Anti-inflammatory clearance of amyloid beta by a chimeric Gas6 fusion protein

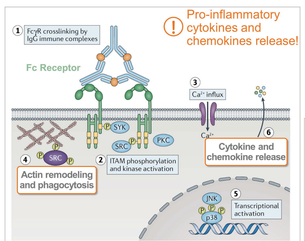
Min Soo Kang[1], Hyuncheol Jung[1,2], Se Young Lee[2], Sukhee Cho[1], Ah-Young Shin[1], Jaekeun Lee[1], Sanghoon Park[1], Won-Suk Chung[2], Chan Hyuk Kim[2]

[1]Illimis Therapeutics, Inc., Seoul, Republic of Korea, [2]Department of Biological Sciences, Korea Advanced Institute of Science and Technology (KAIST), Daejeon, Republic of Korea

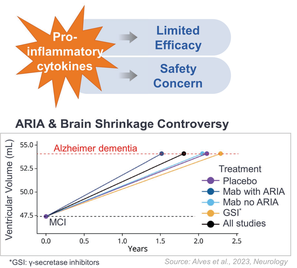
INTRODUCTION

Unmet needs in Aβ-targeting immunotherapy

- Aβ immunotherapy is one of the most promising approaches in treating Alzheimer's disease (AD).
- Although **Aβ-targeting antibodies can substantially reduce Aβ burden in the brain** via Fc receptor-mediated phagocytosis, they **concurrently release pro-inflammatory cytokines and chemokines** which lead to marginal effects on cognitive function and safety concerns.
- **Amyloid-associated imaging abnormalities (ARIA), which are found in various types of brain edema and microhemorrhage, are frequently observed in patients following Aβ-targeting antibody therapy**. These anomalies are intimately linked to immune responses and inflammation, and there have been instances of individuals receiving Aβ-antibody experiencing ventricular enlargement and a sign of white and gray matter shrinkage, which are closely connected to ARIA.
- Innovations for removing Aβ without inflammatory responses are in demand.



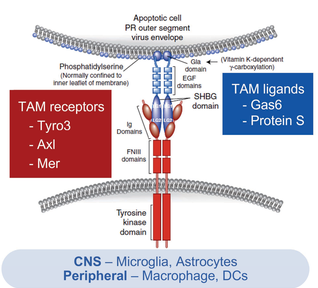
Source: Boumazos et al., 2020, Nature Reviews Immunology

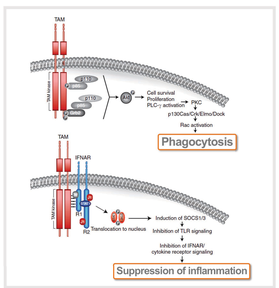


*GSI: γ-secretase inhibitors

Source: Alves et al., 2023, Neurology

Efferocytosis: TAM-mediated anti-inflammatory phagocytosis



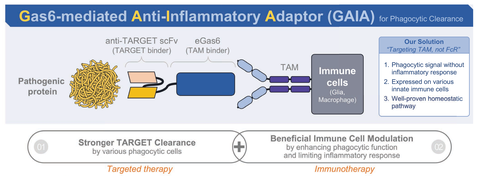


Source: Lemke, 2013. Cold Spring Harb Perspect Biol

- For cellular homeostasis maintenance, phagocytes remove apoptotic cells and unnecessary proteins via **efferocytosis which induces phagocytosis accompanied with suppression of inflammation**.
- **The Tyro3, Axl, and Mer (TAM) receptors and their ligand Gas6 induce efferocytosis**. The ligand Gas6 causes efferocytosis by connecting the TAM receptors and the target, such as phosphatidylserine of apoptotic cells, on a variety of cells that express TAM receptors.

STRATEGY

Illimis' Platform Technology



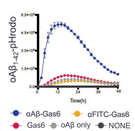
“ Clearing pathogenic protein without unwanted neuroinflammation ”

- 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.

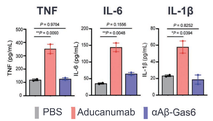
RESULTS

αAβ-Gas6

Phagocytosis

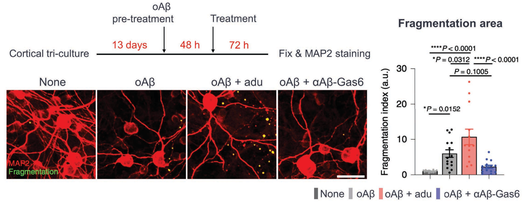


Anti-inflammatory effects



- **Phagocytosis** - to confirm the target-selective uptake, we used oligomeric Aβ conjugated with pHrodo (oAβ-pHrodo) serving as a reporter of phagocytic internalization. Live-cell imaging analysis showed that only αAβ-Gas6-treated cells exhibit a significantly increased phagocytosis of oligomeric Aβ compared to controls.
- **Anti-inflammatory effect** - Quantitative bar graphs show the protein levels of pro-inflammatory cytokines (TNF, IL-6, and IL-1β) were low in αAβ-Gas6 treated groups confirming phagocytic uptake of oligomeric Aβ without inflammation.

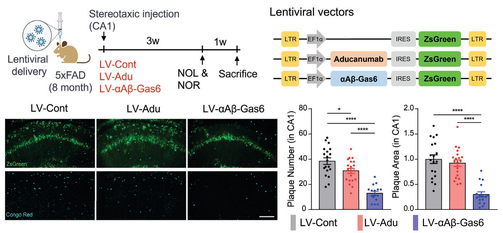
Reduced Neurotoxicity



- **Reduced neurotoxicity -** 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.

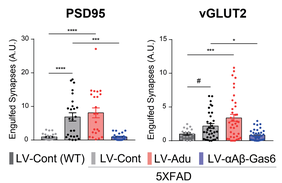
RESULTS

Experimental scheme & Plaque lowering effect

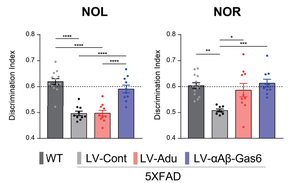


- **Plaque lowering effect** - Both the number and total area of Aβ plaques were significantly decreased in the 5xFAD CA1 injected with LV-αAβ-Gas6, but the decrease was minimal with LV-Aducanumab treatment.

Reduced synapse elimination

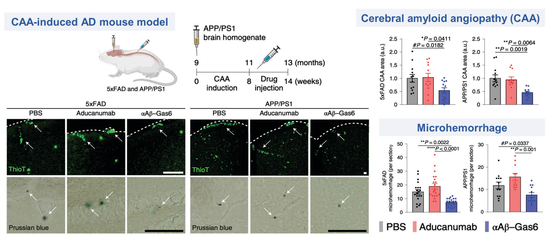


Improved cognitive function



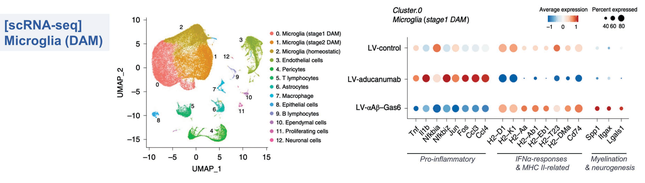
- **Reduced Synapse elimination** - LV-Adu sustained increased synaptic engulfment levels whereas LV-αAβ-Gas6 injection significantly reduced excessive synapse elimination to levels comparable to non-AD WT.
  This suggests αAβ-Gas6 exhibit therapeutic effects against AD not only by eliminating Aβ plaques but also by preventing excessive synapse elimination through reduction of NPAM activation.
- **Improved cognitive function** - LV-αAβ-Gas6 show normal discrimination index similar to WT in novel object location (NOL) and novel object recognition (NOR) tests, suggesting their cognitive functions were rescued.

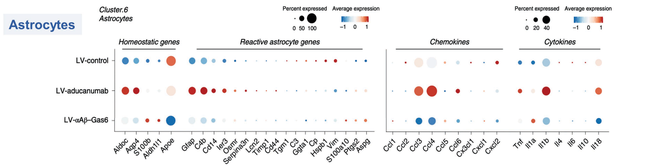
Reduced & minimal ARIA



- **Reduced CAA** - In the CAA-induced AD mouse model, 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.

Anti-inflammatory effects – scRNA-seq





Microglia

- **Pro-inflammatory cytokines (Tnf and Il1b), 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.

CONCLUSIONS

Harnessing the Power of TAM Biology

Improving cognitive function
- ✓ Preventing neuronal death & synaptic loss

More efficient Aβ clearance by utilizing astrocytes
- ✓ Expressing TAM receptor but no FcγR
- ✓ 2X larger number than microglia

Specific targeting of “inflammatory cells in inflammatory site”
- ✓ Only activated with target engaged
- ✓ Axl expression induced by inflammatory stimuli

Anti-Aβ Antibody + GAIA platform

2nd generation Aβ-targeting drug

“Clearing Aβ with anti-inflammatory effects”

Reduced or no ARIA
- ✓ Glial modulation through TAM receptor significantly reduces ARIA

Broad therapeutic window
- ✓ Improved safety profile does not limit dose range anymore

Expanded market
- ✓ Safety profile of GAIA-Aβ suggests it can also treat high-risk patients
  1) with CAA or taking anti-coagulants
  2) APOE4 carrier
  3) showing ARIA after Mab treatment

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