept, and specific requirements for such data will vary among products and clinical indications.

Given all of these considerations, many phase 1 trials of hESC-derived cellular products will have to be capable of measuring some indications of efficacy, or at least desirable therapeutic activity. These considerations of both safety and potential benefit will affect the selection of cell dose, as well as other characteristics of early-phase clinical trials.

DRAFT ADVISORY COMMITTEE DISCUSSION QUESTIONS

The availability and biological properties of human embryonic stem cells (hESCs) have spurred significant interest and effort towards development of new cell therapy products derived from them. Due to the abilities of hESCs to proliferate, differentiate, and form teratomas, the use of cellular therapies derived from hESCs raises several critical issues related to preclinical and product safety testing and patient monitoring. Preclinical evaluation of cellular therapies derived from hESCs should inform the rational, safe design of clinical trials, including identification of potential toxicities as well as the initial doses and dose escalation scheme to be used in a proposed clinical trial. Product characterization should include assessments that provide reasonable assurance of safety, in particular through sensitive measurements of potentially tumorigenic cells in the manufactured product. Patient monitoring should take into account the potential adverse events associated with use of hESCs. The following questions address critical issues related to the clinical use of hESC-derived cell therapy products.

1. Inappropriate Differentiation/Tumorigenicity

Characteristics of undifferentiated hESCs include their proliferative potential, their ability to differentiate, and their capacity to form teratomas. Please discuss optimal study designs for preclinical assessment of inappropriate differentiation, including tumorigenic potential, of an investigational cellular product derived from undifferentiated hESCs. Please consider the following in your discussion:

- Criteria for selection of clinically relevant animal species/models that support engraftment of the administered hESC cells, for example, optimal strategies for evaluating potential host (xeno) rejection of administered hESC-derived products?

- Optimal site of cell implantation in the animals in order to obtain meaningful test results.

- Appropriate study duration.

- Most appropriate dosing method, i.e., absolute undifferentiated hESC number vs. percentage of undifferentiated hESCs present in the product, to extrapolate cell doses tested in animals to planned clinical dose.

2. Characterization of hESC-Derived Cellular Preparations

Cellular products derived from hESCs may consist of heterogeneous cell populations, some that are required for the intended effect, some that may be deleterious, and some that are inert. Thus, detailed characterization of hESC-derived cellular products with respect to identity and purity is important. The goal of product characterization is to establish the relationship between analytical test results used in product characterization and the outcomes for preclinical/clinical studies. Identification of the putative therapeutic as well as undesired cell subtypes present in a cellular preparation is essential in order to extrapolate doses in animals to humans accurately. Please consider the following:

- Please discuss which product characteristics might be predictive of adverse events such as ectopic or inappropriate differentiation, including tumorigenesis or other undesired outcomes. Please include in your discussion the specificity and sensitivity of specific assays used to distinguish undifferentiated, appropriately differentiated, and inappropriately differentiated derivatives within a heterogeneous cell preparation.

3. Patient Monitoring

Safety monitoring of subjects during clinical trials of hESC-derived cellular products may be complicated by several characteristics of the product that may cause various clinical outcomes that could emerge over a protracted period. First-generation hESC-derived cellular products may consist of heterogeneous cell populations comprised of fully differentiated cell types, partially differentiated progenitor cells, and possibly, low levels of undifferentiated hESCs. Accordingly, cell products derived from hESCs may exhibit a variety of properties that reflect the specific cell mixture, including the capacity for proliferation, further differentiation, migration, and functional physiologic integration. Early-phase clinical studies are focused mainly on patient safety but, as described above, it may often be desirable or even necessary to provide for the possibility of some degree of beneficial therapeutic activity as well. Please consider the following:

- Taking into account the capabilities of existing analytical tools and non-invasive monitoring technologies, please discuss features of early phase clinical trial design that will facilitate monitoring of patient safety following administration of hESC-derived cellular products. Please comment on other trial design features, such as cell dosing, that can help to increase the probability of obtaining a measurable therapeutic benefit while ensuring maximum safety.

REFERENCES

1. Przyborski SA. (2005). Differentiation of human embryonic stem cells after transplantation in immune-deficient mice. Stem Cells 23:1242-1250.
2. Blum B and N Benvenisty. (2007). Clonal analysis of human embryonic stem cell differentiation into teratomas. Stem Cells 25:1924-1930.
3. Brederlau A, AS Correia, SV Anisimov, M Elmi, G Paul, L Roybon, A Morizane, F Bergquist, I Riebe, U Nannmark, M Carta, E Hanse, J Takahashi, Y Sasai, K Funa, P Brundin, PS Eriksson and JY Li. (2006). Transplantation of human embryonic stem cell-derived cells to a rat model of Parkinson's disease: effect of in vitro differentiation on graft survival and teratoma formation. Stem Cells 24:1433-1440.
4. Caspi O, I Huber, I Kehat, M Habib, G Arbel, A Gepstein, L Yankelson, D Aronson, R Beyar and L Gepstein. (2007). Transplantation of human embryonic stem cell-derived cardiomyocytes improves myocardial performance in infarcted rat hearts. J Am Coll Cardiol 50:1884-1893.
5. Leor J, S Gerecht-Nir, S Cohen, L Miller, R Holbova, A Ziskind, M Shachar, MS Feinberg, E Guetta and J Itskovitz-Eldor. (2007). Human embryonic stem cell transplantation to repair the infarcted myocardium. Heart.
6. Phillips BW, H Hentze, WL Rust, QP Chen, H Chipperfield, EK Tan, S Abraham, A Sadasivam, PL Soong, ST Wang, R Lim, W Sun, A Colman and NR Dunn. (2007). Directed differentiation of human embryonic stem cells into the pancreatic endocrine lineage. Stem Cells Dev 16:561-578.
7. Oottamasathien S, Y Wang, K Williams, OE Franco, ML Wills, JC Thomas, K Saba, AR Sharif-Afshar, JH Makari, NA Bhowmick, RT DeMarco, S Hipkens, M Magnuson, JW Brock, 3rd, SW Hayward, JCt Pope and RJ Matusik. (2007). Directed differentiation of embryonic stem cells into bladder tissue. Dev Biol 304:556-566.
8. FDA/CBER/OCTGT. (2008). Cellular and Gene Therapy publications http://www.fda.gov/cber/gene.htm.
9. FDA/CBER/OCTGT. (2003). Guidance for Reviewers: Instructions and Template for Chemistry, Manufacturing, and Control (CMC) Reviewers of Human Somatic Cell Therapy Investigational New Drug Applications (INDs)http://www.fda.gov/cber/gdlns/cmcsomcell.htm.
10. Drukker M, H Katchman, G Katz, S Even-Tov Friedman, E Shezen, E Hornstein, O Mandelboim, Y Reisner and N Benvenisty. (2006). Human embryonic stem cells and their differentiated derivatives are less susceptible to immune rejection than adult cells. Stem Cells 24:221-229.
11. Tian X, PS Woll, JK Morris, JL Linehan and DS Kaufman. (2006). Hematopoietic engraftment of human embryonic stem cell-derived cells is regulated by recipient innate immunity. Stem Cells 24:1370-1380.
12. Erdo F, C Buhrle, J Blunk, M Hoehn, Y Xia, B Fleischmann, M Focking, E Kustermann, E Kolossov, J Hescheler, KA Hossmann and T Trapp. (2003). Host-dependent tumorigenesis of embryonic stem cell transplantation in experimental stroke. J Cereb Blood Flow Metab 23:780-785.
13. Lawrenz B, H Schiller, E Willbold, M Ruediger, A Muhs and S Esser. (2004). Highly sensitive biosafety model for stem-cell-derived grafts. Cytotherapy 6:212-222.
14. Shih CC, SJ Forman, P Chu and M Slovak. (2007). Human embryonic stem cells are prone to generate primitive, undifferentiated tumors in engrafted human fetal tissues in severe combined immunodeficient mice. Stem Cells Dev 16:893-902.