Annual Report: Gene Therapy Development for Prion Diseases

Year 1 Progress Report (2024)

Executive Summary: During the first year of the project, significant progress has been made in the development and characterization of AAV vectors expressing dominant negative PrP proteins, although some challenges have been identified that require adjustments to the original timeline.

Key Achievements

1. **Vector Development and Expression Systems**

   - Successfully generated two AAV expression vectors using neuron-specific promoters:
     - Human synapsin promoter-based vector
     - Rat NSE promoter-based vector
   - Developed vectors expressing both wild-type and potential dominant negative PrP from various species
   - Created an additional AAV vector expressing murine GPI-less PrP variant

2. **Expression Level Assessment**

   - Evaluated expression levels following intravenous administration in:
     - PrP knockout mouse models
     - Wild-type mouse models
   - Achieved expression levels ranging from 0.5x to 2.0x of endogenous PrP levels
   - Human synapsin construct showed significantly higher expression compared to rat NSE promoter
   - Demonstrated sustained expression for at least 9 months post-AAV administration

Challenges and Solutions

1. **Expression Level Management**

   - Challenge: Unexpected high expression levels causing toxicity at certain doses
   - Solution: Conducted dose-dependent studies to identify optimal administration levels
   - Current Status: Working on promoter modifications to achieve non-toxic expression levels

2. **Administration Routes**

   - Challenge: Alternative administration routes pending ethics committee approval
   - Plan: Testing different administration routes in 2025 to reduce variability observed with lower doses

3. **Protein Visualization**

- Challenge: Difficulties in membrane-bound PrP visualization due to protein deterioration during fixation
- Progress: Demonstrated functional competence of AAV-expressed PrP through alternative studies

Preliminary Disease Model Studies

- Initiated studies with two animal models:
  - RML model
  - CWD-vole model
- Awaiting results before proceeding with more complex human disease models

Plans for Year 2

1. Optimize expression levels through:
   - Promoter modifications
   - Dose adjustments
   - Cellular localization improvements
2. Complete evaluation of alternative administration routes
3. Proceed with full assessment of negative dominance effects
4. Expand testing to additional disease models

Conclusion: The first year has been highly productive in generating and characterizing AAV vectors expressing different dominant negative proteins. While higher than expected expression levels have required protocol adjustments, the ability to reduce expression levels provides a clear path forward for achieving optimal therapeutic conditions in 2025.