Evaluating the Safety of Stem Cell Therapies: A Regulatory View

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Objectives

- This talk does not contain any clear recommendations
- There are no generalised right or wrong answers or approaches
  - case-by-case
  - dependent on cell, indication, MoA etc.
- Primarily intended to be thought-provoking

***Note**: necessary generalisations made in this talk may not be true in all situations*

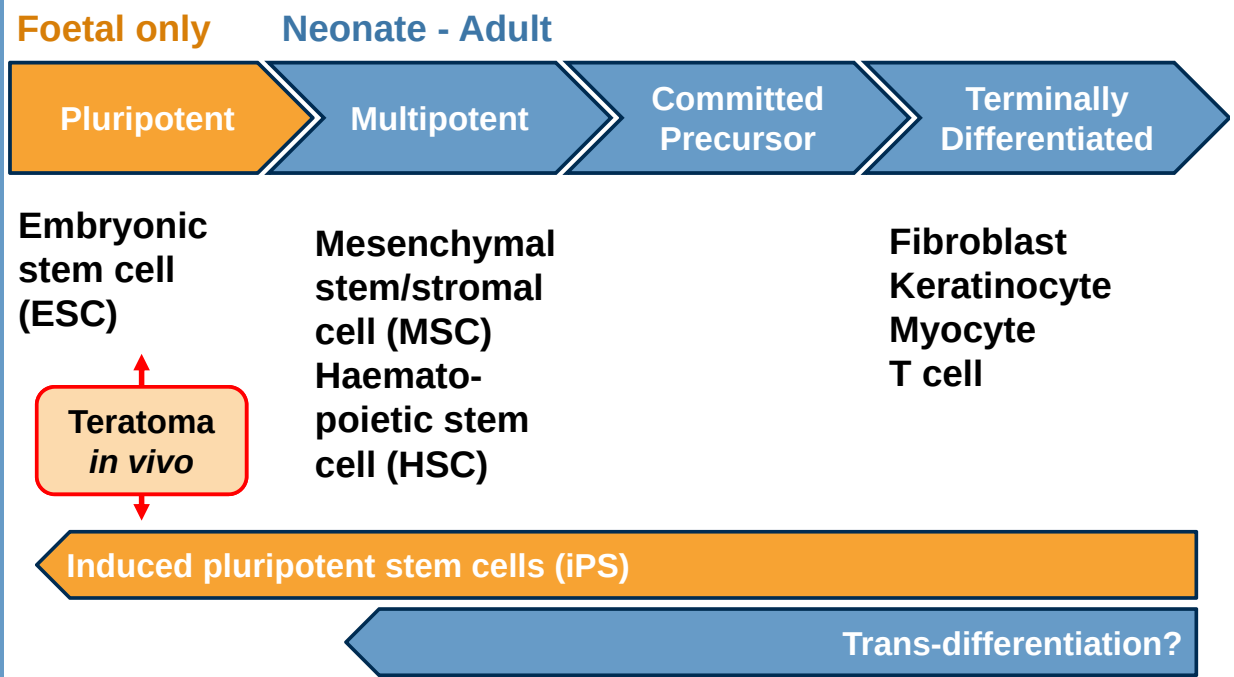
Consulting on Advanced Biologicals

Mechanism/s of Action

For simplicity;

- MoA = assumed or best working theory of the mechanism or mechanisms of action.
  - It is a given these will likely not be known for sure
  - One of your objectives is to generate evidence to support your working theory
  - *Another is not to avoid generating data that don't support it*

When is a cell a stem cell?



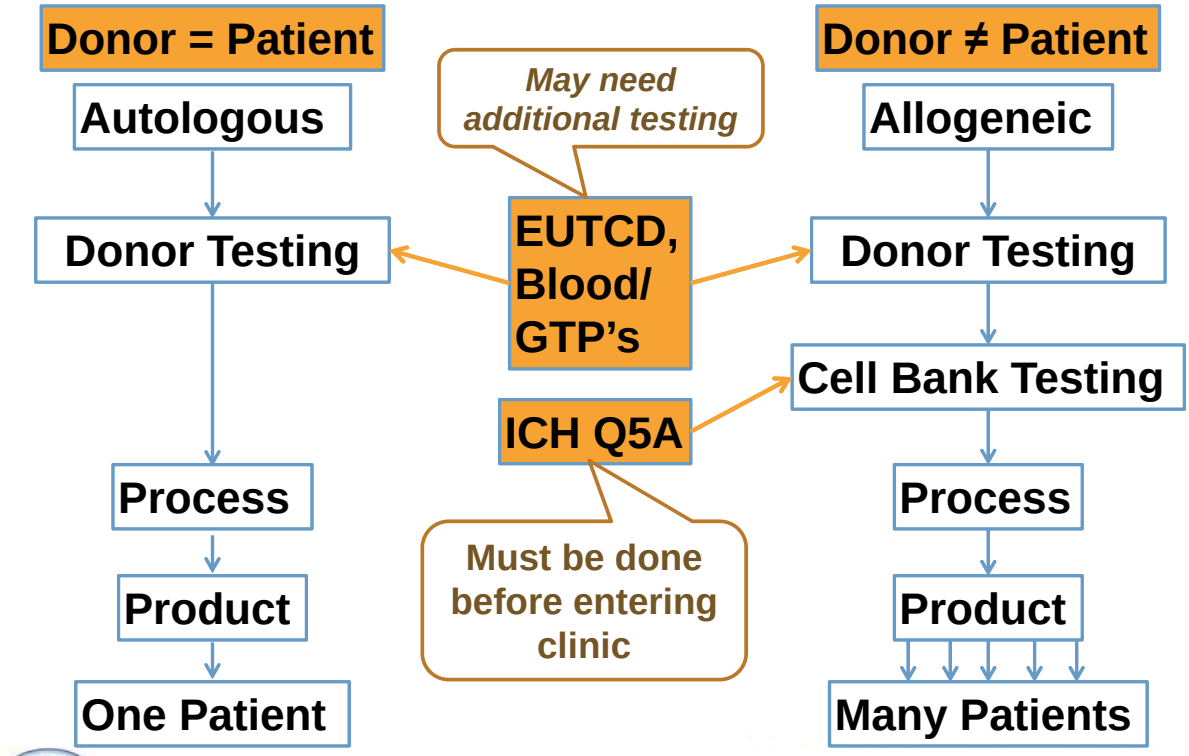
Some unique issues with cells

- Cells are alive
  - Change constantly to environment
- Pose unusual safety issues
  - Cannot be sterilised
  - Cannot incorporate viral clearance steps
- Might persist for the patient's lifetime
- Might form tumours
  - Inherent characteristics
  - As a consequence of manufacturing (e.g. chromosomal damage, growth factor/cytokine exposure

Control at Raw Materials Level

- Need to consider the source of material
- Since raw materials will come in contact with living cell product/intermediates risk of transmission of disease
  - *Appropriate viral testing*
  - *EDQM certificate suitability (TSE risk)*
- *In many cases good quality isn't easily available*
  - *Research grade only*
  - *GMP preferable but not essential*

Starting Material Testing



See also guideline for viral testing of IMPs (not specific to cell products)
http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003798.pdf

Donor Testing Requirements

Screening/Testing	FDA	EMA
Testing Requirements HIV 1&2, HBC, HCV, HTLV 1&2 (leukocyte rich cells only) Chlamydia and Gonorrhea (for reproductive cells only	• Test Methods not specified • Syphilis, West Nile Virus	• EMA Methods Specified (Ab vs. Ag) • Member States additional testing
Donor Test Kits	FDA Approved/Cleared	CE Marked/NCA approved
Travel History	Specific regions identified	General Assessment for risk
Medical History	Assess for risk factors	Additional – Specified chronic autoimmune diseases deferral
Responsible Person	No requirements	Minimal requirements
Collection Site	Registered with FDA	Registered Tissue Establishment with Member States
Donor Record Retention	10 years	30 years
Autologous somatic cells	No donor testing requirements	Same as allogeneic somatic cells

FDA Donor Eligibility - TSE Risk

Guidance for Industry: Eligibility Determination for Donors of Human Cells, Tissues, and Cellular and Tissue-Based Products, FDA 2007

LIST OF BSE-AFFECTED COUNTRIES APPLICABLE TO DONOR DEFERRAL

European Countries to be Used for Deferral of Donors Based on Geographic Risk of BSE

Albania, Austria, Belgium, Bosnia-Herzegovina, Bulgaria, Croatia, Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Liechtenstein, Luxembourg, Macedonia, Netherlands, Norway, Poland, Portugal, Romania, Slovak Republic, Slovenia, Spain, Sweden, Switzerland, United Kingdom[1], and Yugoslavia.

[1]For purposes of this guidance, the United Kingdom should include all of the following: England, Northern Ireland, Scotland, Wales, the Isle of Man, the Channel Islands, Gibraltar, and the Falkland Islands.

Possible Tumorigenicity Risk Factors

- Expansion – high population doubling level (PDL)
- Cumulative cell damage due to repeated enzyme exposure (e.g. passaging)
- Exposure to cytokines and growth factors
  - Minimise exposure
- Presence of undifferentiated pluripotent stem cells (i.e. ESC and iPSC products)
  - Suitably sensitive methods to detect residual stem cells

Exploring Tumorigenicity Risk

- Evidence for eventual cell senescence
  - Fully differentiated cells such a chondrocytes may be sufficient (i.e. senesce at low PDL)
  - No evidence for senescence may be worrying (e.g. pluripotent or transformed cells)
- End of production/post-production cells
  - Tumorigenicity testing in animals
  - *In vitro* methods, e.g. soft agar cultures
  - Chromosomal aberrations

Are animal models suitable for safety testing?

- Animal stem cell niche may not be suitable for human cells
  - Different/incompatible adhesion molecules, cytokines, growth factors
  - Immune clearance before safety issues arise, e.g. tumorigenicity
  - Immunodeficient animals are ‘abnormal’
    - More susceptible tumours
    - Suitable pos/neg control cells

Some of the general arguments

Immunodeficient animals

Mutant allele	Common strain name	Strain nomenclature	Phenotype	Advantages	Disadvantages	Refs
Foxn1^{nu}	C57BL/6-*nu*	B6.Cg-*Foxn1*^{nu}	• Athymic	• Lacks T cells	• High NK-cell activity • Intact humoral immunity • No engraftment of human haematopoietic cells	28,136
Prkdc^{scid}	CB17-*scid*	C.BKa *Igh*^{b}-*Prkdc*^{scid}/IcrSmn	• No mature T and B cells • Radiation sensitive (DNA-repair defect, cannot survive high doses of radiation)	• Lacks mature T and B cells	• High level of innate immunity and NK-cell function • Leaky • Very low level of engraftment of human cells	1
Prkdc^{scid}	NOD-*scid*	NOD.CB17-*Prkdc*^{scid}	• No mature T and B cells • Radiation sensitive • Decreased innate immunity	• Low level of innate immunity • Low NK-cell function • Increased engraftment of human HSCs and PBMCs	• Residual innate immunity • Low but present NK-cell activity • Decreased lifespan owing to thymic lymphomas	9

Shulz *et al Nat Rev Immunol. 2007 Feb;7(2):118-30.*

Homologous Product

- Can then be studies in syngeneic/allogeneic setting.
  - Physiological processes should be intact (PoC)
- The manufacturing process is designed for human cells and may not result in a sufficiently homologous product with animal cells
  - Would need to be altered (GF, cytokines, serum, selection mAbs, culture media etc)
  - Product would therefore not be representative of what will be given to humans

Immunodeficient animals

- Understand the nature of the animal
- Not all arms of the immune system will necessarily be defective
- Innate responses commonly present
- These often quite important for xenogeneic antigens
- You could include immunosuppression
  - May complicate interpretation
  - Best avoided unless intend to use in clinic also

So which model systems should I use?

- Key is to identify which model might be the most predictive of the human situation (for the aspect under study)
- Animals or in vitro or ex vivo organ culture should all be considered

Conclusions

- Cells (including stem cells) pose a range of novel issues for safety testing
- Safety testing starts with appropriate testing of materials
- Using animals to study human cell therapy products comes with many problems
- In vitro bioassays are also artificial but may be more informative in some cases
- There is a need to think outside the box