Exploring the efficacy of dominant negative protein-based gene therapy for different prion diseases: *in vivo* proof-of-concept study

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OBJECTIVES

What important prion-related problem are we trying to tackle?

The challenge: Prion diseases (CJD, FFI, GSS) are fatal with no available treatment

Current reality: No therapeutic options exist to slow or stop disease progression

Urgent need: New treatments that can block prion propagation in the brain

Our goal: Develop a therapy that can slow or halt the pathological process by blocking prion spread

GENE THERAPY USING DOMINANT NEGATIVE PRION PROTEINS

Blocking the spread of misfolded proteins in the brain

What is gene therapy?

- **Uses modified viruses** (AAV vectors) to deliver therapeutic genes directly to brain cells
- **Safe and efficient** way to express protective proteins throughout the brain
- **Can cross the blood-brain barrier** to reach affected neurons

What are dominant negative proteins?

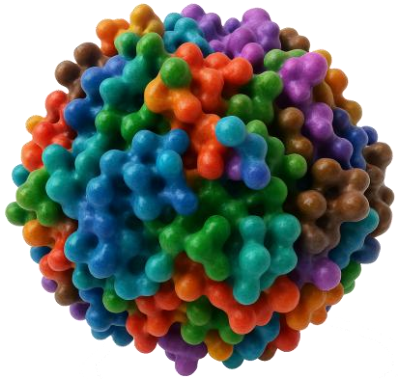
- **Do not misfold spontaneously**
  remain stable
- **Maintain normal cellular function**
  no toxicity to neurons
- **Block prion propagation**
  interfere with the misfolding of endogenous PrPs

How it works together?

AAV gene therapy delivers dominant negative proteins directly where needed in the brain to prevent prion spread

GENE THERAPY USING DOMINANT NEGATIVE PRION PROTEINS

Developing the optimal delivery system

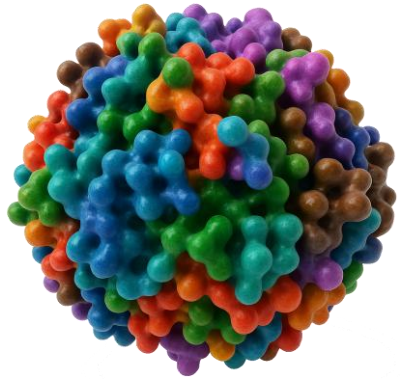


Optimizing AAV vectors for brain-wide expression

Finding the best dominant negative candidates

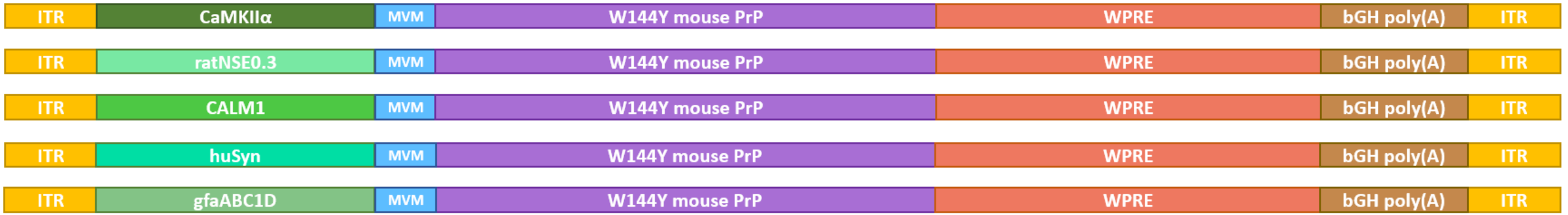
GENE THERAPY USING DOMINANT NEGATIVE PRION PROTEINS

Optimizing AAV vectors for brain-wide expression

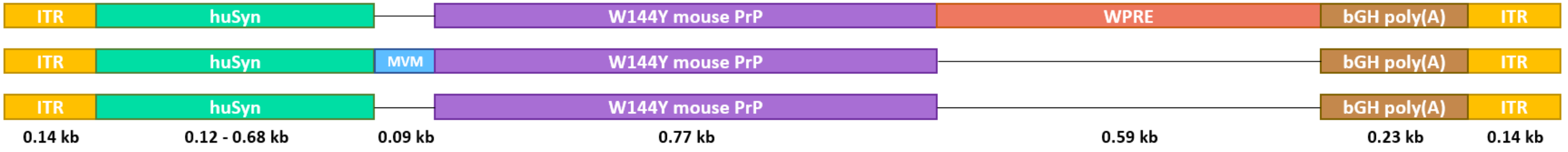


Vector design Tested multiple AAV constructs with different regulatory sequences

Promoter testing



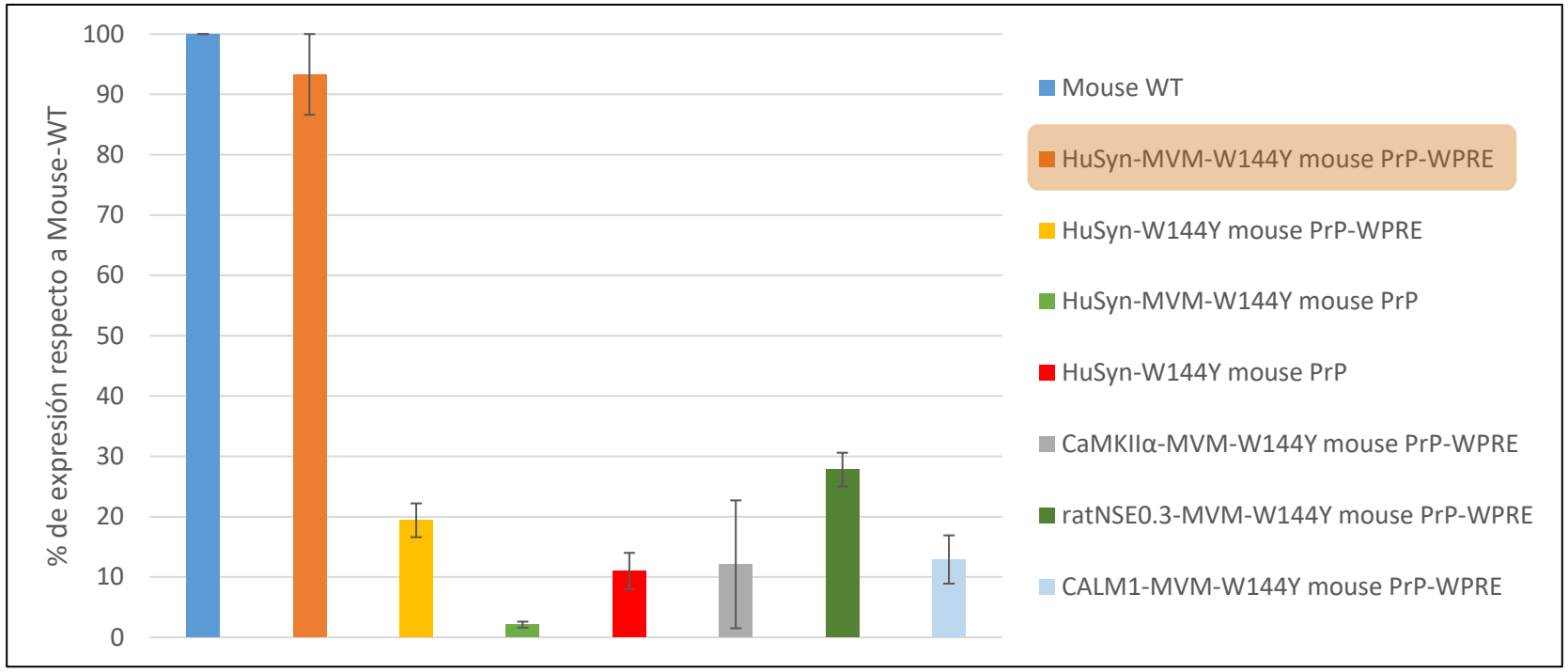
Enhancer combinations



GENE THERAPY USING DOMINANT NEGATIVE PRION PROTEINS

Optimizing AAV vectors for brain-wide expression

Expression analysis Evaluated protein levels 3 weeks after administration



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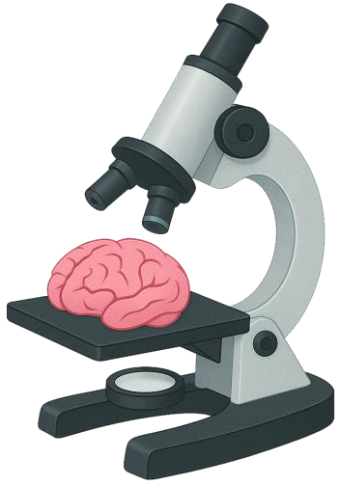
Optimizing AAV vectors for brain-wide expression



Regional distribution Confirmed widespread expression across all brain regions

Sagital view

Olfactory bulb

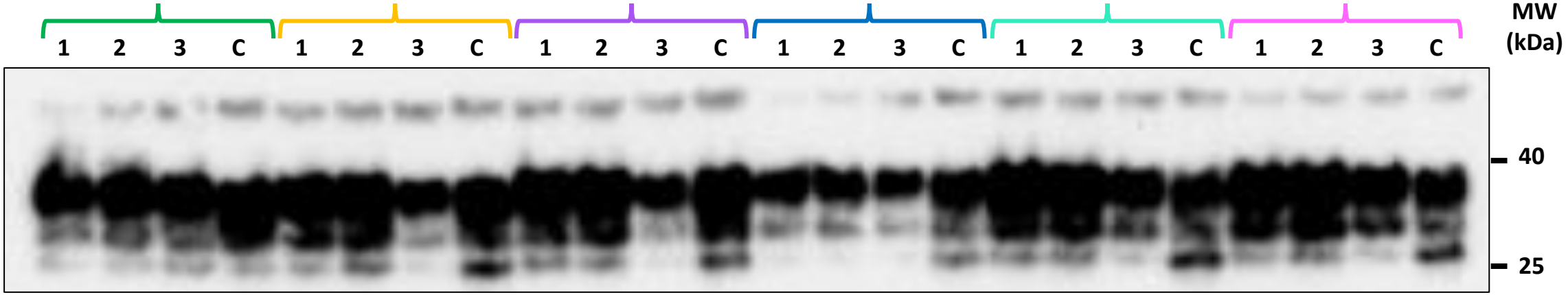
Striatum + Diencephalon + Mesencephalon

Cortex + Hippocampus

Cerebellum

Pons + Medulla oblongata

Spinal cord



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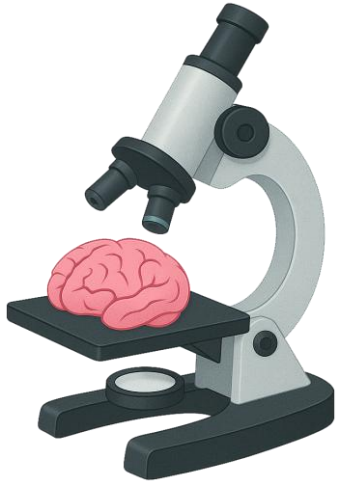
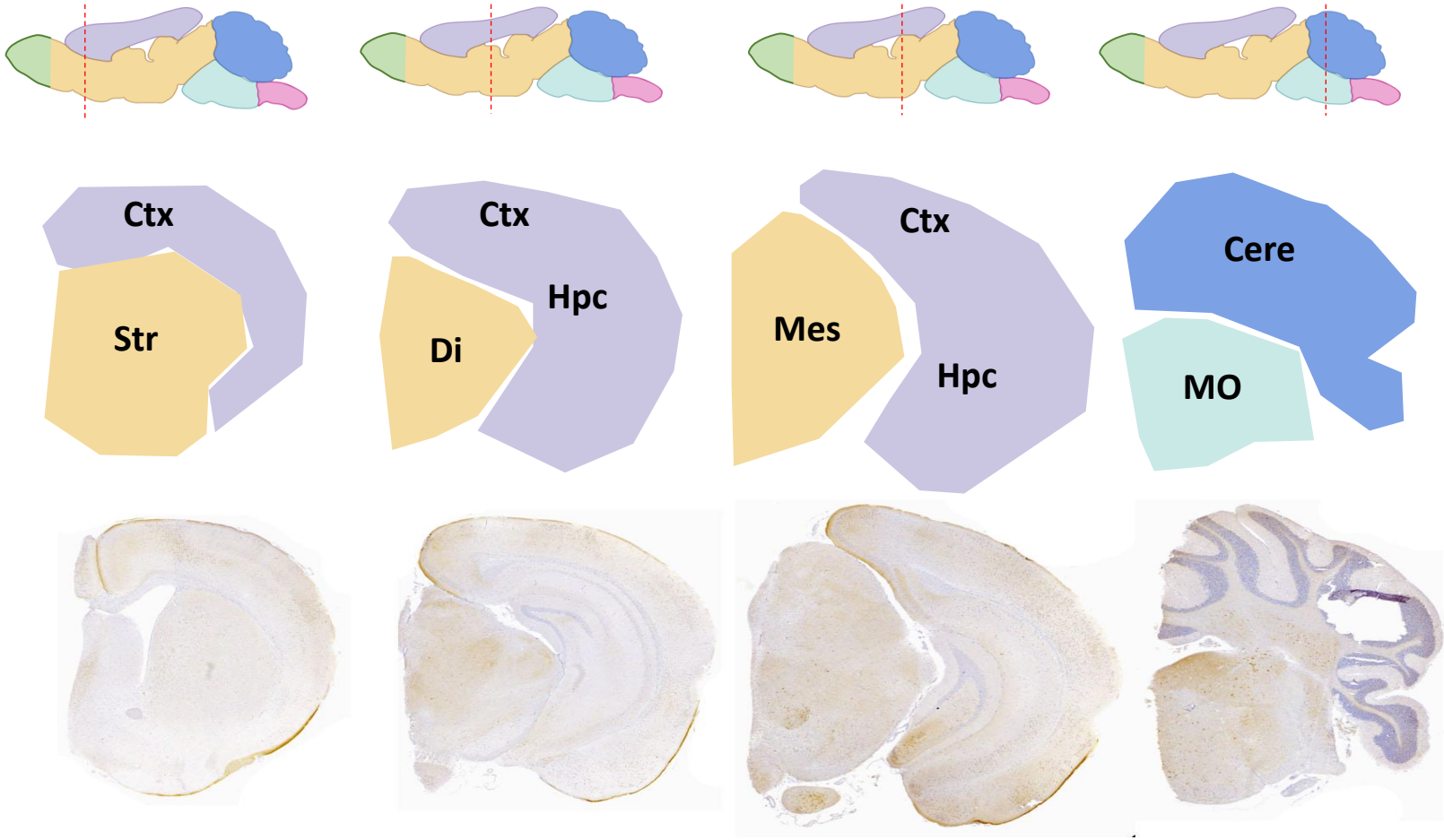
Optimizing AAV vectors for brain-wide expression



Regional distribution Confirmed widespread expression across all brain regions



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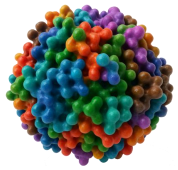
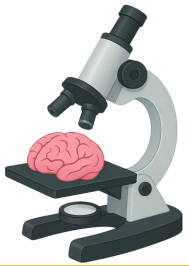
Optimizing AAV vectors for brain-wide expression



Achieved **optimal expression levels** comparable to natural protein levels

Stable, long-lasting expression throughout the brain



Safe delivery with no observed toxicity

GENE THERAPY USING DOMINANT NEGATIVE PRION PROTEINS

Finding the best dominant negative candidates

Approach Selected the most promising variants that could block prion propagation

1º Criteria Proteins that resist misfolding under laboratory conditions

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A "Noah's ark" approach to identify protective proteins

904 species

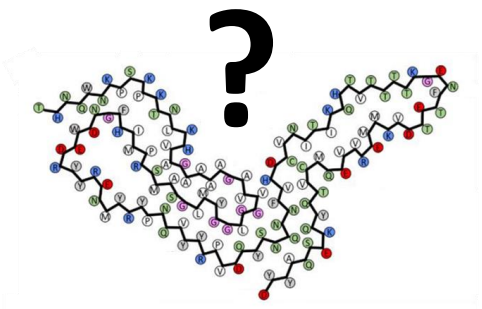
Selection based on sequence

424 different wt PrP

424! recombinant proteins (rec-PrP)

PMSA

Evaluation of the *bona fide* misfolding propensity



Misfolded rec-PrP

Analyzed 904 different species from around the world

Tested 424 different prion protein variants for misfolding resistance

Used advanced laboratory techniques (PMSA) to evaluate each candidate

Eraña et al, Nat. Commun. 2024

GENE THERAPY USING DOMINANT NEGATIVE PRION PROTEINS

Finding the best dominant negative candidates

Approach Selected the most promising variants that could block prion propagation

1º Criteria Proteins that resist misfolding under laboratory conditions

2º Criteria Variants that interfere with normal prion protein conversion

GENE THERAPY USING DOMINANT NEGATIVE PRION PROTEINS

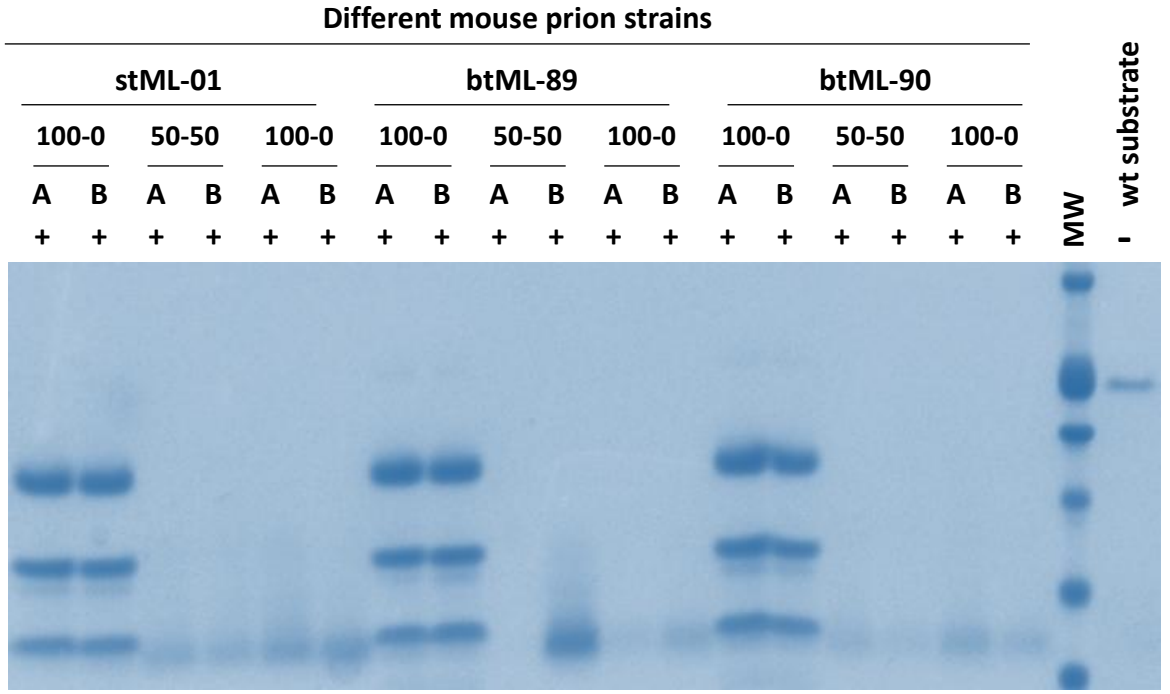
Variants that interfere with normal prion protein conversion

Confirm resistance to **spontaneous misfolding**

Seeded **propagation studies**

Seeded **interference studies**

Selection of the best dominant-negative mutants



Selection of the best dominant negative mutants by PMSA

GENE THERAPY USING DOMINANT NEGATIVE PRION PROTEINS

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2º Criteria Variants that interfere with normal prion protein conversion

3º Criteria Candidates suitable for brain delivery via gene therapy

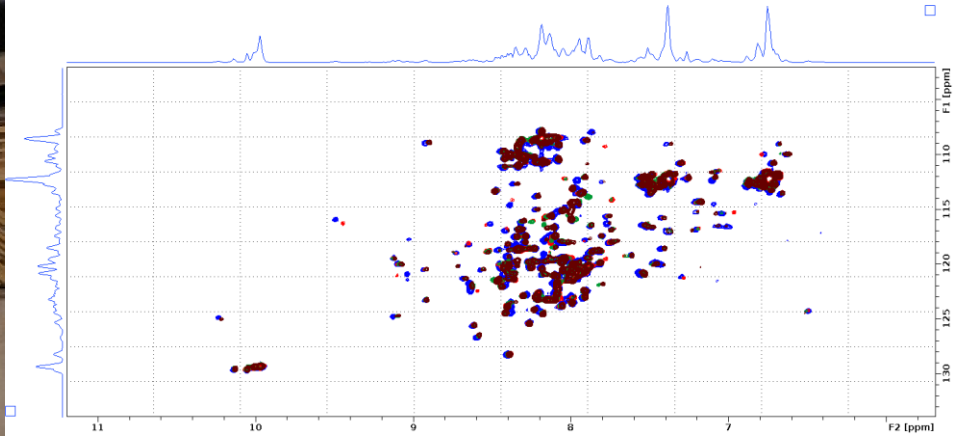
GENE THERAPY USING DOMINANT NEGATIVE PRION PROTEINS

Candidates suitable for brain delivery via gene therapy

Structural characterization using NMR spectroscopy

Computational modeling of potential interference with amyloid fibril formation

Structural characterization for final candidates of dominant negative PrPs



Structural characterization for final candidates of dominant negative PrPs

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Finding the best dominant negative candidates

Approach Selected the most promising variants that could block prion propagation

1º Criteria Proteins that resist misfolding under laboratory conditions

2º Criteria Variants that interfere with normal prion protein conversion

3º Criteria Candidates suitable for brain delivery via gene therapy

This approach identified several highly effective dominant negative proteins for therapeutic testing

GENE THERAPY USING DOMINANT NEGATIVE PRION PROTEINS

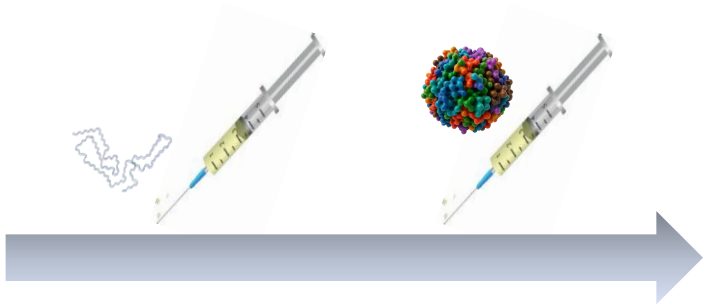
Therapeutic approach and experimental design

C57BL/6

Wild-type mouse

Day 1 — RML prion (ic) inoculation

Day 1 — Tail (iv) AAV inoculation



Day 160-170

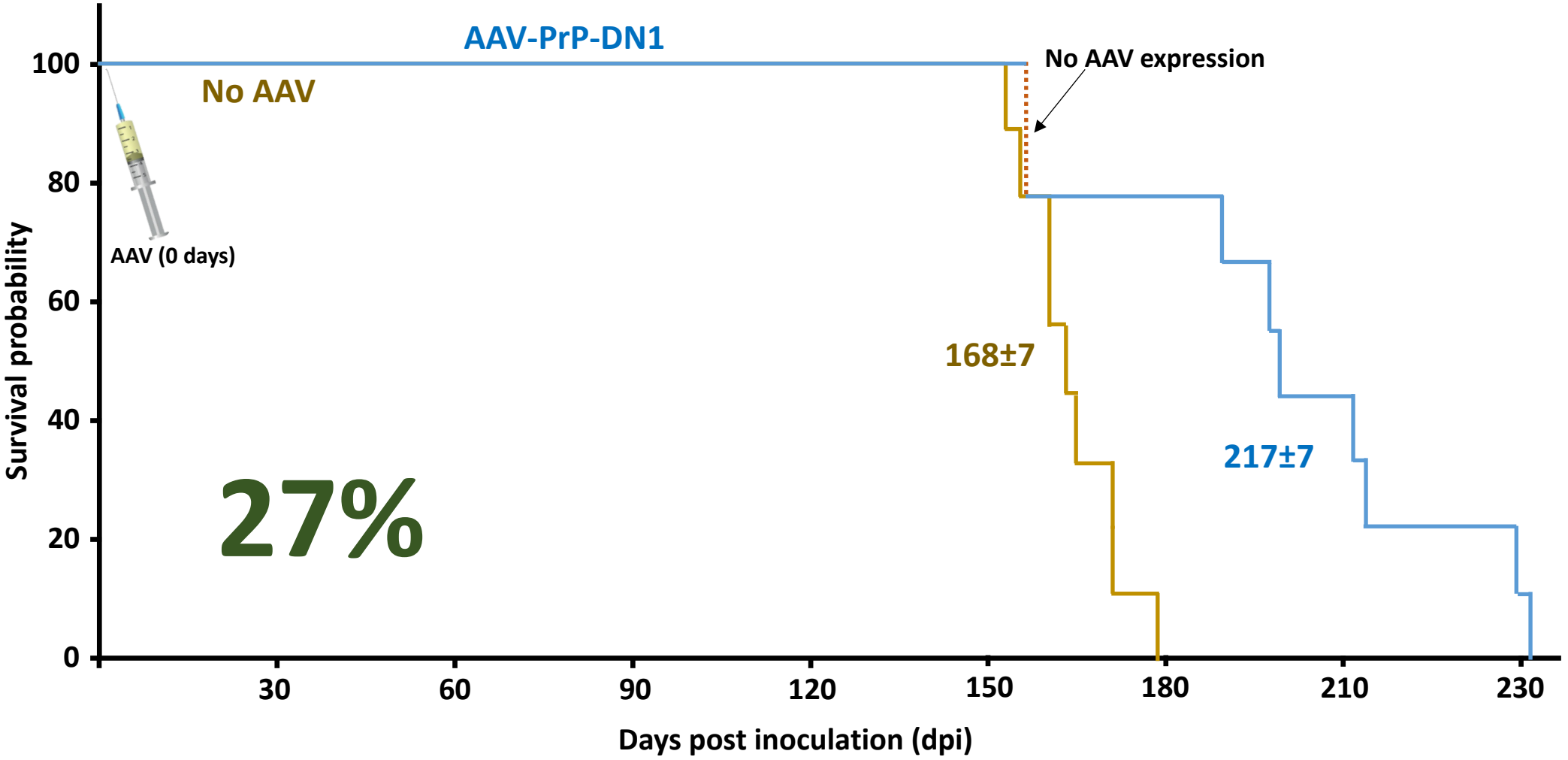
Clinical signs appear, humane endpoint and sample collection

A different dominant negative PrP variant tested

GENE THERAPY USING DOMINANT NEGATIVE PRION PROTEINS

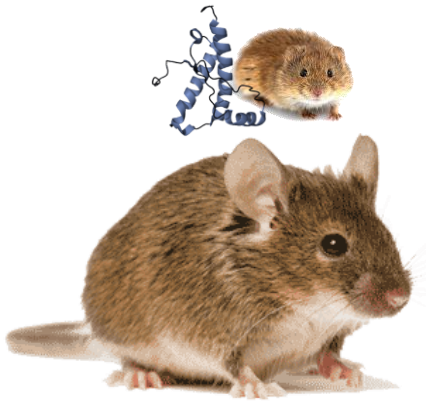
Therapeutic approach and experimental design

RML in C57BL/6



GENE THERAPY USING DOMINANT NEGATIVE PRION PROTEINS

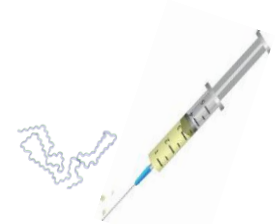
Therapeutic approach and experimental design



TgVole(I109)1x

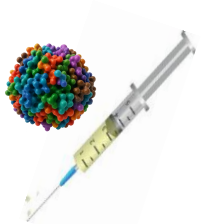
Tg mouse expressing bank vole PrP

Day 1



CWD-vole prion (ic) inoculation

Day 15



Tail (iv) AAV inoculation

Day 65-75

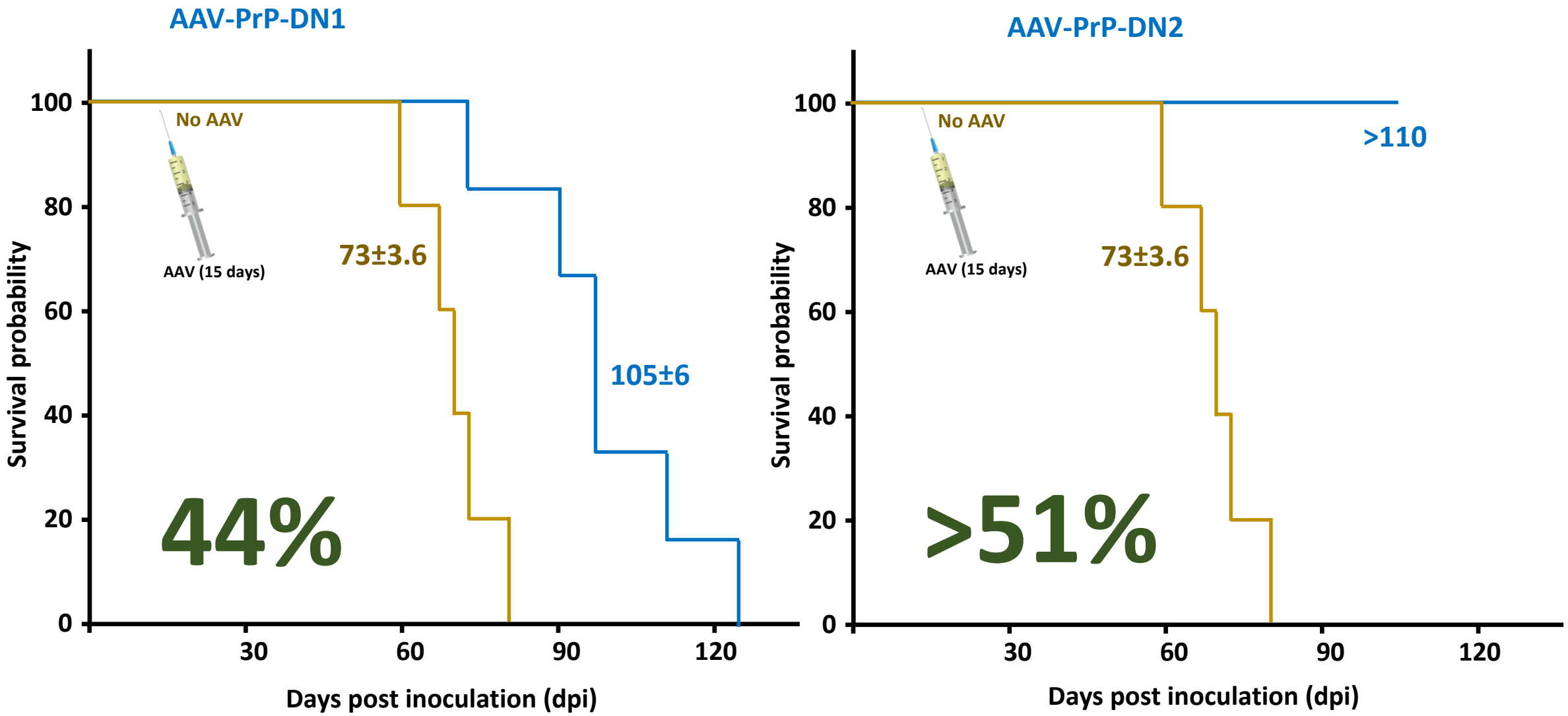
Clinical signs appear, humane endpoint and sample collection

2 different dominant negative PrP variants tested

GENE THERAPY USING DOMINANT NEGATIVE PRION PROTEINS

Therapeutic approach and experimental design

CWD-vole in TgVole(I109)1x



SUMMARY / CONCLUSIONS

- **Successfully identified highly effective dominant negative proteins through screening of 900+ variants from different species worldwide**
- **Achieved remarkable survival extensions of 27-51% in rapid prion disease models**
- **Demonstrated safety and broad brain expression of therapeutic proteins**
- **Validated approach in multiple prion strains**

Impact: This represents the first successful gene therapy showing significant survival extension in prion diseases, providing hope for future treatments for CJD families.

Next Steps: Optimizing dosing for maximum safety and efficacy, testing in human prion disease models (CJD, FFI and GSS), and advancing toward clinical trials.