One-time Delivery of a Vectorized Anti-amyloid Antibody for Increased and Sustained CNS Expression and Target Engagement

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INTRODUCTION

Alzheimer's disease (AD) is characterized by the accumulation of amyloid plaques, leading to cognitive decline. Clinically, passive administration of anti-amyloid antibodies has shown promise in reducing amyloid plaque burden and improving cognitive function in patients with mild cognitive impairment. However, these therapies are limited by poor blood-brain barrier (BBB) penetration and require frequent administration, which can be burdensome for patients and care partners. Here we show that the use of an adeno-associated viral (AAV) vector to deliver a transgene encoding an anti-amyloid antibody can enable sustained expression of the antibody in the brain after a single injection, especially if the vector is delivered via a BBB-penetrant capsid.

METHODS

***In vivo* Studies:**

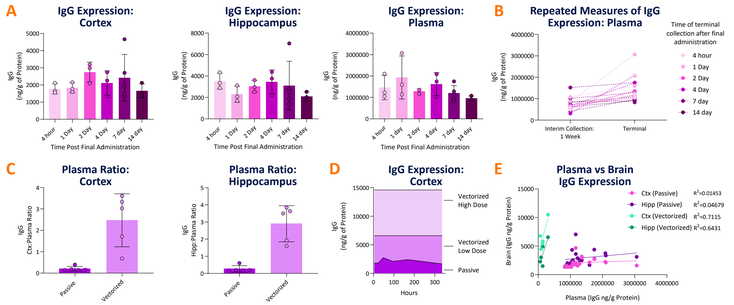
- Passive and vectorized administration of an anti-amyloid antibody in WT animals (Figure 1): WT mice were dosed (IP) weekly with a passive anti-amyloid antibody (24mg/kg) for 4 weeks. CNS tissues and plasma were collected at various time points (as indicated below) to assess IgG levels of exposure. An additional cohort of WT mice were injected once (IV) with a vectorized anti-amyloid antibody (2E13vg/kg). CNS and liver tissues and plasma were collected after 4 weeks and assessed for IgG expression.
- Vectorized anti-amyloid antibody administration in a mouse model of Alzheimer's Disease (AD) (Figures 2-4): Transgenic mice were injected one-time (IV) with a vectorized anti-amyloid antibody at a high (4E13vg/kg) or low (2E13vg/kg) dose. Tissues and plasma were collected at either 1, 2, or 4 months (as indicated) and assessed for VG and IgG expression.
- Anti-amyloid antibody was vectorized in a blood barrier penetrating capsid, VOY101.

Quantification of Vector Genomes (VG): Tissue levels of VG copies were assessed by digital droplet PRC (ddPCR) after DNA isolation. The number of VG copies are recorded per diploid cell (VG/DC).

Quantification of IgG Expression: Immunoassay (IgG ELISA) was used to determine the expression levels in soluble tissue homogenates and plasma.

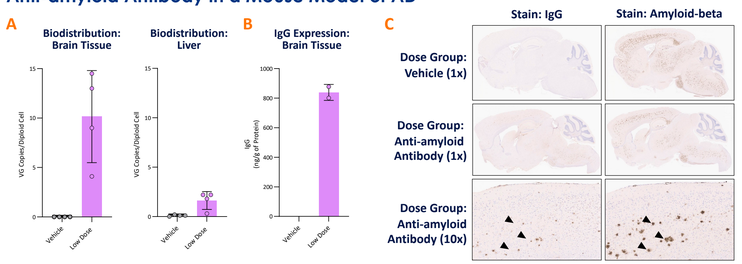
Immuno-histochemical or fluorescent Imaging: CNS tissues were fixed, embedded, sectioned and stained for IgG or amyloid-beta. Immunohistochemical images were stained using DAB chromogenic staining methods, serial sections were stained individually for either IgG or amyloid-beta. Immunofluorescent images were achieved by co-staining IgG and amyloid-beta antibodies within the same section.

Figure 1. Vectorization of an Anti-amyloid Antibody via a BBB-penetrant Capsid Enhances Brain Uptake as Compared to Passive Administration



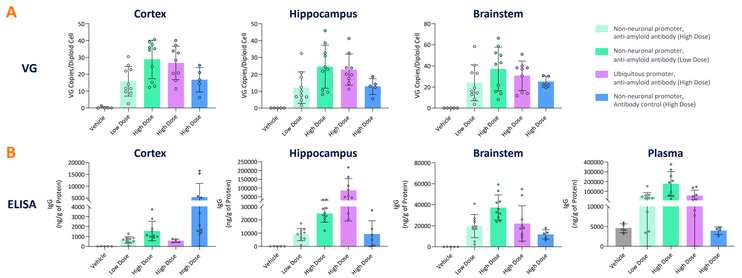
A passively administered anti-amyloid antibody was injected weekly for 4 weeks in WT mice before terminal collection of plasma and brain tissues at 4 hours, 1, 2, 4, 7, and 14 Days after final administration. (**A**) IgG expression in cortex, hippocampus, and plasma of passively administered anti-amyloid antibody. (**B**) IgG expression in the plasma from all groups 1 week post the initial injection compared to terminal collection. Elevation of IgG is observed in all groups after 4 weeks of passive administration. (**C**) IgG brain (cortex and hippocampus) to plasma ratios are over 15-fold greater after single dose of the AAV vectorized anti-amyloid antibody payload. Vectorized data are from 4-weeks post a single IV administration. (**D**) Passive administration IgG expression over time compared to 2 doses of vectorized anti-amyloid antibody. Data show higher consistent expression with vectorization. (**E**) Correlation plots from cortex and hippocampus of passively administered or vectorized anti-amyloid antibody.

Figure 2. Broad Biodistribution and IgG Expression in Brain Tissue 4-weeks After a Single IV Injection of Vectorized Anti-amyloid Antibody in a Mouse Model of AD



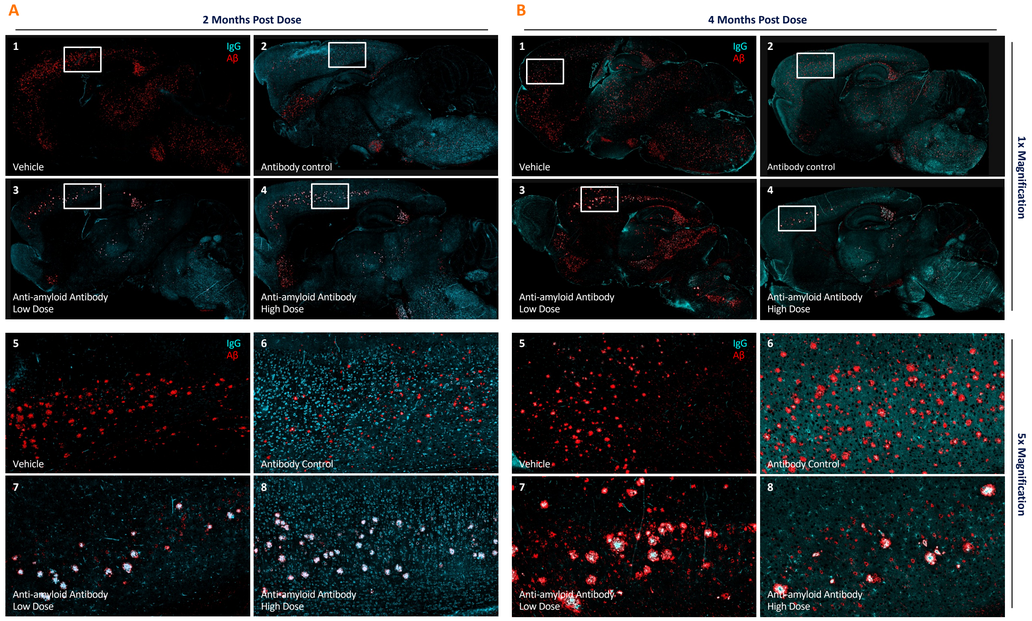
Transgenic animals were injected with a single low dose of a vectorized anti-amyloid antibody to evaluate biodistribution (VG/DC) and IgG protein expression. (**A**) Brain and liver biodistribution (VG/DC) as determined by ddPCR. (**B**) IgG protein expression in brain as determined by ELISA. (**C**) Immunohistochemistry on fixed brains shows overlapping expression of IgG and amyloid-beta in serial sections. This suggests target engagement of the delivered anti-amyloid antibody payload with amyloid-beta plaques. Arrows indicate representative points of overlapping expression of IgG and amyloid-beta.

Figure 3. IV Delivery of Vectorized Anti-amyloid Antibody Shows Sustained Expression of IgG in the Brain After 4 Months



Sustained biodistribution and IgG protein expression up to 4 months post a single IV administration of vectorized anti-amyloid antibody in a mouse model of Alzheimer's Disease. Animals were injected IV in four dose groups: vehicle, vectorized anti-amyloid antibody under either a ubiquitous or non-neuronal promoter (2 doses), and a vectorized antibody control. (**A**) Cortex, hippocampus, and brainstem show trends towards dose dependent biodistribution (VG/DC) after 4 months in-life. (**B**) IgG expression in cortex, hippocampus, brainstem, and plasma show trends toward dose dependent expression of anti-amyloid antibody after 4 months in-life.

Figure 4. IV Delivery of Vectorized Anti-amyloid Antibody Shows Qualitative Reduction in Amyloid-beta After 2 and 4 Months in a Mouse Model of Alzheimer's Disease



Immunofluorescence reveals qualitative reduction in amyloid-beta up to 4 months post single IV injection of vectorized anti-amyloid antibody in a mouse model of Alzheimer's Disease. All dose groups (except vehicle) received a vectorized antibody with expression driven by a non-neuronal promoter. (**A**) Immunofluorescence images of IgG and amyloid-beta at two months post administration. Images 1-4 show whole brain sagittal slices at 1x magnification. Images 5-8 show 5x magnification of the cortex. The magnified images highlight the apparent reduction and target engagement in amyloid-beta detection after administration of a vectorized anti-amyloid antibody. (**B**) Immunofluorescence images of IgG and amyloid-beta at four months post administration. Images 1-4 show whole brain sagittal slices at 1x magnification. Images 5-8 show 5x magnification of the cortex. The magnified images highlight further apparent reduction at 4 months in amyloid-beta detection after a single administration of a vectorized anti-amyloid antibody.

CONCLUSIONS

- In wildtype mice a vectorized anti-amyloid antibody packaged in a BBB penetrant capsid has over 15-fold greater brain:plasma ratios as compared to a passively administered antibody over 4 weeks.
- In a transgenic mouse model of AD, there is broad biodistribution of vector genomes and sustained antibody expression in the brain after a 1-month in-life period. Immunohistochemical evidence in the cortex and hippocampus reveal that the vectorized antibody co-localizes with amyloid-beta plaques, indicating successful target engagement.
- In a transgenic mouse model of AD, there is sustained biodistribution of vector genomes and antibody expression in the brain over a 4-month period. Immunofluorescence analysis of the cortex and hippocampus reveal high degree of co-localization of the vectorized antibody and amyloid-beta. Qualitatively, there is a notable reduction in amyloid-beta staining after a 1-time administration of a vectorized anti-amyloid beta antibody.
- These results demonstrate that vectorization of an anti-amyloid antibody using a BBB penetrant capsid offers a promising strategy for sustained and enhanced, brain-wide antibody expression, overcoming the limitations of passive antibody therapies in AD.