Preclinical Biodistribution of Cell and Gene Transfer Vectors

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Disclosure

• Received consulting fee from Plulristem Therapeutics as a Member of SAB
Preclinical Safety Studies

• Identify target organs for toxicity...
  – If it doesn’t go to an organ, not likely to be toxic
• Wide variability in vectors and cell products which can impact localization and integration into distant tissues
• Must assess the distribution, in vivo, of the gene transfer vector or cellular agent among tissues for (unanticipated) tissue integration
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Biodistribution

• Where does it go?

  and

• When does it get there?
• How long does it stay there?
  – Corollary: does it hurt anything while it's there? (toxicology)

Always remember the gonads!
Biodistribution

• Assess for the presence of a cellular agent or gene therapy vector sequence in a panel of tissues collected at multiple time points ranging from a few days to several months post administration.
Biodistribution: FDA Principles

- The Good Laboratory Practice (GLP) regulations (21 CFR Part 58) apply to the conduct of preclinical laboratory safety studies that are intended to support INDs.
- We recommend that all preclinical toxicity and biodistribution/persistence studies evaluate the formulation and method of administration proposed for the clinical study.
- We recommend that you consult with CBER prior to submission of your IND to discuss the adequacy of your preclinical safety studies.
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Biodistribution: Assay

Five Components

• Gene transfer vector or cellular agent
• Animal model
• Method of analysis
• Tissue panel
• Time course
Biodistribution: Assay

Target for Analysis

• Gene Therapy
  – Assess for the vector/transgene sequence

• Cell Therapy
  – Human cells in immunocompromised rodent
    – use human specific sequences
  – Animal cells in animals as preclinical model—use Y chromosome in female hosts if possible
    N.B.-this will not be the clinical formulation
Biodistribution: Assay

Selection of your animal model

• Rodents are acceptable (also large animals)
• Disease model ideal, but not necessary
• May use animal tissue from efficacy studies
• Must be able to obtain panel of tissues
• Must have sufficient number of animals to be meaningful (exact number depends on findings, often 10 mice acceptable)
VIII. PRECLINICAL EVALUATION OF CELLULAR AND GENE THERAPIES

B. Animal Species Selection and Use of Alternative Animal Models

It is recognized that animal models of disease may not be available for every cellular or gene therapy system. Preclinical pharmacologic and safety testing of these agents should employ the most appropriate, pharmacologically relevant animal model available. A relevant animal species would be one in which the biological response to the therapy would be expected to mimic the human response. For example, a vector expressing a human cytokine would best be tested in an animal species in which that cytokine binds to the corresponding cytokine receptor with affinity comparable to that seen with human receptors, and initiates a pharmacologic response comparable to that expected in humans.
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Biodistribution: Assay

Method for Analysis

• QPCR

• Others
  – Non-quantitative or semi-quantitative PCR
    • Often acceptable, especially if all negative (sensitivity)
  – FISH
  – Southern blot
  – Reporter gene expression (e.g. GFP, LacZ, luciferase)...cannot be clinical formulation
Biodistribution: Assay

QPCR

• Validate assay: known pos, known neg
• Determine for sensitivity, specificity, and absence of inhibitors,
• Positive control should be a spiked, inherently negative sample (best to prove no inhibitors) in contrast to a separate sample or only using β-actin or GAPDH (still should use these controls)
Biodistribution: Assay

QPCR

What is adequate sensitivity?
Gene transfer: <100 copies of vector/μg DNA

When in doubt...contact the FDA before developing the assay to ensure your data will satisfy reviewers
## Biodistribution: Tissue panel

- Brain
- Heart
- Lung
- Liver
- Spleen
- Kidney
- Blood
- Bone marrow
- Ovaries/Testes
- Draining lymph nodes (from injection site)
- Injection site (muscle or subcutis tissue)
- Protocol specific tissues, e.g. thymus, gut
  - Talk to the FDA in advance, if uncertainty.
Gonads and Vertical Transmission

- Assess Testes and Ovaries
- If negative → study complete (?-sensitivity)
- If positive, breed treated subjects and assess offspring using biodistribution assay (if vector integrated into sperm or ova, all cells in the offspring should contain the vector sequence).
- Consult with FDA if concerns
Biodistribution: Time Course

Time points should be selected, in part, based on knowledge of your vector or cell product...

...and on your desired goal

- Short term persistence if you want to induce a short term effect, e.g. stimulate an immune response as a cancer vaccine, stimulate neovascularization, then prove non-persistence.

- Long term persistence if you want to permanently express your product, e.g. correct a monogenic disorder, then prove persistence.
Case Study

Safety and biodistribution studies of an HSV multigene vector following intracranial delivery to non-human primates

— Wolfe et al. 2004

NUREL-C2
Case Study: Intracranial HSV

- Rhesus macaques, n=5
- Craniotomy
- Injected 10 x 0.01 ml doses of vector prep in circular pattern to simulate circumferential treatment of a tumor
- Assessed animals at 4 and 35 days
Case Study: Intracranial HSV

- QPCR Assay specific for vector sequence
- Validated
- Sensitivity was 35 copies/100 ng genomic DNA
- 3 different primer/probe sets
- Different QPCR instruments—all in agreement
- Assessed injection site at 4 days
- Assessed panel of tissues at 35 days
Case Study: Intracranial HSV
## Case Study: Intracranial HSV

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<thead>
<tr>
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<th>4 d</th>
<th>35 d</th>
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<tbody>
<tr>
<td>Injection site</td>
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<td>+ (10-fold less)</td>
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<tr>
<td>Contralateral cortex</td>
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<td>-</td>
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<td>Spinal cord (C, T, L)</td>
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<td>Heart</td>
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<tr>
<td>Skeletal muscle</td>
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Case Study: Intracranial HSV

Conclusions of Biodistribution Studies

• Vector sequences persist for at least 35 days at the injection site (with 10-fold reduction)
• Viral vector spread either does not occur or occurs at very low levels (below detection) by this route of administration.
Case Study: Intracranial HSV

Summary of the Five Components

• Gene Transfer Vector: HSV multigene NUREL-C2
• Animal model: Rhesus macaques
• Method of analysis: QPCR - validated, sensitive
• Tissue panel: injection site, protocol specific sites, general body tissues
• Time course: 4 and 35 days