Preclinical Considerations for Stem Cell Therapies: Pluripotent Stem Cells

September 28, 2010

CARPENTER GROUP
Stem Cell Strategies

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

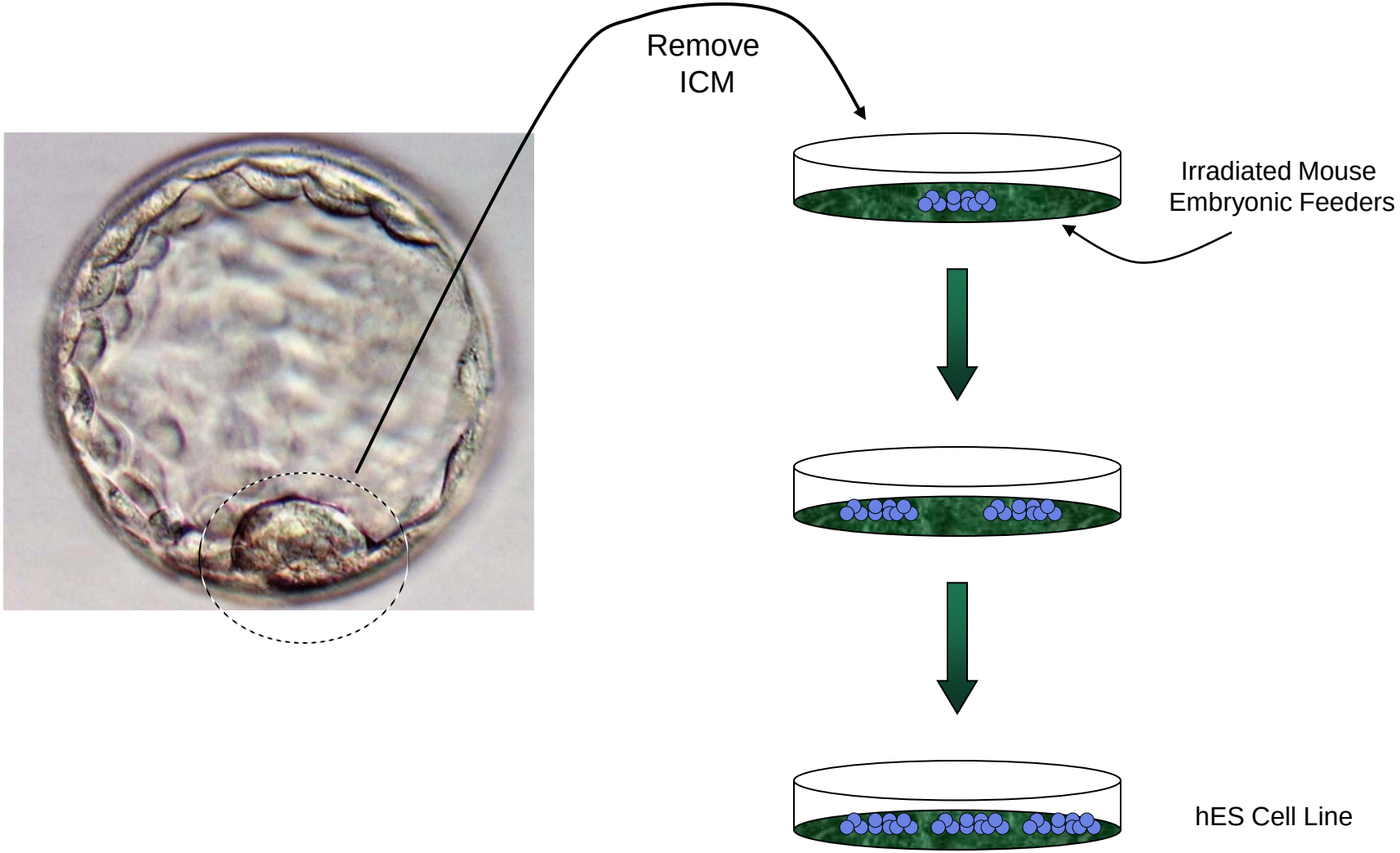
- Allogeneic
  - MCBs used for multiple targets and multiple donors
    - hESC
    - iPSC
- Autologous
  - “MCBs” used for individuals, personalized medicine
    - Nuclear transfer hESCs
    - Patient specific iPSCs

Pluripotent Stem Cells

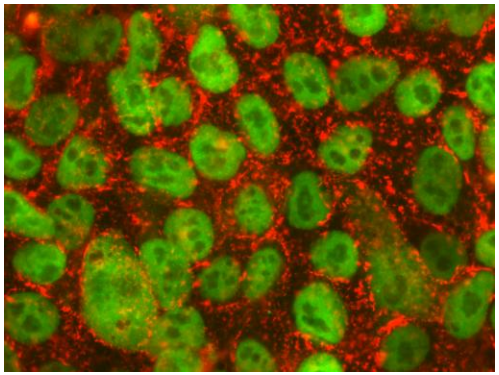
- Embryonic carcinoma cells
- Embryonic germ cells
- Epiblast cells
- **Embryonic stem cells**
- **Induced pluripotent cells**

Considerations for Human Embryonic Stem Cell Products

Human Embryonic Stem Cells: Derivation



Pluripotent Stem Cells Differentiate into 3 Germ Layers



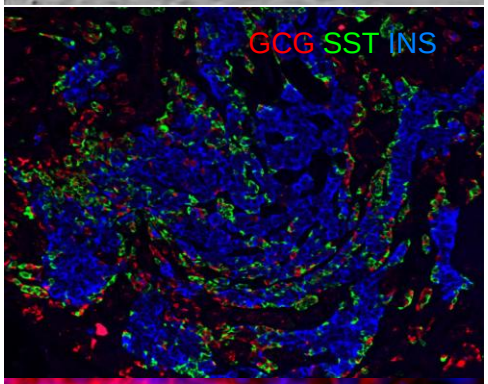
Undifferentiated hESCs

Mesoderm

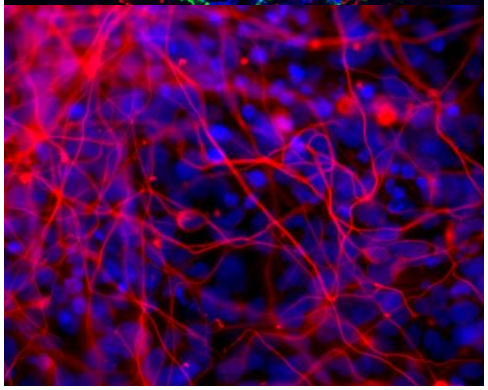
Endoderm

Ectoderm

Cardiomyocytes



“Islets”



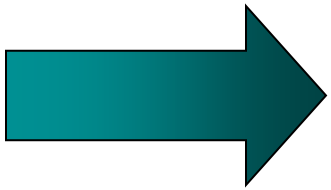
Neurons

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

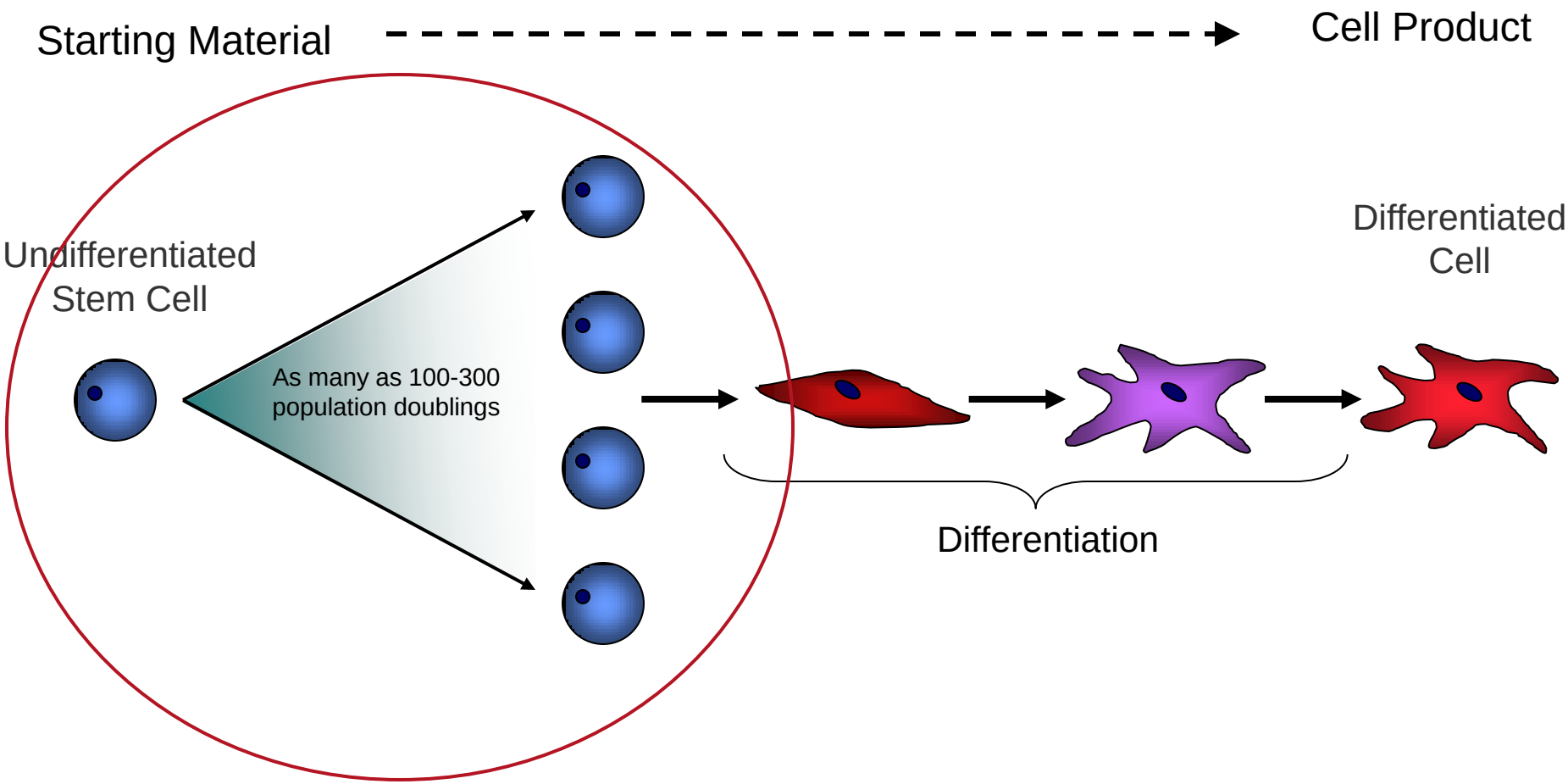
Defining Characteristics of hESCs Result in Safety Concerns

- Unlimited Proliferative capacity
  - Concerns about stability over long term culture
- Pluripotency
  - Concerns about teratoma formation



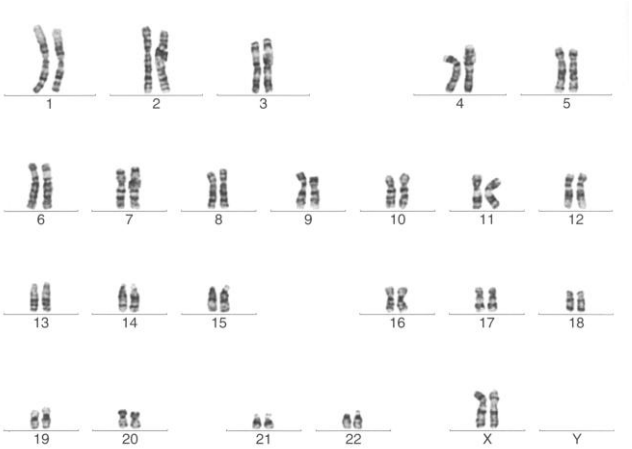
Safety Concerns

Expansion & Differentiation of Stem Cells

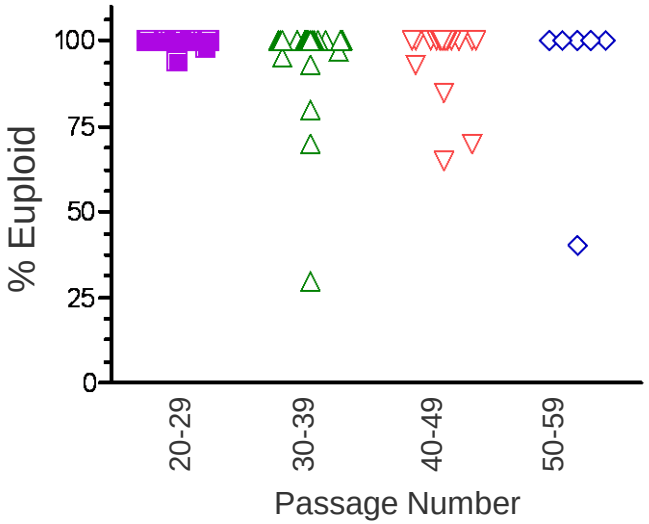


Cytogenetic Analysis of hESCs

H9 p98



H1



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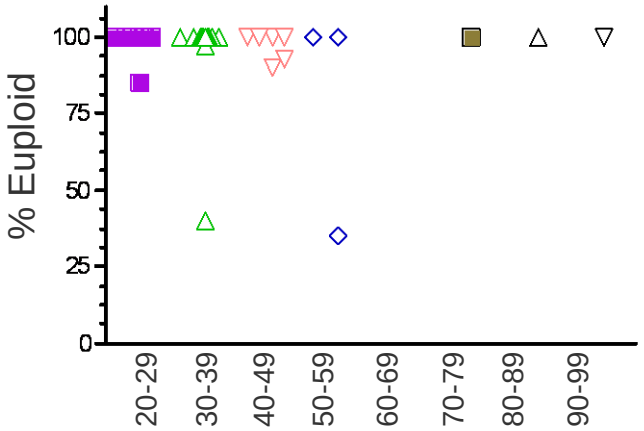
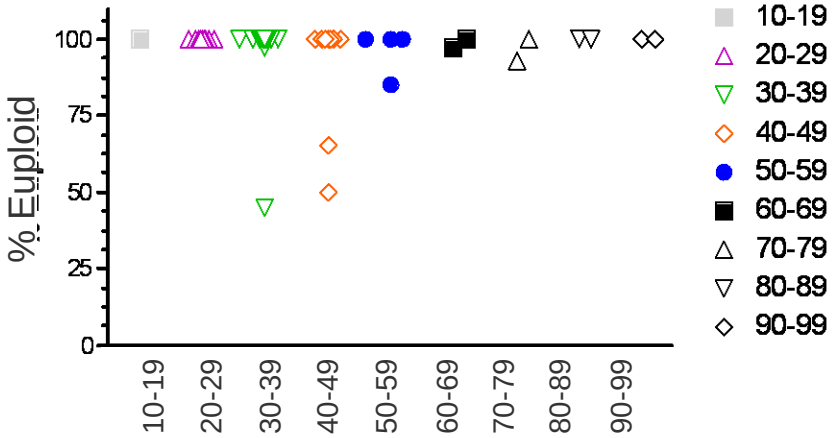


H9



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

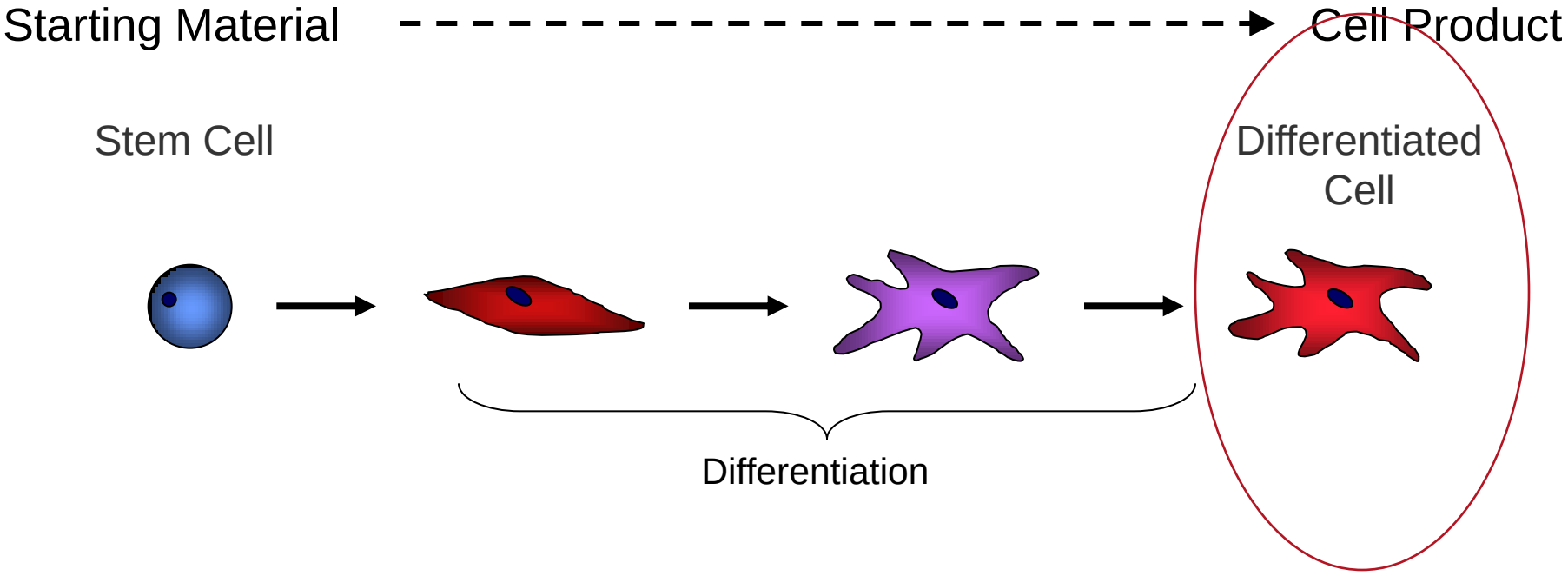
Cytogenetic Analysis of hESCs in Long Term Culture

- References demonstrating stable phenotype and karyotype over long-term culture
  - Rosler et al Dev Dyn 229, 259-274 (2004)
  - Brimble et al Stem Cells 13, 585-597 (2004)
  - Draper et al Nature Biotech 22 (2004)

- References demonstrating that hESCs acquire abnormal karyotypes similar to human embryonic carcinoma cells
  - Rugg-Gunn et al Nat Genet 37, 585-587 (2005)
  - Sun et al Hum Mol Genet 15, 65-75 (2006)
  - Draper et al Nature Biotech 22 (2004)

- References which identified recurrent chromosomal abnormalities associated with oncogenic transformation
  - Lefort et al Nat Biotech 26, 1364-1366 (2008)
  - Spits et al Nat Biotech 26, 1361-1363 (2008)

Stages of Characterization of Cell Product

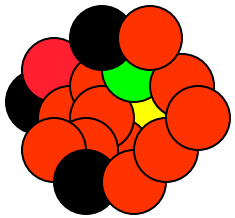


- Marker expression
- Viability
- Consistent Composition
- Stable Karyotype

- 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**

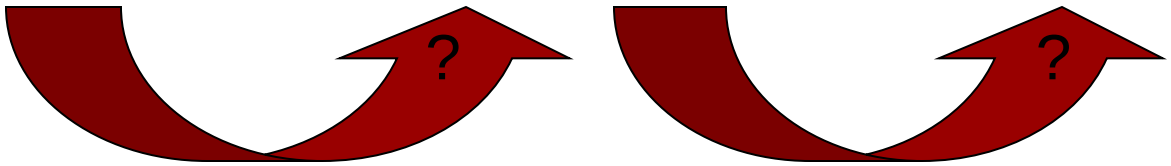


In Vivo Evaluation of Cell Product

- 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

	Mouse ESC	Human ESC
Morphological Character	**Rounded colonies**	**Flat colonies**
Growth Requirements	**LIF, BMP**	**bFGF, activin**
Marker Expression	**SSEA-1**	**SSEA-4**
Spontaneous Trophoblast Differentiation	**no**	**yes**

Considerations for cell lines that are tumorigenic or tumor-derived

Guidance for Industry

Characterization and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infectious Disease Indications

Additional copies of this guidance are available from the Office of Communication, Outreach and Development (OCOD) (HFM-40), 1401 Rockville Pike, Suite 200N, Rockville, MD 20852-1448, or by calling 1-800-835-4709 or 301-827-1800, or email ocod@fda.hhs.gov, or from the Internet at http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/default.htm

For questions on the content of this guidance, contact OCOD at the phone numbers listed above.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
[February 2010]

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).

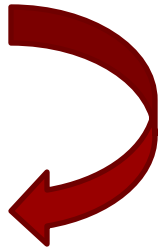
Tumorigenicity is defined as the process by which cells form tumors when inoculated into animals (generally a syngeneic, an immunosuppressed allogeneic or an immunosuppressed xenogeneic host).

The goal … is to determine whether your cell substrate is capable of forming tumors after inoculation into animals.

From Joy Cavagnaro, ISCT meeting Sept 27, 2010

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]



From Joy Cavagnaro, ISCT meeting Sept 27, 2010

Tumorigenicity: What is the Appropriate Assay for hESC products?

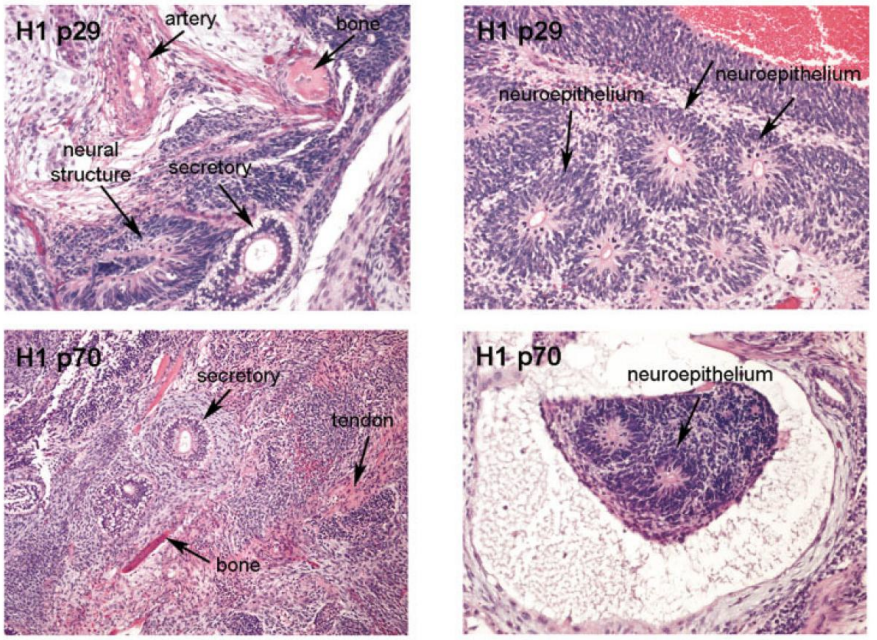
- How many ES cells does it take to make a teratoma?
  - Is there an absolute number of cells required?
  - Is there a frequency required (percentage of cells)?
  - Needs to be measured for each cell line, each product?
- What is the effect of implant site on teratoma formation?
  - Are some sites more 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

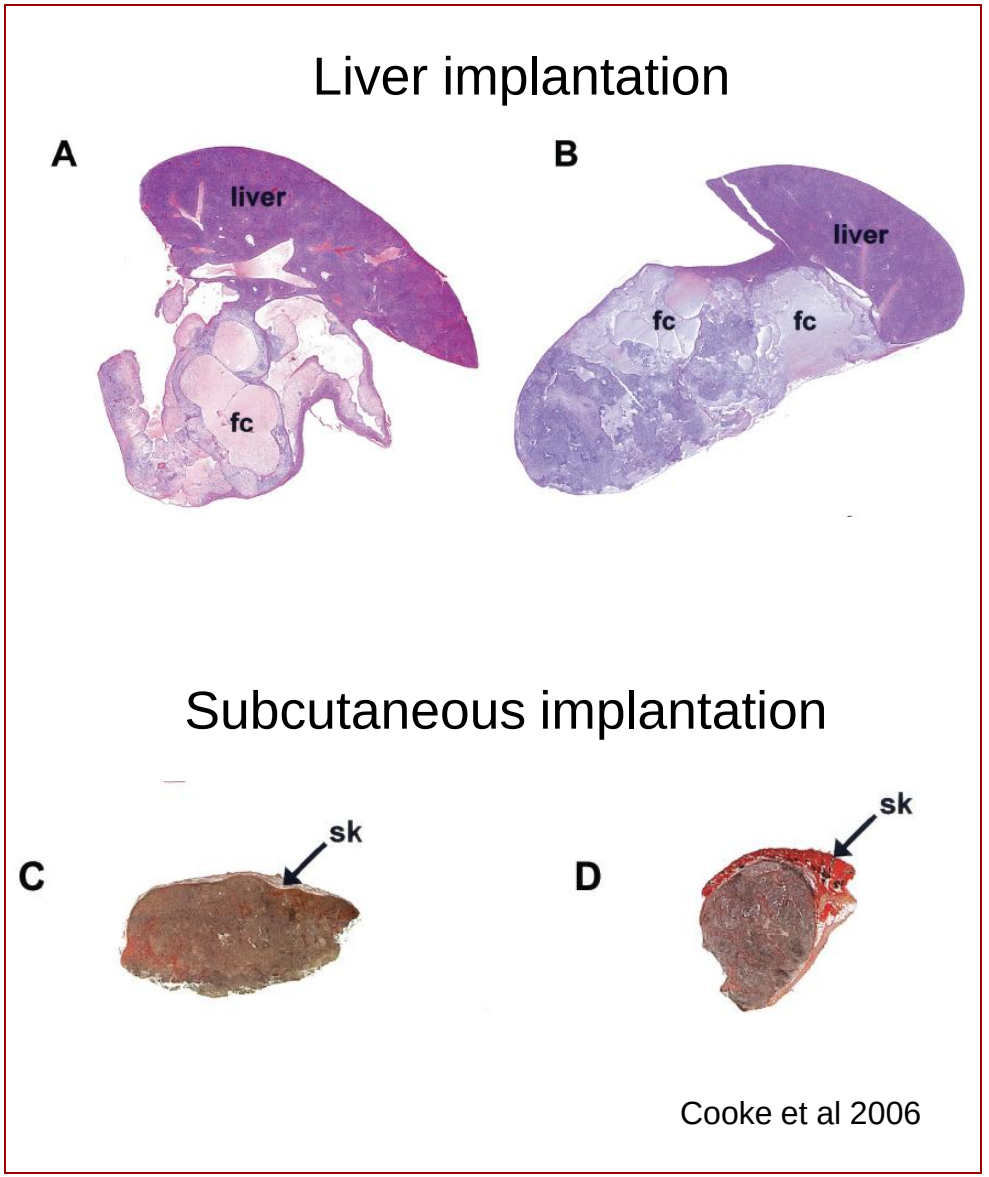
- Risk of teratoma formation will be balanced with patient population and implant site



Rosler et al 2004

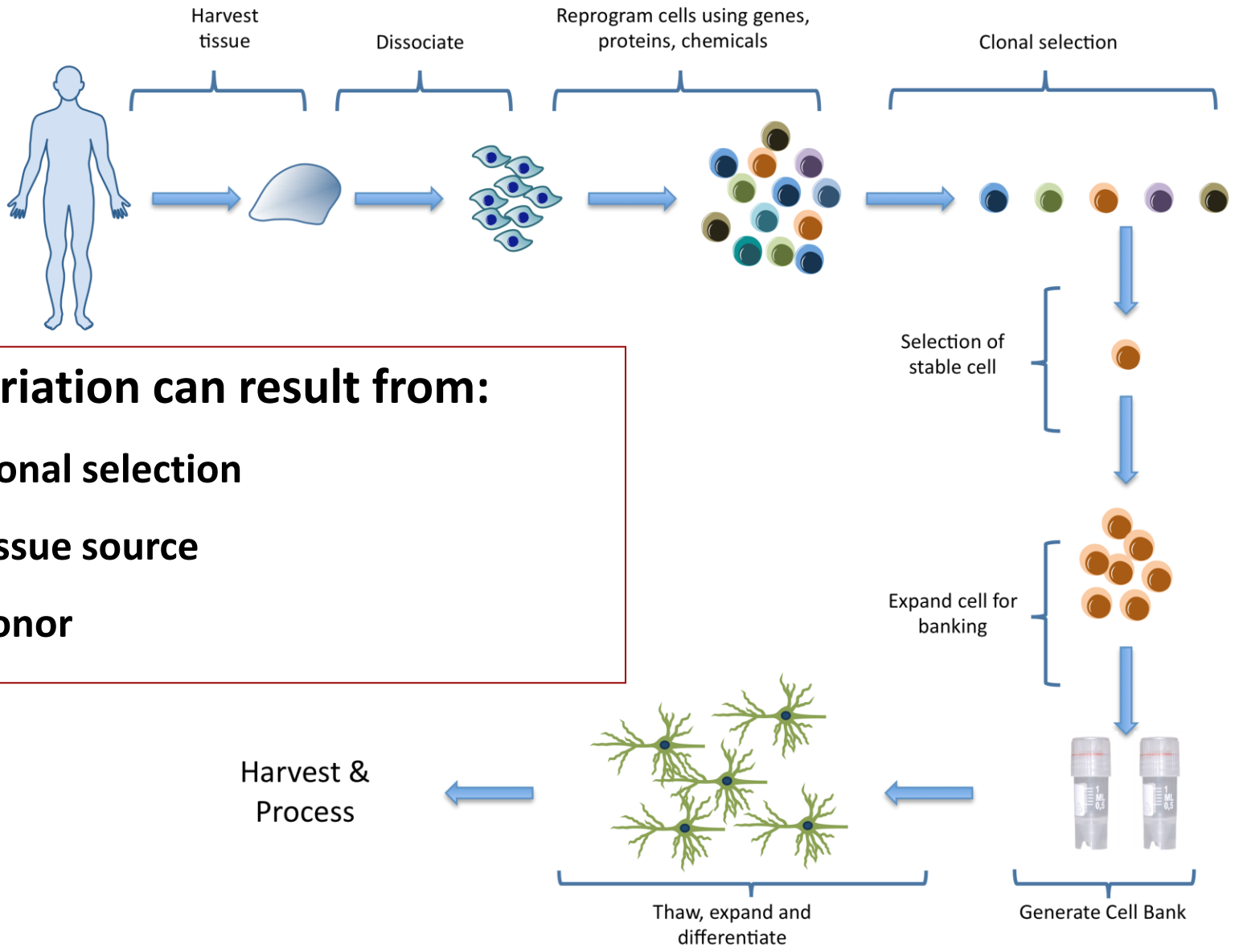
Influence of Environment on Teratoma Formation

- Effect cell survival
- Effect cell differentiation



Considerations for Human Induced Pluripotent Stem Cell Products

Generating Autologous Cell Products from iPSCs



Variation can result from:

- **Clonal selection**
- **Tissue source**
- **Donor**

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