Australian Government
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TGA considerations for preclinical studies of cell therapy products

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TGA Health Safety Regulation

- Cell therapy is a broad field:
  - Large range of applications/indications
    - E.g. Bone marrow transplants, neural cell replacement, heart repair, cartilage and/or bone replacement or cancer treatment (CAR-T-cells)
  - Large range of cell types:
    - E.g. If stem cells: Mesenchymal Stem Cells (MSCs), Haematopoietic Stem Cells (HSCs), Neural Stem Cells (NSCs), Embryonic Stem Cells (ESCs), Induced Pluripotent Stem Cells (iPSCs), or large range of progenitor or differentiated cell fates
  - Each indication and/or each cell type carries unique and context-related challenges
- Cell therapy is multidisciplinary:
  - Therefore, difficult to take issues in isolation
- Cell therapy is fast paced and dynamic

Challenges: Preclinical Evaluations (Overview)

- Knowledge gap:
  - Multidisciplinary convergence
  - Dynamic, fast-paced clinical development (increases gap between fundamental and translational research)
  - Complacency
- Suitability of animal models:
  - Immunological response; can impact on safety and efficacy
    - Even in absence of immune response, susceptible to species specific differences
  - Biological context; cells are live entities, therefore understanding biological context important for their utility

Challenges: Preclinical Evaluations (Overview)

- Biologically active dose
  - Identification of biological active dose complicated; influenced by factors such as:
    - Indication (location)
    - Mechanism of action (often, not well characterised)
    - Type of cell therapy (i.e. differentiated cell fates, progenitor cells or naïve stem cells)
    - Stochastic nature of cell proliferation and differentiation (depends on final resting position of cells)
    - Stochastic nature of distribution (post infusion/transplant)
    - Stochastic nature of cell survival (post infusion/transplant)

The Knowledge Gap

The ‘Knowledge Gap’

Multidisciplinary convergence

Molecular Biology

Cell culture

Proteomics

From www.123rf.com

Developmental Biology

Immunology

Genomics

‘Convergence’

The ‘Knowledge Gap’

- So what is a knowledge gap (in the context of cell therapies)?
  - It is essentially ‘gaps’ in knowledge between the deciplines
  - The ‘knowledge gaps’ have implications for safety and efficacy assessments
    - Often incumbent on the nonclinical evaluator to accommodate the ‘knowledge gaps’ when performing an evaluation
- Reason for the ‘knowledge gap’
  - Different rates of progress between fields
  - Different rates of progress within fields
    - i.e. fundamental ➡ translational research transitions are out of step
  - Insufficient technological progress
    - Analytical/detection methods
    - Tissue culture techniques

The 'Knowledge Gap'

- Reason for the 'knowledge gap' (continued)
  - Complacency
    - Due to convergence of entire disciplines it is sometimes "just too hard" to cover *all* essential aspects
      - "just too hard" = cost prohibitive?
  - Over-reliance on limited published data to bridge gap between "therapeutic potential" ➡ clinical application
    - Most published data demonstrate "potential", but lack sufficient depth in safety and efficacy findings
  - Over-reliance on evolutionarily conserved cellular response
    - Cells demonstrate robust survival and differentiation potential many circumstances
      - E.g. in bone marrow transplants
    - Other circumstances require precise handling and manipulation of cells to achieve desired results
      - Where this is not possible, the field as a whole, tends to imply the cells can 'compensate' for precise handling and manipulation (i.e. "*The cell knows what to do*")

The ‘Knowledge Gap’

The notion of “The cell knows what to do” is insufficient to bridge knowledge gap and thoroughly evaluate safety and efficacy aspects of pre-clinical studies.

The 'Knowledge Gap'

Exampled 1: Using the 'homing' potential of Mesenchymal Stem Cells (MSCs) to treat various diseases:

- Published evidence of 'homing'
- Homing mechanisms not extensively characterised or clearly defined
- When 'homing' does occur, characterisation is limited:
  - i.e. Quantification within target tissue, distribution relative to damaged cells/tissue sections, long-term integration and/or propagation or phenotype characterisation
- The fact that 'homing' happens is often assumed to be sufficient for clinical application
  - However, in the absence of accurate MOA, quantification and characterization, non-clinical evaluation of safety and efficacy is challenging.

The 'Knowledge Gap'

Exampled 2: Cell replacement therapies (e.g. diseases of the central nervous system)

- Published evidence of limited replacement potential
- Replacement mechanisms not extensively characterised (*in vivo*)
  - Number of cells requiring replacement
  - Appropriateness of neural connections
  - Longevity of replaced cells/neurons
- Lack of characterisation sometimes due to technological limitations
  - Quantification of cells and connections within target tissue difficult
  - Assessing accuracy of replacement connections difficult
    - Since original connections haven't necessarily been appropriately resolved
- Therefore, difficult to reconcile animal model outcomes with histology data

Appropriate Animal Models

Appropriate animal models

- Common issue; immunogenic responses (with clinical product)
  - Can use immune compromised animals
    - However, doesn't always allow for appropriate disease model to be used
- Often overlooked; biological context in which model is used
  - Classical toxicological studies – consider pharmacology, pharmacodynamics, ADME, carcinogenicity etc
  - In cell therapies – concepts such as molecular signalling also need to be considered
- Temporal regulation, not discussed in cell therapy models
  - Concept especially relevant to stem/progenitor cell therapies
  - Lack of consideration of temporal regulation by-product of the 'knowledge gap'
    - Not necessarily a shortcoming of animal models *per se*
    - However concept required addressing when using animal models

Appropriate animal models

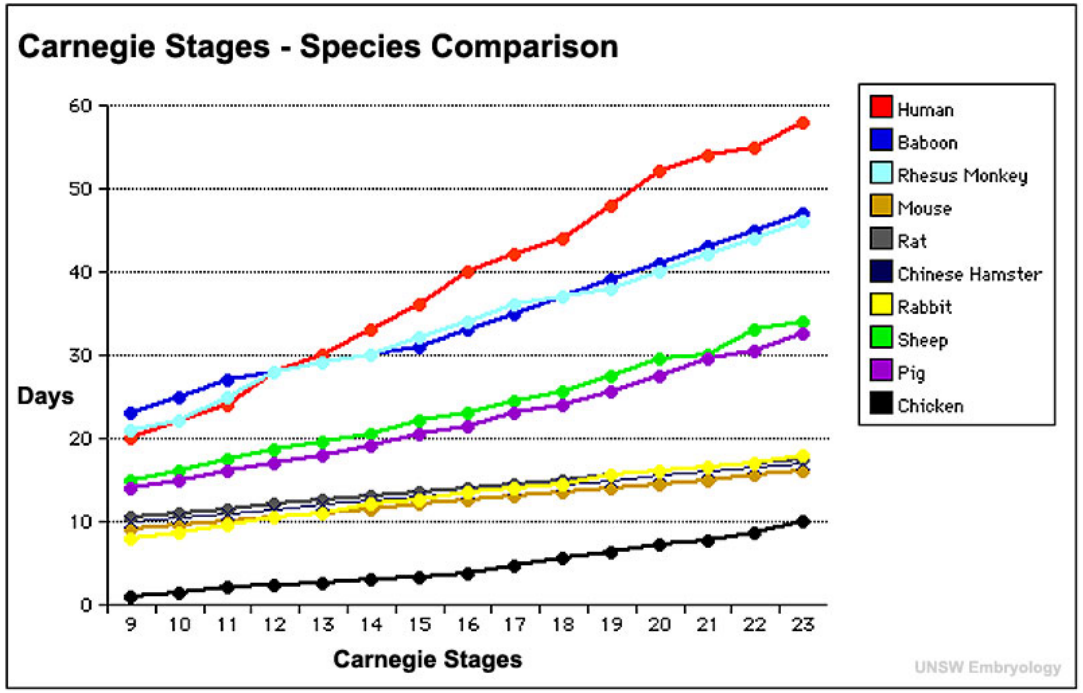
Temporal regulation

- Gestation periods of common pre-clinical animal models:
  - Mouse 19 days (~85% genome conservation with humans)
  - Rat 21-23 days
  - Canine Av 61 days
  - Monkey 164 days (95% genome conservation with humans)
  - (Humans 259-280 days)
- Increased gestation time likely due to increased cell count in larger animals
  - There is also increased molecular signalling coordination required with increasing size
    - This process requires additional time
      - This is an example of temporal regulation

Appropriate animal models

Temporal regulation (Carnegie staging of development)

- Staging of development not based on size, but on evolutionarily conserved structures
  - i.e. same signals, same structures formed at different times during gestations

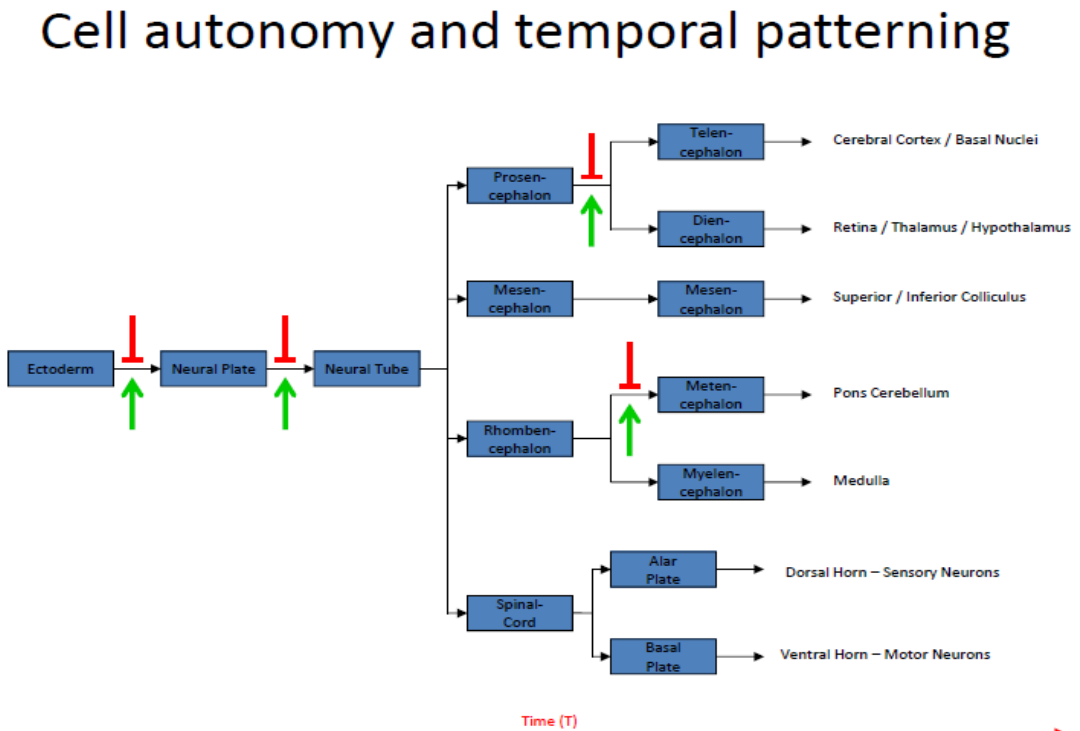


From: embryology.med.unsw.edu.au

Appropriate animal models

Temporal regulation (CNS patterning as an example)

- Duration of molecular signals important for final cell fate



Appropriate animal models

Temporal regulation

- Following xeno-transplant, which temporal mechanisms take precedence?
  - The host (animal model) or donor (clinical product)?
    - Data on such temporal regulatory mechanisms limited
- Is understanding temporal regulation relevant?
  - Yes it is! Because…
    - May have implications for duration of safety studies
    - May have implications for efficacy studies
      - Will human cells be less efficacious in animals models as opposed to the clinic.
    - May have broader implications in naïve stem/progenitor studies *cf.* differentiated cell fates

Appropriate animal models

Temporal regulation

- Can temporal regulation be accommodated in animal models?
  - Not in all circumstances
    - Most developmental processes follow liner-non repetitive time-line
      - Therefore, difficult to replicate host developmental signals in cell-replacement models (e.g. CNS)
  - There is no accommodation of developmental timelines in most cell replacement models
  - It is often 'implied' the naïve cells can compensate for variations in developmental signals
    - i.e. "The cell knows what to do"
      - This notion is likely fueled by the presence of differentiated stem cells in adult models
        - Due to random differentiation or
        - Secondary (but limited) differentiation pathways
          - In the absence of verified MOAs, remains speculative

Biologically Active Dose

Biologically active dose

- Selection of biologically active dose is context dependant
  - Indication/application (local/systemic)
  - Choice of cells; differentiated cells (local/systemic transplants) or naïve stem cells (local/systemic)
    - Differentiated cells; need to consider proliferative potential (intrinsic/extrinsic regulation)
      - E.g. Immune cells transplants (chimeric antigen receptor T-cells (CAR-T cells) in cancer therapies)
    - Naïve stem/progenitor cells: differentiation and proliferative potential
      - Regulation of differentiation potential (intrinsic/extrinsic regulation)
      - Proliferative potential (intrinsic/extrinsic regulation)
        - E.g. MSCs in GVHD or NSCs in neurodegenerative diseases
        - Due to proliferative potential, biologically active dose can significantly increase over time
- Selection of biologically active dose is influenced by cell survival rates
  - Cell therapy transplants/infusions are often associated with high levels of cell death
    - Usually a by-product of misaligned biological context (which cells die is a random process)

Biologically active dose

- Influenced by residual cell phenotype
  - Related to differentiation potential
    - Balance of target cell phenotypes Vs residual cell phenotypes can impact safety and efficacy
      - Quantification and characterisation difficult due to limitations of lineage tracking capabilities
        - Technological limitation
- Complicated by need for mixture of cells
  - Cell replacement not always ‘1:1 replacement’ of single cell fates (active cells Vs support cells)
  - Support cells secrete trophic factors
    - Impact of trophic factors difficult to quantify
      - Effect can be influenced by distance from target cells/tissues
      - Effect can be influenced by signalling from local environment

Biologically active dose

- Influenced by location/final resting position of cells
  - Important in therapies where cells administered systemically or locally, but require homing and/or migration to reach target site
    - Appropriately differentiated cells may not be ‘biologically (therapeutically) active’ if
      - Not ‘replacing’ diseased cell or
      - Providing trophic support for diseased cells/tissues (requires close proximity)
  - Final resting position is a random process
    - i.e. difficult to quantify homing/integration mechanisms
      - e.g. “MSC homing” or neural cell transplants to CNS(technological limitation)

Biologically active dose

- Influenced by biological context
  - Using cell replacement in neurological disorders as an example:
    - Neural replacement only successful if appropriate connections are established & maintained
      - Local environment should be conducive to differentiation (i.e. appropriate molecular signals)
      - Assuming appropriate differentiation, accurate neural connections requires establishment
        - i.e. Transplantation of stem cells with differentiation potential alone is insufficient
  - Using homing as an example (assuming cells migrated to damaged tissue)
    - Infiltration of target tissue required
      - Either infiltration in sufficient numbers or through proliferation post integration
      - With target tissue, local migration required to elicit action according MOA
        - Must be assessable or quantifiable
- Biologically active dose is a dynamic value

Summary

Challenges for preclinical studies of cell therapy

- Knowledge gap:
  - Causes and examples
- Animal models
  - Suitability based on compatibility and biological context
- Biologically active dose
  - Intricacies of defining dose as it pertains to cell therapies

"...What gets us into trouble is not what we don't know. It's what we know for sure that just *ain't* so..."

Mark Twain