Development and characterization of the fastest animal model able to propagate GSS prions faithfully

Joaquín Castilla, PhD

15th July 2023

OBJECTIVES

The development of therapies for any disease requires prior assessment of their safety and efficacy in animal models. To accurately evaluate the potential effectiveness of treatments, it is crucial to have animal models that faithfully reproduce the disease observed in humans

This project aims to develop and characterize new animal models that are directly susceptible to human prions, specifically those causing Gerstmann-Sträussler-Scheinker disease

GERSTMANN-STRÄUSSLER-SCHEINKER (GSS)

Gerstmann-Sträussler-Scheinker (GSS) is an autosomal dominant hereditary disease, characterized by a slow progressive nature. It is also the first recognized human transmissible spongiform encephalopathy (TSE) associated with a mutation in the gene responsible for encoding the prion protein (PrP)

Among prion diseases, GSS is considered rare, with approximately 60 families reported worldwide. The age of onset can vary between 30 and 60 years old, and the duration of the disease can range from 3.5 to 9.5 years

The clinical manifestation primarily includes cerebellar ataxia, pyramidal signs, and dementia. However, it's important to note that GSS is characterized by both genotypic and phenotypic heterogeneity, indicating variability in its genetic makeup and resulting clinical features

Several mutations on *PRNP* gene are involved: P102L, P105L, A117V, Y145STOP, F198S, D202N, Q217R, Y218N and also octa repeats (OR) insertions (8 OPRI, 6OPRI...)

For decades, it was considered a non-infectious and non-transmissible prion disease

ANIMAL DISEASE MODELS

What are the desired characteristics of a disease model?

It should exhibit pathological hallmarks of the neurodegenerative disease

It should display clinical signs that resemble the symptoms observed in humans with the neurodegenerative disease

It should exhibit a progressive course of the disease, mirroring the temporal progression of pathology

It should help to evaluate the efficacy of potential therapies and study underlying mechanisms of action

It should be easily accessible, reproducible, and manageable in terms of breeding, housing, and experimental procedures

Models that possess these critical characteristics can serve as valuable tools for understanding disease mechanisms, testing potential therapies, and advancing our knowledge of neurodegenerative disorders

SIX (6) DIFFERENT STUDIES

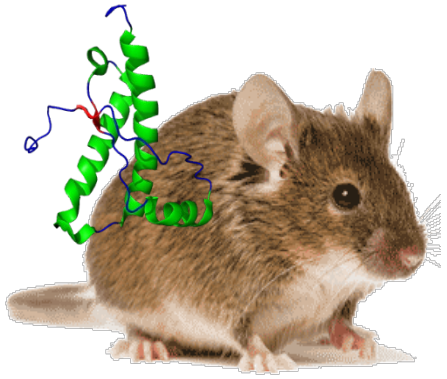
- ✓ **Comparative study of different animal models**
- ✓ **Specific infectivity**
- ✓ **Study to assess the impact of PrP quantity**
- ✓ **Kinetics for the study of biomarkers**
- ✓ **Kinetics to understand the temporal progression of the disease**
- ✓ **Assessing the utility of this model for evaluating antiprion therapies**

GSS SAMPLES

A117V

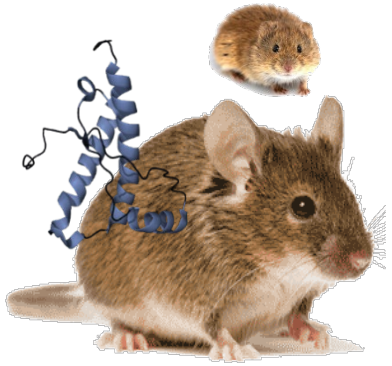
P102L

ANIMAL MODELS



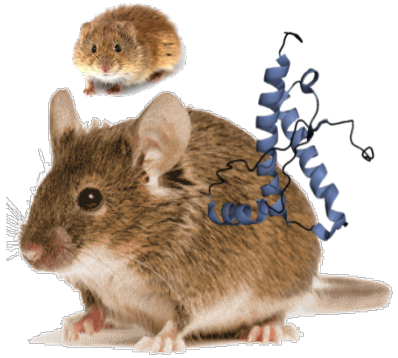
Wild-type mouse (1x)

Tg mouse expressing human PrP (6-8x)



Tg mouse expressing bank vole PrP (~1x)

Tg mouse expressing mouse PrP (~3x)



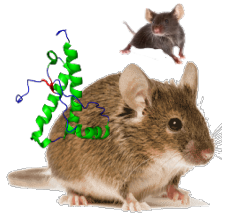
Tg mouse expressing bank vole PrP (~4x)

COMPARATIVE STUDY OF DIFFERENT ANIMAL MODELS

Intracerebral inoculations

50 dpi | 100 dpi | 200 dpi | 400 dpi | >500 dpi

Tg mouse expressing mouse PrP (1x)



P102L M129V

A117V

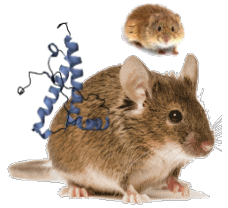
NO DISEASE

Tg mouse expressing human PrP (6-8x)

P102L M129V

A117V

Tg mouse expressing bank vole PrP (~1x)



P102L M129V 135 dpi

P102L V129V 244 dpi

A117V 78 dpi

A117V 67 dpi

A117V 79 dpi

Tg mouse expressing mouse PrP (~3x)

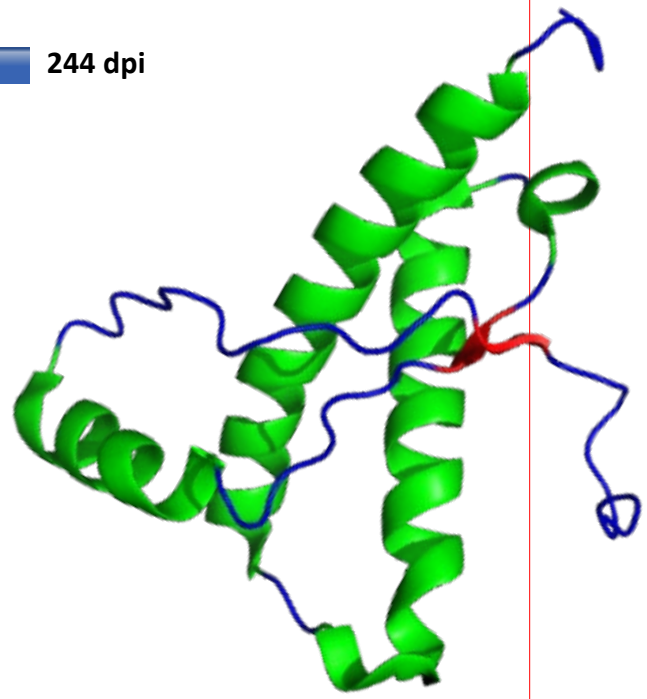
A117V 57 dpi

Tg mouse expressing bank vole PrP (~4x)

P102L V129V 65 dpi

A117V 41 dpi

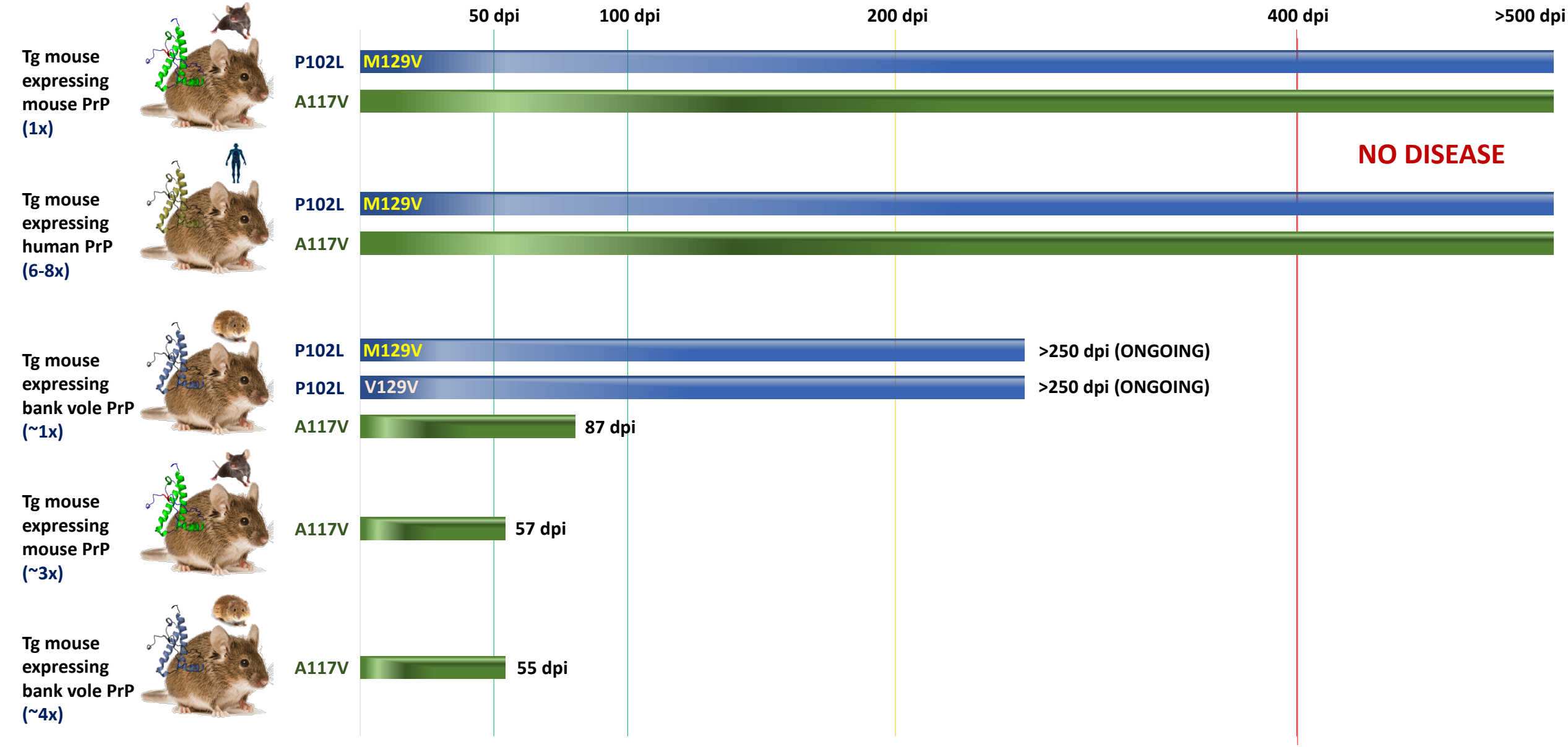
A117V 38 dpi



Isoleucine 108/109

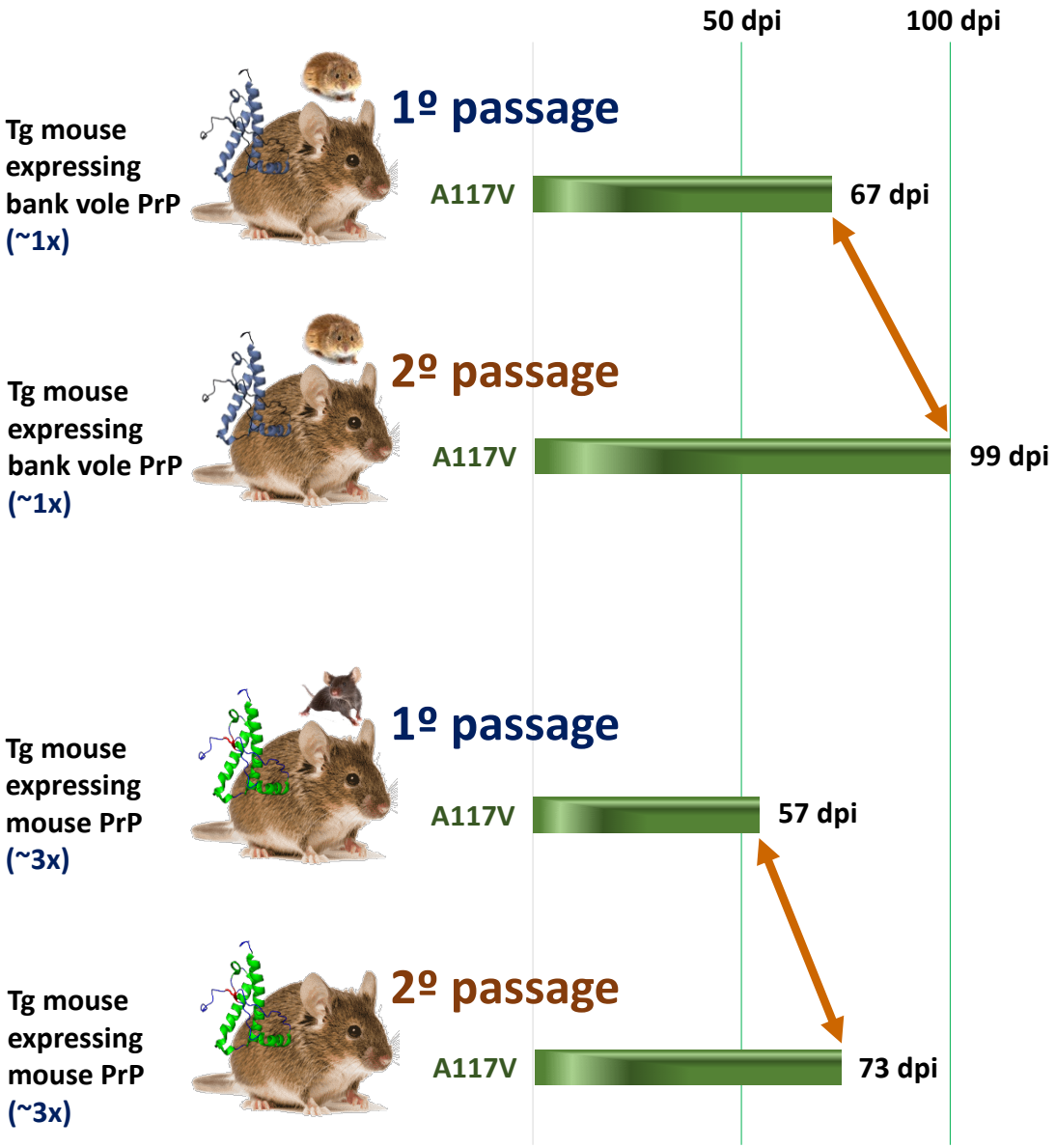
COMPARATIVE STUDY OF DIFFERENT ANIMAL MODELS

Intraperitoneal inoculations



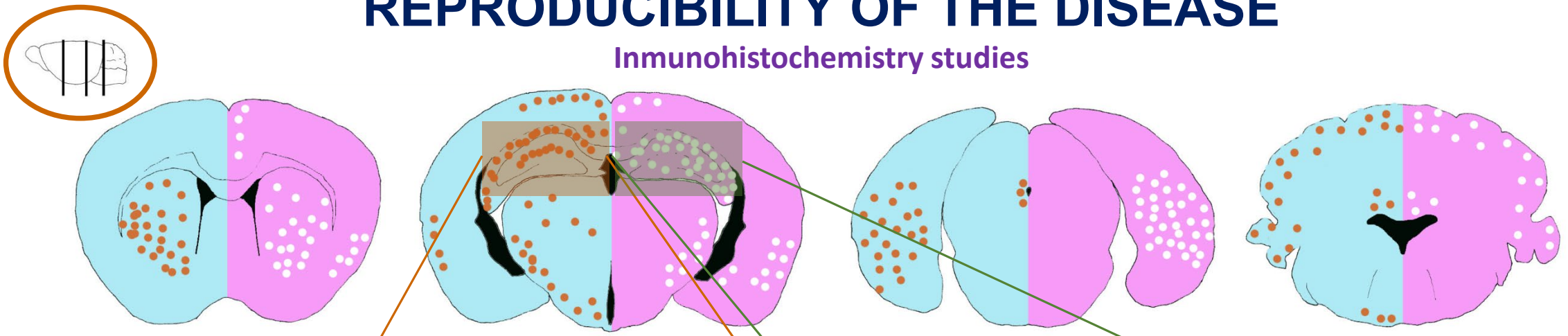
COMPARATIVE STUDY OF DIFFERENT ANIMAL MODELS

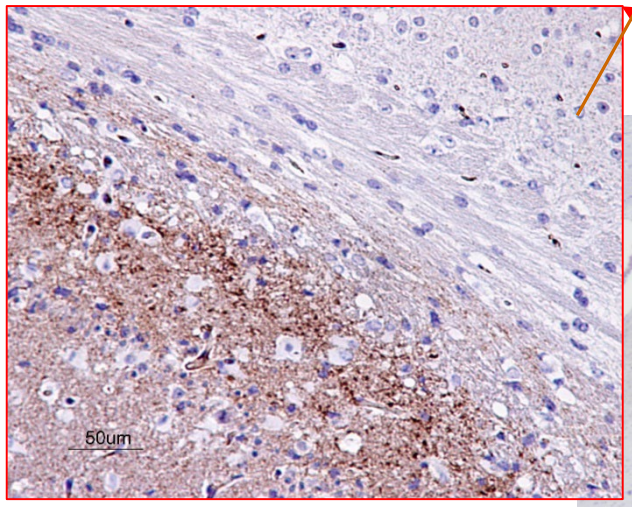
Serial passages (intracerebral inoculacions)

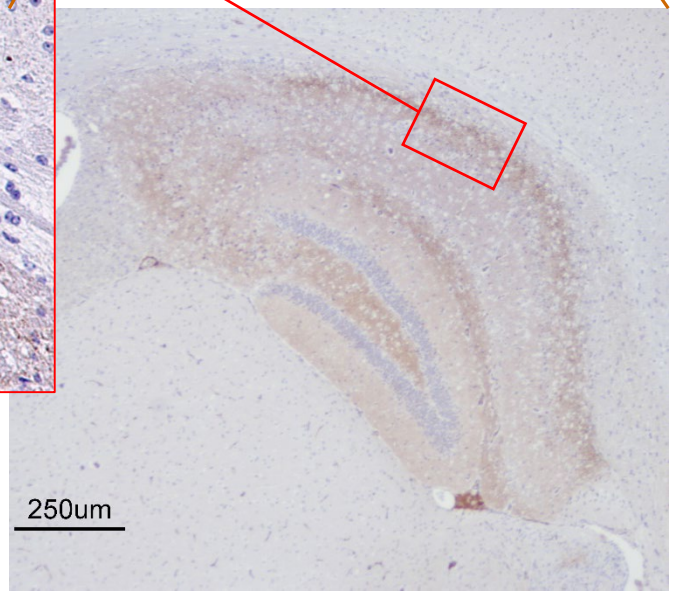


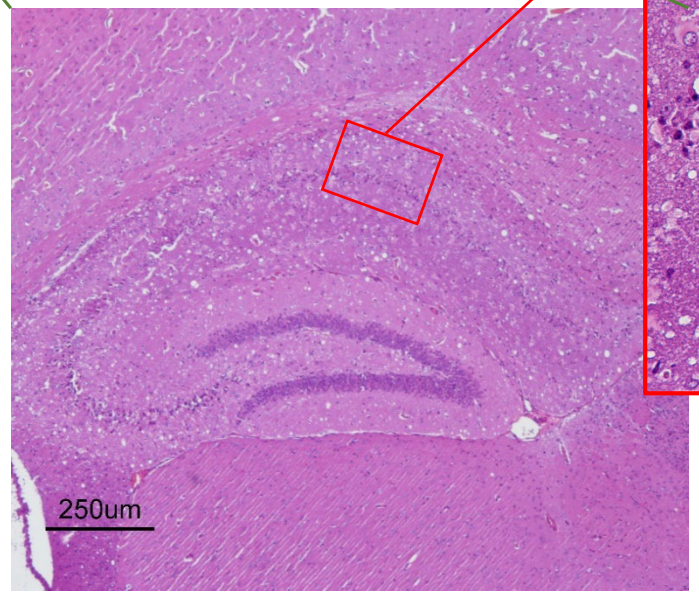
REPRODUCIBILITY OF THE DISEASE

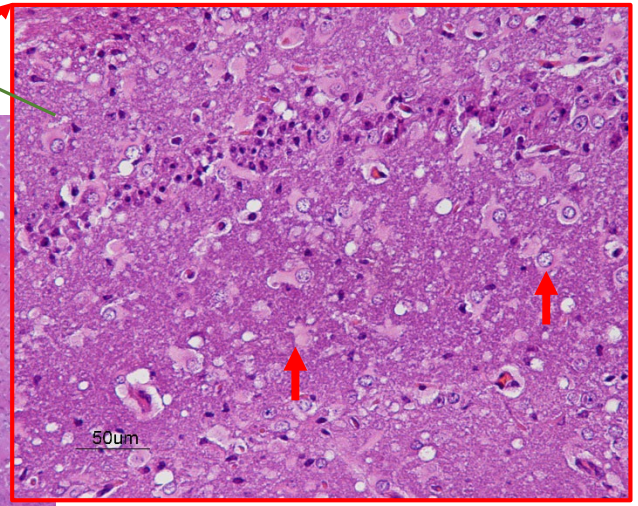
Inmunohistochemistry studies









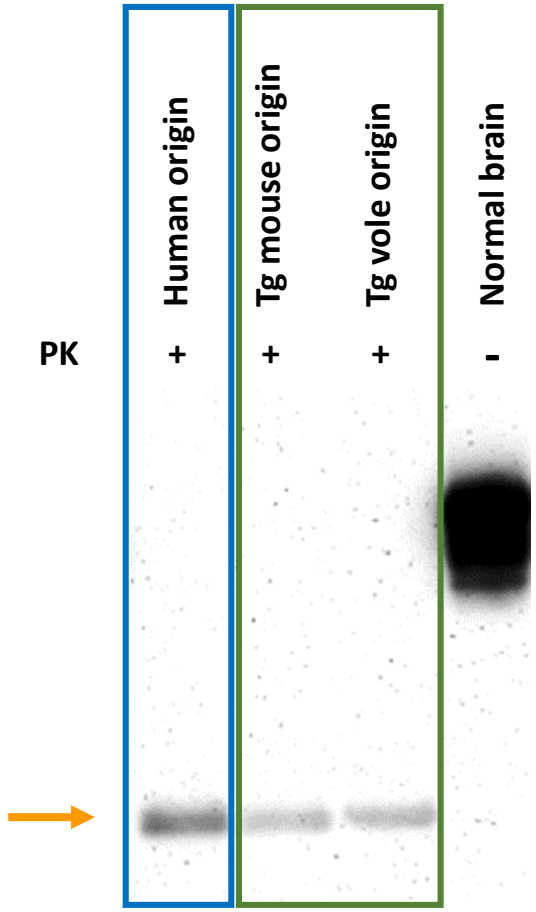


The deposition of PrP^{res} is of a fine punctate /granular type, preferably in the white matter

The lesion is centered in the hippocampus, striatum (basal nuclei), and occipital cortex. There is some involvement in the frontal, parietal, and temporal cortex, as well as the cerebellum.

REPRODUCIBILITY OF THE DISEASE

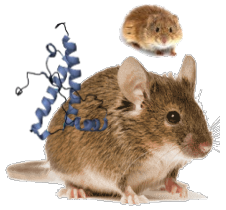
Biochemical studies



SPECIFIC INFECTIVITY

Intracerebral inoculation

Tg mouse expressing bank vole PrP (~1x)



50 dpi | 100 dpi | 200 dpi | 400 dpi

10^{-1} A117V — 67 dpi

10^{-2} A117V — 66 dpi

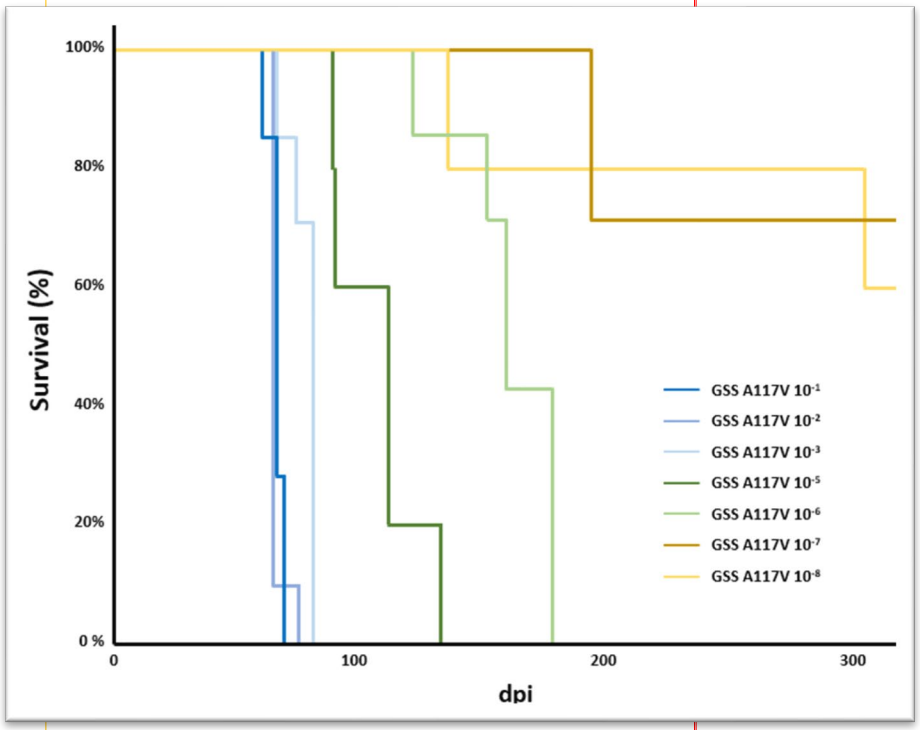
10^{-3} A117V — 79 dpi

10^{-5} A117V — 108 dpi

10^{-6} A117V — 163 dpi

10^{-7} A117V — 277 dpi

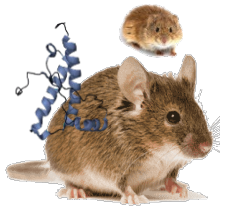
10^{-8} A117V — 310 dpi



SPECIFIC INFECTIVITY

Intracerebral inoculation

Tg mouse expressing bank vole PrP (~1x)



50 dpi 100 dpi 200 dpi 400 dpi

10^{-1} A117V 67 dpi

10^{-2} A117V 66 dpi

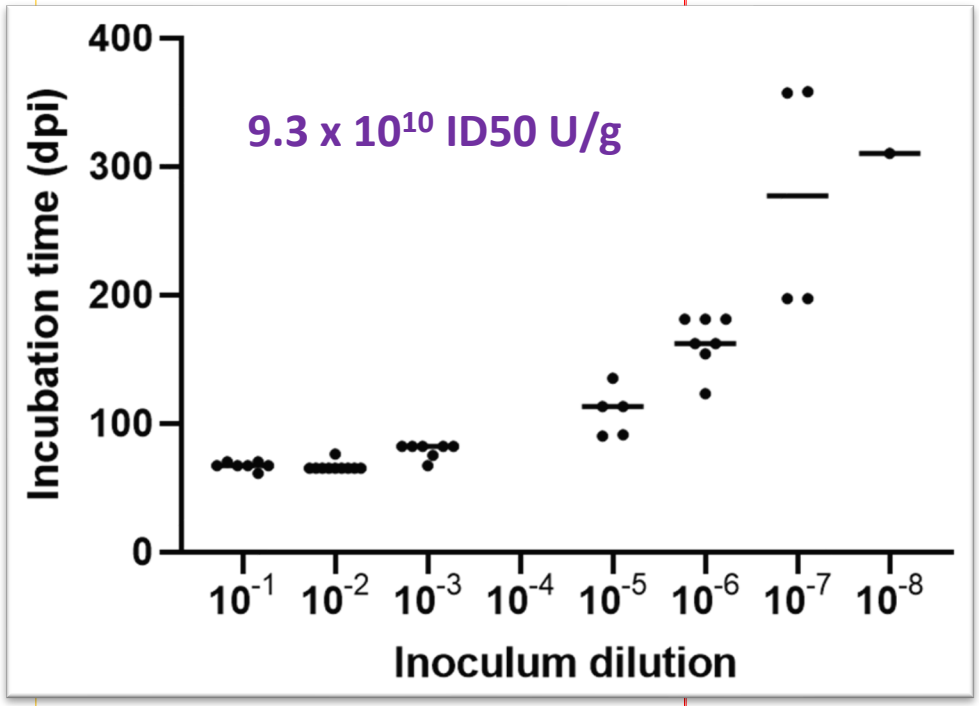
10^{-3} A117V 79 dpi

10^{-5} A117V 108 dpi

10^{-6} A117V 163 dpi

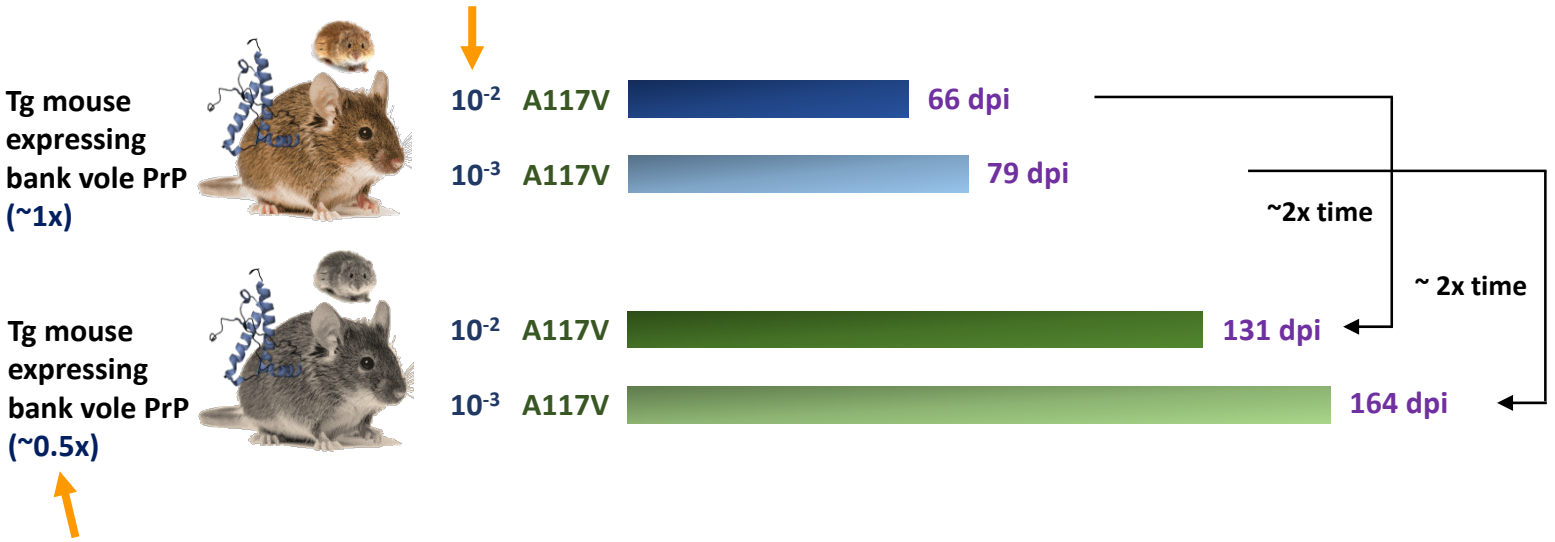
10^{-7} A117V 277 dpi

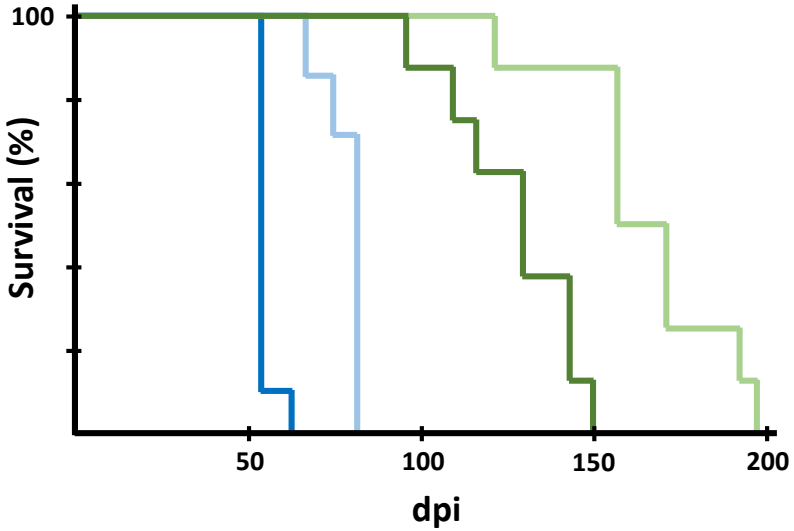
10^{-8} A117V 310 dpi



STUDY TO ASSESS THE IMPACT OF PrP QUANTITY

Intracerebral inoculations





JCI insight RESEARCH ARTICLE

Antisense oligonucleotides extend survival of prion-infected mice

Gregory J. Raymond,[1] Hien Tran Zhao,[2] Brent Race,[1] Lynne D. Raymond,[1] Katie Williams,[1] Eric E. Swayze,[2] Samantha Graffam,[3] Jason Le,[3] Tyler Caron,[3] Jacquelyn Stathopoulos,[3] Rhonda O'Keefe,[3] Lori L. Lubke,[1] Andrew G. Reidenbach,[3] Allison Kraus,[1] Stuart L. Schreiber,[3] Curt Mazur,[2] Deborah E. Cabin,[4] Jeffrey B. Carroll,[5] Eric Vallabh Minikel,[1,3,6,7] Holly Kordasiewicz,[2] Byron Caughey,[1] and Sonia M. Vallabh[1,3,6,7]

Engineered Zinc Finger Transcriptional Regulators Specifically Reduce Prion Expression and Extend Survival in an Aggressive Prion Disease Model

Abstract #490

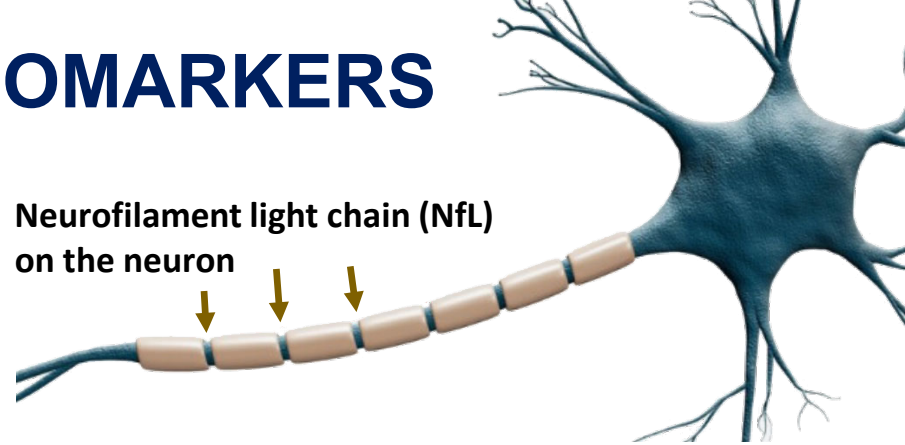
Bryan Zeitler[1], Meredith A Mortberg[2], Shih-Wei Chou[1], Mohad Mehrabian[1], Kimberly Marlen[1], Michael Howard[2], Samantha Graffam[2], Kenney Lenz[2], Tyler Caron[2], Qi Yu[1], Jing Hu[1], Sarah Hinkley[1], Alicia Goodwin[1], Asa Hatami[1], Alaric Falcon[1], Toufan Parman[1], Jason Fontenot[1], Amy M Pooler[1], Eric Vallabh Minikel[2], Sonia M Vallabh[2]

[1] Sangamo Therapeutics, Inc., Richmond, CA
[2] Broad Institute of MIT and Harvard, Cambridge, MA

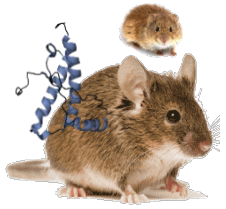
Sangamo THERAPEUTICS

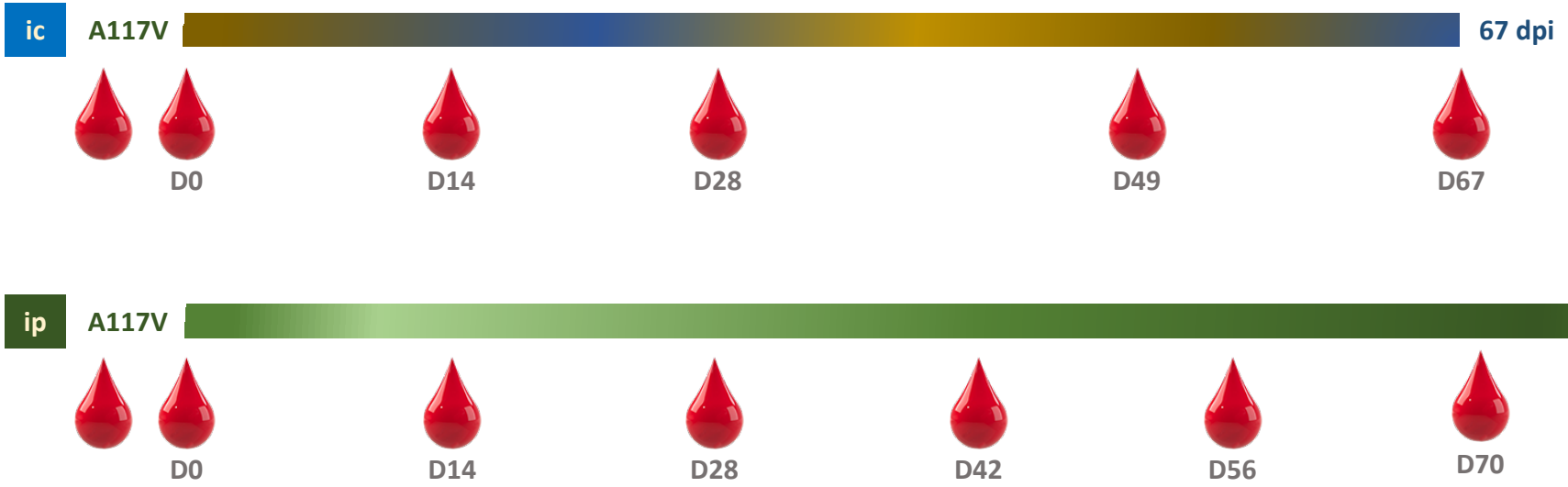
KINETICS FOR THE STUDY OF BIOMARKERS

Blood-based biomarker



Tg mouse expressing bank vole PrP (~1x)



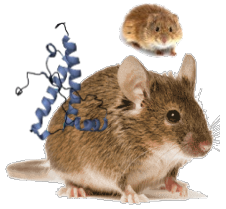


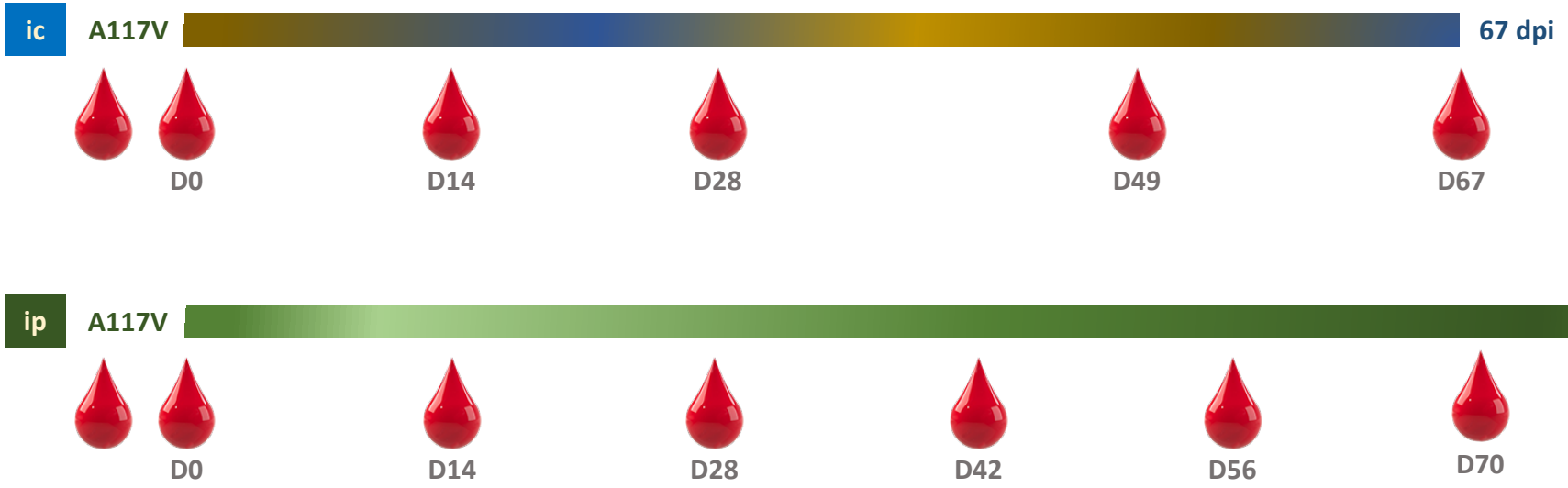
KINETICS FOR THE STUDY OF BIOMARKERS

Blood-based biomarker

SIMOA Technology

Tg mouse expressing bank vole PrP (~1x)

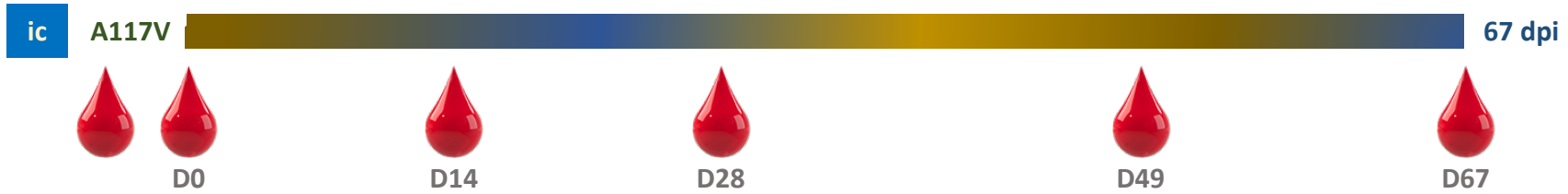




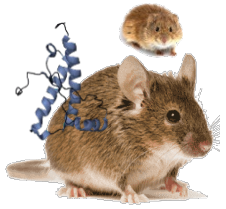
KINETICS FOR THE STUDY OF BIOMARKERS

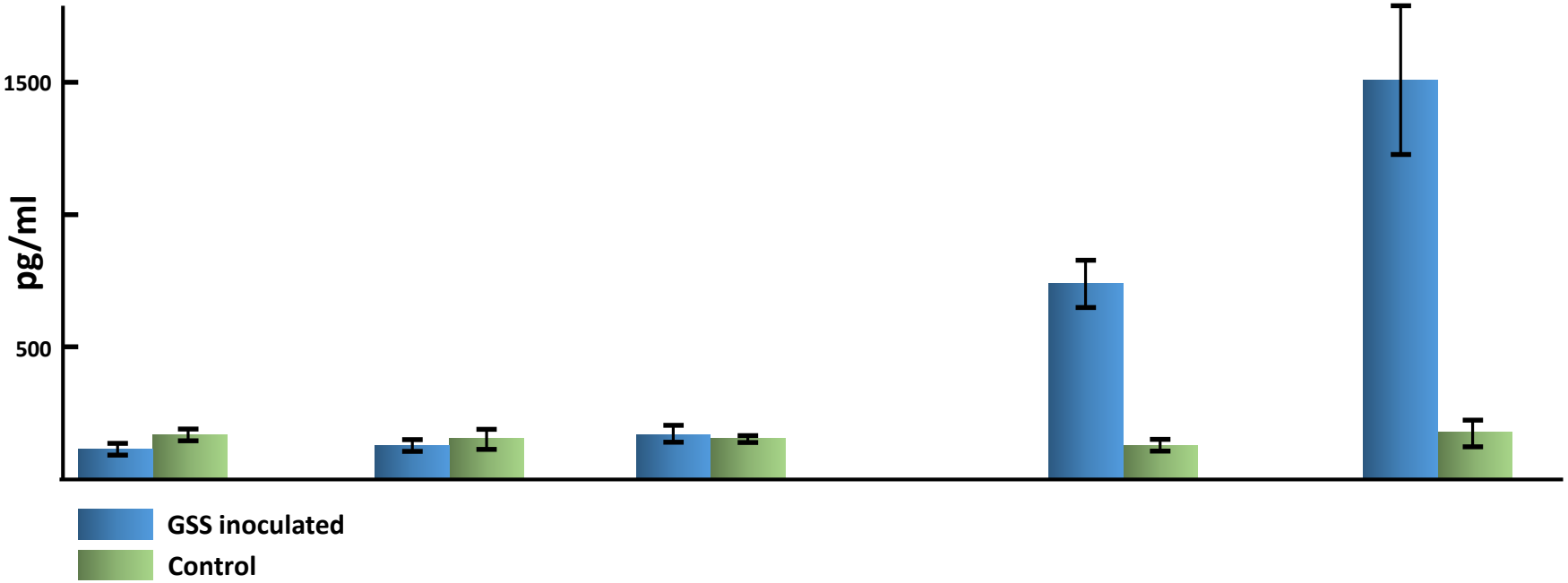
Blood-based biomarker

SIMOA Technology



Tg mouse expressing bank vole PrP (~1x)



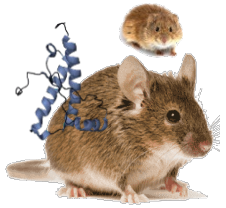


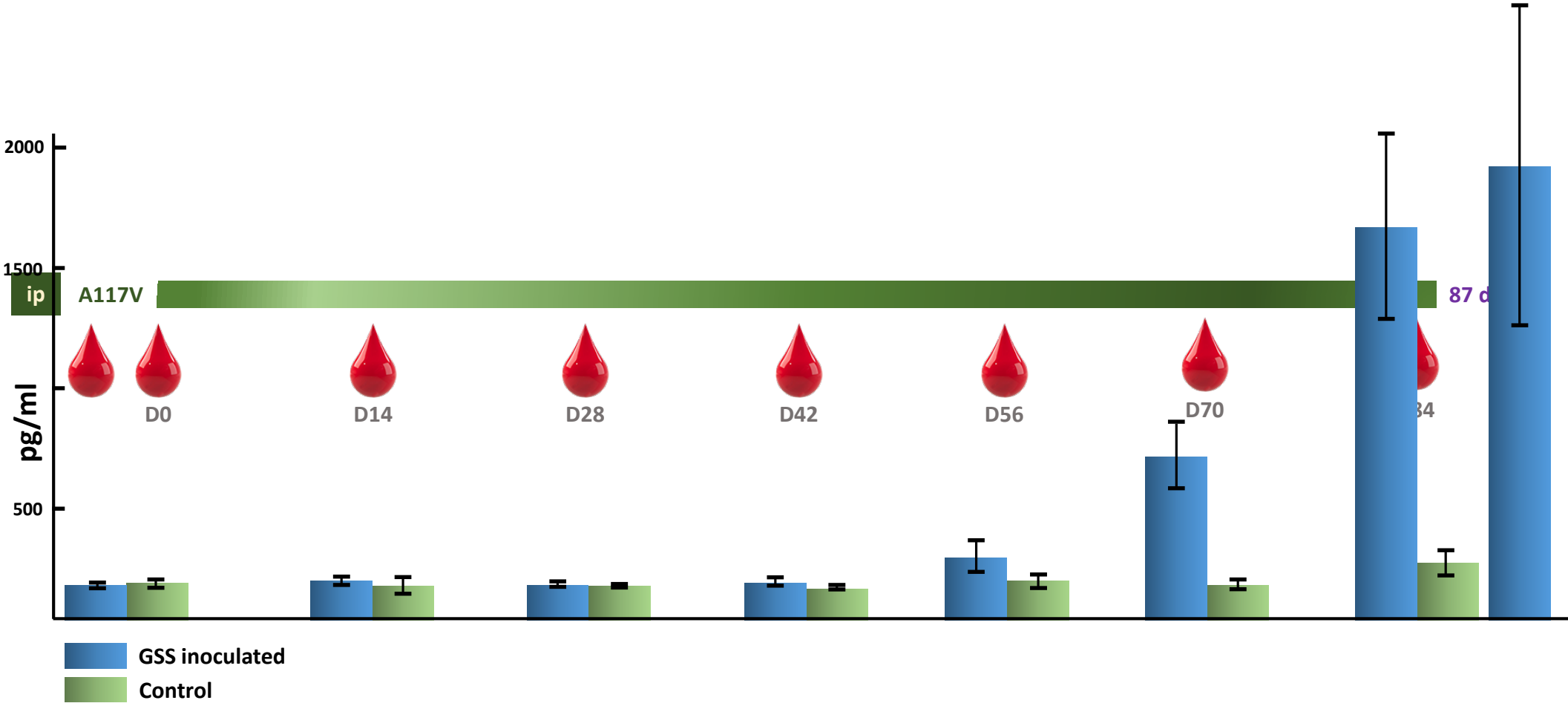
KINETICS FOR THE STUDY OF BIOMARKERS

Blood-based biomarker

SIMOA Technology

Tg mouse expressing bank vole PrP (~1x)



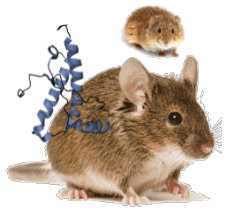


KINETICS TO UNDERSTAND THE TEMPORAL PROGRESSION OF THE DISEASE

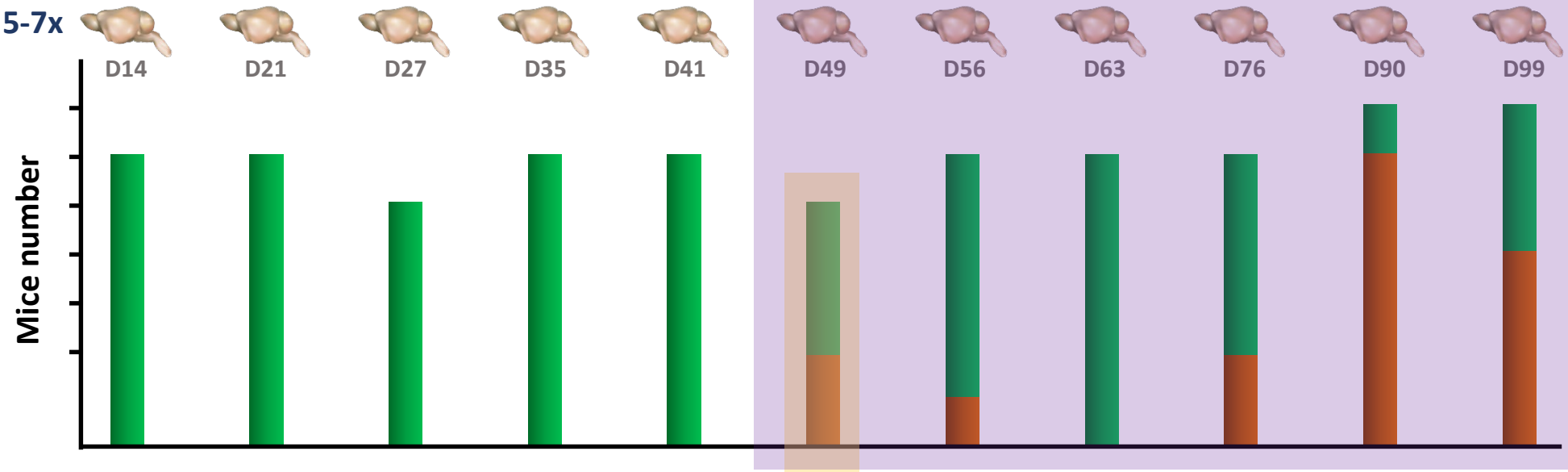
Intraperitoneal inoculation

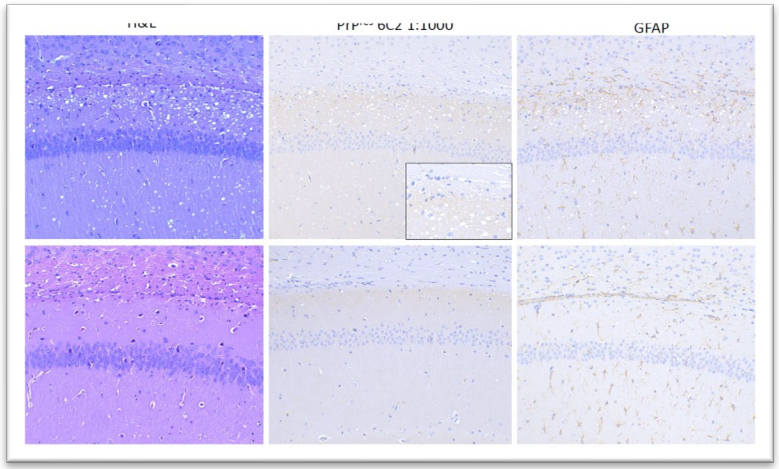
10^{-1} A117V — 99 dpi

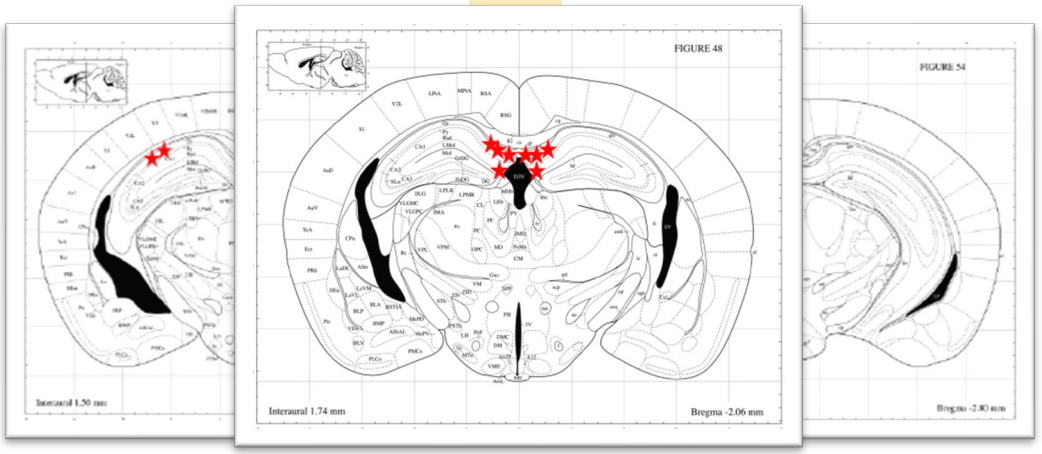
5-7x



Tg mouse expressing bank vole PrP (~1x)







Unilateral spongiform lesion located in the hippocampus, affecting the alveus, oriens and, to a slightly lesser extent, the stratum radiatum of hippocampal CA1 horn."

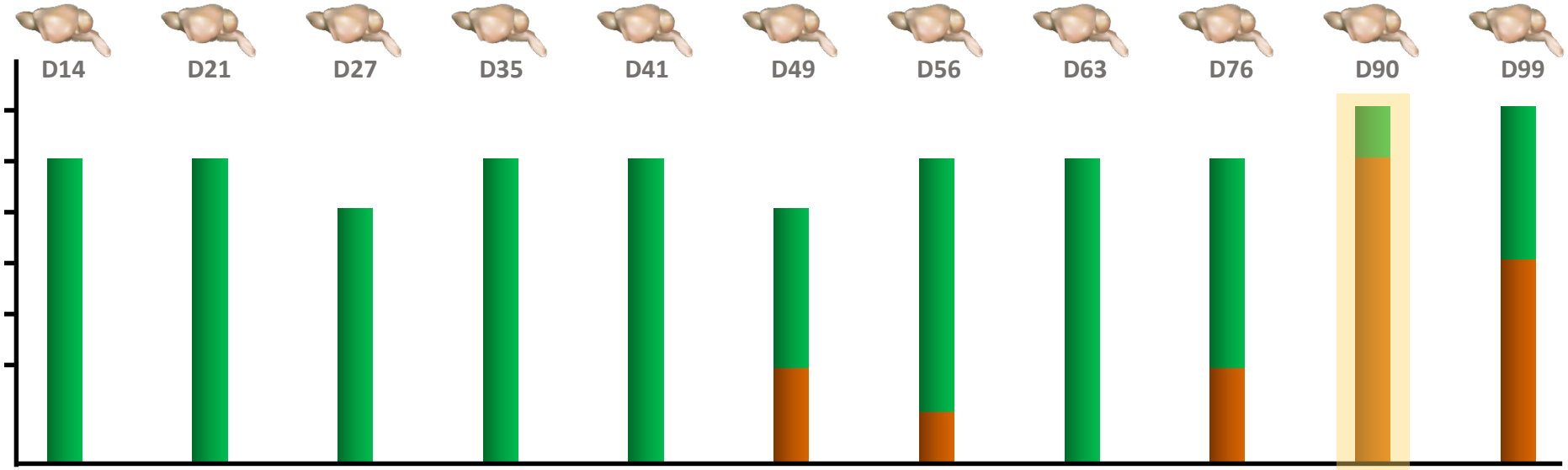
Bilateral symmetric spongiform lesion, located in the hippocampus, affecting the medial oriens of CA1.

KINETICS TO UNDERSTAND THE TEMPORAL PROGRESSION OF THE DISEASE

Intraperitoneal inoculation

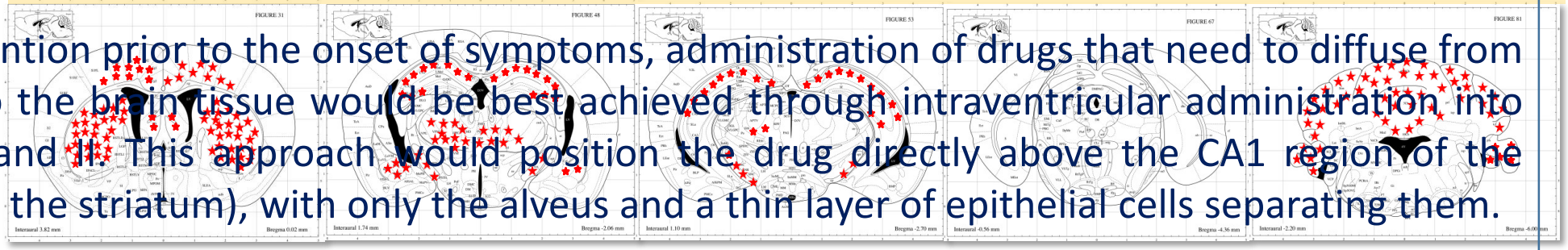
10^{-1} A117V — 99 dpi

5-7x



Tg mouse expressing bank vole PrP (~1x)

Widely distributed bilateral spongiform lesion throughout the brain, especially affecting the striatum, frontal cortex, amygdala, and rostrodorsal aspect of the hippocampus (CA1, CA2, and CA3).



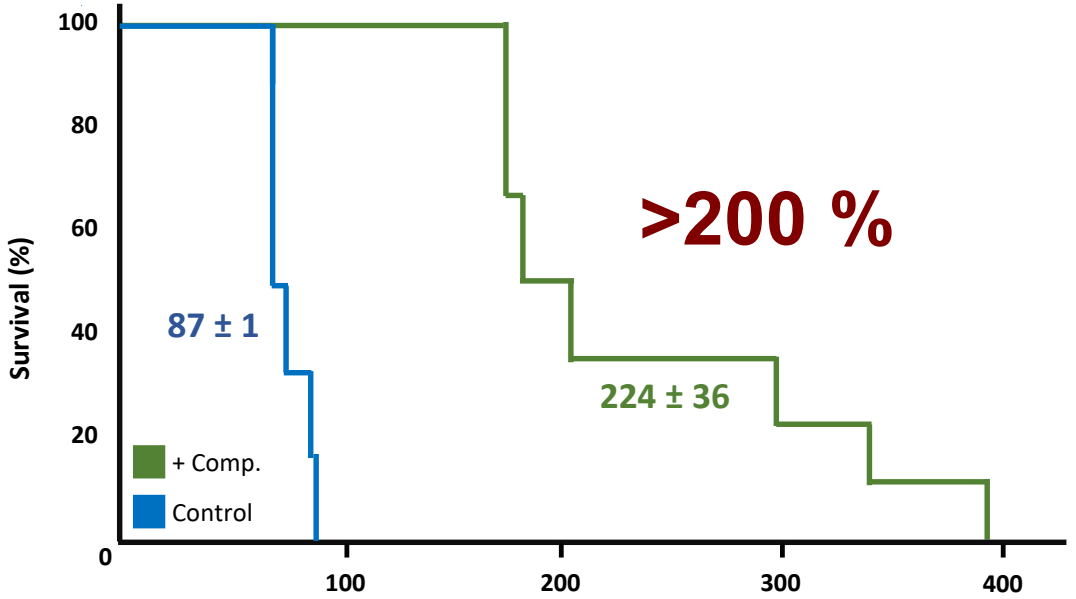
In a hypothetical intervention prior to the onset of symptoms, administration of drugs that need to diffuse from the brain ventricles into the brain tissue would be best achieved through intraventricular administration into the lateral ventricles I and II. This approach would position the drug directly above the CA1 region of the hippocampus (as well as the striatum), with only the alveus and a thin layer of epithelial cells separating them.

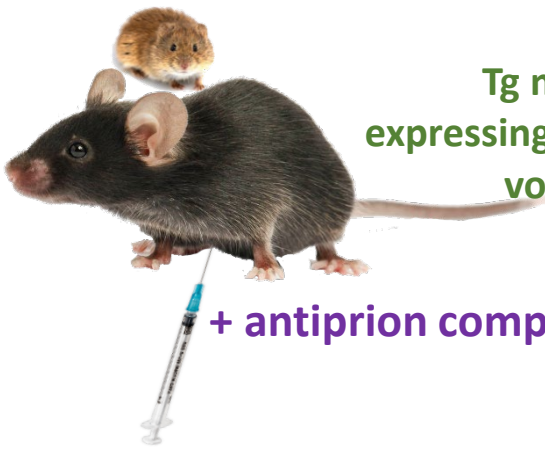
ASSESING THE UTILITY OF THIS MODEL FOR EVALUATING ANTIPRION THERAPIES

Intraperitoneal inoculation

Tg mouse expressing bank vole PrP (~1x)

Control





Tg mouse expressing bank vole PrP (~1x)

+ antiprion compound

SUMMARY / CONCLUSIONS

The development of therapies for any disease requires prior assessment of their safety and efficacy in animal models. To accurately evaluate the potential effectiveness of treatments, it is crucial to have animal models that faithfully reproduce the disease observed in humans

We have developed and characterized new animal models that are directly susceptible to human prions, specifically those causing Gerstmann-Sträussler-Scheinker disease

- We have developed the fastest animal model to date for a human prion disease, capable of propagating prions in less than 40 days.
- By evaluating different inoculation routes, we have made this animal model suitable for testing treatments.
- We have thoroughly examined the brain lesions that develop in these animals. This study has provided valuable insights into the temporal progression of the disease to focus treatment where most needed.
- We have analyzed the arousal of a biomarker in blood, the levels of which increase weeks before the onset of neurological clinical signs.
- Additionally, our research has effectively demonstrated the utility of this animal model in assessing the efficacy of two potential treatments. One treatment aims to reduce PrP^C levels, while the other utilizes a molecule with anti-prion activity.

ACKNOWLEDGMENTS

The “Mercies in Disguise Research Grant, in Honor of the Baxley Family”, contributed by Kathy Baxley and Family

The José A. Piriz and Sonia E. Piriz Memorial Research Grant, contributed by Karla and Lauren Piriz

The Thomas Lord Charitable Trust

The Strides for CJD Grant and CJD Foundation Grant, contributed by the Families of the CJD Foundation

Acknowledgments

Hasier Eraña
Jorge Moreno
Cristina Sampedro
Carlos Díaz
Nuno Anjo
Maitena San Juan
Josu Galarza
Eva Fernández
Patricia Piñeiro
Mirta García

Former lab members:

Natalia Fernández
Saioa Elezgarai
Chafik Harrathi
Maite Pérez
Vanessa Venegas
Nagore Sacristán
Ezequiel González
Miguel Ángel Pérez
Sandra García
Rafael López
Tomás Barrio

Collaborators:

Mariví Geijo
Glenn C. Telling
Steffen Halbgebauer
Enric Vidal
Samanta Giler
Jesús R. Requena
Nuria López
Markus Otto

Funding:

Thanks!

Gracias!

Eskerrik asko!