Toxicological Assessment of Cell Therapy

NCAC 2020 Spring Virtual Symposium on Next Generation Tools, Technologies and Surrogate Endpoints in Toxicological Assessments

25 June 2020

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The information within this presentation is based on the presenter's expertise and experience and represents the views of the presenter for the purposes of a training.
Outline

- Background of cell therapy
- Cell differentiation and sources of cells
- Manufacturing and Product characterization
- Objectives of preclinical studies
- Animal models
- Proof-of-Concept (POC) Studies
- Biodistribution
- In Vivo Tumorigenicity
- Toxicology Studies
- Dose extrapolation in clinic
- Concluding Remarks
The human body contains over 200 different cell types (example, bone or brain cells).

Injury, disease or ageing: lead to the loss of specialized cells from the body, which may be irreversible in many cases.

Objective: Introduce new, healthy cells (mostly human) into a patient’s body, to
- Replace
- Regenerate
- Repair
the diseased or missing ones.

An emerging technology except for bone marrow transplantation for hematopoietic reconstitution.

Often indicated for rare or life threatening diseases, hence, have the capacity to fill unmet medical needs.

Can be extremely efficacious; can have unique safety challenges & concerns.
## Background of cell therapy (2)

<table>
<thead>
<tr>
<th>Small molecule</th>
<th>Monoclonal antibody</th>
<th>Cell (T cell)</th>
</tr>
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<tbody>
<tr>
<td>261 Da Cyclophosphamide</td>
<td>~10 nM, 148 kDa ~570X small molecule Adalimumab</td>
<td>~10 uM, ~1000X mAb T lymphocyte</td>
</tr>
<tr>
<td>Non-living</td>
<td>Derived from living cell, final product is non-living</td>
<td>Final product is living</td>
</tr>
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</table>
Cells differentiation

**Totipotent stem cells:**
- Differentiate into *embryonic and extraembryonic cell types.*

**Pluripotent stem cells:**
- Form cells of *all germ layers.*

**Multipotent stem cells:**
- Differentiate into *closely related family* of cells.

**Oligopotent stem cells:**
- Differentiate into only *a few cell types.*

**Unipotent cells:**
- Produce only *one cell type,* their own.
**Source of Cells**

**Source:**
- Autologous (self), Allogenic (intraspecies), Xenogeneic (interspecies)
- iPSCs (Induced Pluripotent Stem Cells): Genetically modified stem cells (reprogrammed from somatic adult cells)

<table>
<thead>
<tr>
<th>Stem cell-derived (Undifferentiated)</th>
<th>Functionally mature cells (differentiated)</th>
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<tbody>
<tr>
<td>- Embryonic, Fetal, Perinatal, Adult</td>
<td>- Adult</td>
</tr>
<tr>
<td>- Capacity for self-renewal</td>
<td>- No capacity of self-renewal and</td>
</tr>
<tr>
<td>- Capacity for differentiation</td>
<td>differentiation</td>
</tr>
</tbody>
</table>
**Donor eligibility of human cells:**
- Access to reliable donors is critical in the development.
- A donor is eligible only if screening shows that the donor is free from infection communicable diseases.

**Tissue collection and apheresis techniques:**
- Greatly impact the quality and potency of starting material.

**Cell Isolation and Processing:**
- Purification, isolation, and cryopreservation of cells performed using standardized protocols to ensure consistent high-quality cells.
- The process is the product. Change in process have impact on product attribute. Hence, consistent process as per GMP is required.

Product characterization:

- **Identity, composition**: To identify cells, biomarkers are developed. All non-cellular components appropriately characterized.
- **Purity and Cell number**: Unwanted cells controlled, free from adventitious microbial agents.
- **Viability and stability**: A shelf life under specified storage conditions determined.
- **Sterility**: Parenteral administration

**Potency testing**: *

- Potency testing or assays are required to assess intended biological effect.
- Performed as part of product conformance testing, comparability studies, and stability testing.
- Tests may be *in vitro* or *in vivo*, and may include surrogate markers such as protein expression profiles, flow cytometry assays, etc.

### Objectives of preclinical studies

- Establishing “Proof-of-concept” (POC), Pharmacologically effective dose(s)
- Optimization of route of administration
- Potential target tissue(s) of toxicity/ activity
- Recommend initial safe dose & dose escalation scheme in humans
- Parameters to monitor clinically

<table>
<thead>
<tr>
<th>Pivotal toxicity</th>
<th>Yes</th>
</tr>
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<tbody>
<tr>
<td>Genetic toxicity</td>
<td>Generally not required</td>
</tr>
<tr>
<td>Safety pharmacology</td>
<td>Some, as a part of toxicity</td>
</tr>
<tr>
<td>Exposure</td>
<td>Biodistribution</td>
</tr>
<tr>
<td>DART and Tumorigenicity</td>
<td>May be</td>
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**Preclinical data required** depends on:
- Amount of published preclinical or clinical safety information,
- Previous preclinical/ clinical experience,
- Minimal vs significant ex vivo manipulation.

Animal Model

- The use of multiple species or nonhuman primates is not a default.
- Biologically relevant species, comparable physiology/pathophysiology to human population.

- Cross-species immunogenicity necessitate use of immunocompromised animal model.
  - Immunosuppressive agents in immune-competent animals (e.g. large animals)
  - Genetically immunodeficient animals (rodents - SCID mice, Balb-c nude mice)
- Humanized (transgenic) animals
- Surgically altered /Tumor models
- Administration of analogous cellular products in the preclinical studies.
Pharmacology/ efficacy studies:
- Evaluate reversing or slowing disease progression.
- Markers of biological activity employed when available.
- **In vitro**: biological activity
- **In vivo**: animal models of disease/injury

**Study Design Example:**
- Group size: 5/sex for rodents; 3 to 5/sex for nonrodent.
- Surgically altered /Tumor models
- Provide preliminary data on
  - Maximum tolerated dose, cell survival
  - Selection of animal model
  - Proposed mechanism of action

**Potential use of cell therapy in:**
- Cancer
- Blood
- Cardiovascular
- Eye
- Nervous system
- Respiratory track
- Skin and connective tissue
- Viral and
- Congenital diseases
Cell Fate Following In Vivo Delivery:

- Not subject to conventional chemical analyses/ metabolism.
- Cells have an inherent potential to distribute to sites other than to the target organ/ tissue.
- Understanding survival, proliferation, distribution, differentiation and integration is a key safety concern.
- Sensitive methods used to detect the implanted cell.

- Imaging method:
  - Detection of radioisotope-labeled cells or cells tagged with diI (fluorescent) label.
  - Non-invasive; hence, the same animal can be evaluated over time.

- Polymerase chain reaction (qPCR) analysis and immunohistochemistry:
  - Identify cells of human origin or cells of a karyotype different than the host.
**Study Design (qPCR analysis) Example:**
Single Dose Biodistribution Study in the Mouse with 3 /6-month Observation Period

<table>
<thead>
<tr>
<th>Group</th>
<th>Time point</th>
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<td></td>
<td>24 h</td>
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**In-life phase evaluations:**
- Clinical observations, injection site scoring, Body weights, Food consumption.

**qPCR analysis for detection of human-specific DNA:**
- Blood samples and selected major tissues and injection site collected and processed for qPCR analysis.
In Vivo Tumorigenicity

- Phenotypic stability (tumorigenic potential or ectopic tissue formation) is a serious concern for pluripotent/multipotent stem cell products or when karyotypic changes seen during manufacture.
- Relevant animal model (immunocompromised SCID mouse), 10/sex/group
- Dose level(s): Maximum feasible dose, positive and negative controls
- Cellular engraftment via intended clinical route.
- Tumor formation is monitored during over 6 months and microscopic pathology is done for all tumors.

Cell administration

No effect  
Benign tumour/teratoma  
Malignant tumour

Potential outcome

Tumor are evaluated to identify the source of mass i.e. host (spontaneous tumors) or cell product.
Factors considered in the design of the toxicology study:

- **Animal model:** one or more relevant models (mostly immunocompromised)
- **Test article:** human cells or comparable analogous cells
- **Dose levels:** Multiple dose levels, in order to determine the NOAEL (Minimum effective dose, multiples of proposed human dose; controls)
- **Number of animals:** 5/sex/group for rodents; 3 to 5/sex for nonrodent.
- **Route of administration:** As per clinical indication. IV, SC routes are common. Uncommon route may be required based on indication (example, intrathecal).
- **Chronic studies may be needed, if cells persisted in the body, beyond one month as demonstrated in biodistribution study.**
- **Additional requirement for cell therapy with Noncellular Constituents:**
  - If medical device is used, assess biocompatibility with cells as per ISO-10993
  - Formulation (e.g. DMSO), contaminant may need to be evaluated.
Parameters:
- General health status, food consumption, body weights, clinical laboratory parameters (hematology, coagulation, serum chemistry), local tolerance.
- Assess macroscopic pathology (gross observations) and microscopic pathology (histopathology) at terminal sacrifice.
- Analyze multiple sections from site of transplantation. Consider specific IHC staining in addition to standard staining.

Interpretation:
- Assess host immune response (inflammatory response in target/nontarget tissue, auto-antibodies, sensitized cells against normal tissues/organs).
- Assess functional and morphologic response in target/nontarget tissue.
- Identify target tissues.
- Characterize toxicities (delayed toxicity/ reversibility of toxicity).
Tissue Specific Considerations
HPC (hematopoietic progenitor cells), Cord Blood Injectable Suspension for Intravenous Use

<table>
<thead>
<tr>
<th>Type</th>
<th>Indication</th>
<th>Proposed Mechanism</th>
<th>Preclinical studies</th>
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</thead>
<tbody>
<tr>
<td>Allogeneic cord blood hematopoietic</td>
<td>Hematopoietic system disorders</td>
<td>Cord Blood may migrate to the bone marrow, where the cells divide and mature, and</td>
<td>Not conducted due to the previous human experience with HPC, Cord Blood and minimal manipulation. Embryofetal development study reported.</td>
</tr>
<tr>
<td>hematopoietic progenitor cell therapy</td>
<td></td>
<td>are then released into the bloodstream, to restore blood counts and function (including immune function) of blood-borne cells of marrow origin.</td>
<td></td>
</tr>
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The most common infusion-related (clinical) adverse reactions are:
• Allergic reactions, anaphylaxis, Infusion Reactions, Graft failure - immunologic rejection
Principle of CAR (chimeric antigen receptor) T cell therapy

- A type of treatment in which a patient's T cells are changed in the laboratory so they will attack cancer cells.
- The gene for a special receptor, chimeric antigen receptor, CAR (that binds to a certain protein on the patient’s cancer cells) is added to the T cells in the laboratory.
- Large numbers of the CAR T cells are grown in the laboratory and given to the patient by infusion.
• **Cytokine release syndrome (CRS):** Immune activation resulting in elevated inflammatory cytokines. Severity varies from organ toxicity to potentially life threatening.

• **Neurological toxicity:** It is plausible that elevated cytokine levels are partly responsible for the neurologic sequelae.

• **On-target/off-tumour recognition:** Most targets of CAR T cells have shared expression on normal tissues. The severity of reported events has ranged from manageable lineage depletion (B-cell aplasia) to severe toxicity (death).

• **Anaphylaxis:** Antigen-recognition domains derived from murine mAb. Hence, both cellular and humoral rejection of CAR T cells have been demonstrated due to the immunogenicity of foreign protein.

• **Insertional oncogenesis:** Due to viral vector, low risk.
### CAR T cell therapy - YESCARTA® and KYMRIAH™

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<tr>
<td>CD19-directed genetically modified autologous T-cell immunotherapy</td>
<td>Treatment of adult patients with relapsed or refractory large B-cell lymphoma</td>
<td>Binds to CD19 (B lymphocyte antigen)-expressing cancer cells and normal B cells. (Killing of CD19-expressing cells)</td>
<td>Toxicology studies referenced.</td>
</tr>
</tbody>
</table>

- **YESCARTA®** (axicabtagene ciloleucel) suspension for IV infusion.
- **KYMRIAH™** (tisagenlecleucel) suspension for intravenous infusion.
- The common adverse effects in animals: B cells decreased/aplasia.
- Clinical adverse reactions include:
  - Cytokine release syndrome: fever, hypotension, tachycardia, hypoxia, and chills.
  - Neurologic Toxicities: encephalopathy, tremor, dizziness.
  - Hypersensitivity Reactions, Infections, Prolonged Cytopenia, Hypogammaglobulinemia.
### Cellular Immunotherapy - PROVENGE®

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<th>Proposed Mechanism</th>
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</thead>
<tbody>
<tr>
<td>An autologous cellular immunotherapy</td>
<td>Metastatic castrate resistant (hormone refractory)</td>
<td>Induce an immune response targeted against PAP (Prostatic acid phosphatase), an antigen expressed in most prostate cancers</td>
<td>Not conducted due to the autologous nature of Sipuleucel-T and the patient population of focus evaluated.</td>
</tr>
</tbody>
</table>

- PROVENGE® (sipuleucel-T) Suspension for Intravenous Infusion.
- PROVENGE consists of autologous peripheral blood mononuclear cells, including antigen presenting cells (APCs).
- Cells are activated with a recombinant human protein, PAP-GM-CSF.
- The most common clinical adverse reactions are chills, fatigue, fever, back pain, nausea, joint ache, and headache.
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<td>Genetically modified oncolytic viral therapy</td>
<td>Patients with melanoma recurrent after initial surgery</td>
<td>Replicate within tumours and to produce the immune stimulatory protein GM-CSF</td>
<td>Biodistribution (qPCR) Toxicity, Embryofetal development</td>
</tr>
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**IMLYGIC** (talimogene laherparepvec) Suspension for intralesional injection.

**Animal:** No adverse effects in immunocompetent mice.
- Severe combined immunodeficient (SCID) mice administered repeat intra-tumoral injections of IMLYGIC at 30-fold MRHD developed systemic viral infection (viral inclusion bodies or necrosis in tissues).

**Clinical:** May cause life-threatening herpetic infection in immunocompromised patients.
- IMLYGIC-treated patients were fatigue, chills, pyrexia, nausea, influenza-like illness, and injection site pain.
### Skin and Cartilage Indications

<table>
<thead>
<tr>
<th><strong>Product name</strong></th>
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<th><strong>Indication</strong></th>
<th><strong>Proposed Mechanism</strong></th>
<th><strong>Preclinical studies</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>LAVIV® (azficel-T) Suspension for Intradermal Injection</td>
<td>Autologous cultured human fibroblasts</td>
<td>Nasolabial fold wrinkles in adults</td>
<td>Increase the synthesis of extracellular matrix/collagen</td>
<td>Not conducted due to the autologous nature and the previous human experience. Literature reference cited.</td>
</tr>
</tbody>
</table>
• Preclinical studies provide the scientific basis to conduct the proposed clinical investigations.
• Justification for proceeding with clinical trials is based on
  o establishment of a rational scientific basis,
  o demonstrated proof of concept,
  o an adequate demonstration of safety in relevant animal model(s).
• Conventional allometric scaling methods for cell therapy products may be less precise.
• Dose exploration that includes identification of the MTD is generally recommended.
• For some cell therapy products, toxicity is not expected to be substantial in the predicted therapeutic range.
• In this situation, dose exploration is done by determining the range of biologically active or optimal effective doses (Minimum Anticipated Biological Effect Level - MABEL).
Tissue Specific Guidance

- Guidance for Industry, Cellular Therapy for Cardiac Disease, October 2010.
- Guidance for Industry, Preparation of IDEs and INDs for Products Intended to Repair or Replace Knee Cartilage, 2011.
• Cell therapy products are live and dynamic, hence, requires customized case-by-case preclinical program.

• Studies conducted in suitable animal models (mostly immunocompromised) to assess mechanism of action, biodistribution and safety of the product.

• Novel cell therapy requires guidance from agencies and experts.

• Cell therapies are the emerging therapeutic advance that will cure, rather than just treat some of serious human diseases.
Thank you for your attention.