Final project report

Gene therapy development for prion diseases

(Project Period: 2024-2025)

Executive summary

This final report summarizes the complete achievements of the gene therapy development project for prion diseases (2024-2025). The project has successfully achieved results in developing and validating AAV-based gene therapy approaches using dominant negative prion proteins. This represents a demonstration of therapeutic efficacy in prion disease models, with survival extensions ranging from 35% to over 100% in different disease models. The project has systematically advanced from vector development and optimization through comprehensive *in vivo* efficacy testing, establishing a robust platform for translational development toward clinical applications.

Project objectives

The primary objectives of this project were:

- Develop and optimize AAV vectors for efficient brain-wide delivery and expression of dominant negative prion proteins
- Characterize expression levels, temporal stability, and subcellular localization of AAV-delivered proteins
- Assess therapeutic efficacy in multiple prion disease models (RML, CWD, human prion diseases)
- Validate dominant negative candidates through structural and functional characterization

Accomplishments

3.1. Development and optimization of AAV expression systems

Vector development with multiple promoter configurations

We systematically evaluated multiple AAV constructs incorporating different CNS-specific promoters and regulatory elements to achieve optimal expression. The comprehensive testing included:

- Multiple neuron-specific promoters: CaMKIIα, rat NSE (ratNSE0.3), CALM1, human synapsin (huSyn), and gfaABC1D
- Systematic evaluation of regulatory elements: MVM (Minute Virus of Mice) enhancer, WPRE (Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element), and combinations thereof
- Expression of diverse PrP variants from multiple species, including both wild-type and potential dominant negative candidates
- Development of GPI-less PrP variant to assess extracellular expression capabilities

Through systematic comparison, we identified the huSyn-MVM-WPRE configuration as optimal, achieving expression levels comparable to endogenous PrP while maintaining neuron-specific targeting. This optimized construct was used in all subsequent therapeutic efficacy studies.

Expression level assessment and temporal stability

Following intravenous administration in both PrP-knockout and wild-type mouse models, we achieved:

- Expression levels ranging from 0.5x to 2.0x of endogenous PrP levels, with dose-dependent control
- Significantly higher expression with the human synapsin promoter compared to other neuron specific promoters
- Sustained stable expression for at least 9 months post-administration, demonstrating long-term transgene stability
- Dose-dependent relationship between viral load and expression levels, enabling titration to optimal therapeutic ranges

Alternative administration routes

To expand therapeutic delivery options, we successfully evaluated additional administration routes:

- Retroorbital injection: Achieved comparable brain-wide transduction to intravenous delivery
- Intraperitoneal injection: Demonstrated efficient CNS targeting with similar expression patterns

More complex direct CNS administration routes were evaluated but ultimately not pursued due to their inability to achieve comprehensive brain-wide distribution across all neuroanatomical regions.

Functional validation and localization

The correct expression and localization of AAV-delivered PrP was validated through its ability to generate functional disease models in knockout mice. This proof-of-concept, described in a manuscript currently under review, demonstrates that the AAV system expresses PrP with the same biochemical properties and subcellular localization as standard transgenic models. This validation confirms that the dominant negative protein will be correctly delivered to the appropriate cellular compartments to exert its therapeutic effect.

3.2. Therapeutic efficacy assessment in prion disease models

Selection and characterization of dominant negative candidates

Through a comprehensive systematic screening approach, we identified highly effective dominant negative PrP variants:

- Analyzed over 900 species worldwide from diverse mammalian and vertebrate taxa
- Tested 424 distinct recombinant PrP variants for resistance to spontaneous misfolding using PMSA (Protein Misfolding Shaking Amplification) technology
- Evaluated candidates for ability to interfere with endogenous PrP conversion in seeded propagation and interference assays

- Selected lead candidates based on: (1) resistance to spontaneous misfolding, (2) dominant negative interference with prion propagation, and (3) suitability for AAV-mediated brain delivery

Therapeutic results

The therapeutic efficacy studies yielded very promissing results, representing a successful demonstration of gene therapy efficacy in prion diseases:

RML prion model in C57BL/6 mice (AAV-PrP-DN1):

- Control mice (no AAV): 161 ± 5 days survival
- **AAV-treated mice: 217 ± 7 days survival**
- **Result: 35% survival extension (49 days)**

CWD-vole prion model in TgVole(I109)1x mice (AAV-PrP-DN1):

- Control mice (no AAV): 73 ± 4 days survival
- **AAV-treated mice: 105 ± 6 days survival**
- **Result: 44% survival extension (32 days)**

CWD-vole prion model in TgVole(I109)1x mice (AAV-PrP-DN2):

- Control mice (no AAV): 73 ± 4 days survival
- **AAV-treated mice: 160 days survival**
- **Result: >100% survival extension (87 days and counting)**

These results demonstrate that gene therapy can significantly extend survival in rapidly progressive prion disease models. The >100% extension with PrP-DN2 is particularly remarkable, indicating profound disruption of prion propagation mechanisms.

Ongoing studies in human prion disease models

Building on the success in animal prion models, we have initiated therapeutic efficacy studies in human prion disease models:

- Creutzfeldt-Jakob Disease (CJD MM1): Currently testing both dominant negative candidates in the Tg650 mouse model expressing human PrP. Studies are ongoing due to the slower disease progression in this model (expected timeline: 200-240 days).
- Gerstmann-Sträussler-Scheinker (GSS): Studies planned with GSS-A117V prions to assess efficacy against this inherited prion disease.
- Fatal Familial Insomnia (FFI): Future studies planned using our proprietary FFI mouse model.

Structural validation of lead therapeutic candidate

To ensure the therapeutic potential and translational viability of one of our lead candidate, we conducted comprehensive structural characterization based on Nuclear Magnetic Resonance (NMR) spectroscopy. We have confirmed that the dominant negative variant maintains the native PrP fold with no significant structural perturbations.

These structural data provide critical validation that the therapeutic efficacy stems from a dominant negative mechanism rather than non-specific protein aggregation or cellular toxicity. The preservation of native structure is essential for translational development toward human clinical applications.

Conclusions

This project has achieved success in developing AAV-based gene therapy for prion diseases. The demonstration of significant therapeutic efficacy—including the >100% survival extension with our lead candidate PrP-DN2—represents an important advance in the field. For the first time, we have shown that dominant negative gene therapy can profoundly disrupt prion propagation *in vivo*, providing hope for future treatments for currently untreatable fatal neurodegenerative diseases.

The systematic optimization of AAV vectors, comprehensive screening of >900 species to identify optimal candidates, and structural validation establish a robust platform for clinical translation. The sustained 9-month (at least) expression stability provide flexibility for therapeutic development. Functional validation of AAV-expressed PrP confirms that this delivery system maintains proper localization and biological activity for PrP variants, ensuring therapeutic candidates will be correctly positioned to exert their protective effects.

The ongoing studies in human prion disease models (CJD MM1, with GSS and FFI planned) will provide critical proof-of-concept for translational development. The preservation of native structure in our lead candidate, combined with the remarkable therapeutic efficacy observed, positions this approach favorably for progression toward clinical trials.