# Intrathalamic Delivery of AVB-101 Rescues Pathology in Progranulin Deficient Mice and Achieves Widespread Cortical Expression in Two Large Animal Models

AVIADOBIO

**Poster #370**

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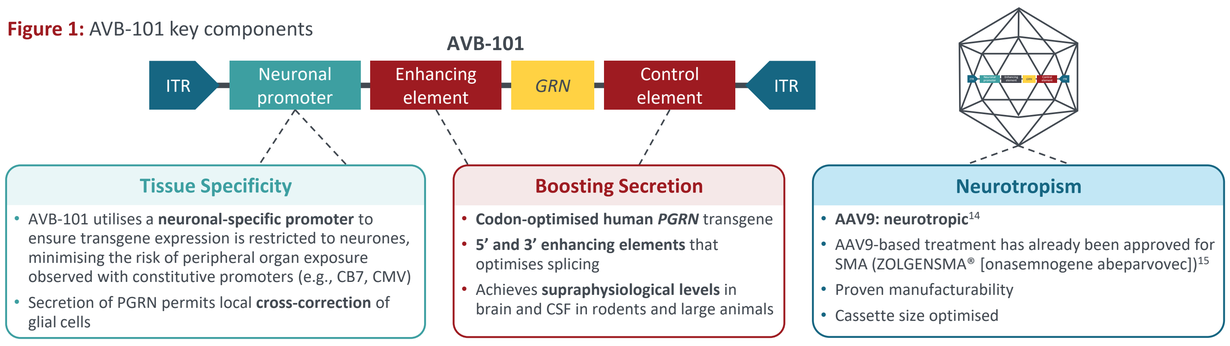
## Introduction

- Frontotemporal dementia (FTD) affects ~30,000 patients in the United States of America and ~76,000 patients in Europe* causing a progressive decline in behaviour, personality, executive function and/or language, with death 6–12 years after initial symptom onset[1–7]
- The burden associated with FTD is significant and costly, and currently no disease modifying therapies are available[8]
- FTD caused by loss-of-function mutations in the *GRN* gene (FTD-*GRN*) leads to deficient progranulin (PGRN) production that increases microglial activity, accelerates neurodegeneration, and leads to pathogenic transactive response DNA-binding protein 43 (TDP-43) accumulation[9–12]
- PGRN supplementation has been demonstrated to correct the pathological phenotype in rodent models of FTD-*GRN*, suggesting that it is a valid therapeutic target for patients with FTD-*GRN*[13]
- AVB-101 is an adeno-associated virus serotype 9 (AAV9)-based gene therapy that encodes PGRN, and is in development to treat patients with FTD-*GRN*
- AVB-101 has been designed with efficacy and safety in mind (**Figure 1**) and here we present preclinical data from a range of animal models to provide rationale for its application in clinical trials

**Figure 1:** AVB-101 key components



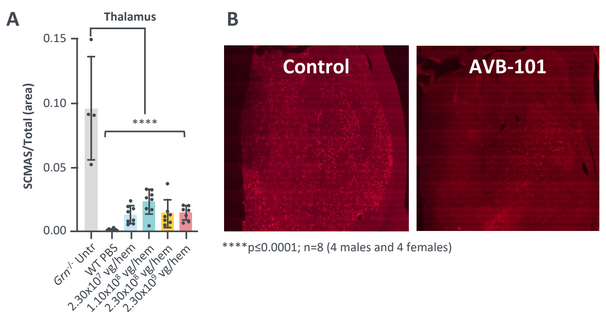

AAV9, adeno-associated virus serotype 9; CB7, chicken β-actin 7; CMV, cytomegalovirus; CSF, cerebrospinal fluid; GRN, granulin precursor; ITR, inverted terminal repeat; PGRN, progranulin; SMA, spinal muscular atrophy.

## Results

### AVB-101 substantially reduces pathology in *Grn* knock-out mice

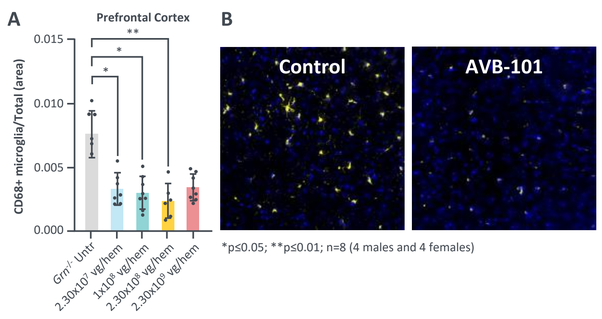
- AVB-101 was administered intrathalamically to *Grn*$^{-/-}$ mice to assess impact on FTD-*GRN* disease pathology
  - Without intervention, *Grn*$^{-/-}$ mice develop extensive lipofuscinosis and age-dependent neuroinflammation
- Intrathalamic delivery of AVB-101 in young *Grn*$^{-/-}$ mice (6 weeks) restored wild-type levels of lipofuscinosis (subunit C mitochondrial adenosine triphosphate synthase [SCMAS]) across all doses (**Figure 2**)
- Intrathalamic delivery of AVB-101 in older *Grn*$^{-/-}$ mice (28 weeks) reduced levels of microglial activation, as measured by cluster of differentiation 68 (CD68) expression, throughout the central nervous system (CNS) (**Figure 3**)

**Figure 2:** Quantification (**A**) and visualisation (**B**) of SCMAS (lipofuscinosis) in the thalamus of control and AVB-101-treated young *Grn*$^{-/-}$ mice. Infused at 8 weeks, imaged 12 weeks post AVB-101 delivery (representative images).




****p≤0.0001; n=8 (4 males and 4 females)

**Figure 3:** Quantification (**A**) and visualisation (**B**) of neuroinflammation (CD68) in the thalamus of control and AVB-101-treated old *Grn*$^{-/-}$ mice. Infused at 40 weeks, imaged 12 weeks post AVB-101 delivery (representative images).



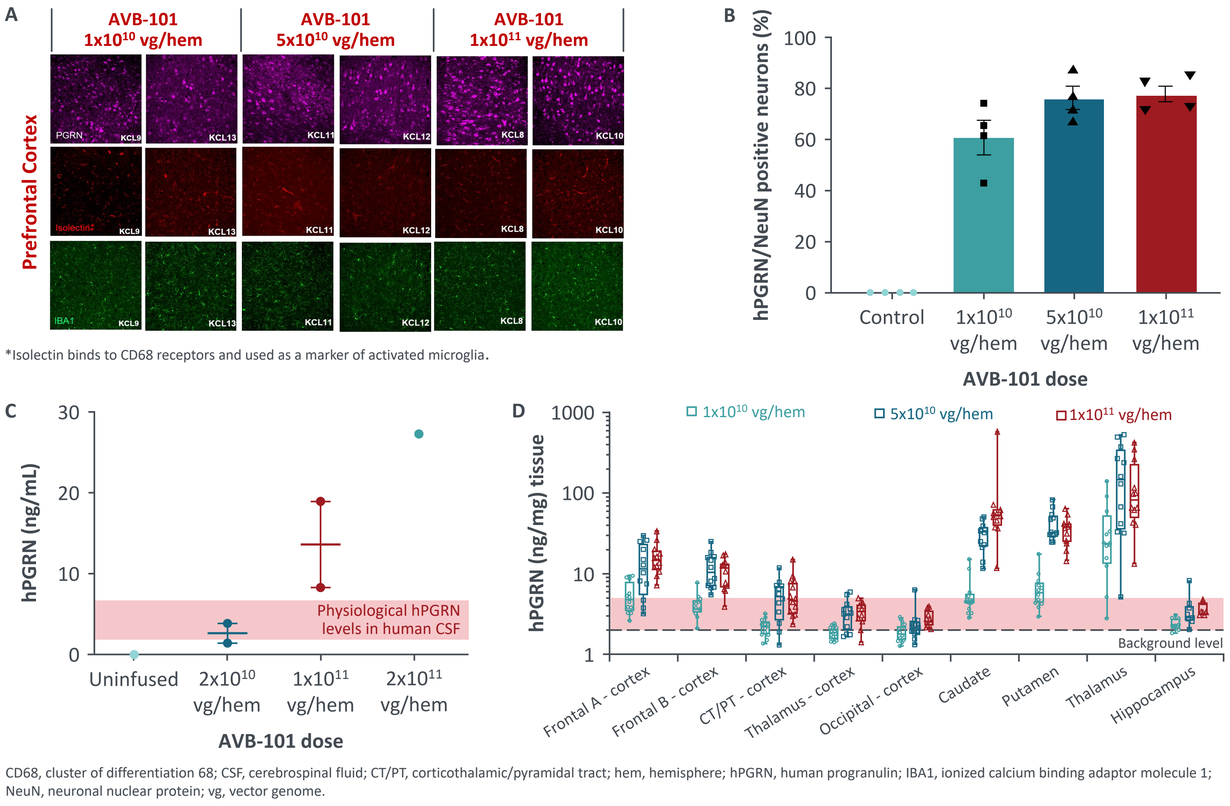

*p≤0.05; **p≤0.01; n=8 (4 males and 4 females)

CD68, cluster of differentiation 68; hem, hemisphere; PBS, phosphate buffered saline; SCMAS, subunit C mitochondrial ATP synthase; Untr, untreated; vg, vector genome; WT, wild type.

### Intrathalamic administration of AVB-101 to sheep led to targeted and robust cortical human progranulin (hPGRN) expression without microglial activation

- AVB-101 was administered to sheep via convection-enhanced delivery under stereotactic guidance. Animals were sacrificed after four weeks in life. The treatment was dosed with or without gadolinium-based contrast agent
  - The ovine model was chosen as sheep brains are more comparable to the human brain in size versus the brain of a macaque and sheep are widely used as a model in neurological research
- AVB-101 achieved robust hPGRN expression in the prefrontal cortex without microglial activation (**Figure 4A**) and hPGRN was detected in 60–75% of neuronal nuclear protein (NeuN) positive neurons in the sheep prefrontal cortex (**Figure 4B**)
- Intrathalamic delivery of AVB-101 results in a dose-dependent increase of hPGRN in the cerebral spinal fluid (CSF) and normal to supraphysiological levels of the hPGRN protein in various brain areas affected in FTD-*GRN* (**Figure 4C** and **4D**)
- hPGRN levels in serum remained undetectable before and after AVB-101 treatment

**Figure 4:** Visualisation of hPGRN expression and microglia activation in the prefrontal cortex (**A**), quantification of hPGRN/NeuN positive neurons (**B**), and hPGRN levels in the CSF (**C**) and across brain regions (**D**) in AVB-101-administered sheep.



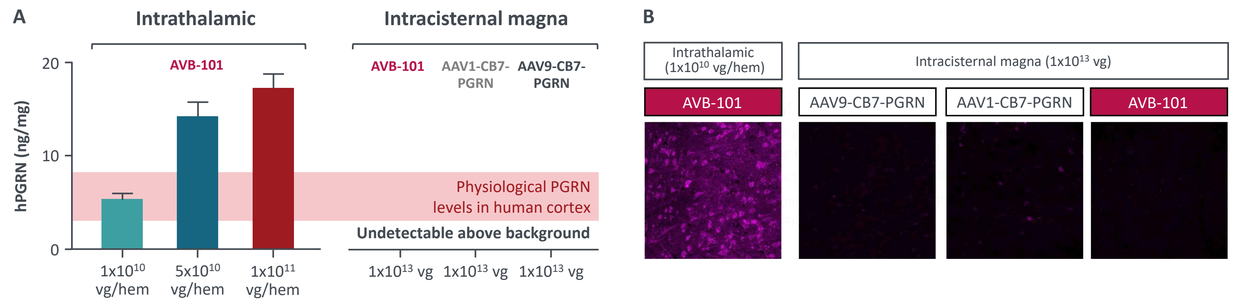

*Isolectin binds to CD68 receptors and used as a marker of activated microglia.

CD68, cluster of differentiation 68; CSF, cerebrospinal fluid; CT/PT, corticothalamic/pyramidal tract; hem, hemisphere; hPGRN, human progranulin; IBA1, ionized calcium binding adaptor molecule 1; NeuN, neuronal nuclear protein; vg, vector genome.

### Intrathalamic administration of AVB-101 led to superior biodistribution and expression versus intracisternal magna delivery in sheep

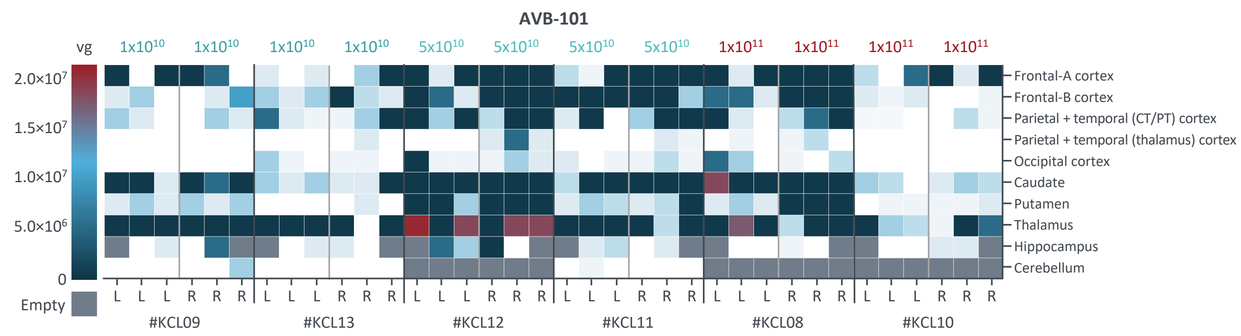
- Intrathalamic and intracisternal magna administration routes for AVB-101 were compared in adult sheep
- Intrathalamic delivery achieved extensive neuronal expression throughout the cortex and subcortex including non-neuronal cells; whereas intracisternal magna delivery resulted in negligible levels of hPGRN throughout the CNS in all treated animals (**Figure 5A** and **5B**)
- The lowest-tested dose of AVB-101 1x10$^{10}$ vector genome/hemisphere (vg/hem) administered via the intrathalamic route achieves PGRN levels observed in healthy human cortices (**Figure 5A**) while intracisternal magna distribution of AVB-101 at a dose of 1x10$^{13}$ vg, 500 times greater than the intrathalamic dose, was undetectable (**Figure 5B**)
- Intrathalamic delivery of AVB-101 resulted in broad vector distribution across brain regions (**Figure 6**)
- The intrathalamic delivery route decreased liver exposure and shedding versus intracisternal magna delivery as determined by quantitative polymerase chain reaction with undetectable levels of AVB-101 vector in liver biopsies following intrathalamic administration

**Figure 5:** hPGRN levels in the cerebral spinal fluid (**A**) and immunostaining representative visualisation of hPGRN in the prefrontal cortex (**B**) according to the administration route used in sheep.




AAV[X], adeno-associated virus serotype [X]; CB7, chicken β-actin 7; hem, hemisphere; hPGRN, human progranulin; PGRN, progranulin; vg, vector genome.

**Figure 6:** AVB-101 distribution across brain regions according to dose in sheep following intrathalamic administration.

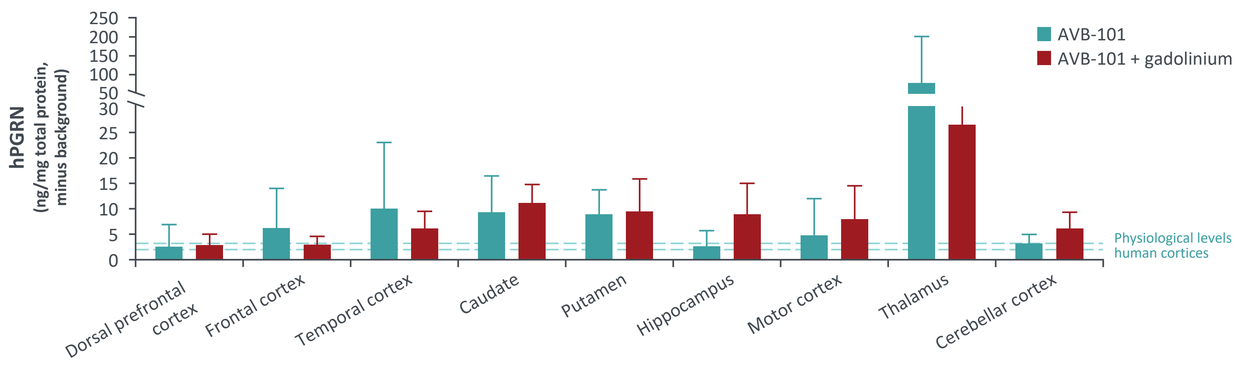



#KCL denotes animal number.
CT/PT, corticothalamic/pyramidal tract; L, left; R, right; vg, vector genome.

### Biodistribution study confirms broad brain distribution of hPGRN following AVB-101 delivery to non-human primates (NHP)

- AVB-101 was administered to cynomolgus monkeys by single bilateral intrathalamic injection using convection enhanced delivery
  - AVB-101 biodistribution was tested with and without a gadolinium-based contrast agent added to the formulation buffer
- After 8 weeks in life, there was widespread brain distribution of hPGRN, achieving physiological levels of human cortices (**Figure 7**)
  - The highest levels of hPGRN were observed in the thalamus with an increase in cortical areas and other areas receiving thalamic projections
  - There was no negative impact of gadolinium on hPGRN distribution

**Figure 7:** hPGRN biodistribution in NHP brain following intrathalamic AVB-101 administration.




hPGRN, human progranulin; NHP, non-human primate.

## Conclusions

- AviadoBio is developing novel gene supplementation and silencing therapies for neurodegenerative disorders including AVB-101, which has been engineered for specific, effective and targeted expression of hPGRN in patients with FTD-*GRN*
  - AVB-101 is designed to normalise cortical hPGRN levels in patients with FTD due to *GRN* mutations, while restricting PGRN expression to neurons and enhancing secretion efficiency to minimise the required dose of vector
- AVB-101 administered to *Grn*$^{-/-}$ mice representative of FTD-*GRN* pathology successfully suppressed neuronal lipofuscinosis and reactive microglia, key hallmarks of disease
- Following administration to sheep, AVB-101 led to targeted and robust hPGRN expression in a dose-dependent manner
- The intrathalamic route was demonstrated to be superior to intracisternal magna delivery when compared in the sheep model
  - Intrathalamic administration of AVB-101 was well tolerated, with widespread cortical and subcortical biodistribution that reached normal to supraphysiological levels of hPGRN with minimal levels of vector in the liver, confirming minimal vector shedding from the CNS
- Intrathalamic AVB-101 administration to NHPs resulted in widespread cortical hPGRN levels. The use of gadolinium-based contrast agent had no overt effect on hPGRN expression
- Intrathalamic administration has been well tolerated across pre-clinical studies. This includes a total of 58 animals (20 sheep, 38 NHP) to date, with additional NHP Good Laboratory Practice (GLP) toxicology studies ongoing

### AVB-101 delivered by intrathalamic infusion constitutes a novel and promising approach to address unmet medical needs in FTD-*GRN*, with clinical trials due to be initiated in late 2022

**References: 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.** Korneyenkov MA and Zamyatnin Jr AA. Pharmaceutics. 2021;13(5):750; **15.** Zolgensma. Prescribing information. Bannockburn, IL: Novartis Gene Therapies, Inc. March 2022.

**Notes:** *US data are an estimate of cognitive syndromes of frontotemporal lobar degeneration.[1] European data are an estimate of behavioral variant FTD in the European Union of 2013, Norway, Iceland and Lichtenstein.[2]

**Acknowledgements:** The authors thank the following for their contribution to the data presented: UK Dementia Research Institute, King's College London, Van Geest Foundation, My Name'5 Doddie Foundation, Neurochase, University of Bristol, and the University of Lincoln.

The authors thank Aleksandra Kaliszewska, Natalia Arias Del Castillo and Katharine Zhang for their contribution to this work. dna Communications, London, UK, provided editorial and layout support, sponsored by AviadoBio per Good Publication Practice 3 (GPP3) guidelines.

**Disclosures:** CES, DYL, YB, PH, RJ, ZW, JF, AF, CJM, and LYB are employees of AviadoBio. JA, MG, and OB are employed by Kings College London and under a scientific research agreement with AviadoBio.