Discussion Agenda

- Allogeneic vs Autologous Cell Products
- Pluripotent Stem Cells
- Human embryonic stem cells
  - Characterization of starting material and cell product
  - In vivo assessment of safety – tumorigenicity
- Induced pluripotent stem cells
  - The autologous cell therapy model
Pluripotent Stem Cells: Allogeneic vs Autologous Therapy

- Allogeneic
  - MCBs used for multiple targets and multiple donors
    - hESC
    - iPSC

- Autologous
  - “MCBs” used for individuals, personalized medicine
    - Nuclear transfer hESCs
    - Patient specific iPSCs
Pluripotent Stem Cells

- Embryonic carcinoma cells
- Embryonic germ cells
- Epiblast cells
- Embryonic stem cells
- Induced pluripotent cells
Considerations for Human Embryonic Stem Cell Products
Human Embryonic Stem Cells: Derivation

- Remove ICM
- Irradiated Mouse Embryonic Feeders
- hES Cell Line
Pluripotent Stem Cells Differentiate into 3 Germ Layers

- Undifferentiated hESCs
- Mesoderm
- Endoderm
- Ectoderm
- Cardiomyocytes
- “Islets”
- Neurons
Regulatory Issues Result from Fundamental Characteristics of Living Cells

- Cells change over time in vitro and in vivo
- Cells exist in a heterogeneous environment
- Cells integrate and migrate after transplantation
- Cells will interact with host system
Defining Characteristics of hESCs Result in Safety Concerns

- Unlimited Proliferative capacity
  - Concerns about stability over long term culture
- Pluripotency
  - Concerns about teratoma formation
Expansion & Differentiation of Stem Cells

Starting Material → Undifferentiated Stem Cell → Differentiation → Cell Product

As many as 100-300 population doublings

Differentiated Cell
Cytogenetic Analysis of hESCs

H9 p98

H1

H7

H9

Rosler et al., 2004
Cytogenetic Analysis

- **G-Banding**
  - Allows detection of numerical abnormalities, inter-chromosomal abnormalities, intra-chromosomal abnormalities
  - Performed in cytogenetics lab
  - 20 cells or more examined
  - Clinically correlated

- **Fluorescence In Situ Hybridization**
  - Screen for microdeletions/duplication of known targets

- **Spectral Karyotype (SKY) analysis**
  - Allows detection of unknown rearrangements

- **Comparative Genomic Hybridization**
  - Detects submicroscopic abnormalities (<5Mb)
  - Genomic copy number variation
Cytogenetic Analysis of hESCs in Long Term Culture

- References demonstrating stable phenotype and karyotype over long-term culture

- References demonstrating that hESCs acquire abnormal karyotypes similar to human embryonic carcinoma cells

- References which identified recurrent chromosomal abnormalities associated with oncogenic transformation
Stages of Characterization of Cell Product

Starting Material → Cell Product

Stem Cell → Differentiated Cell

Differentiation

- Marker expression
- Viability
- Consistent Composition
- Stable Karyotype

What will be your release criteria?

What will be on your CofA?
Identity Analysis Includes Assessment of Different Populations in Product

- Cell Product might be a heterogeneous population

- Cell Product assessment will include:
  - “Functional” cell
  - Accessory cells
  - Inappropriate cells
    - Undifferentiated cells
    - Cytotoxic cells
  - “Bystander” cells
In Vivo Evaluation of Cell Product

- Efficacy
  - Disease models

- Safety
  - Dosing/Toxicity
  - Biodistribution
    - Where do the cells go?
    - Maintain identity if found in other tissues?

- Stability
  - Functional stability
  - De-differentiated cells?

- Tumorigenicity
What is the Relevant Animal Model?

- Many cell based products are species-specific

- Will large animal studies be meaningful?
  - Is there a suitable large animal model?
All Pluripotent Stem Cells are NOT Equal: Origin May Influence Tumorigenicity

- Human ESC does not equal mouse ESC
  - Single cell cloning
  - Requirements for self-renewal are different
  - Efficiency of teratoma formation
  - Ability to Differentiate
- Human ESC does not equal human iPSC

<table>
<thead>
<tr>
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<th>Mouse ESC</th>
<th>Human ESC</th>
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<tr>
<td>Morphological Character</td>
<td>Rounded colonies</td>
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<td>Spontaneous Trophoblast Differentiation</td>
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Considerations for cell lines that are tumorigenic or tumor-derived

You should assess cell lines that are tumorigenic or tumor-derived for potential oncogenic viruses and oncogenic substances (including nucleic acids) which could be associated with induction of a neoplastic process in a vaccine recipient.

Test strategies …may be determined on a case-by-case basis, depending on the tissue type, source species, passage history, and extent of knowledge of the transforming event(s).

Tumorigenicity is defined as the process by which cells form tumors when inoculated into animals (generally a syngeneic, an immunosuppressed allogeneic or an immunosuppressed xenogeneic host).

The goal … is to determine whether your cell substrate is capable of forming tumors after inoculation into animals.
Considerations associated with tumorigenicity testing of cell substrates

- **Choice of appropriate animal models**
  - Known to be susceptible to tumor formation by tumorigenic cells
    - Most commonly used nude (nu/nu) mice; newborn nude mice might be best choice for weakly tumorigenic phenotype

- **Definition of a positive result**
  - Progressive tumor formation at the site of injection
  - Some cell types may also cause tumors at distant sites
    - Confirm at necropsy by molecular or immunological methods

- **Determination of appropriate duration of testing**
  - Balance increased sensitivity of longer test, against likelihood of false positive
  - *Weekly tumorigenic cells* might require between 4 and 7 months to form tumors

- **Determination of appropriate numbers of cells to be tested**
  - $10^7$ test cells or positive control cells in 0.2mL (0.1mL newborns) via sc
  - **10 animals/test group** [at least 9/10 positive control animals must be positive]
Tumorigenicity: What is the Appropriate Assay for hESC products?

- How many ES cells does it take to make a teratoma?
  - Is there an absolute number of cells required?
  - Is there a frequency required (percentage of cells)?
  - Needs to be measured for each cell line, each product?

- What is the effect of implant site on teratoma formation?
  - Are some sites more permissive?
  - Do the neighboring cells (from graft or from implant site) influence teratoma formation?

- Are other cell types tumorigenic?

- Does the immune status of the recipient affect teratoma formation?

- What does a negative result mean?
Teratoma vs Teratocarcinoma

- **Teratoma** = benign tumor

- **Teratocarcinoma** = malignant tumor
  - Primitive embryonic cells
  - Usually neuroepithelium
  - Extraembryonic cell types
  - Absence of a clear capsule or boundary

- Risk of teratoma formation will be balanced with patient population and implant site

Rosler et al 2004
Influence of Environment on Teratoma Formation

- Effect cell survival
- Effect cell differentiation

Cooke et al 2006
Considerations for Human Induced Pluripotent Stem Cell Products
Generating Autologous Cell Products from iPSCs

Variation can result from:

• Clonal selection
• Tissue source
• Donor

Harvest tissue → Dissociate → Reprogram cells using genes, proteins, chemicals → Clonal selection

Selection of stable cell → Expand cell for banking → Generate Cell Bank

Harvest & Process → Thaw, expand and differentiate
Considerations for Using iPSCs

- iPSCs from different tissues sources are not equivalent
  - Different gene expression patterns by genome-wide transcriptional analysis
  - Different methylation patterns
  - Show differences in differentiation – cell lines show bias toward cell types of origin
  - Different efficiencies for teratoma formation

- iPSCs show different methylation patterns than ESCs or ntESCs
  - iPSCs appear to have “epigenetic memory”
  - Cells generated by nuclear transfer are “closer to the ground state of pluripotency”

- These patterns change over time in culture
  - Continuous passaging eliminates the transcriptional, epigenetic and differentiation differences
Summary

- Development of cell products from pluripotent stem cells has unique challenges
  - Stability of starting material
  - Stability of cell product

- Tumorigenicity can be impacted by
  - Cell number
  - Implant site
  - Cell line and cell type

- Autologous cell therapies using iPSC cells will require development of predictive assays