Transferrin receptor-binding blood-brain barrier shuttle enhances brain delivery and efficacy of a therapeutic anti-Aβ antibody

Authors
Allison Butt[1], Laura M. McNair[1], Marta Ramos Vega[1], Henrik H. Hansen[1], Camilla Stampe Jensen[2], Evdoxia Alexiou[1], Jacob Lercke Skytte[1], Martin R Madsen[1], Florence Sotty[2], Franziska Wichern[1], Casper Graversen Salinas[1], Allan Jensen[2], Sandra Vergo[2], Jacob Hecksher-Sørensen[1]

[1]Gubra, Hørsholm, Denmark;
[2]Biotherapeutic Discovery, H. Lundbeck A/S, Valby, Denmark

Corresponding author
Jacob Hecksher-Sørensen, jhs@gubra.dk

Background & Aim

Improved CNS access of amyloid β (Aβ)-directed antibodies may increase therapeutic benefits while also reducing dose-dependent adverse effects of this drug class. Using light sheet fluorescence microscopy (LSFM), we 3D mapped and quantified brain delivery, distribution and efficacy of a blood-brain barrier (BBB) shuttle-enhanced therapeutic anti-Aβ antibody in a mouse model of Alzheimer's disease (AD).

Methods

See study outline in Fig. 1. Transgenic APP/PS1 [APP(KM670/671NL(swe)/PS1(L166P))] mice were administered (i.p.) standard human IgG (Ctrl-hIgG, 50 nmol/kg), Aducanumab (Adu, 10 or 50 nmol/kg) or Aducanumab fused with mTfR1 binder as BBB-shuttle (TfR1-Adu, 10 nmol/kg) once weekly for 12 weeks. The left whole hemibrain was co-stained with antibodies against hIgG and hAβ. The right whole hemibrain was stained with Congo Red. Samples were optically cleared and scanned on a LSFM. Advanced computational analysis allowed for simultaneous, automated mapping and quantification of compound distribution (hIgG fluorescent signal) and plaque counts (Aβ+ plaques, Congophilic plaques) using a custom mouse brain atlas.

Conclusion

+ **TfR1 BBB-shuttle enhances brain delivery and distribution of Aducanumab**
+ **TfR1 BBB-shuttle improves plaque-clearing efficacy of Aducanumab**
+ **TfR1 BBB-shuttle mitigates vascular labelling of Aducanumab, potentially translating to reduced risk of ARIA adverse effects**

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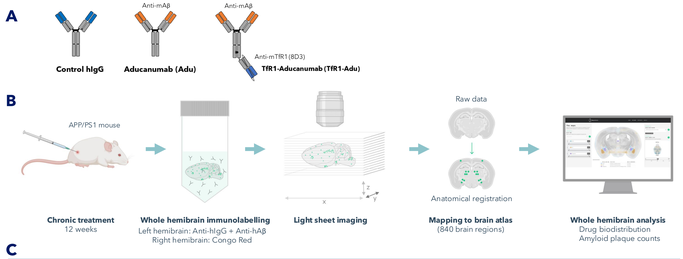
1 Quantitative 3D LSFM brain imaging pipeline



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Study design	Group	Treatment	Number of animals	Age (weeks)
Chronic treatment (i.p., once weekly for 12 weeks)	1	Control hIgG 50 nmol/kg	8	44 ± 2
	2	Aducanumab 10 nmol/kg	8	43 ± 3
	3	Aducanumab 50 nmol/kg	10	43 ± 2
	4	TfR1-Aducanumab 10 nmol/kg	9	41 ± 4

Figure 1. Study outline and automated quantitative 3D light sheet fluorescence microscopy imaging workflow. **(A)** Design of humanized IgG antibodies. Schematic illustration of control human IgG (Ctrl-hIgG), Aducanumab (Adu) and Aducanumab fused with anti-mouse transferrin receptor (TfR1-Adu). **(B)** AI-based automated whole-brain imaging pipeline applied for comparative evaluation of whole hemibrain distribution and therapeutic efficacy of Adu vs. TfR1-Adu following chronic treatment (12 weeks) in transgenic APP/PS1 mice. **(C)** Treatment groups and average age at treatment start. Mice were terminated 72h after last dosing.

2 TfR1 BBB-shuttle enhances brain delivery and mitigates vascular labelling of Aducanumab

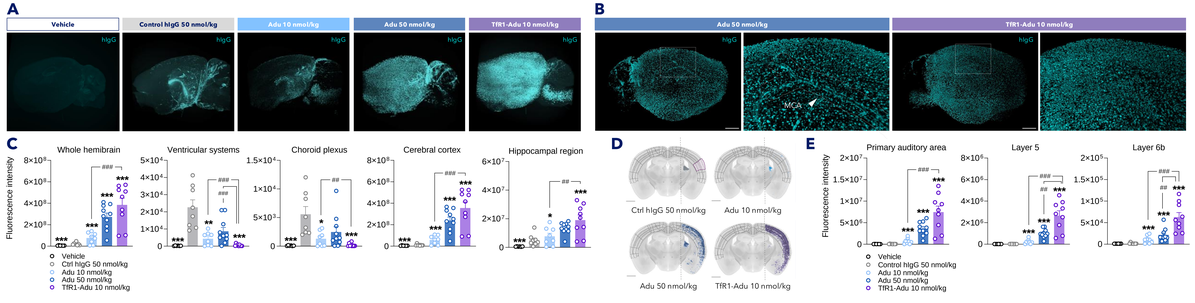

Figure 2. TfR1 BBB-shuttle enhances brain delivery and distribution of Aducanumab in AD mice. (A) Representative 3D LSFM raw images of anti-hIgG stained whole hemibrains (sagittal view). TfR1-Adu shows more homogeneous and deeper brain distribution compared to unmodified Adu. **(B)** hIgG signal distribution of Adu (50 nmol/kg, left panels) and TfR1-Adu (10 nmol/kg, right panels), revealing that the TfR1 BBB-shuttle mitigates vascular labelling of Adu (MCA, middle cerebral artery). **(C)** Accumulated fluorescent signal intensity (arbitrary units) in the whole hemibrain, ventricular systems, choroid plexus, cerebral cortex and hippocampus. **(D)** TfR1-Adu demonstrates substantially deeper cortical distribution as compared to unmodified Adu. Virtual 2D coronal sections from group-averaged 3D LSFM-imaged hemibrains. Cortical layers 1-6 are indicated. **(E)** Accumulated fluorescent signal intensity (arbitrary units) of individual mAbs in selected subdivisions of the cortical primary auditory area. *p<0.05, **p<0.01, ***p<0.001 vs. Ctrl hIgG 50 nmol/kg; ##p<0.01, ###p<0.001 (Dunnett's test negative binomial generalised linear model).

3 Brain-wide biodistribution of TfR1-Aducanumab

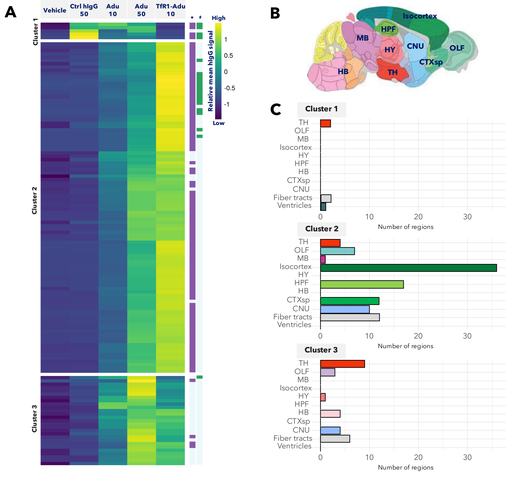

Figure 3. TfR1 BBB-shuttle enhanced brain delivery of Aducanumab in AD mice. (A) Heatmap of a total of 132 brain regions with increased fluorescence signal of Aducanumab (Adu, 10 nmol/kg, 50 nmol/kg) or TfR1-Aducanumab (TfR1-Adu, 10 nmol/kg) compared to Ctrl hIgG (50 nmol/kg). Colors indicate relative mean hIgG signal. The heatmap is segmented into three major clusters using hierarchical clustering according to highest hIgG signal for Ctrl hIgG (Cluster 1, n=5 brain regions) TfR-1Adu (Cluster 2, n=100 brain regions), and Adu (cluster 3, n=27 brain regions). *p<0.05 TfR1-Adu (10 nmol/kg) vs. Adu (10 nmol/kg); #p<0.05 TfR1-Adu (10 nmol/kg) vs. Adu (50 nmol/kg), Dunnett's test negative binomial generalized linear model. **(B)** Major brain divisions, including the thalamus (TH), olfactory areas (OLF), midbrain (MB), isocortex, hypothalamus (HY), hippocampal formation (HPF), hindbrain (HB), cortical subplate (CTXsp), cerebral nuclei (CNU), fiber tracts and ventricles. **(C)** Major brain divisions represented in the three clusters.

4 Therapeutic Aβ antibody biodistribution vs. plaque-clearing efficacy

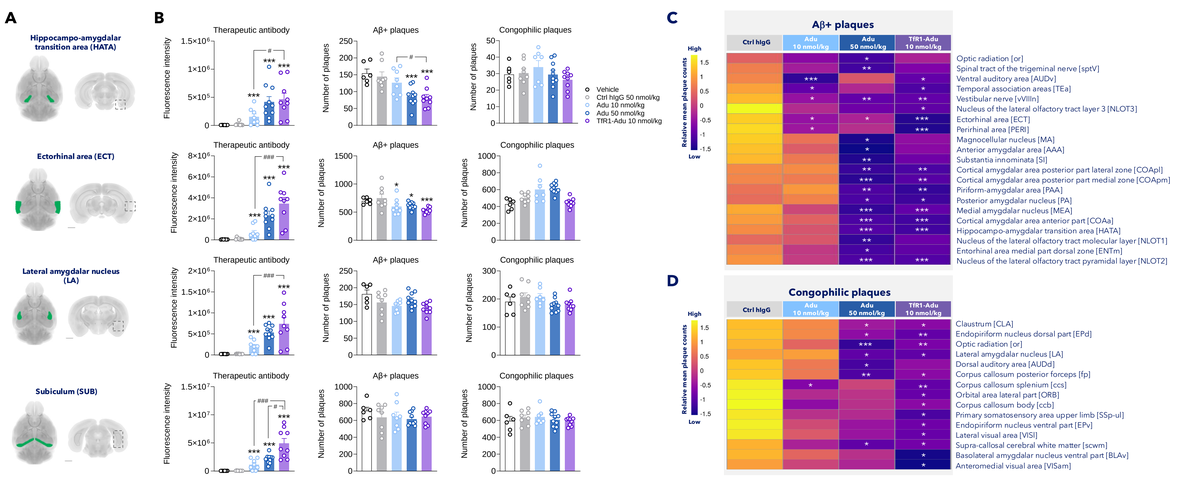

Figure 4. Simultaneous global mapping of therapeutic Aβ antibody biodistribution and plaque load in AD mice. (A, B) Selected brain regions, including hippocampo-amygdalar transition area (HATA), ectorhinal area (ECT), lateral amygdala nucleus (LA) and subiculum (SUB) represent brain areas with markedly higher IgG signal of low-dose TfR1-Adu (10 nmol/kg) compared to low-dose Aducanumab (Adu, 10 nmol/kg). **(A)** Dorsal and coronal view of the four selected brain regions. **(B)** Comparative quantitative analysis of drug distribution, Aβ+ plaques and congophilic plaques, respectively, in each region. The left hemibrain was co-stained with anti-human IgG (therapeutic antibody distribution, accumulated fluorescence signal intensity) and anti-human Aβ (Aβ+ plaques). The right hemibrain was stained with Congo Red (Congophilic plaques). *p<0.05, **p<0.001 vs. Control hIgG (50 nmol/kg); #p<0.05, ###p<0.001 for indicated group comparisons. Dunnett's test negative binomial generalized linear model). **(C, D)** Global comparative analysis of Aβ+ and congophilic plaque burden after chronic dosing with TfR1-Adu or Adu. Brain regions included in the heatmaps showed significant reduction of plaque counts following Adu or TfR1-Adu treatment. *p<0.05, **p<0.01, ***p<0.001 vs. Ctrl hIgG (Dunnett's test negative binomial generalized linear model).