AVIADOBIO

Delivering Curative Genetic Medicines for Neurodegenerative Disorders

Intrathalamic Delivery of AVB-101 Rescues Pathology in *Grn* Null Mice and Achieves Widespread Cortical Expression in a Large Animal Model

Speaker : Youn Bok Lee (Co-founder & Head of Discovery)

Lee YB[1,2], Lee DY[1,2], Allison J[1], Kaliszewska A[1], Arias Del Castillo N[1], Zhang K[1], Bekele Y[1,2], Hosseini P[1,2], Joubert R[1,2], Walker Z[1,2], Furtado J[1,2], Gumus M[1], Fernandes A[1,2], Brock O[1], Shaw CE[1,2]

[1] United Kingdom Dementia Research Institute, Department of Basic and Clinical Neuroscience, Institute of Psychiatry, Psychology and Neuroscience, King's College London, 5 Cutcombe Road, London SE5 9RT, UK

[2] AviadoBio Ltd, 86 Hatton Garden, Floor 5, London, United Kingdom, EC1N 8QQ

AviadoBio Focus: Enhanced Biodistribution From Precision Dosing

History:
Formed on pioneering research by **King's College London** and the **UK Dementia Research Institute**

Mission:
Develop **transformative gene therapies** for **neurodegenerative diseases**

Method:
Combining **next-generation vector design** with **neuroanatomy-led delivery**

Delivery:
Precise dosing with **extensive biodistribution** in the brain and spinal cord

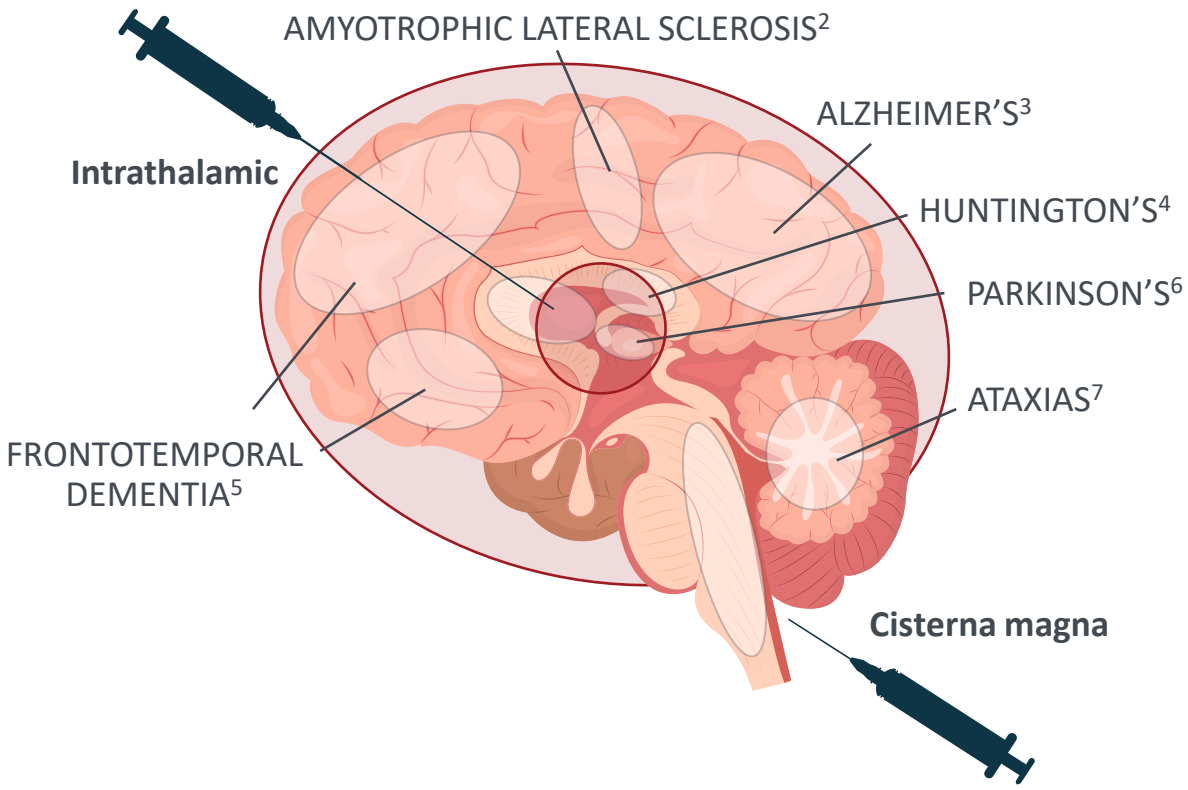
Platforms:
Optimised for **gene supplement** and **miRNA knockdown**

Our lead programme uses **AAV** to deliver a **progranulin transgene (AVB-101)** to patients with **frontotemporal dementia** due to loss-of-function mutations in the *GRN* gene encoding progranulin (FTD-*GRN*)

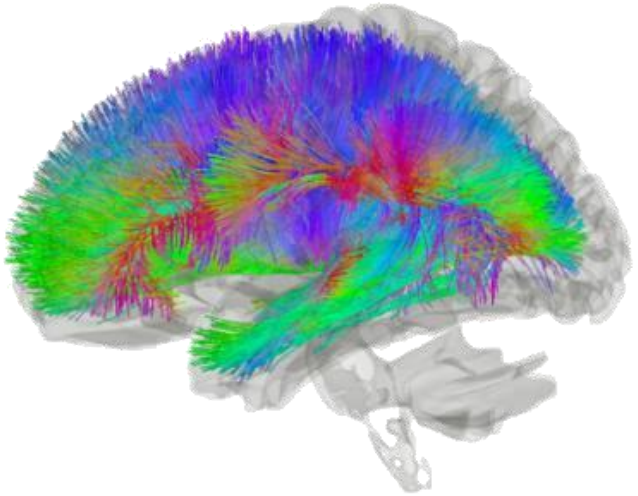
Phase I/II trial to be initiated late **2022**

AAV, adeno-associated virus; FTD, frontotemporal dementia; miRNA, microRNA.

Biodistribution is the Key Challenge for CNS Gene Therapies[1]



Intrathalamic AAV delivery exploits afferent and efferent connections between the thalamus and cortex



MRI tractography depicting the thalamo-cortical network[8]

Minimal amount of **intrathalamic AAV** achieves **broad brain distribution**

AAV, adeno-associated virus; CNS, central nervous system; MRI, magnetic resonance imaging.

1. Kimura S and Harashima H. Pharmaceutics. 2020;12(12):1216; 2. Masrori P and Van Damme P. Eur J Neurol. 2020;27(10):1918–1929;
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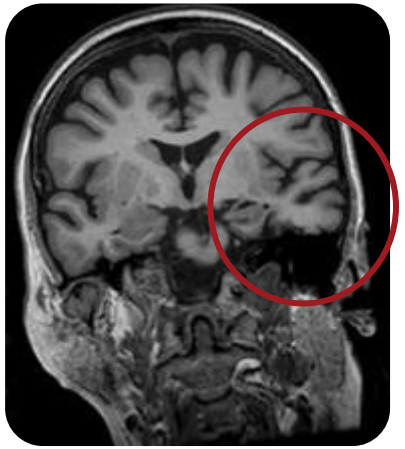
FTD-*GRN*: A Clear Target for Gene Therapy

Significant Unmet Need

- **~30,000 patients in US and ~76,000 patients in Europe*** experience **progressive decline in behaviour, personality, executive function** and/or **language,** and **death 6–12 years after initial symptoms of FTD**[1–7]
- **Burden is significant and costly** with **no disease-modifying therapies** available[8]



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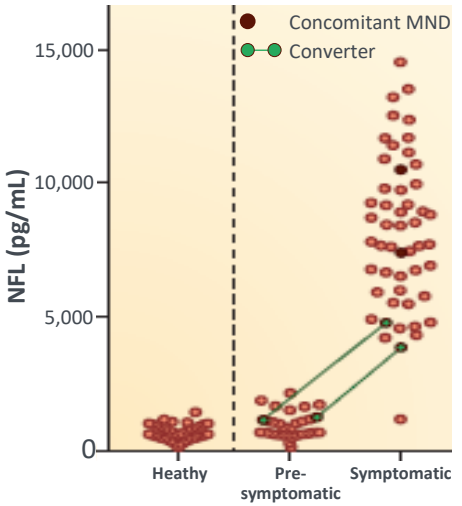
Established Disease Biology

- **Loss-of-function mutations in *GRN* gene are causative for FTD-*GRN***[9]
- **Deficiency in PGRN increases microglial reactivity, accelerates neurodegeneration and TDP-43 accumulation**[9–12]
- **PGRN supplementation corrects pathological phenotype in rodent models**[13]



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Tractable Clinical Development

- Established **natural history cohorts** and **registries**[14,15]
- Rate of **atrophy progression is measurable by MRI**[14]
- **NFL is a blood biomarker** for **neurodegeneration risk**[16]
- **Clinical endpoints** available to **support regulatory approval**[14,15,17]

*US data is an estimate of cognitive syndromes of frontotemporal lobar degeneration. Europe data is an estimate of behavioural variant FTD in the EU27 of 2013, Norway, Iceland and Lichtenstein.
EU, European Union; FTD, frontotemporal dementia; NFL, neurofilament light chain protein; PGRN, progranulin; symp, symptom; US, United States of America.

1. Knopman DS and Roberts RO. J Mol Neurosci. 2011;45(3):330-335; 2. European Medicines Agency. Public summary of opinion on orphan drug designation: Methylthionium for the treatment of behavioural variant frontotemporal dementia. 2013; 3. Greaves CV and Rohrer JD. J Neurol. 2019;266(8):2075–2086; 4. Olney NT et al. Neurol Clin. 2017;35(2):339–374; 5. Young JJ et al. Ther Adv Psychopharmacol. 2018;8(1):33–48; 6. Boxer AL and Miller BL. Alzheimer Dis Assoc Disord. 2005;19 Suppl 1:S3–6; 7. Kansal K et al. Dement Geriatr Cogn Disord. 2016;41(1-2):109–122; 8. Galvin JE et al. Neurology. 2017;89(20):2049–2056; 9. Chang MC et al. J Exp Med. 2017;214(9):2611–2628; 10. Root J et al. Neurobiol Dis. 2021;154:105360 11. Yin F et al. J Exp Med. 2010;207(1):117–128; 12. Ahmed Z et al. Am J Pathol. 2010;177(1):311–324; 13. Arrant AA et al. Brain. 2017;140(5):1447–1465; 14. Rohre JD et al. Lancet Neurol. 2015;14(3):253–262; 15. Poos JM et al. Alzheimers Res Ther. 2022;14(1):10; 16. Swift JI et al. J Neurol Neurosurg Psychiatry. 2021;92(2):204–215; 17. Staffaroni AM et al. Brain. 2019;142(2):443–459; 18. Boeve BF et al. Lancet Neurol. 2022;21(3):258–272; 19. Meeter L et al. Nature. 2017;13:406–419.

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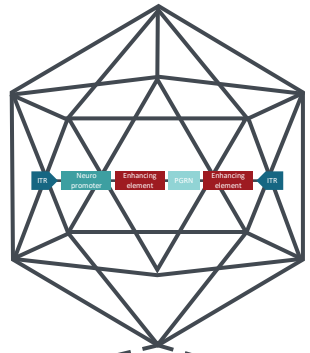
AVB-101: Gene Supplementation Therapy for FTD-*GRN*

AVB-101



Tissue Specificity

- AVB-101 utilises a **neuronal-specific promoter** to ensure transgene expression is restricted to neurones, minimising the risk of peripheral organ exposure observed with constitutive promoters (e.g., CB7, CMV)
- Secretion of PGRN permits local **cross-correction** of glial cells

Boosting Secretion

- **Codon-optimised human *PGRN*** transgene
- **5' and 3' enhancing elements** that optimises splicing
- Achieves **supraphysiological levels** in brain and CSF in rodents and large animals

Neurotropism

- **AAV9 serotype: neurotropic**[1]
- AAV9-based treatment has already been approved for SMA (ZOLGENSMA®)[2]
- Proven manufacturability
- **Cassette size** optimised

Designed with efficacy and safety in mind

AAV9, adeno-associated virus serotype 9; CB7, chicken β-actin 7; CMV, cytomegalovirus; CSF, cerebrospinal fluid; FTD, frontotemporal dementia; ITR, inverted terminal repeat; hPGRN, human progranulin; SMA, spinal muscular atrophy.

1. Korneyenkov MA and Zamyatnin Jr AA. Pharmaceutics. 2021;13(5):750; 2. Zolgensma. Prescribing information. Bannockburn, IL: Novartis Gene Therapies, Inc. March 2022.

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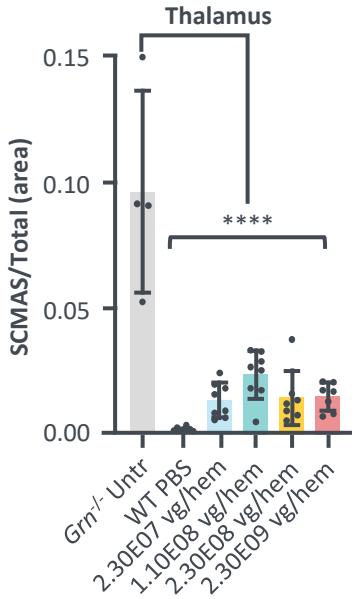
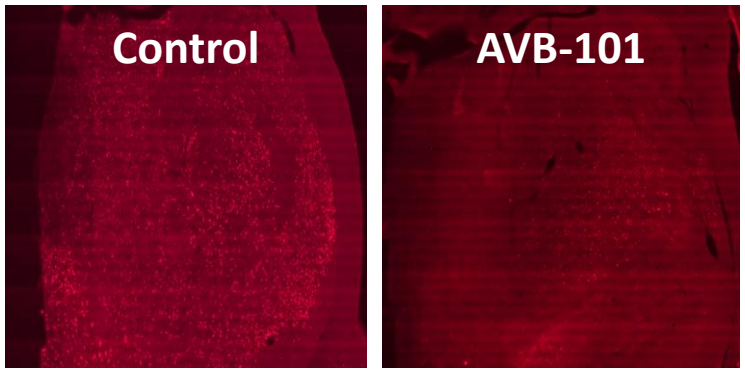
AVB-101 Substantially Reduces Pathology in *Grn* Knock-Out Mice

- *Grn*$^{-/-}$ mice develop extensive lipofuscinosis and age-dependent neuroinflammation

Lipofuscinosis (SCMAS)

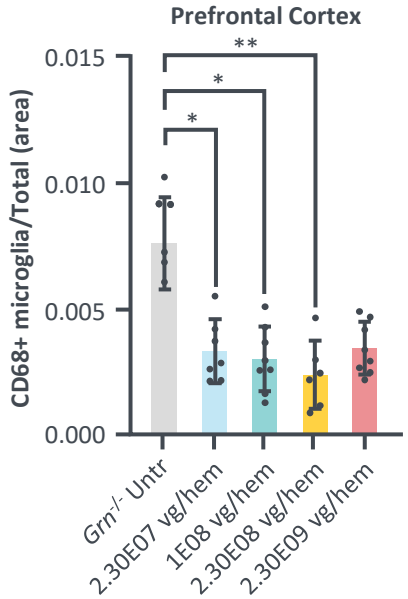


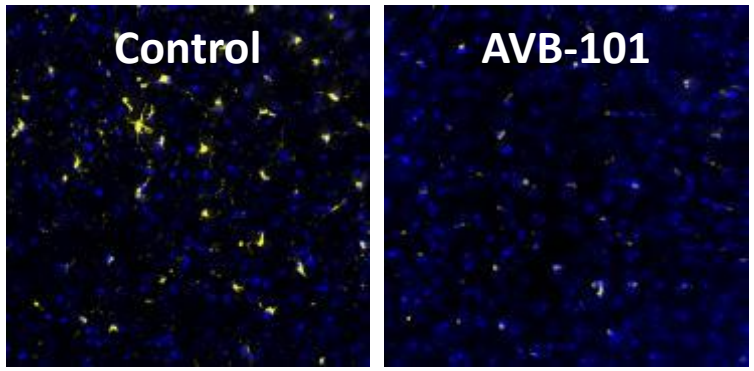


Visualisation and quantification of **SCMAS (lipofuscinosis)** in the thalamus of control and treated young *Grn*$^{-/-}$ mice. Infused at 8 weeks, imaged at 20 weeks

****p≤0.0001

Neuroinflammation (CD68)





Visualisation and quantification of prefrontal cortex **CD68+ve microglia** in control and treated old *Grn*$^{-/-}$ mice. Infused at 40 weeks, imaged at 52 weeks

*p≤0.05; **p≤0.01

- **Intrathalamic delivery of AVB-101 in young *Grn*$^{-/-}$ mice (6 weeks) restored wild-type levels of lipofuscinosis (SCMAS) across all doses**
- **Intrathalamic delivery of AVB-101 in older *Grn*$^{-/-}$ mice (28 weeks) reduced levels of microglial activation throughout the CNS**

+ve, positive; CD, cluster of differentiation; CNS, central nervous system; hem, hemisphere; PBS, phosphate-buffered saline; SCMAS, subunit C mitochondrial ATP synthase; untr, untreated; vg, vector genome; WT, wild type.

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AVB-101 Sheep Study: Overview

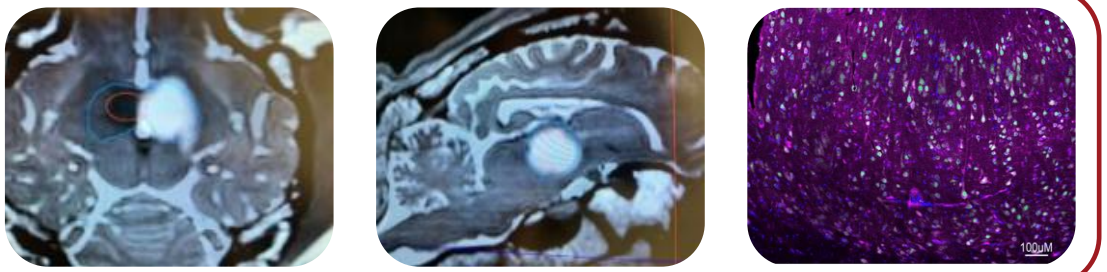
Thalamus:
Chosen as **centralised hub** connected to almost **all cortical and sub-cortical regions**

AAV9:
Capsid chosen as it is **neuronotropic** and **facilitates anterograde and retrograde transport**

Sheep:
Chosen as brain size is **twice that of a macaque**

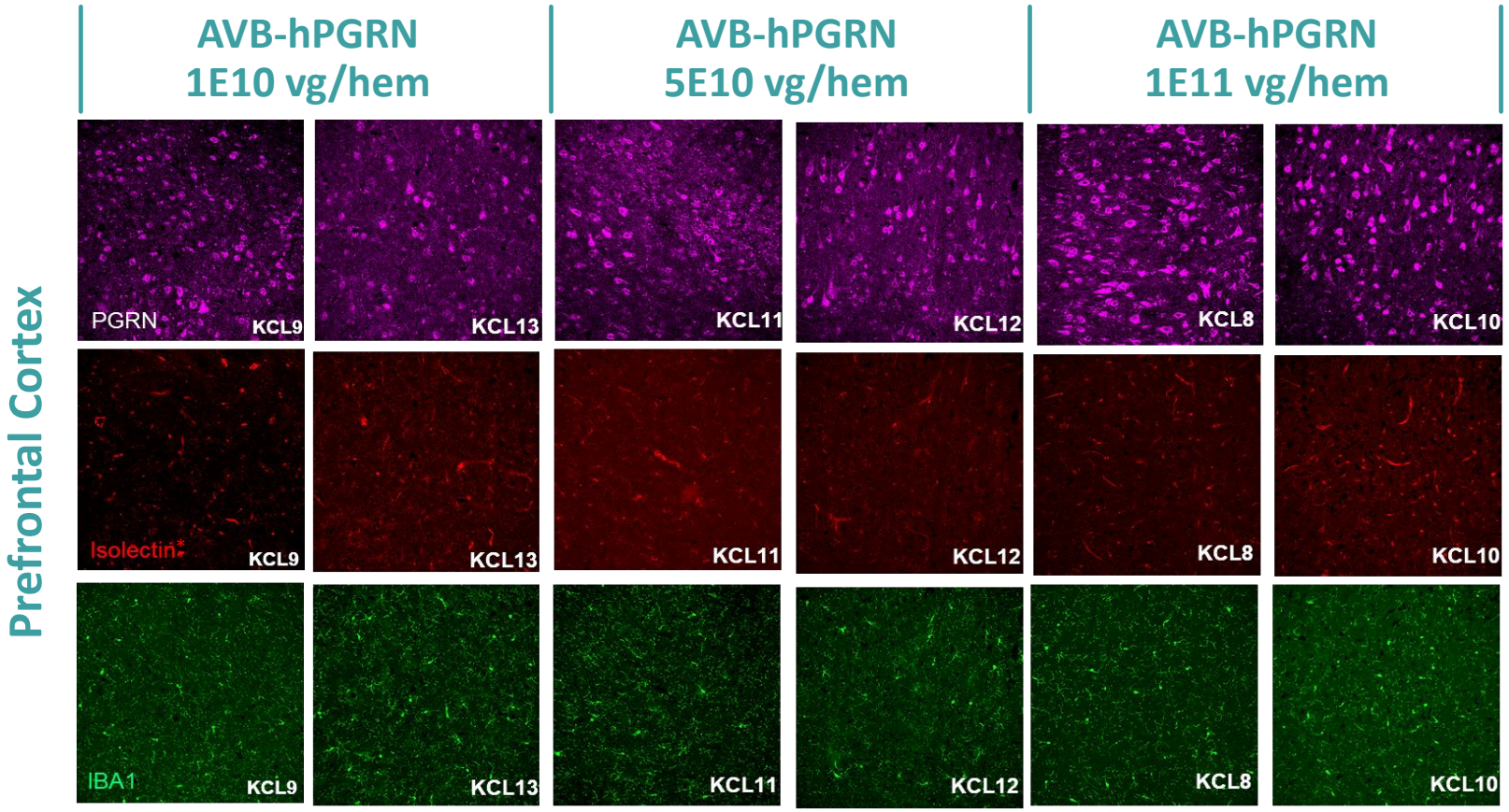
Administration:
Convection-enhanced delivery catheter placed in thalamus under stereotactic guidance
AVB-101 +/- gadolinium infused, and animals sacrificed after four weeks in life

MRI scans are used to define anatomy and select optimal trajectory and have shown **hPGRN** expression in cortical layers I–VI

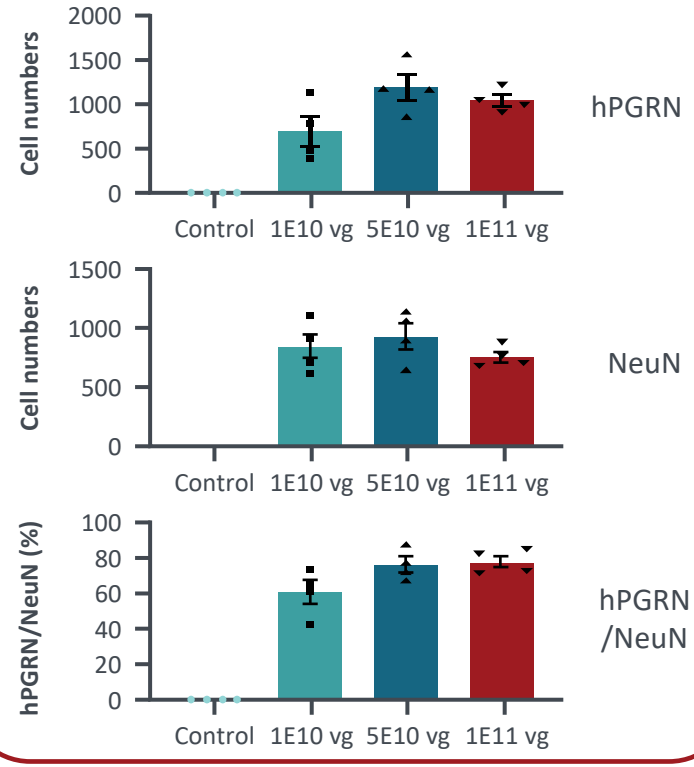


AAV9, adeno-associated virus serotype 9; hPGRN, human progranulin; MRI, magnetic resonance imaging.

Robust Cortical hPGRN Expression Without Microglial Reactivity



Quantification of hPGRN/NeuN Neurons



PGRN detected in 60–75% of NeuN positive neurons in the sheep prefrontal cortex

*Isolectin binds to CD68 receptors and used as a marker of activated microglia.
CD, cluster of differentiation; hem, hemisphere; hPGRN, human progranulin; IBA1, ionized calcium binding adaptor molecule 1; PGRN, progranulin; NeuN, neuronal nuclear protein; vg, vector genome.

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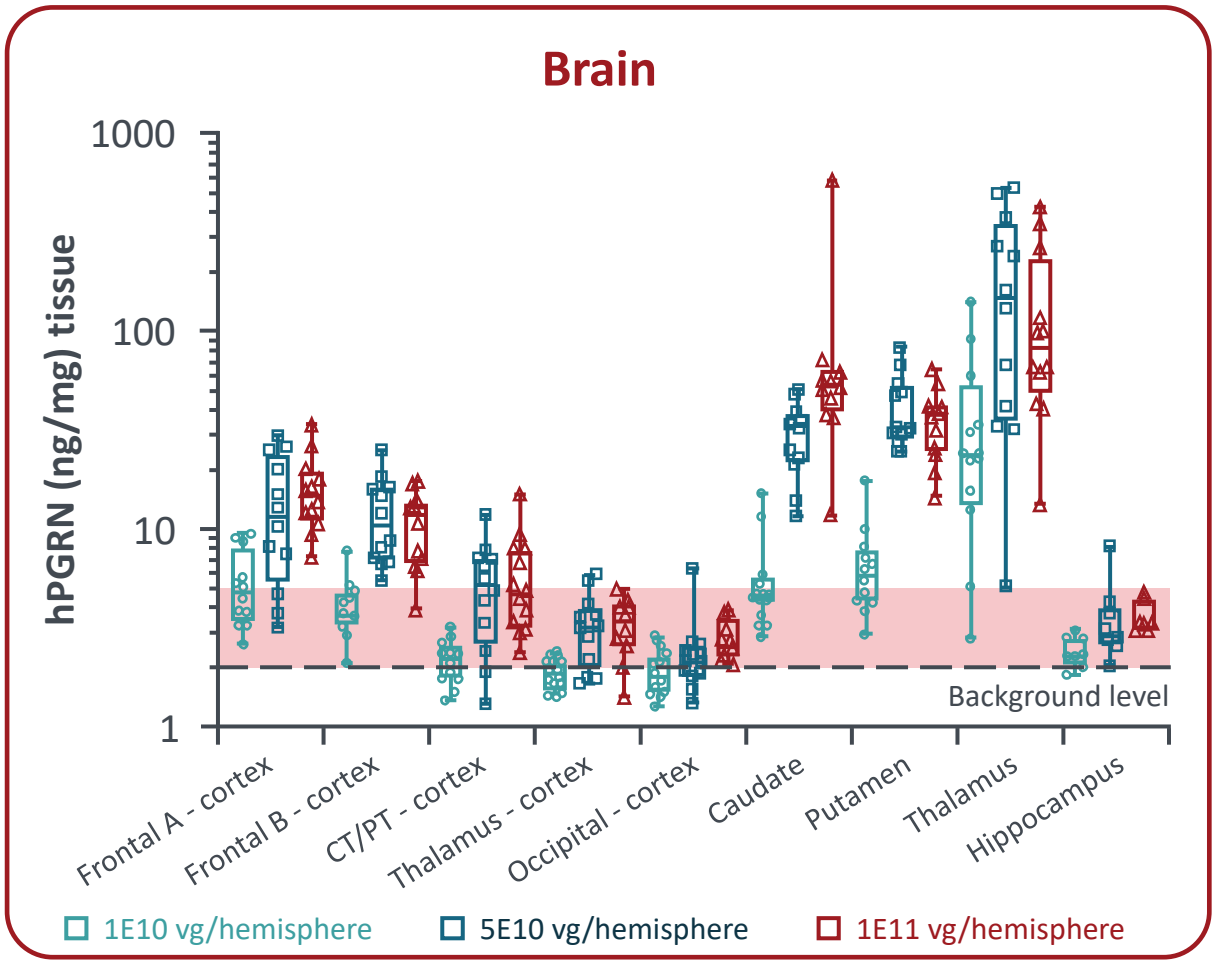
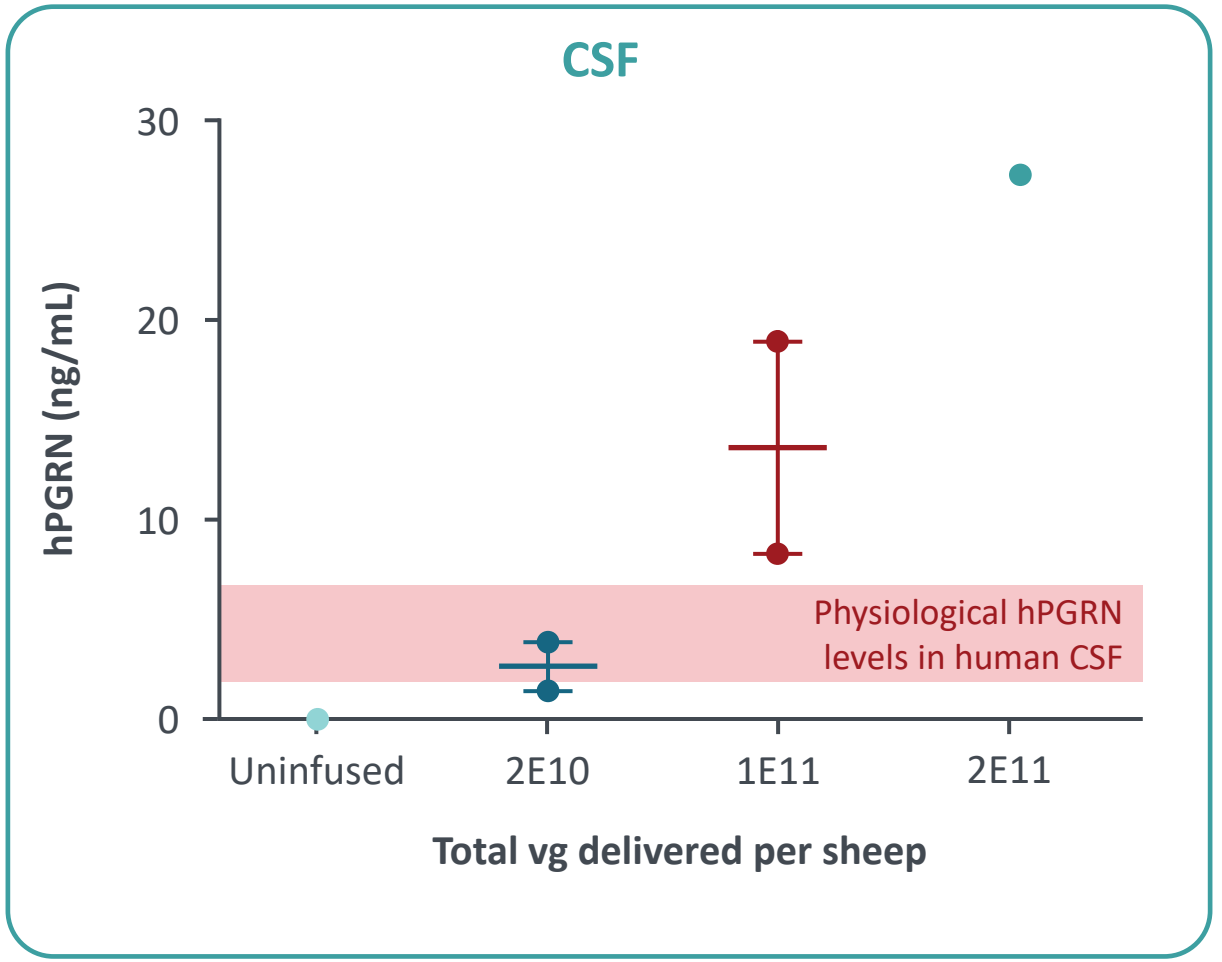
Dose-dependent Increase of hPGRN in Brain Reflected in CSF After Intrathalamic AVB-101





Normal to supraphysiological levels of hPGRN **achieved across the brain and CSF** at **very low vector doses**

CSF, cerebrospinal fluid; CT, corticothalamic; hPGRN, human progranulin; PGRN, progranulin; PT, pyramidal tract; vg, vector genome.

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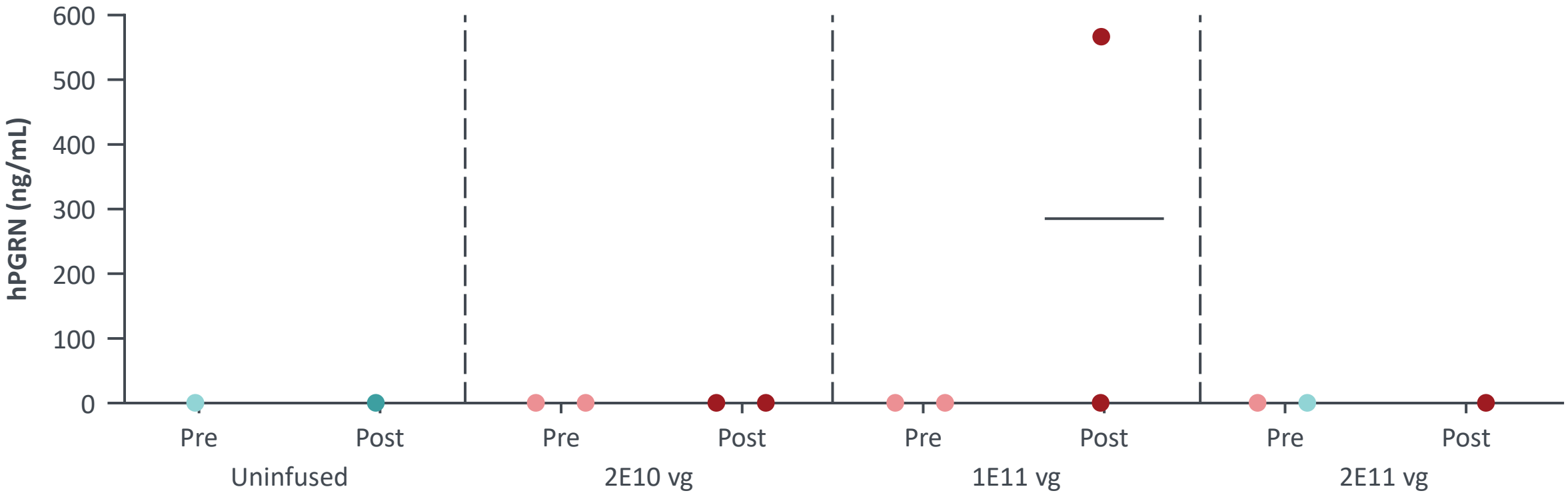
Negligible Levels of hPGRN in Sheep Serum Demonstrates CNS-Restricted Expression



*Single outlier result likely due to contamination

Serum hPGRN was **undetectable** before and four weeks after intrathalamic infusion of **AVB-101**

hPGRN, human progranulin; vg, vector genome.

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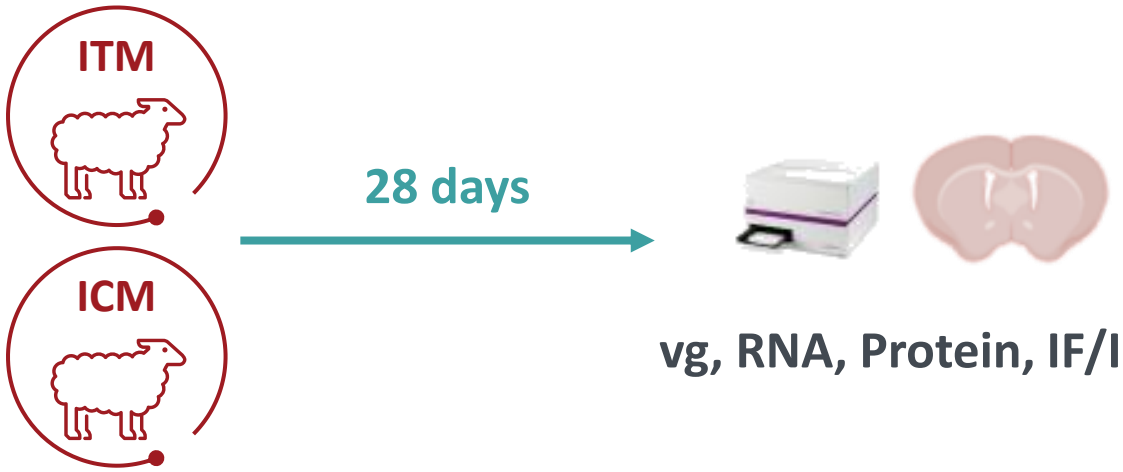
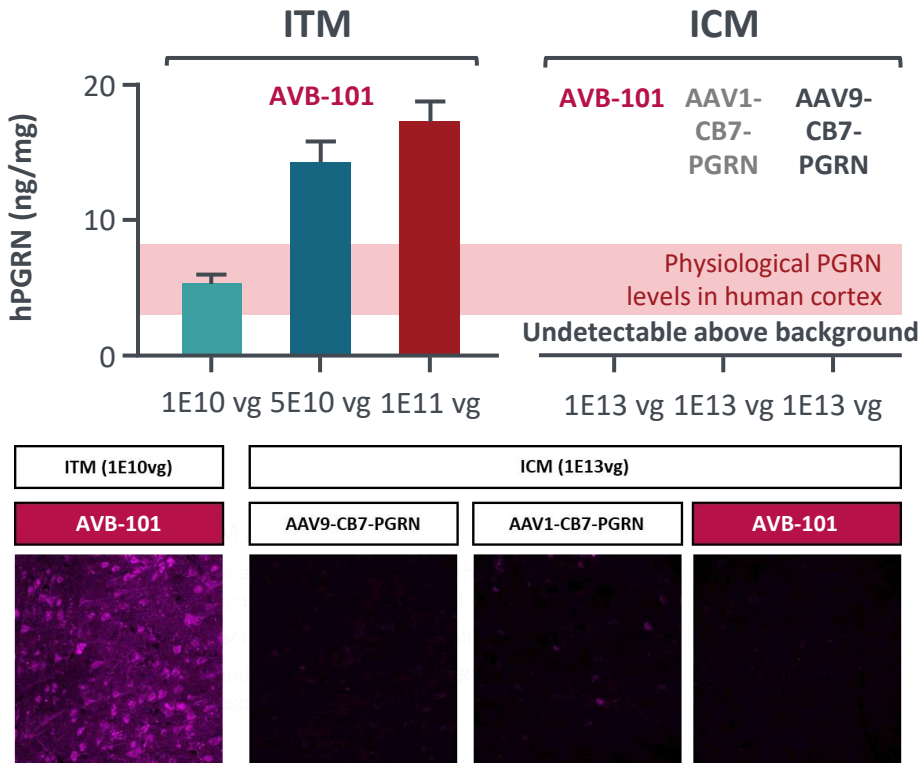
Intrathalamic vs. Intracisternal Magna Shows Intrathalamic Superiority



- **Intrathalamic** delivery achieved extensive neuronal expression throughout the cortex and subcortex including non-neuronal cells
- **Intracisternal magna** delivery results in negligible levels of hPGRN throughout the CNS in all intracisternal magna treated animals



hPGRN immunostaining in the prefrontal cortex of ITM and ICM infused animals

Minimal amount of **intrathalamic** AAV administration achieves **broad brain distribution**

AAV1, adeno-associated virus serotype 1; AAV9, adeno-associated virus serotype 9; CB7, chicken β-actin 7; CNS, central nervous system; hPGRN, human progranulin; ICM, intracisternal magna; IF, immunofluorescence; IHC, immunohistochemistry; ITM, intrathalamic; PGRN, progranulin; RNA, ribonucleic acid; vg, vector genome; WT, wild type.

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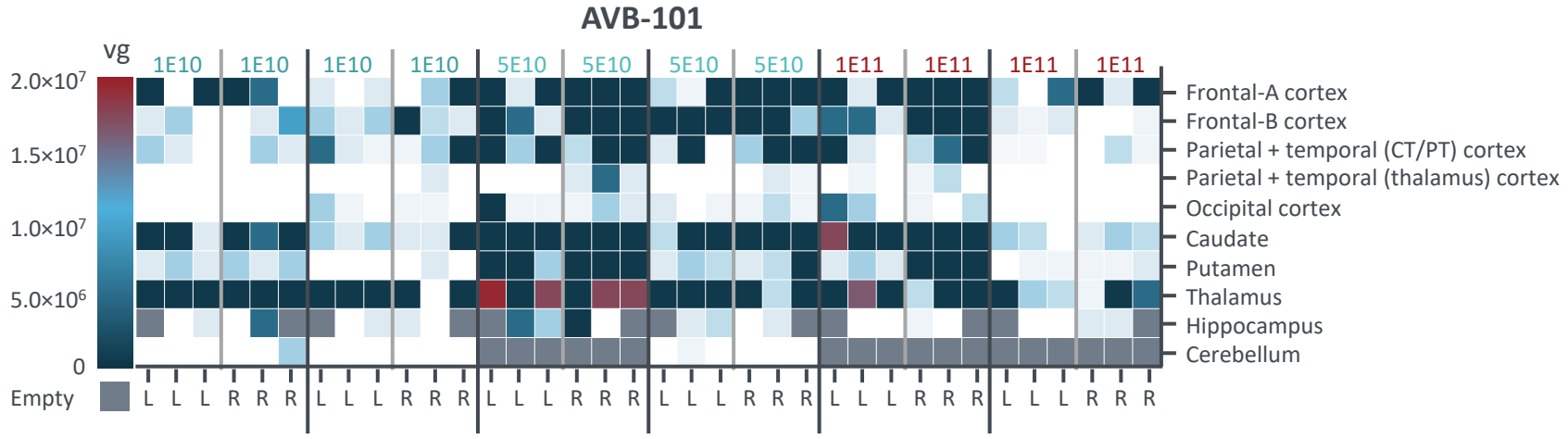
Superior Vector Distribution Using Intrathalamic vs. Intracisternal Magna Delivery

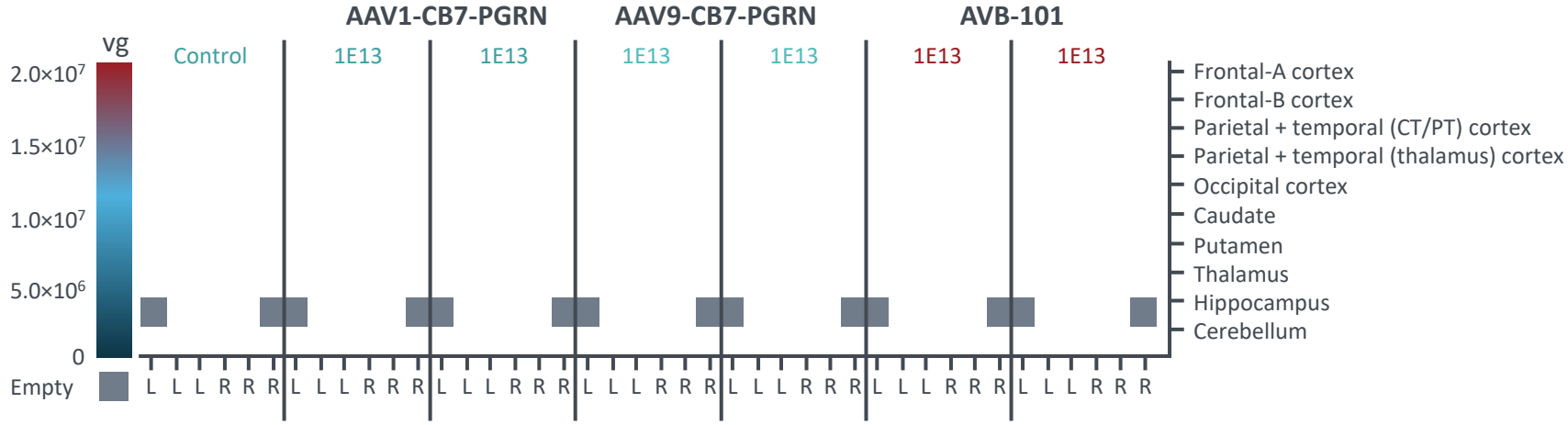
Intrathalamic

AVB-101



Intracisternal Magna

AAV1-CB7-PGRN
AAV9-CB7-PGRN
AVB-101



AAV1, adeno-associated virus serotype 1; AAV9, adeno-associated virus serotype 9; CB7, chicken β-actin 7; CT, corticothalamic; L, left; PGRN, progranulin; PT, pyramidal tract; R, right; vg, vector genome

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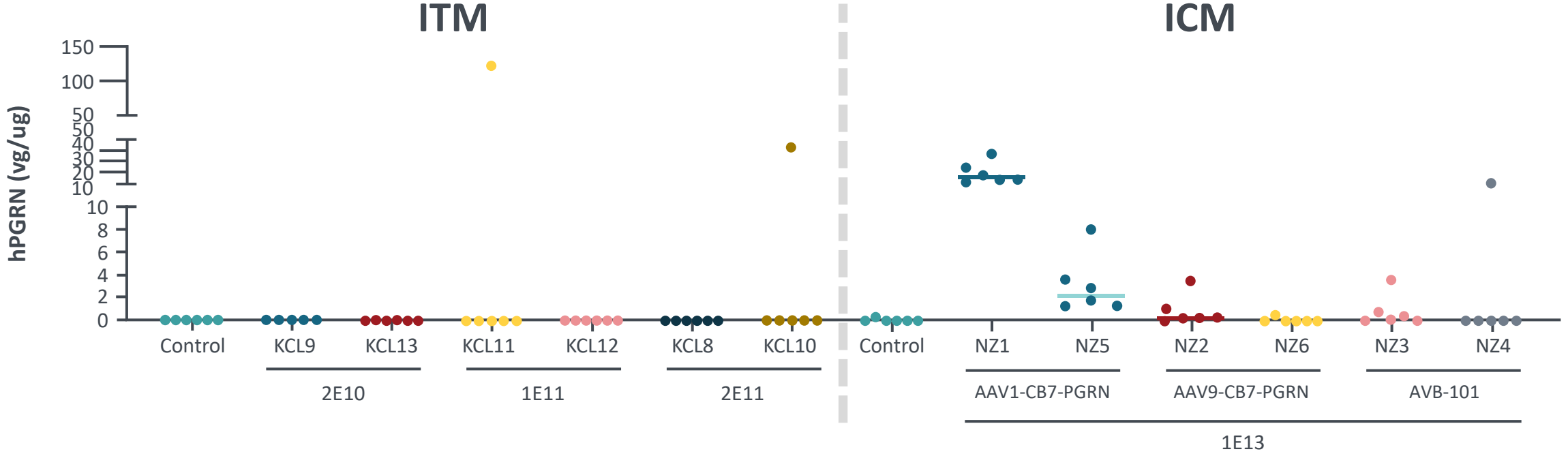
Intrathalamic Delivery Minimises AAV Liver Exposure and Shedding vs. Intracisternal Magna

Liver vg Quantification (qPCR)



AVB-101 vector was **undetectable** in liver biopsies following intrathalamic delivery but was detected following intracisternal magna delivery of three different AAV constructs

Undetectable AAV liver levels confirm **minimal vector shedding** from CNS following intrathalamic delivery

AAV, adeno-associated virus; CB7, chicken β-actin 7; CNS, central nervous system; hPGRN, human progranulin; ICM, intracisternal magna; ITM, intrathalamic; PGRN, progranulin; qPCR, quantitative polymerase chain reaction; vg, vector genome.

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Summary

- **AviadoBio is developing novel gene supplementation and silencing therapies for neurodegenerative disorders**
- **AVB-101** is designed to **normalise cortical PGRN levels** in patients with FTD due to *GRN* mutations while restricting PGRN expression to neurons and enhancing secretion to lower vg dose

AVB-101 in ***Grn*-/- mice suppressed neuronal lipofuscinosis and reactive microglia**

AVB-101 in **sheep was well tolerated, with minimal liver exposure and widespread cortical and subcortical biodistribution**

AVB-101 delivered by intrathalamic infusion constitutes a novel and promising approach to address unmet medical need in FTD-*GRN*

Coming soon: GLP toxicology studies in nonhuman primates are ongoing and clinical trials are due to be initiated late 2022

FTD, frontotemporal dementia; GLP, good laboratory practices; PGRN, progranulin; vg, vector genome.

Acknowledgements

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