DEVELOPMENT OF CELL THERAPIES FOR PARKINSON'S DISEASE: LESSONS LEARNED AND CHALLENGES AHEAD

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DEVELOPMENT PATHWAY

- Selection of candidate cell line
- Manufacturing/testing plan
- Preclinical studies
- Interactions with FDA
- Phase 1 First-in-human study
- Continued clinical development

SELECTION OF CELL LINE

- hESC
  - Large scale MCB can be generated
- hiPSC
  - Avoids ethical concerns with hESCs
  - Autologous or allogeneic strategies possible
  - Minimizes immune effects and need for immunosuppression
- Combination cell/gene therapy
  - Synergistic effect with gene of interest (e.g., growth factors)

- GOAL IS TO GENERATE FUNCTIONAL DOPAMINERGIC NEURONS

MANUFACTURING PLAN

- Identification of scale
  - Adequate product for quality testing, nonclinical studies, clinical studies + stability program and retains
- Selection of GMP manufacturing site
- Process transfer/process development
  - Scale up considerations
    - Adherent culture systems limited in scale; suspension adaptation necessary
  - Avoidance of products of animal origin
  - Identify key process parameters of potential sources of variability
- Concomitant assay development
  - Identify critical quality attributes and set specifications/limits

KEY PROCESS PARAMETERS FOR hiPSC MANUFACTURE

- Harvest tissue
- Dissociate
- Reprogram cells using genes, proteins, chemicals
- Clonal selection
- Selection of stable cell for expansion and banking
- Establish/qualify Master Cell Bank
- Thaw, expand, and differentiate
- Harvest and delivery to patient

PRECLINICAL STUDIES

- Goals
  - Demonstrate Proof of Concept in animal model(s) of disease
  - Determine the Minimal Effective Dose and Maximum Feasible Dose for use in Safety/Tox and Tumorigenicity Studies
  - Establish safety profile for cell treatment
  - Confirm lack of tumorigenicity

PRECLINICAL STUDIES ANIMAL MODELS

- Neurotoxin models
  - Acute toxic effect -> Stable lesion
  - Non-pathogenic mechanism
    - Rodent (6-OHDA or MPTP)
    - Non-Human Primate (MPTP)
  - Suitable for restorative interventions
- Genetic models
  - Progressive pathology
  - May replicate a pathological mechanism of PD
- Aged animals
- Non-Human Primates >15-20 years of age present pathological changes in nigro-striatal neurons

ASSAY DEVELOPMENT

- In vitro characterization for preclinical testing
  - Neurophysiological profile
  - Dopamine production and metabolites (DOPAC and HVA)
  - Assays for assessment of cell survival, engraftment, migration
- Other assessments for evaluation biological/functional responses in animal models
- Biological assays for release of product
  - Potency – must correlate to predictable functional outcomes

NON-HUMAN PRIMATE MPTP-LESION

- Gold standard PD model
- Intravenous or intracarotid artery delivery
  - Pros
    - Parkinsonian motor deficits with established rating scales
    - Stable pathology from mild to extensive loss of dopaminergic neurons
    - Anatomical organization of NHP brain is consistent with human brain
  - Cons
    - Intra-animal variability in MPTP sensitivity
    - Less suitable for investigating neuro-protective interventions
    - Expensive, need personnel experienced with the model

SCALING CONSIDERATIONS

- Scaling factors for Rodent -> NHP -> Human
  - Volume of target structure
    - Mouse striatum 12 mm^3
    - Rat striatum 25 mm^3
    - NHP Putamen 1200 mm^3
    - Human Putamen 4000 mm^3
  - Brain volume and architecture present a challenge in scaling up local delivery procedures from small animal models to the clinical reality
    - Dose based on volume of target structure
    - Consider both concentration and volume
    - Balance increased distribution vs risk of off-target delivery

DELIVERY CONSIDERATIONS

- What is the clinical target structure?
  - Consideration of pathological changes
  - Degeneration of nigro-striatal fibers
  - Putamen, Caudate Nucleus and/or Substantia nigra
- How much coverage of the target is required?
  - Broad distribution within the target maybe necessary for clinical effect. Early PD gene therapy studies had limited distribution
  - Focus on specific areas of the target structure. Post-commissural putamen is more affected in PD than anterior putamen or caudate nucleus but more challenging to target
- Are there adverse effects from delivery to non-targeted regions?
  - Off-target effects of GDNF in the mid-brain included weight loss in NHP

DELIVERY CONSIDERATIONS

- Is there a suitable delivery device for clinical use?
  - Development of specialized reflux-resistant cannula for infusion of large volumes of gene therapy vectors
- How accurate is the delivery device?
  - Surgical targeting error is a significant issue that can be minimized by intra-operative imaging.
  - Development of neuro-navigational devices for use with real-time MRI guidance and monitoring
- How much coverage can be achieved from a single target site?
  - Targeting large structures (e.g. putamen) requires multiple delivery sites to optimize target coverage
  - How far will the cells migrate beyond the initial site of delivery
  - Can intra-operative imaging be used to visualize distribution
- How many sites can be safely targeted?
  - Targeting multiple sites in the putamen and/or substantia nigra

DELIVERY SYSTEM

- How will the cell therapy be handled at the clinical site/in the OR?
  - Stability of the product
  - Final formulation at clinical site
  - Quantity required to account for dead-volumes and priming of delivery devices
  - Procedures for maintaining sterility during loading
- Are there surgical constraints?
  - Duration of surgery
  - Resources required to support surgical procedure
  - Biosafety considerations

DELIVERY SYSTEM OPTIONS

- Hand injections
  - Simple, "low tech"
  - Easiest to implement, feeling of control on the part of the surgeon
  - Not consistent delivery - cannot control or know the precise infusion rate
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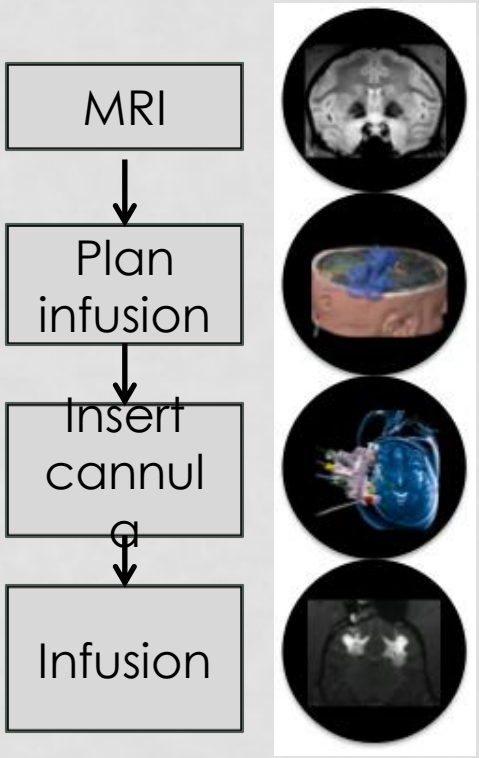
  - Not capable of doing true convection enhanced delivery
  - No additional regulatory considerations

- Infusion pumps
  - Most consistent method, offers most control over infusion rate
  - True convection enhanced delivery
  - Can be tedious to program and operate
  - Off label use

STATE OF THE ART DELIVERY

- Integrated CED platform for delivery of viral vectors to the brain developed in support of ongoing AAV2-AADC and AAV2-GDNF clinical trials in PD



Imaging

Diagnostic pre-infusion
Measurement of porous expansion

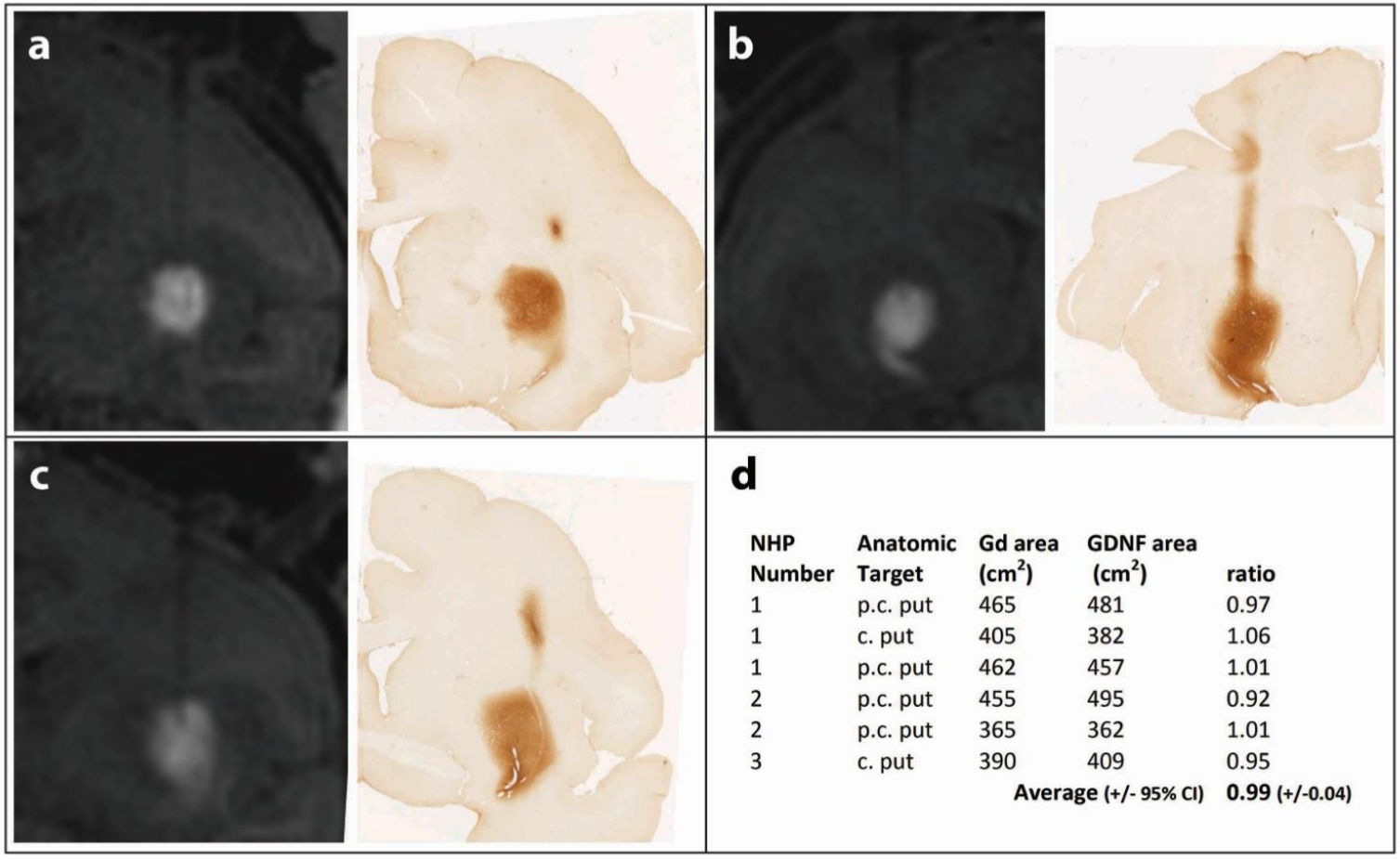
Software

DTI-based simulation
Shape fitting for inverse planning

Hardware

Acute iMRI CED (MRII, BrainLab)

CORRELATION BETWEEN PUTAMINAL DISTRIBUTION OF GD TRACER AND GDNF EXPRESSION



NHP Number	Anatomic Target	Gd area (cm^2)	GDNF area (cm^2)	ratio
1	p.c. put	465	481	0.97
1	c. put	405	382	1.06
1	p.c. put	462	457	1.01
2	p.c. put	455	495	0.92
2	p.c. put	365	362	1.01
3	c. put	390	409	0.95
			Average (+/- 95% CI)	**0.99** (+/-0.04)

Richardson et, al., 2011

INTERACTIONS WITH FDA

- Early consultation with Pharm/Tox
  - Present draft Clinical Synopsis and well thought out Preclinical Plan
  - Agree on design, timepoints and endpoints of preclinical studies (in vitro and in vivo)
  - Agree on scaling factors between animal models and human
  - Identify the need for consults with other Centers
    - Off label use of other regulated products; novel products
- Pre-IND
  - Present Manufacturing/Testing Plan
  - Present/discuss any relevant nonclinical findings
  - Present planned Clinical Synopsis

INTERACTIONS WITH FDA (CON'T)

- Agree on Patient Enrollment Criteria
  - First-in-human studies with significant risk therapeutic strategies will begin in later stage patients; subsequent studies will gradually include those subjects with greater chance of clinical response once safety is established
- Agree on interval between individual subjects/dose cohorts
- Agree on definition of Dose Limiting Toxicity
- Agree on Stopping Rules
- Agree on intervention/mitigation strategy for significant risks

CLINICAL STUDY SAFETY CONSIDERATIONS

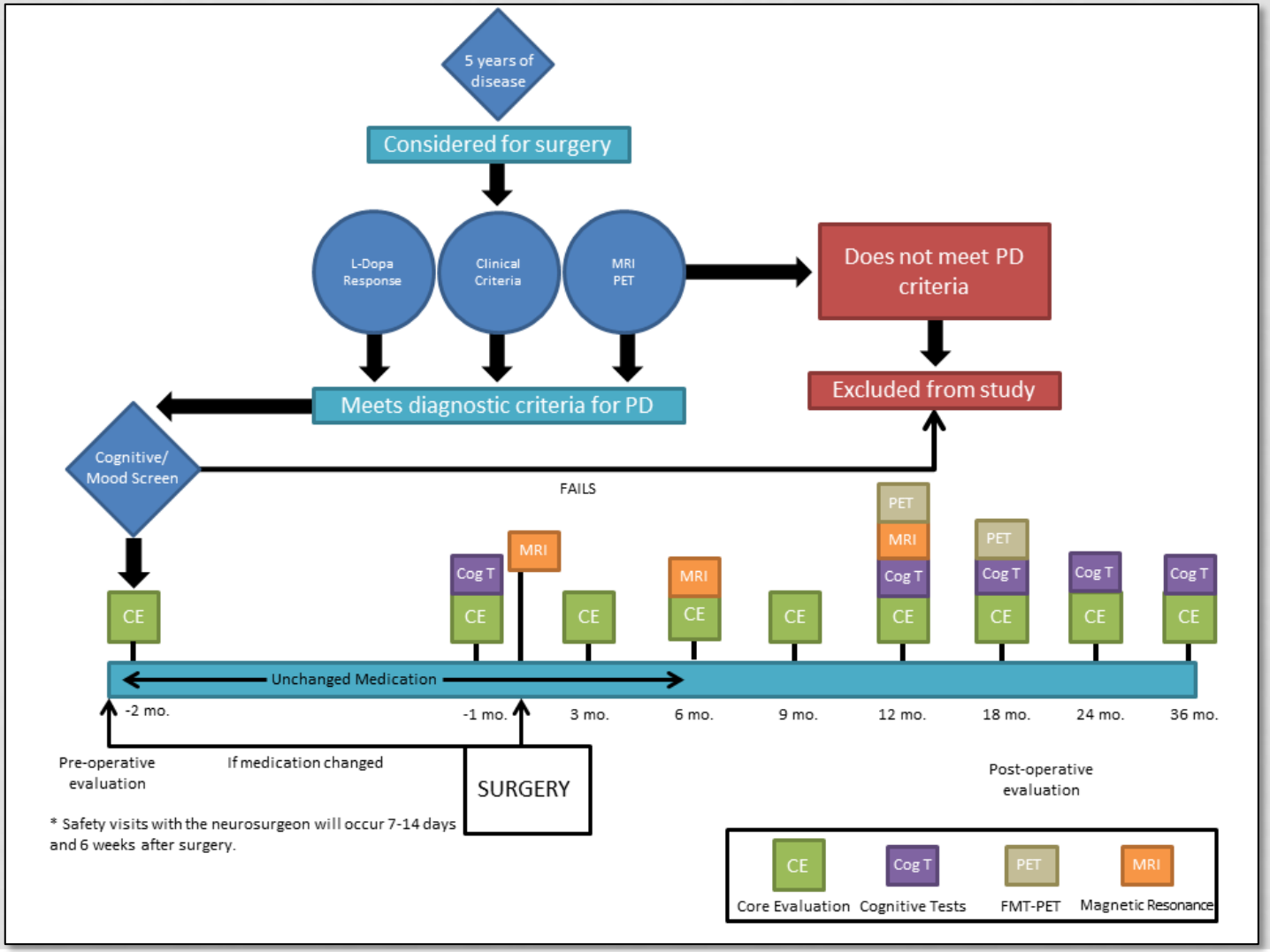
- Risks
  - Intracranial hemorrhage - probably similar to DBS, 2-3%
  - Infection - similar to brain biopsy, approx 1%
  - Reflux/non-targeted delivery - depends on cannula design, infusion rate, local tissue characteristics, cannula stability in tissue
  - Emergent psychopathology
  - Dyskinesias

- Prospectively design the plan for monitoring and intervention as necessary

CELL AND GENE THERAPY AND DEEP BRAIN STIMULATION (DBS)

- DBS is the current gold standard for the surgical treatment of Parkinson's disease
  - Can deter patients from investigational clinical studies since it provides relatively reliable and predictable improvement in PD symptoms
  - Can also "reassure" patients in investigational studies, as they know they can eventually chose to have DBS if investigational therapy is unsuccessful
  - Confounder in clinical study assessments

GENERAL SCHEMA FOR PHASE 1 CLINICAL STUDY



FINANCIAL CHALLENGES

- Surgery
- Biomarkers
  - MRI – $6,5000
  - PET/FMT - $17,000 for pre and post

CHALLENGES FOR SUBJECTS

- Lengthy surgery
- Long term commitment to Study Visits (~3 years)
- Lengthy Study Visits for PD assessments
  - Cognitive
  - Functional
    - Both in ON and OFF states
  - Biomarkers (e.g., PET, response to L-Dopa challenge)
    - May require travel
    - May be uncomfortable
- Patient diaries
- Delaying decision for DBS

CHALLENGES FOR FURTHER CLINICAL DEVELOPMENT

- Moving quickly to younger/less advanced Parkinson's patients
- Identification of the appropriate biomarkers/outcome measures (MRI, PET) to be used in selection of dose levels for further clinical studies
- Need for sham surgeries in later stage controlled clinical studies
- Large number of patient/subjects required to meet clinical benefit target of 60% improvement in UPDRS Motor Score
- ? Identification of Surrogate Endpoints
- Co-development of novel delivery system (Combination Product)

COLLABORATORS

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