EVALUATION OF STEM CELL-DERIVED CELLULAR THERAPY PRODUCTS

Alys Bradley, Director of Pathology - Charles River Laboratories Edinburgh
# EVALUATION OF STEM CELL-DERIVED CELLULAR THERAPY PRODUCTS

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**EVERY STEP OF THE WAY**

*charles river*
INTRODUCTION – STEM CELL DEFINITION

Role of the Pathologist
EVALUATION OF STEM CELL-DERIVED CELLULAR THERAPY PRODUCTS

KEY FEATURES OF STEM CELLS

1. The capacity to self-renew
2. Extensive proliferation potential
3. Capacity to generate differentiated progeny
Potential Therapies:
• Repair
• Replace
• Restore
• Regenerate

Potential Mechanisms Of Action:
• Cells incorporated into & become part of the recipient/targeted tissue.
• Cells direct or facilitate regeneration.
  • secreting factors
  • intercellular signalling and interactions
EVALUATION OF STEM CELL- DERIVED CELLULAR THERAPY PRODUCTS

Potential sources:
• Pluripotent Stem Cells (hESC and iPS)
• Adult stem cells (mesenchymal, hematopoietic, etc)
• Functionally mature/differentiated and structural cells
  • chondrocytes, RPE cells, pancreatic islets, cardiomyocytes etc.

1. Human tissue donor
2. Animal source
3. Tissues generated/grown ex vivo on scaffolds
**EVALUATION OF STEM CELL-DERIVED CELLULAR THERAPY PRODUCTS**

**TYPES OF STEM CELLS - EMBRYONIC**

- ESC lines are derived from the inner cell mass of a blastocyst.
- ESC first isolated from mice in 1981.
- Human embryonic stem cells (hESC) were first derived in 1988.
- They are pluripotent cells that have the ability to differentiate into derivatives of all three germ layers; endoderm, mesoderm and ectoderm.
- Two distinctive properties:
  1. Pluripotency
  2. Ability to replicate indefinitely


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TYPES OF STEM CELLS - ADULT

- Multipotent Somatic Adult stem cells (ASC) are found naturally in differentiated tissue throughout the body, where they function to maintain tissue by replacing damaged or lost cells.

- ASC can only differentiate into cell types of the tissue from which they originated and tend to have limited self renewal.

- Examples of ASC include mesenchymal stem cells (MSC), Hematopoietic Stem Cells (HSC) and endothelial progenitor cells (EPC).

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TYPES OF STEM CELLS - INDUCED PLURIPOTENT

- Induced pluripotent (iPSC) cell lines are derived from a non-pluripotent adult somatic cell, through the forced expression of specific genes.

- Takahashi et al (2006) showed that pluripotent stem cells could be obtained by transducing/reprogramming mouse embryonic and adult fibroblasts with a limited set of transcription factors inc. Oct3/4, Sox-2 and Nanog.

- Various methods exist to revert adult cells to pluripotency including viral mediated transduction, epigenetic reprogramming and protein mediated transduction.

- iPSC - can be patient-specific, so address some potential issues of cell rejection.

- Large scale iPSC therapies are likely to be allogeneic so have similar safety concerns to hESC.


Reprogramming

Fibroblasts

iPSC

Mesenchymal to Epithelial Transition
CHALLENGES

Role of the Pathologist
EVERY STEP OF THE WAY

• Huge public, scientific and clinical expectation for stem cell therapeutics as treatments for genetic and inflammatory diseases.

• Heightened concern for the safety of these promising therapies due to the potential of adverse effects in the patient.
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Challenges include

• Relevant animal model(s) selection.
• Study Design.
• Few guidelines available.

• Guidance in stem cell derived therapy by FDA and European regulators is vague and case by case assessment.
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• Safety Issues to be addressed:
  • cell survival and differentiation,
  • cell distribution
  • potential for tumor formation.

• Cells must be well-characterized, as the extent of the preclinical program necessary is contingent upon any genetic manipulations and modifications of the cell line.
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Current therapeutic areas – Repair of Disease processes

• Stem cell derived products:
  • AMD
  • Spinal cord injury (rat spinal cord injury model)
  • Stroke injury (rat stroke cerebral artery occlusion model)

• Stem cell transplantation:
  • Autologous neural stem cell
  • Autologous olfactory ensheathing cell
  • Autologous bone marrow cell:
    • Intrauterine stem cell/blood transfusion from mother to 2\textsuperscript{nd} trimester fetus: to treat αthalassemia, CIRM clinical trial
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Current therapeutic areas

Stem cell therapies investigated in preclinical phase in 2017; UK Stem Cell Catapult data
PROGRAMME DESIGN

Role of the Pathologist
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PROGRAMME
KEY ENDPOINTS
Role of the Pathologist
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Preclinical Questions - Similar to Drugs/Biologics

- What are they?
  - Cell Characterization
- What do they do?
  - Activity
- What is the best way to test?
  - Animal Model
- Where do they go?
  - Biodistribution
- How long do they stay?
  - Biodistribution
- Are they safe?
  - Toxicity
- What can they turn into?
  - Tumorigenicity
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Pluripotent Stem Cells (hESC and iPS); Adult stem cells (mesenchymal, hematopoietic, etc)

CONCERNS:
- Potential inflammatory/immune response to injected/implanted cellular product
- Inappropriate cell proliferation (i.e., tumor formation)
- Inappropriate cell differentiation (i.e., ectopic tissue formation)
- Cell migration to non-target tissues
- Interactions with concomitant therapies

AIMS:
- Cells incorporated into and become part of the recipient/targeted tissue (repair, replace, or restore injured/diseased cells)
- Cells direct or facilitate regeneration by secreting factors or through intercellular signaling and interactions
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POTENTIAL SAFETY CONCERNS FOR CELL THERAPY PRODUCTS

Sponsor Questions/Study Objectives:

- Risks of the injection/implantation procedure
- Potential inflammatory/immune response to the injected/implanted cellular product
- Inappropriate cell proliferation (i.e. tumor formation)
- Inappropriate cell differentiation (i.e. ectopic tissue forms)
- Cell migration to non target areas/tissues
- Interactions with concomitant therapies
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INTERDISCIPLINARY CELL THERAPY TEAM APPROACH

Customize programs around the novel properties of each cellular product.

PRECLINICAL AND DISCOVERY SCIENTISTS
REGULATORY SPECIALISTS
PATHOLOGISTS

= STUDY DESIGN

Charles River Navigators
EVERY STEP OF THE WAY

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INTERDISCIPLINARY CELL THERAPY TEAM APPROACH

PRECLINICAL

Shawna Jackman
PA

Doug Learn
PA

Mary McElroy
EDI

CELL CHARACTERISATION

Margaret McCartney
EDI

PATHOLOGY

Alys Bradley
EDI

Stuart Naylor
EDI

Lyndsay Brogan
EDI

Julie Schwartz
NV

Curtis Chan
NV

NAVIGATORS

Lauren Black
NAV

Charles River
STUDY DESIGN

Role of the Pathologist
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CELL CHARACTERISATION
CELL DETECTION METHODS
BIODISTRIBUTION/CELL FATE
ANIMAL MODEL SELECTION
PRODUCT ACTIVITY/EFFICACY
TOXICITY/SAFETY

PRECLINICAL CELL THERAPEUTIC PROGRAM
EVERY STEP OF THE WAY

PRECLINICAL PROGRAMME CHALLENGES

• Programs must support clinical trials.
• Consistent and well-characterized product.
• Biomarkers related to pharmacology.
• Biocompatible formulation.
• Route of administration and regimen should mimic the proposed clinical program.
• Evaluate local and chronic toxicity, including off-target effects.
• Demonstrate Dose-response, biodistribution.
  • assays based on PCR and Immunohistochemical stains
• Tumor formation potential.

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Cell products must be well characterized before GLP safety studies.

Strategic program planning and design: model, dose, timepoints.

Cell detection methods (PCR/IHC) must be robust, reliable and sensitive.
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STUDY DESIGN

Preclinical Study Objectives:

- Evidence that cell therapy is safe and effective.
- Dose regime must be clinical relevant.
- Use *intended clinical product*.
- Administer by the *intended clinical route*.
- Administer with the *intended delivery device*.
- *GMP and GLP studies*.
- Minimally active dose.
- No adverse effect level dose (NOAEL).
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**STUDY DESIGN**

**Nonclinical Study Should Help the Clinical Study Design**

- Program customized to the particular cell, its actions and the intended clinical use, at each step.
- Communicate with regulatory agencies on nonclinical program designs and animal model selections **before** studies are initiated.
- Provide Proof Of Concept (POC) in the selected animal model(s).

**Study POC/Draft design should provide data on the following variables:**

- Dose levels (min and max tolerated dose).
- Route of administration.
- Cell survival/Duration in host ? Patient?
- Number of dose occasions.
- Monitoring for potential toxicities (e.g. biomarker selection)
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STUDY DESIGN

Use of Pilot Studies

• Demonstrate that persistence is associated with efficacy (biodistribution assays).
• Determine dose levels and time points (if cells don’t persist then shorter time study times possible).
• Timing of administration in relationship to progression of disease (e.g. acute or subacute phase of pulmonary fibrosis models).

Differences from ‘Routine’ Toxicology Studies:

• Irreversible – no recovery animals as cells can not be removed.
• Animal model of disease, but still to GLP.
• Immunogenicity concerns/infection for immunocompromised.
• Combined safety/efficacy endpoints.
• Tumorigenic potential/ectopic tissue formation.
• Tissues routinely retained for PCR and/or IHC evaluations.
OPTIMAL SPECIES

Role of the Pathologist
**EVALUATION OF STEM CELL-DERIVED CELLULAR THERAPY PRODUCTS**

**CHOICE OF ANIMAL MODEL**

**Choice of Species**
- No default species; provide scientific justification for the species/model(s) used.
- Pharmacologically sensitive species.
- Progressive host for the cellular therapeutic, with consideration given to the intended clinical route of administration.
- Animal models/strains with normal life span required for studies up to 3-12 months duration.

**Immunocompetent and immunocompromised test systems:**
- Rodents (including neonates), nonhuman primates, pigs and surgically altered animals.
- Animals with competent immune systems can be treated with immunosuppressant agents to enable cell survival after implantation.

**Experimental variables considered:**
- 3Rs.
- Animal details (source, numbers, age, weight, sex etc.).
- Adequate group sizes (statistical power).
- Detailed welfare assessments before and during study at several time-points.
- Details of dosing (e.g., infusion rates, timing of cell delivery, delivery system, acute/local effects).
- Anatomical location – diseased/injured areas.
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CHOICE OF ANIMAL MODEL

Relevance to human disease
- May be necessary to perform in an animal model of efficacy.
- Immunodeficient or immunocompromised animals (steroid co-treatment) to prevent rejection.
- Cell potency and durability affected by local tissue environment.

Rodents:
- Larger Group sizes.
- Genetically-susceptible strains are available e.g., some mouse strains are more susceptible to bleomycin-induced fibrosis.
- NOD/SCID mouse.
- NOG mouse.
- SCID mouse.
- NIH III mouse.
- NIH Nude rat.

Non-Rodents:
- Organ/tissue size comparable – able to administer human dose, scaffold/delivery device.
- Pigs, Primates, Sheep.
COMMON ANIMAL MODEL – Immunocompromised Mice

Immunocompromise

Pros:
• Consistency and ease of use.
• Allows certain disease/injury modelling.
• Defined degree of immunodeficiency with various genetic rodent models.

Cons:
• Limited to using rodents.
• Does not predict immuno-reactivity to transplanted cells.
• Physically fragile/susceptible to disease.
• Limited pathology database.
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NUDE MICE

• Mutation of the FOXN1 gene (causes a deteriorated or absent thymus)
• Inhibited immune system as greatly reduced number of mature T lymphocytes.
• Absence of functioning T cells means can receive many different types of tissue and tumor grafts as cannot reject allografts or xenografts.
• NB: Most strains of nude mice are slightly "leaky" and do have a few T cells, especially as they age.

• The life span normally 6-12 months, but in controlled environments can live almost as long as normal mice (18 months - two years).
EVERY STEP OF THE WAY

Often referred to using their branded name "NSG™"

Extremely immunodeficient as carry two mutations on the NOD/ShiLtJ genetic background:

- Severe combined immune deficiency (scid) in the DNA repair complex protein \(Prkdc\), so B and T cell deficient.
- Complete null allele of the IL2 receptor common gamma chain (\(IL2rg^{null}\)) prevents cytokine signaling through multiple receptors, leading to a deficiency in functional NK cells.

Can be humanized by engraftment of human CD34+ hematopoietic stem cells (HSC), peripheral blood mononuclear cells (PBMC), patient derived xenografts (PDX), or adult stem cells and tissues.
EVERY STEP OF THE WAY

EXPOSED DORSAL SPINAL CORD SURFACE SUBJECTED TO WEIGHT DROP IMPACT USING A 10 g ROD (2.5 mm DIAMETER) DROPPED AT THE HEIGHT OF 25 mm.

24 HRS AFTER INJURY, ANIMALS DEMONSTRATING COMPLETE BILATERAL HINDLIMB PARALYSIS ARE ASSIGNED TO TREATMENT GROUPS TO RECEIVE A SPINAL GRAFT OF STEM CELLS OR MEDIA/SCAFFOLD ONLY.

SECTIONS STAINED WITH H&E, LUXOL FAST BLUE/CRESYL VIOLET, AND MASSON’S TRICHROME STAIN TO ASSESS: DEMYELINATION, GITTER CELL ACCUMULATION, NECROSIS, CHROMATOLYSIS, GLIOSIS AND FIBROSIS/FIBROPLASIA.

CHRISTOPHER REEVE FOUNDATION/ANDERSON: HUMAN NEURAL STEM CELLS (HuCNS-SCs). STEMCELLS INC. IS CONDUCTING A PHASE II PROOF OF CONCEPT TRIAL USING HuCNS-SC IN CERVICAL SPINAL CORD INJURY WITH PARTICIPANTS BEING TREATED BETWEEN 10 TO 23 MONTHS POST-INJURY.
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Disease/Injury models - Rat Spinal Cord Injury - Dorsal laminectomy at T9-T10

Location of sections for histopathology: 100um, 300um, 1mm and 3mm from injection site, on each side

4 sections x 2 (rostral and caudal halves) = 8 sections per rat
EVERY STEP OF THE WAY

**TBE induced by injection of autologous blood clots (Busch et al, Brain Res. 1997 Dec 5;778(1):16-24).**

- Femoral arterial blood from donor rat collected into 20 cm long catheter and retained for 2 hours at room temperature and subsequently at 4°C for 22 hours to allow clot formation.

- Clots are rinsed several times with (PBS, pH 7.4) to remove blood cells and obtain a white clot, which is then cut into 2 mm-long pieces and transferred to 1 mg/mL albumin in PBS to allow clot retraction.

- 2 hours later, 20 fragments are embolized into Middle Cerebral Artery via one meter-long catheter, with ~3 cm distance between clots to keep them apart.

- 7 days after Middle Cerebral Artery Occlusion (stroke), brains are removed, assessed grossly for size of infarction and stored in 4% PFA at +4°C until processed for histopathological evaluation.

**Disease/Injury models - Rat Thromboembolic Stroke Injury - tMCAO**

*Figures not shown in this text.*
EVERY STEP OF THE WAY

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*Disease/Injury models* - Rat Stroke Injury - tMCAO


- Edema, hemorrhage, gliosis (astrogliosis/microgliosis), cavitation, necrosis, inflammation, vascularisation, vasculitis, foreign body macrophage reaction.
CELL MANUFACTURING & CHARACTERIZATION

Role of the Pathologist
• Characterization of cells should include, but not be limited to:
  1) Identity & Composition;
  2) Viability & Stability;
  3) Purity & Sterility.

• Starting hypothesis - Cell-based therapeutics are a heterogeneous mixture of cells.

• Qualified Biomarkers to identify the cells should include: morphology, surface, and genetic markers.
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CHARACTERIZATION OF CELL LINE

**Identity and composition**


**Stability and homogeneity**

- Storage conditions, growth pattern stability through derivation, split ratio, consistent production capacity over long term storage and lot expiration.

**Purity and sterility**

- Lack of contaminants and impurities such as adventitious viruses, mycoplasma, nucleic acids, bacteria, fungi, retroviruses and endotoxins. Detection of biomarkers for undifferentiated stem cells.
PATHOLOGY ENDPOINTS

Role of the Pathologist
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STUDY PROGRAMME

- Pilot Tolerability, Viability and Biodistribution Study.
- GLP Biodistribution Studies:
  - No default species - scientific justification for the species/model(s) used.
  - Demonstrate toxicity.
  - Demonstrate efficacy (in studies using animal models).
- Tumorgenicity studies.
  - Assess tumorigenic potential.
- GMP Product release studies.
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PATHOLOGY AIMS - Biodistribution

**Aim** - Evaluate local migration and systemic distribution, i.e. determine cell fate.

**Key concerns to be addressed in Pathology Report:**
- Site of activity/inoculation local toxicity.
- Migration at local Site.
- Distribution outside of target.
- Integration in target and non-target tissues.
- Ability to endure in the animal model at the target site
- Surrogate species with reporter gene e.g. GFP pig stem cells/Landrace pigs.
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PATHOLOGY AIMS - Biodistribution

- **Duration** – dependent on predicted persistence of products.
  - Transient products need to confirm clearance, e.g. MSC immunomodulatory therapies,
    - 4-12 weeks
  - Multi-dose or products expected to engraft, dependent on product and life span of test species.
    - 3-12 months
  - Acute disease models (Ensure evaluated during early stages of disease, not after start to be debilitated).
    - 1 month

- **Time points** - e.g:
  - Sacrifice Time point 1 - 48h or 1 week post dose.
  - Sacrifice Time point 2 – 2 weeks or 3 months post dose.
  - Sacrifice Time point 3 – 4 weeks or 6 months post dose.
  - Evaluate one time point before processing the next (may allow less aggressive sampling protocol).
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PATHOLOGY AIMS - Biodistribution

- GLP-compliant.
- Rodents: 5-10/sex/group.
- Non-rodents: 3-5/sex/group
- Test groups: sham and/or untreated control; clinical or maximal feasible dose.
- Pathology from all animals; Full Tissues retained.
- H&E sections
- PCNA/Ki67 to assess proliferation.
- Administration site and regional lymph nodes
- Major organs (liver, lung, spleen, heart, kidney, brain, bone marrow, gonads, gross lesions, other therapy specific targets based on mode of action and likely distribution)
- BWt, Clin Obs.
- Conduct PCR as first screen then confirm positives with IHC and/or ISH.
Immunohistochemistry (IHC)

In Situ Hybridization (ISH)

- IHC: Detection of human cells with anti-human mitochondria antibody
- ISH: Detection of human cells with specific probes
- Provides cellular distribution
- Semi-quantitative or advanced quantifiable morphometric techniques

hESCs stained with TRA-1-60 marker (green punctate staining) to identify undifferentiated stem cells.

Nuclei counter-stained with DAPI (blue)
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**PATHOLOGY AIMS - Biodistribution**

**Whole Animal Imaging**
- Provides cellular distribution
- Expensive
- Specialist equipment

**Fluorescence**
- Sensitive
- Reporter genes e.g. GFP
Case Study – Surrogate Species for RPE product

- Stem cell product cells not readily identifiable on H&E.
- Regulator requested biodistribution data on surrogate species with reporter gene.
- Stem cell therapy product manufactured to GMP using GFP (Green Fluorescent Protein)-pig stem cells.
- GFP stem cell product implanted into Gottingen minipig retina.
- Tissues collected from major organs and inoculation site (eye).
- Histopathology using fluorescent microscope to look for migration/differentiation of pig stem cell product with GFP reporter gene.
Case Study – Surrogate Species for RPE product

- 2 time points: 3 weeks and 13 weeks.
- 5 dose groups with differing concentrations, from $250 \times 10^3/100\mu l$ - $10x 10^6/100 \mu l$.
- Brain, Heart, Kidney, Liver, Lung, Lymph Nodes, Pancreas, Spleen, Thymus evaluated for presence of fluorescent cells.
- Minipig eye injected with GFP-pRPCs (Green Fluorescent Protein transduced pig Retinal Progenitor Cells) used as a positive tissue control.
- Slides qualitatively assessed using the 5-point grading criteria:

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<tr>
<td>0</td>
<td>Negative – No fluorescent cells present</td>
</tr>
<tr>
<td>+</td>
<td>Minimal</td>
</tr>
<tr>
<td>++</td>
<td>Mild</td>
</tr>
<tr>
<td>+++</td>
<td>Moderate</td>
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<td>++++</td>
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Case Study – Surrogate Species for RPE product

- Minipig eye implanted with GFP-pRPCs (Green Fluorescent Protein transduced pig Retinal Progenitor Cells) tissue control.

Positive Control:
Implant stained with Chicken anti GFP 1:750 x 400 magnification

Negative Control:
Implant stained with Chicken anti GFP 0:0 x 400 magnification
SAFETY ASSESSMENT

Role of the Pathologist
Patient considerations:

- Could the cell surface antigens and/or secreted products induce a polyclonal response?
- Location of inoculation - How immune privileged are the stem cells?
- Maturation status?
  - Does this alter with timepoints?
  - Does this alter in response to inflammation?
- Cell source autologous/allogeneic
- What is the effect of an aging immune system?
- Will there be a need for prolonged immune-suppression?
- Function of the delivered cell?
Safety Assessment

Immunogenicity considerations:

ALLOGENEIC ESCs

• Standard immunosuppressive drug regimens can significantly reduce immune response to prolong survival of hESC derived xenografts, but can’t prevent eventual immune rejection.
• Human ESCs express MHC Class I, and can’t directly activate T cells in vitro or in vivo.
• **BUT** MHC Class I and Oct4 are immunogenic antigens that **indirectly** activate T cells through antigen presenting cells.
• NK cells eliminate mouse and hESCs in vitro.

• After transplantation, both mESCs and hESCs can’t maintain pluripotent state and undergo spontaneous differentiation into various cell types leading to T-dependent allogeneic rejection; also teratoma risk?

• Human immune system of humanized mice can reject allogeneic cells derived from human embryonic stem cells (hESCs), and thus can be used to vigorously test immune tolerance strategy’
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Safety Assessment

Immunogenicity:

• Hypothesis: patient-specific iPSCs are autologous, so won’t be rejected by the patient.............BUT

• hiPSCs can be rejected by allogeneic and autologous NK cells.

• Need presence of functional antigen presenting cells at the transplantation site are to reveal the immunogenicity of iPSC-derived cells.

• iPSC-derived RPE cells aren’t immunogenic to autologous immune system.
Test System considerations:

• Disease model:
  • immune competent.
  • Relevant to clinical disease.
  • Humanised.
  • HCD available.

• Adequate and enough Controls animals:
  • For Disease = littermates or parental unaffected (WT) line.
  • For Surgery = sham surgery and vehicle only recipients.
  • For device implanted = device with no cells.
Study Design

- GLP-compliant.
- Male and Female immune-deficient mice (7-9 weeks old).
- 25/sex/group
  - Sacrifice Group 1: @ 1 month; 10/sex/group.
  - Sacrifice Group 2: @ ≥ 6 month; 15/sex/group.
- Test groups:
  - sham surgery and/or untreated negative control.
  - Low dose stem cell therapeutic.
  - Mid dose stem cell therapeutic.
  - clinical or maximal feasible dose stem cell therapeutic.
  - Positive control group(s)
- May need DRF if cellular product secretes pharmacologically active substances e.g. islet cells
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Safety Assessment

• Pathology from all animals.
• Local toxicity - such as injection-site reaction.
• Systemic toxicity less of a concern for many cellular therapeutic products, e.g. stem cells implanted in the retina – immune privileged site.
• Immunogenicity concerns and irreversibility of engraftment must be considered.
• Standard toxicological evaluations are performed, including clin obs, bwt, feed consumption, ophthalmology, clin path, necropsy, organ wt, histopath.
• Satellite group for clinical pathology/biomarkers.
• Conduct PCR as first screen then confirm positives with IHC and/or ISH.
• PCNA/Ki67 to assess proliferation.
• Need to use full cellular product at human scale.
• Large animals must be immunosuppressed.
EFFICACY

Role of the Pathologist
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Safety Assessment

Efficacy/Proof of Concept

- Combine with Tox Study - 2 armed approach.
- Appropriate control groups – type and number.
- Local toxicity.
- GLP or GLP voluntary.
- Diseased models.
- Repair of disease process-associated damage.
- Preservation of normal structure.
- Structural integration of cells.
- Necropsy blinded.
- Histopathology un-blinded.
- Currently CBER don’t need SEND format data – may change.
TUMORIGINIC POTENTIAL

Role of the Pathologist
Tumour Risk

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Tumorigenic potential

- Pluripotent stem cells - Teratomas
- Continuous Culture/Passage – selective pressure for genetic change
- Secondary tumours from inoculated cells-
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Tumorigenic Potential

• Assess potential for cell phenotypic stability, tumour or ectopic tissue formation in an immunocompromised rodent: NOD SCID or Nude mouse.

• Single subcutaneous or clinical route administration with at least three dose groups (including positive control).

• Animals palpated weekly for mass presence.

• Tissues are retained for histopathology.

• Study duration is typically the lifespan of the animal.
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Tumorigenic Potential

• Intended clinical product with the intended route of administration.
• Maximum feasible dose.
• Controls – positive, spiking, assurance of engraftment, spontaneous tumors.
• Sufficient numbers of animals (up to 20-25/sex/group to assess rare events).
• Sufficient study duration………………as long as possible.
• Multiple time points.
• Specific cell markers unlikely to lose activity if cells differentiate.
• Anatomical location/size/incidence of tumor.
• Type of tumor/Origin of tumor cells.
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Tumorigenic potential

Positive control

HeLa cell carcinoma:
REGULATORY ISSUES

Role of the Pathologist
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USA Regulation

- Office of Tissues and Advanced Therapies (OTAT)
  - Functionally mature/differentiated cells e.g. retinal pigment epithelial cells
  - Stem cells/stem cell-derived
    - Adult (e.g. haematopoietic, neural, mesenchymal)
    - Perinatal (e.g. placental, umbilical cord blood)
    - Fetal (e.g. neural)
    - Embryonic
    - Induced pluripotent stem cells (iPSCs)
EU Regulation


• Cell therapies = defined as medicinal products when there is more than minimal manipulation of any cell type destined for clinical application or where the intended use of the cells is different to their normal function in the body.

• Same safety and efficacy rules as for all medicinal products (GLP); the quality and manufacturing to good-manufacturing-practice (GMP) requirements.
EVALUATION OF STEM CELL- DERIVED CELLULAR THERAPY PRODUCTS

UK Regulation
EVALUATION OF STEM CELL-DERIVED CELLULAR THERAPY PRODUCTS

Regulation

• No “one size fits all” approach.
• Data needed depends on the characteristics of the product.
• Approach based on balance of risk and benefit.
  • GLP and GMP studies
  • Can reference data in peer-reviewed Journals
  • Cross reference to similar products
  • Clinical trial reports
• FDA : non-binding informal scientific discussions between CBER/OTAT and Sponsor “INTERACT” – Initial Targeted Engagement for Regulatory Advice on CBER products (previously pre-pre-IND meeting) mercedes.serabian@fda.hhs.gov
SUMMARY

Role of the Pathologist
EVERY STEP OF THE WAY

EVALUATION OF STEM CELL-DERIVED CELLULAR THERAPY PRODUCTS
PRE CLINICAL STEM CELL THERAPEUTIC PROGRAM

CELL CHARACTERISATION

CELL DETECTION METHODS

BIODISTRIBUTION/CELL FATE

ANIMAL MODEL SELECTION

PRODUCT ACTIVITY/EFFICACY

TOxicITY/SAFETY
CASE REPORT

Adult Stem Cells

Role of the Pathologist – GLP Study
EVERY STEP OF THE WAY

Multipotent Somatic Adult Stem Cells (ASC)

- Adult Stem cell derived medicinal product for use as a skin cell therapy.
- Human Dermal Microvascular Endothelial Cells (HDMECs).
- Mixed test cell line: keratinocytes, fibroblasts, melanocytes and HDMECs; pool of several donors; several passages.
- Test system: NOD-SCID Gamma (NOD.Cg-Prkdc^scid^Il2rg^tm1Wjl^SzJ), 6-8 weeks old at start of dosing; single sex (female).
- Combined immune deficiency and IL2 receptor gamma chain deficiency, so good model for investigating human neoplastic disease.
- End points were evaluated in this study: mortality, clinical observations, palpable masses, body weights, food consumption, organ weights, gross necropsy and histopathology.
- Cell therapy given by a single subcutaneous injection followed by a 26 week observation period.

EVALUATION OF STEM CELL-DERIVED CELLULAR THERAPY PRODUCTS
EVERY STEP OF THE WAY

Multipotent Somatic Adult Stem Cells (ASC)

- Malignant squamous cell carcinoma present at the inoculation site.
- Tumours characterised by cords and trabeculae of polygonal epithelial cells with large vesicular nuclei, prominent nucleoli, and basophilic cytoplasm. There was a high mitotic index, anisocytosis, and loss of cohesion (acantholysis) between the tumour cells.

- Tumours were considered of test item origin due to their location (subcuticular).
- Squamous cell carcinomas are derived from squamous epithelial cells (keratinocytes) which are not found in the mouse subcutis/dermis, but were part of the test item cell lineage (Human keratinocytes, fibroblasts, melanocytes and Human Dermal Microvascular Endothelial Cells).

EVALUATION OF STEM CELL-DERIVED CELLULAR THERAPY PRODUCTS
CASE REPORT

Induced Pluripotent Stem Cell

Role of the Pathologist – GMP study
EVERY STEP OF THE WAY

Research at UCL/Moorfields Eye Hospital NHS Foundation Trust

- Stem cell derived medicinal product to treat macular degeneration.
- Pluripotent human embryonic stem cells (hESC) induced to differentiate into retinal pigmented epithelial (RPE) cells.
- IHC and ISH to GMP standards.
- Final product = Fixed patch in phosphate buffered saline
- Other half implanted into patient to replace RPE cells lost during disease progression
- The first surgery successfully performed in summer of 2015, no complications to date.

“There is real potential that people with wet age-related macular degeneration will benefit in the future from transplantation of these cells,” Professor Lyndon Da Cruz from Moorfields Eye Hospital, London (retinal surgeon)
EVERY STEP OF THE WAY

Research at UCL/Moorfields Eye Hospital NHS Foundation Trust

- In a two hour operation, patch inserted under the retina of each patient, who were then monitored for a year.
- Both went from not being able to read at all, to reading up to 80 words-a-minute using normal reading glasses.
- Douglas Waters, 86, from Croydon, London, was one of two people who had received the treatment at Moorfields Eye Hospital. He developed severe wet AMD in July 2015 and received the treatment three months later in his right eye.
- “I was struggling to see things clearly, even when up-close. After the surgery my eyesight improved to the point where I can now read the newspaper and help my wife out with the gardening. It’s brilliant what the team have done and I feel so lucky to have been given my sight back.”

“The results suggest that this new therapeutic approach is safe and provides good visual outcomes”
Professor Lyndon Da Cruz from Moorfields Eye Hospital, London (retinal surgeon)
REFERENCES
EVALUATION OF STEM CELL-DERIVED CELLULAR THERAPY PRODUCTS

Support Information

Summary report on the EMA workshop on stem cell based therapies

Guideline on human cell-based medicinal products

Guideline on the non-clinical studies required before first clinical use of gene therapy medicinal products.

Points to consider on xenogeneic cell therapy medicinal products

http://www.thelancet.com/pdfs/journals/lancet/PIIS0140-6736(10)61249-4.pdf
EVALUATION OF STEM CELL-DERIVED CELLULAR THERAPY PRODUCTS

Support Information


EVERY STEP OF THE WAY