Anti-inflammatory clearance of Aβ by a chimeric Gas6 fusion protein

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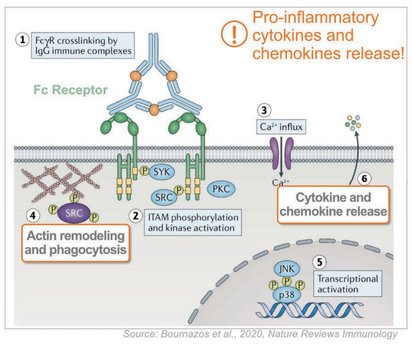
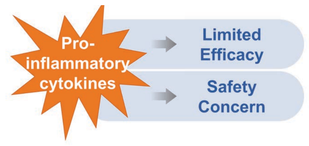
INTRODUCTION

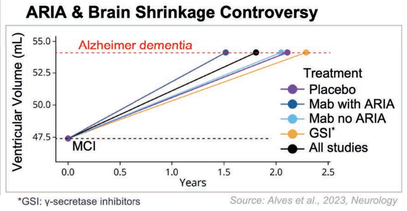
- Aβ immunotherapy is one of the most promising approaches in treating Alzheimer's disease (AD).
- Although Aβ-antibodies can substantially reduce Aβ burden in the brain via Fc receptor-mediated phagocytosis, they concurrently release pro-inflammatory cytokines and chemokines which leads to limited effects on cognitive function and safety concerns.
- Patients receiving Aβ-antibody treatment often experience brain edema and microhemorrhage (ARIA) closely associated with immune reactions and inflammation, and there have been reports that patients treated with Aβ-antibody had ventricular expansion, a sign of shrinking white and gray matter, and it is tightly correlated with ARIA.
- Innovations for removing Aβ without inflammatory responses are in demand.



Source: Bournazos et al., 2020, Nature Reviews Immunology



ARIA & Brain Shrinkage Controversy

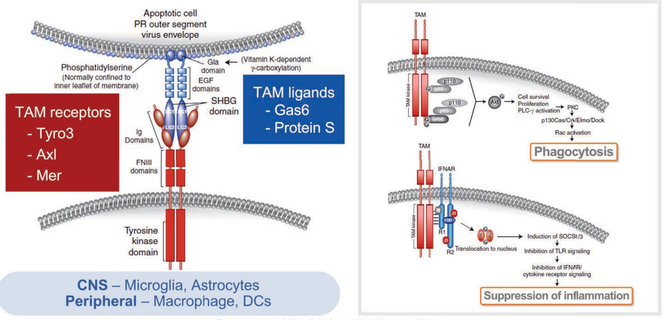


*GSI: γ-secretase inhibitors

Source: Alves et al., 2023, Neurology

STRATEGY

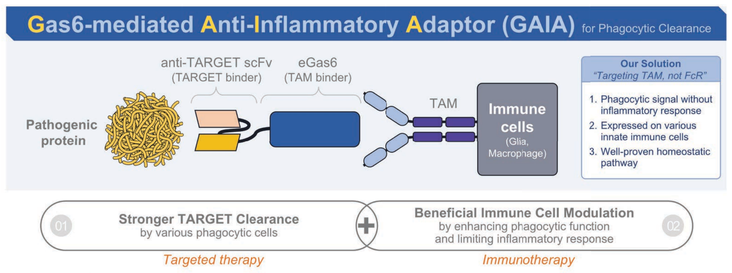
Efferocytosis – TAM-mediated anti-inflammatory phagocytosis



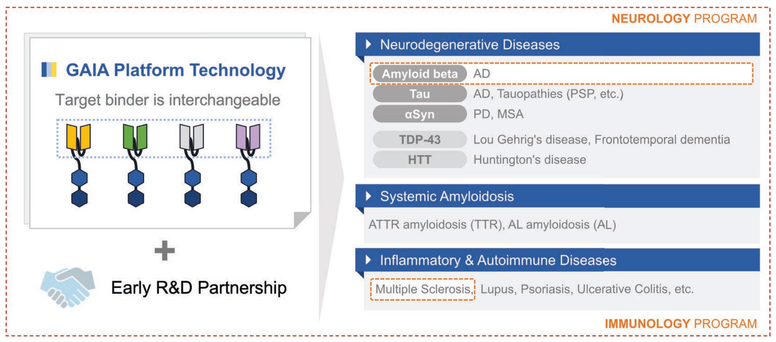
Source: Lemke, 2013. Cold Spring Harb Perspect Biol

- For homeostasis maintenance, phagocytes remove apoptotic cells and unnecessary proteins via efferocytosis which induces phagocytosis accompanied with suppression of inflammation.
- Efferocytosis is mediated by TAM receptors and their ligand Gas6. TAM receptors are expressed on various glial and myeloid cells, and the ligand Gas6 induces efferocytosis by bridging the TAM receptors and the target such as phosphatidylserine of apoptotic cells.

Illimis' Platform Technology

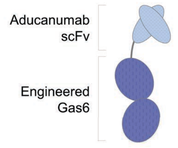


- By using minimal active domain of Gas6, we developed a novel fusion protein platform "GAIA" which enables the target-directed phagocytosis without unwanted neuroinflammation.
- GAIA consists of two parts: 1) TARGET binder and 2) TAM binder. Once GAIA binds both the pathogenic protein (e.g. Aβ) and TAM receptors on glial cells, it induces efferocytosis enabling efficient removal of the target proteins and limiting inflammatory responses simultaneously.
- As the target binder is interchangeable, target indication can be easily expanded from neurodegenerative diseases to various immune-related diseases.
- We constructed αAβ-Gas6 as a prototype in AD to confirm proof-of-concept of GAIA.

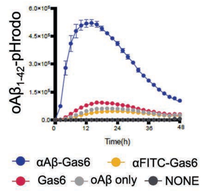


RESULTS

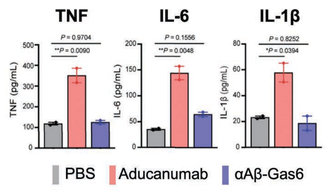
αAβ-Gas6



Phagocytosis

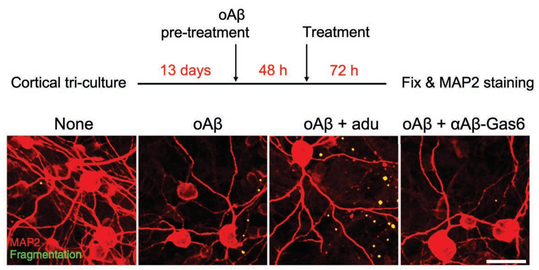


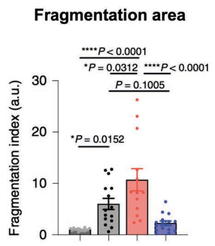
Anti-inflammatory effects



- **αAβ-Gas6 construct** - aducanuamb scFv was used as a Aβ-binding domian.
- **Phagocytosis** - to confirm the target-selective uptake by HMC3 cells, we used oligomeric Aβ conjugated with pHrodo (oAβ-pHrodo), that emits a bright red fluorescent signal under acidic conditions, such as in lysosomes, serving as a reporter of phagocytic internalization.
- Live-cell imaging analysis showed that only αAβ-Gas6-treated cells exhibit a significantly increased fluorescent signal compared with Gas6- and αFITC-Gas6-treated cells.
- **Anti-inflammatory effect** - Quantitative bar graphs show the protein levels of pro-inflammatory cytokines (TNF, IL-6, and IL-1β) in THP-1^{Axl} measured by Cytometric Bead Array at 18 hr.

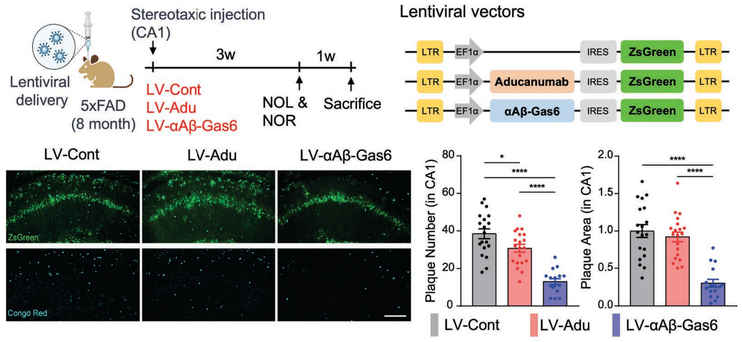
Reduced neurotoxicity





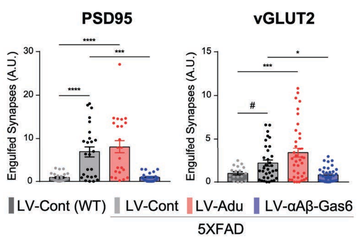
- In tri-culture system, cortical neurons are co-cultured with microglia and astrocytes.
- Aducanumab significantly exacerbated oAβ-induced neuronal toxicity, resulting in increased neuronal fragmentation. We found that αAβ-Gas6 treatment was able to largely rescue oAβ-induced neuronal fragmentation to levels similar to the oAβ-nontreated control group.
- This in vitro results suggest that αAβ-Gas6 can circumvent the side effects of conventional antibody therapy, such as pro-inflammatory responses and resulting neuronal toxicity.

Experimental scheme & Plaque lowering effect

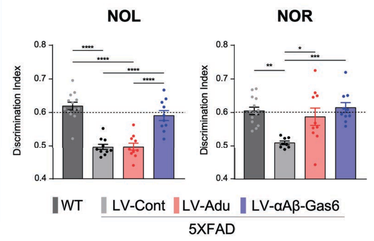


- LV-aducanumab and LV-αAβ-Gas6 were bilaterally injected into the hippocampal CA1 regions of 8-m-old 5xFAD mice.
- Different LV-injected groups had a similar number of ZsGreen-expressing cells in the CA1 (comparable expression levels were also confirmed by a dot blot assay).
- Both the number and total area of Aβ plaques were significantly decreased in the 5xFAD CA1 injected with LV-αAβ-Gas6, but the decrease was minimal with LV-aducanumab treatment.

Reduced Synapse elimination



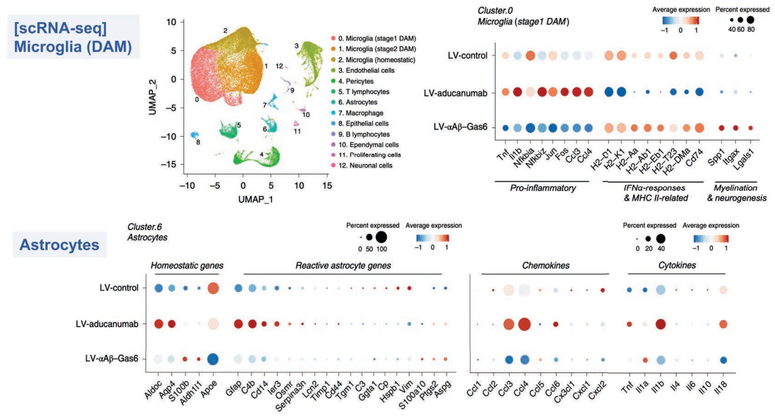
Improved cognitive function



- **Reduced Synapse elimination** - PSD95- or vGLUT2-positive excitatory synaptic puncta engulfed by microglia were evaluated. LV-αAβ-Gas6 injection significantly reduced excessive synapse elimination to levels comparable to non-AD WT, whereas LV-Adu showed increasing trends, suggesting αAβ-Gas6 exhibit therapeutic effects against AD not only by eliminating Aβ plaques but also by preventing excessive synapse elimination through reduction of NPAM activation.
- **Improved cognitive function** - LV-αAβ-Gas6 show normal discrimination indexes similar to WT in Novel Object Location (NOL) and Novel Object Recognition (NOR) tests, suggesting their cognitive functions were rescued. In contrast, LV-adu showed a rescue only in NOR.
- Habituation (10min), Training (10min), Testing (10min) and intervals were 24h and 3h, respectively.

RESULTS

Anti-inflammatory effects – scRNA-seq



- After quality control using Seurat analysis, unsupervised clustering analysis separated all 74,889 single cells into 12 clusters. Based on the plaque-associated microglial gene set, microglia were segregated into 1) homeostatic 2) Stage1-damage-associated microglia (DAM), and 3) Stage2-DAM.

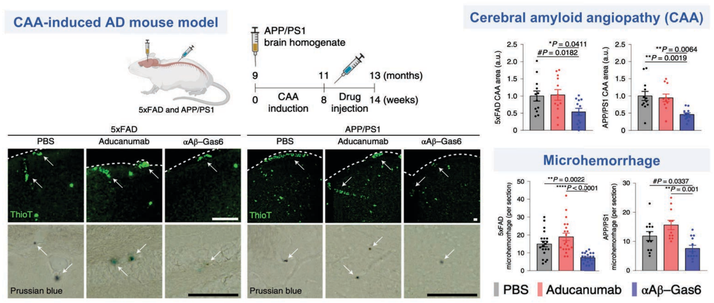
Microglia

- **Tnf and Il1b levels** were significantly upregulated in DAM clusters of the LV-aducanumab group but were suppressed in the LV-αAβ-Gas6 group to levels even lower than the LV-control-injected group.
- **Inflammatory chemokines (Ccl3, Ccl4, and Ccl6) as well as the molecules involved in NF-kB signaling (Nfkbia and Nfkbiz) and stress responses (Jun and Fos)** were all significantly upregulated in the LV-aducanumab group but were further suppressed in the LV-αAβ-Gas6-injected group.
- Microglia from the LV-αAβ-Gas6-injected group upregulated different gene sets related to **interferon-alpha responses and MHC class II-related genes (Cd74, H2-Aa, H2-D1, H2-k1, H2-Eb1, and so on)**. Furthermore, **neonatal microglial genes (Spp1, Itgax, Lgals1)** known to deliver signals for **myelination and neurogenesis** were also upregulated in the LV-αAβ−Gas6-injected group.

Astrocytes

- Similar to microglia, we found that astrocytes from the LV-aducanumab-injected group significantly upregulated **several reactive astrocyte genes (Gfap, C4b, and Cd14)** as well as **pro-inflammatory chemokines and cytokines** compared with the LV-αAβ-Gas6-injected group.

Reduced & minimal ARIA

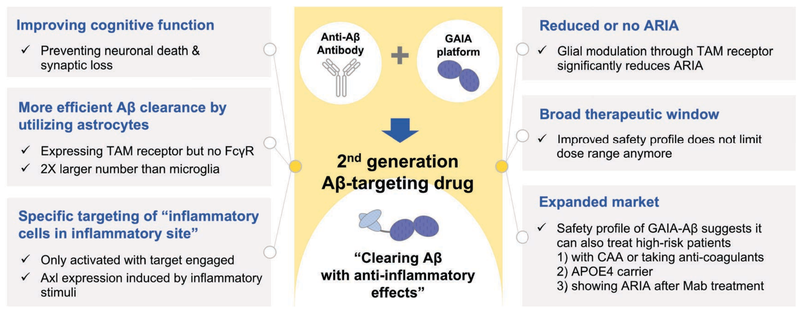


- CAA was induced in 9-m-old APP/ PS1 and 5xFAD mice by injecting brain homogenate obtained from 20-m-old APP/PS1 mice into their ventricles. Treatments were intrathecally administered twice a week for 6 weeks.
- The amount of Aβ associated with blood vessels and microhemorrhage were measured by detecting hemosiderin deposits with Perl's Prussian blue staining. Only the αAβ-Gas6-injected group showed a significant decrease in both CAA and microhemorrhage, whereas the aducanumab-injected group showed an increasing tendency of microhemorrhage.
- The results suggest that αAβ-Gas6 eliminates Aβ plaques without inducing CAA-related side effects of conventional Aβ antibody therapy, such as ARIA.

*** The data presented here have been previously published in (Chung et al., Nature Meidicine, 2022, PMID: 35927581). We are excited to share our results and encourage interested readers to refer to the publication for more detailed information.**

CONCLUSIONS

Harnessing the Power of TAM Biology



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