

ASGCT Translational Sciences Training Course

Design of Preclinical Safety and Efficacy Studies: The Basics of Cell, Gene, and Oligonucleotide-Based Therapies

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Favorite Sponsor Quotes as a Regulator

“We’re talking regulatory now...not science.”??

“My study is 70% GLP is that OK?”

Most Frequent Question as a Consultant

“What is the least amount I have “To DO” to get into the clinic?”

Agenda

- “Case-by-case” approach
- Scope of Products
- Product Attributes
- Principle vs. practice
- Defining the question
- Specific safety concerns based upon product attributes

“Case-by-case” approach

- Is not ...
 - a **minimalist** approach
 - consistent with “**traditional practices**” for pharmaceuticals –i.e. checklist
 - **Easy to predict** if “acceptable” to regulatory authorities
- Must...
 - establish effective dialogue between developer and regulator
 - to ensure success
- Is...
 - **science-based, questions-based**, data driven, practical
 - consistent with “**traditional principles**”
 - **targeted based upon product attributes**
 - designed to obtain maximum information with judicious animal use
 - rational, **limitations/** knowledge gaps are **identified**
 - **flexible**, based on knowledge base
 - **innovative**, as new models to replace “outdated” models to answer new questions are ongoing activities

What is the Question?

- What is the optimal procedure/route/anatomical site for product delivery?
- What is the optimal timing for product delivery?
- Where does the product go?
- Will repeat administration be needed?
- Will chronic immune suppression be needed?
- What is the risk/benefit for the planned patient population?
 - Is there potential to see any activity in early trials?
- Is the proposed FIH in a vulnerable population?

Specific product attributes defined within and across product classes

- **Cell-based therapies**

- Peripheral- & cord blood-derived progenitor cells
- Progenitor cells, e.g. stem cells derived from various types of human tissues, embryos; iPSCs
- Modified cells (e.g. engineered T cells)
- Differentiated cells, e.g. islet cells, cartilage cells etc.

- **Gene-based therapies**

- Vectors:
 - Replication deficient viral vectors, e.g. retroviruses, Ad, AAVs, vaccinia/fowlpox viruses, HSVs, lentivirus, viral particles; bacterial vectors...
 - Plasmid DNA vectors
- Various types of transgenes delivered by various vectors
- Oncolytic viruses (OVs) – Conditional replication competent viruses, e.g. Ad, vaccinia, HSV, NDV - /transgene

Specific product attributes defined within and across product classes

- Oligonucleotide-based therapies (ODNs)
 - Antisense (ASOs)
 - Apatmer
 - siRNA
 - Immunostimulatory (IS) ODNs
 - Other types (e.g. ribozyme, microRNA, hairpin, decoys etc)

Recent arguments against labeling ODNs as gene therapy

- Unable to integrate into genome
- Cannot be autonomously replicated in cells
- Do not comprise a gene-no promoter/enhancer
- Cannot be translated into protein
- Interact with mRNAs, not nuclear DNA
- Reversible as ODN is cleared

***NIH and EMEA currently exclude ASOs and siRNA (non-vector mediated) as gene therapies**

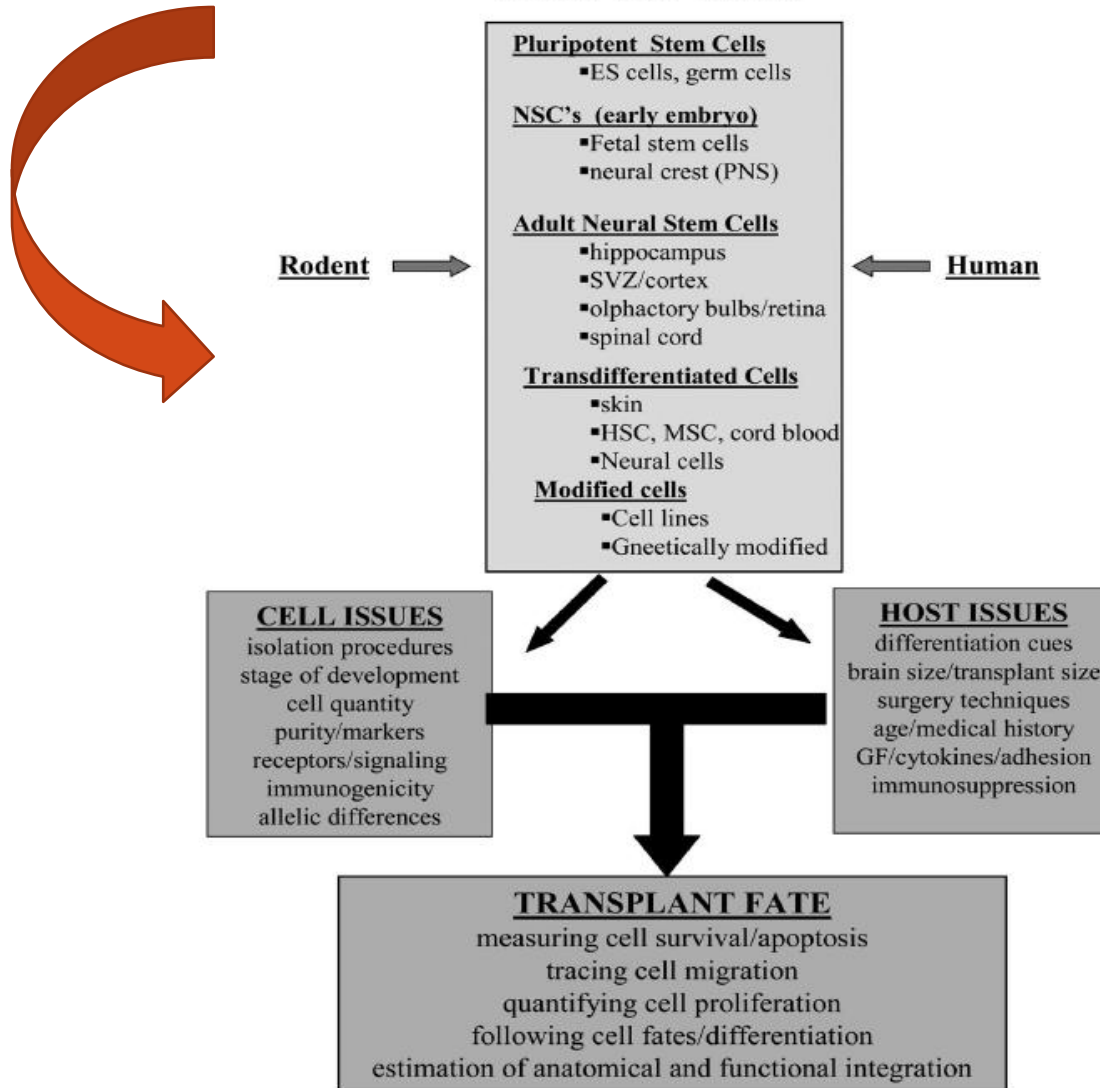
Case-by-case:

based on product attributes



- **Product attributes vary within product class**
 - Source of cells/donor tissue
 - Heterogeneity of cell cultures; cell products
 - X potency; X differentiated or “stemness”
 - Toti, Pluri, Multi
 - Un, Partially, Fully
 - Degree of foreignness
 - Autologous; Allogeneic; Xenogeneic
 - Reactivity to environment
 - Disease specificity
 - Dependency on survival for function?
 - Uncontrolled proliferation?

TRANSPLANTATION THERAPY: CELLS AND HOSTS



Case-by-Case
Considerations per
Disease Indication

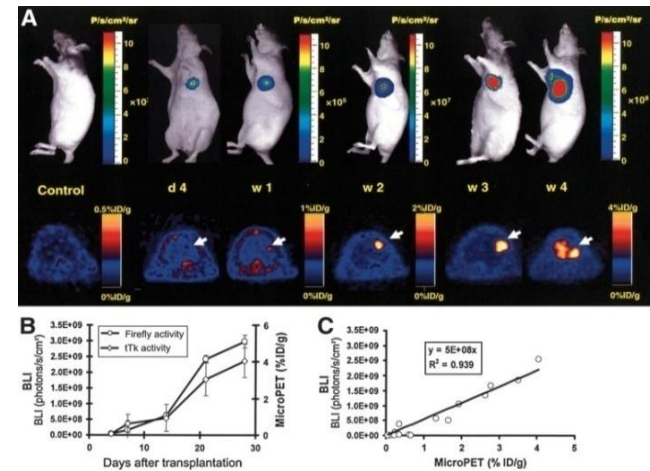
Neurodegenerative
Diseases

Product Attribute	Cell Therapy	Gene Therapy	ODN Therapy
Manufacture	Biological	Biological	Chemically synthesized
Purity	"Heterogeneous"	"Homogeneous"	Homogeneous single entity
Impurities	Difficult to qualify	Difficult to qualify	Easy to qualify
Potency	Needed (Difficult)	Needed	Not needed
Delivery System	Sometimes device	Sometimes device	Novel formulation
Dose Interval	Once, intermittent	Once	Daily, intermittent
Half-life	Months/years/ lifetime	Months/years/ lifetime	Minutes, hours
Species Specificity	Often	Sometimes	Sometimes
Toxicity	Usually related to MOA and/or host response	Usually related to MOA and/or host response	Often related to chemistry/product class
Immunogenicity	Sometimes	Sometimes	NA

Principle	Practice Cell Therapy	Practice Gene Therapy	Practice ODN Therapy
Test article	Clinical candidate; sometimes animal analogue	Clinical candidate; sometimes comparative vector; homologous transgene	Clinical candidate; one or more animal analogues
PK/ADME	Cell migration; cell trafficking; site for intended activity, distribution outside target site, migration at local site, time course	Biodistribution; level and persistence of viral gene expression; integration; vector shedding	Parent drug, catabolites
Species specificity	Access of anatomic site with intended delivery device, chemically modified or genetically immunodeficient	Sensitive to infections and the pathological consequences induced by wild type; sensitive to biology of transgene	Hybridization dependent and independent effects
Toxicity [High Dose]	Safety margin; maximum feasible dose	Safety margin; maximum feasible dose	Safety margin; MTD

PK/ADME: Cell Therapy

- Biodistribution or “cell trafficking”
 - Sufficient cells at the site
 - Migration at local site
 - Migration over time; sequential monitoring
- Systemic vs. local/targeted delivery
 - Inherent homing to tissues e.g. lung via IV ROA?
- Methods for cell tracking [in vivo, ex vivo; antemortem; postmortem]
 - Preclinical [e.g. 5/sex/group/time point]
 - e.g. fluorescence, magnetic particle-based imaging, isotopic imaging-PET-CT, SPECT, LacZ, GFP, B-gal, Dil-CM, human Alu ; Y chromosome; in situ hybridization, qPCR, qRT-PCR, IHC
 - Clinical [“antemortem”]
 - e.g. plain films; CT; Optical bioluminescence or fluorescence; ultrasound/echo; SPECT; PET; MRI; MRI/fluorescence; MRI/fluorescence/ultrasound



Principle	Practice Cell Therapy	Practice Gene Therapy	Practice ODN Therapy
Dose	Viable cell number Enumeration of specific cell pop Total DNA Total protein	Particle Number Transducing unit (DNA hybridization assay) Total Protein HPLC assay using authenticated ref std	Formulated - complex weight HPLC or CE using; authenticated ref std
Dose Administration	Concentration, volume, rate of delivery, location of injection, number of injections, cell stability	Volume, location of injection, number of injections, vector/transgene stability	Simple; complex formulations to enhance delivery
Dose Extrapolation	Number of cells delivered, initially retained, or eventually incorporated (BW, BSA, or target organ); cross species validation; previous human experience similar products	BW, BSA, or target organ); cross species validation; previous human experience similar products	Generally based on BW (mg/kg)



Extrapolation of Dose

Algorithm Based on Product Attributes



Comparison of Rat and Human Infusion Parameters

	Rat	Human
Efficacious Dose	3 10^6 cells/0.3 kg	$\sim 7 \cdot 10^8$ cells/70 kg (est.)
Body Wt Relative Dose	1 10^7 cells/kg	1 10^7 cells/kg
Infusion Volume	0.3 mL to 3 mL	~ 70 mL
Blood Volume	~ 18 mL	~ 5000 mL
Infusion Rate	0.1 mL/min (3 to 30 min)	3.3 to 6.7 mL/min ~ 100 mL/15-30 min
Body Wt Relative Infusion Rate	0.1 mL/min/0.3 kg ~ 0.33 mL/min/kg	3.3 to 6.7 mL/min/70 kg ~ 0.05 to 0.1 mL/min/kg
Body Wt Relative Infusion Rate (Cell)	3.3 10^6 cells/min/kg	5 10^5 to 1 10^6 cells/min/kg

IV ROA

Neural progenitors for spinal cord injury-base dose comparison on cross sectional area of spinal cord

Pancreatic progenitor cells –inform target dose based on animal models human cadaveric transplants

Demonstrating Pharmacodynamics (PD); Proof-of-Concept (POC)...and safety

- **In vitro/ ex vivo** studies
- Perform studies in **animal models of human disease/injury**
 - Measurement of biomarkers of activity/ *safety*
 - Results serve to support a rationale for conducting clinical trial
- Provide information concerning feasibility
 - Facilitate route of administration optimization
- Explore **dose-response relationship** between product and an activity/ ***safety outcome***
- Assess characteristics of animal model to humans
 - Cell biology
 - Anatomy
 - Biomechanics
 - Pathophysiology
- **Identification of abilities and limitations of the animal model(s)**



Toxicology Study Design Considerations- "The Practice" Cell and/or Gene Therapies

- Normal animals or animal models of disease
- Appropriate controls
 - Placebo, sham, positive
- Mimicking clinical treatment as closely as possible
 - Product, ROA, formulation including cell concentration (cells therapy), device, dose regimen etc.
 - Timing of administration relative to disease/injury
 - "window of opportunity"
- Consider interim, term, recovery assessments
- Reasonable group size
 - Generally 5-10 sex/timepoint [rodents];
4-6/sex/timepoint non-rodents
 - Plan for attrition based upon surgical procedure/disease model-
if applicable [could be as high as 50%]

Specific Toxicological Endpoints Included in Study Designs

- Endpoints –“over time” [early, mid, late]
 - Mortality, clinical observations, BW, food consumption, specific assessment of site of delivery, clinical labs, specific biomarkers, specific functional assessments, non-invasive imaging modalities, gross pathology, histopathology (special stains e.g. HuNA, Ki67)
 - Morphological alterations in either target/non-target tissues
 - Macroscopic and microscopic
- Tumorigenicity potential [cell therapies]

Toxicology Study Design Considerations- “The Practice” OND Based Therapies

- Safety pharmacology
 - In vitro (hERG relevance-size?)
 - In vivo (CV and respiratory –repeat dose tox; CNS-relevance if not transferred across BBB?)
- General toxicity (generally mice and NHP)
 - Use of homologous molecule(S)
- Genotoxicity (currently expected)
- Immunotoxicity (especially proinflammatory)
- Reproductive toxicity
 - Clinical candidate
 - Homologous molecule?
- Carcinogenicity (based on indication- design?)

Making the Case for Animal Models of Disease

- “Case-by-case”/Science-based/Questions-based
 - Product-specific design of programs
 - Defined by studies to ask specific questions
 - To support clinical decision-making
 - To obtain maximum information
 - Judicious use of animals
 - Modified, based upon knowledge base
 - NOT a minimalist approach
 - Limitations/knowledge gaps are identified
 - New models are encouraged to replace ‘outdated’ models to answer new questions

Thank you for your attention!



Relevant Guidance

<http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/default.htm>

- Guidance for Industry: Guidance for Human Somatic Cell Therapy and Gene Therapy (March 1998)
- Guidance for Industry: Gene Therapy Clinical Trials – Observing Subjects for Delayed Adverse Events (November 2006)
- Guidance for Industry: Preparation of IDEs and INDs for Products Intended to Repair or Replace Knee Cartilage (July 2007)
- Guidance for Industry: Considerations for Plasmid DNA Vaccines for Infectious Disease Indications (November 2007)
- ICH S6 document: Preclinical Safety Evaluation of Biotechnology Derived Pharmaceuticals (July 1997)

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- J. Cavagnaro (2008) Considerations in Design of Preclinical Safety Evaluation Programs to Support Human Cell-Based Therapies in *Preclinical Safety Evaluation of Biopharmaceuticals: A Science-based Approach to Facilitating Clinical Trials*, John Wiley & Sons, NJ. pp 749-781.

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