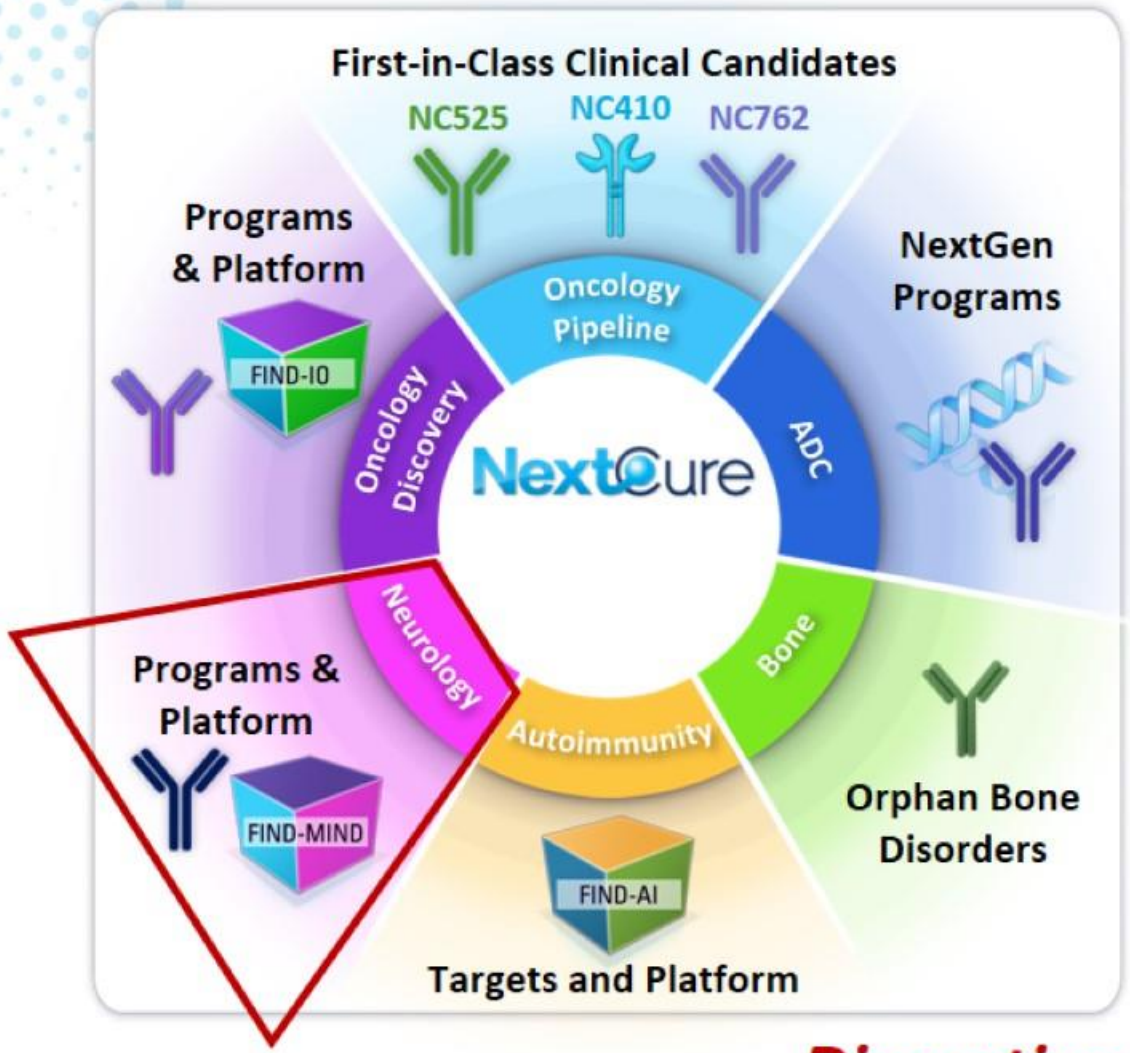
NextCure

Targeting Pathogenic APOE4 in Alzheimer's Disease

Thomas Schaffer, PhD

NextCure – Who We Are?



Team

- Experienced management
- Passionate
- Collaborative network

Washington University in St. Louis

GMP Manufacturing

- Dedicated
- State-of-the art
- 2,000L capacity

Expertise

- Multiple scientific disciplines
- Product development experience
- New MOA

Disrupting Standard of Care

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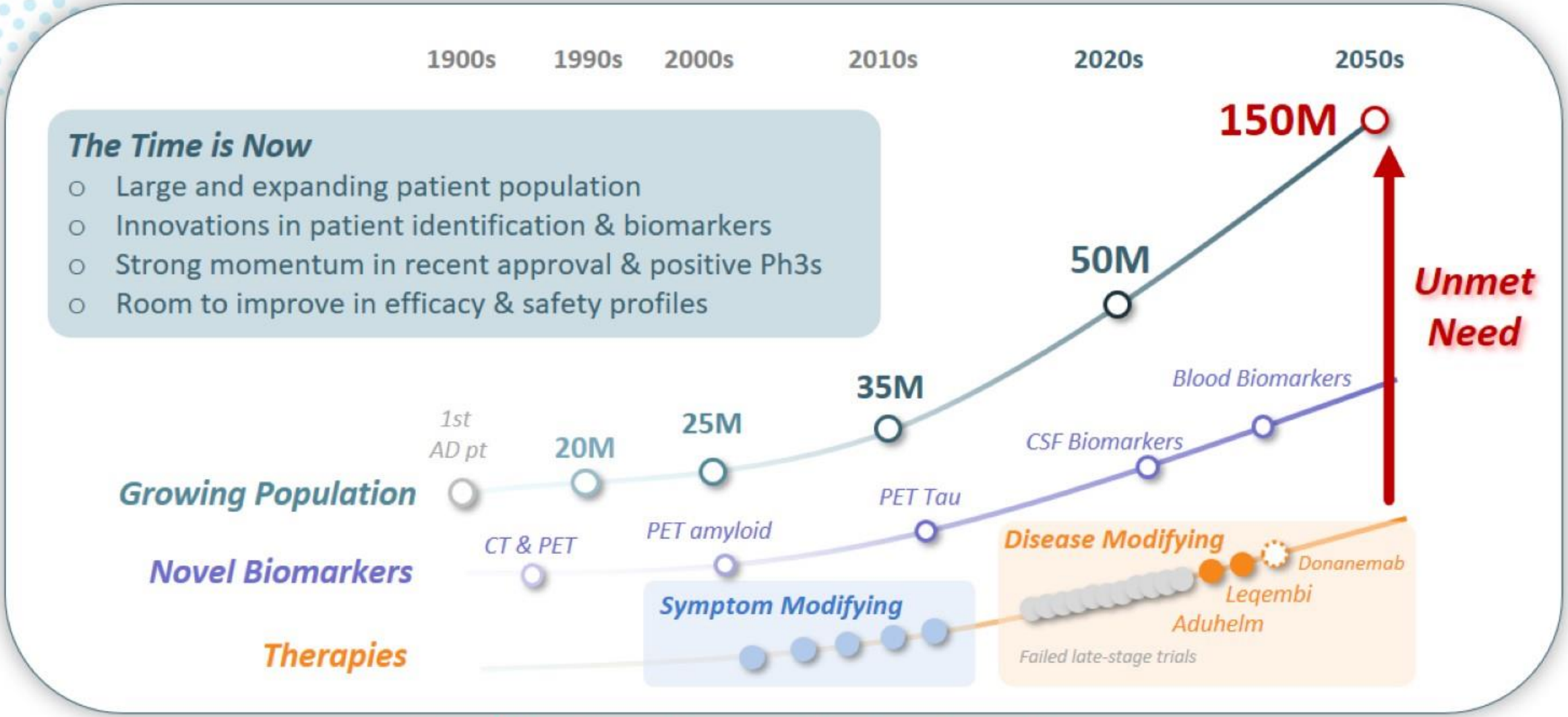
Growing Understanding in Alzheimer's Disease but Still Large Unmet Need



Need for New Targets To Continue Progress

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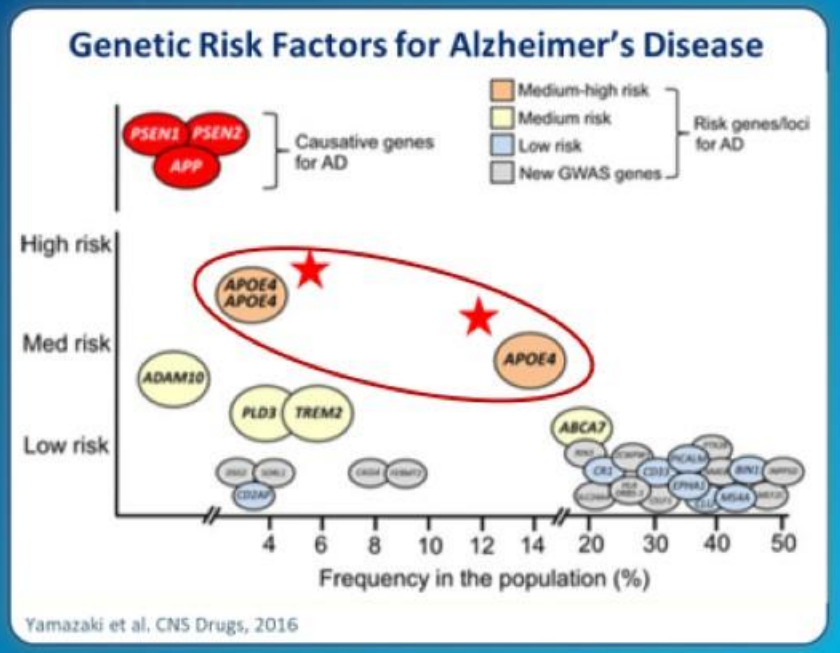
Targeting Major Genetic Risk Factor – APOE4



Yamazaki et al. CNS Drugs, 2016

APOE4 Major Role in Alzheimer's Disease

50-60% of AD Patients are APOE4 carriers despite being found in 15-25% of the total population

APOE4 Carriers have **increased risk** to current therapies

Known role in **multiple pathologies** impacting tau and amyloid clearance as well as vascular function

Potential **opportunity** in several indications as APOE4 association found in all dementia patients

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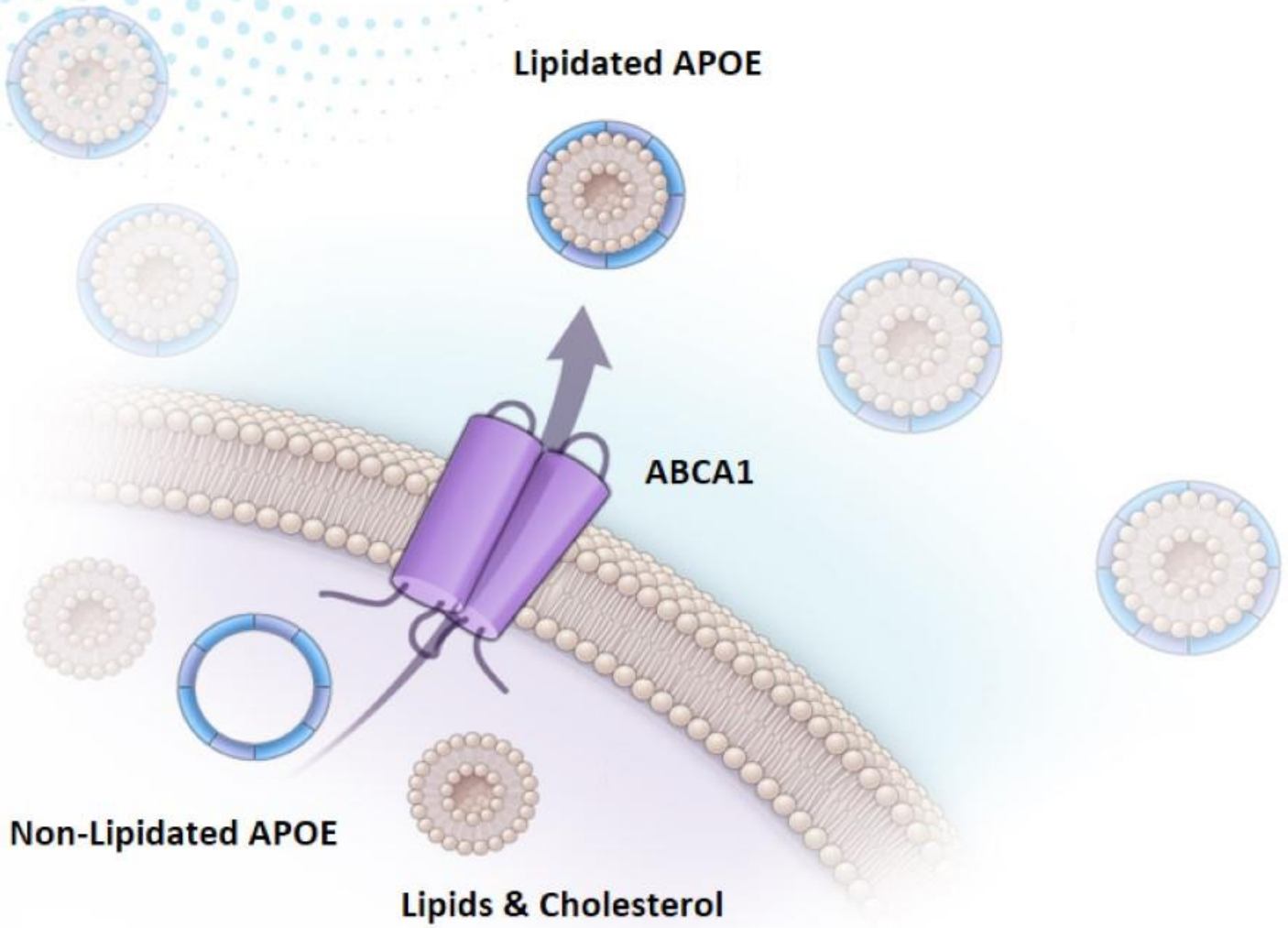
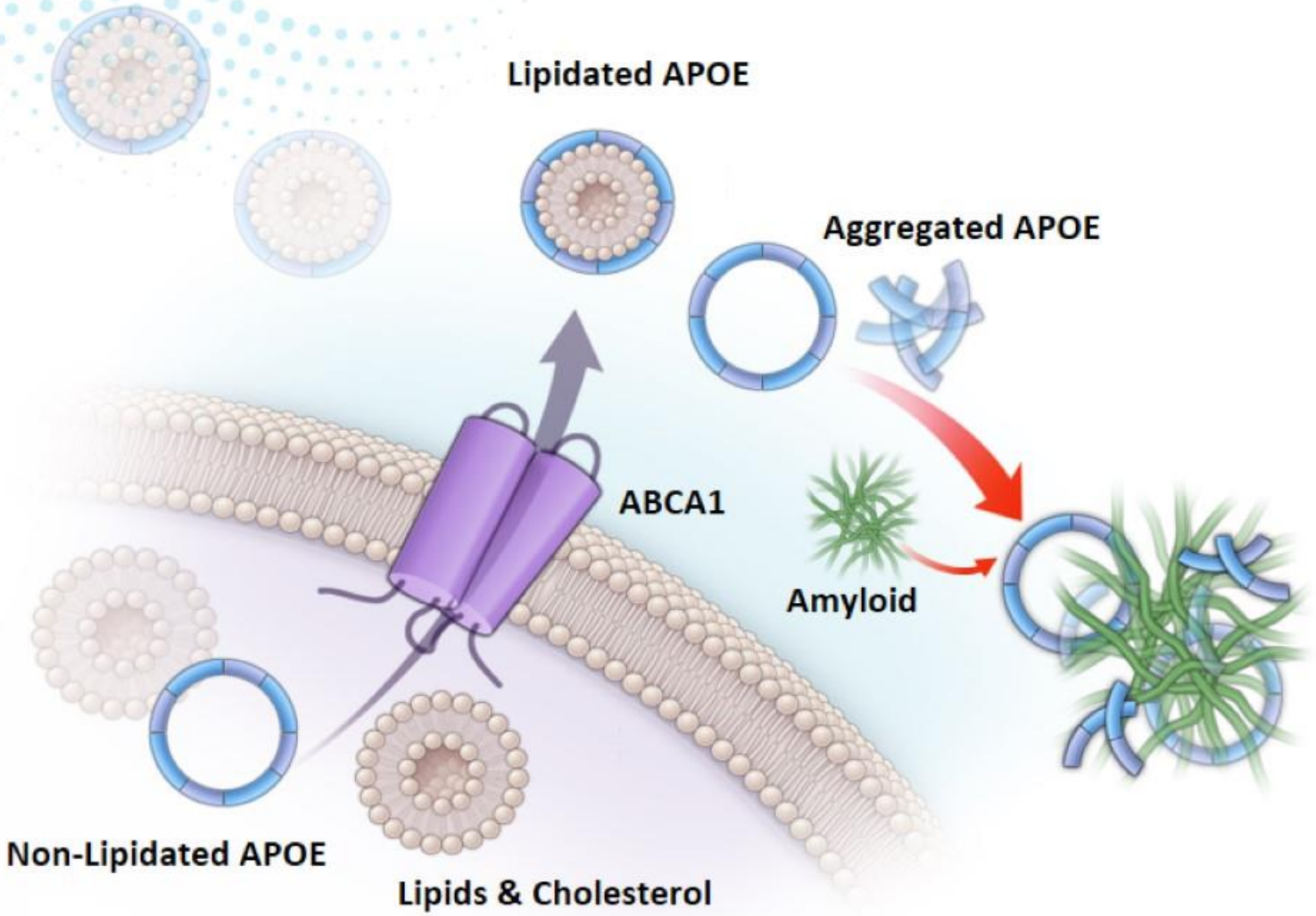
APOE Biology Overview



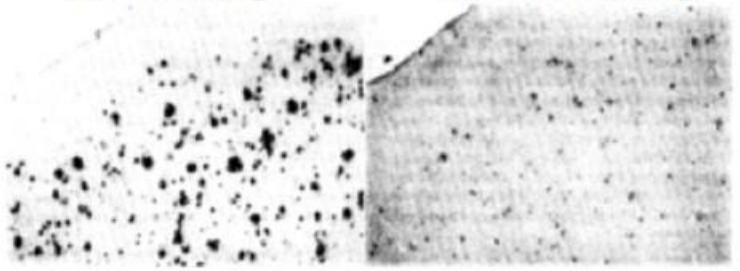
Background

- Apolipoprotein E is a 34kDa glycoprotein, largely produced in the brain by astrocytes and activated microglia
- Three isoforms E2, E3, & E4, with homozygous frequencies of ~7%, ~78%, and ~15% respectively
- Functions to transport lipids within the CNS
- Lipidated by ATP-binding cassette (ABC) transporters ABCA1, which transfer cholesterol and other phospholipid
- Also takes up lipids generated after neuronal degeneration and redistributes them to other cells

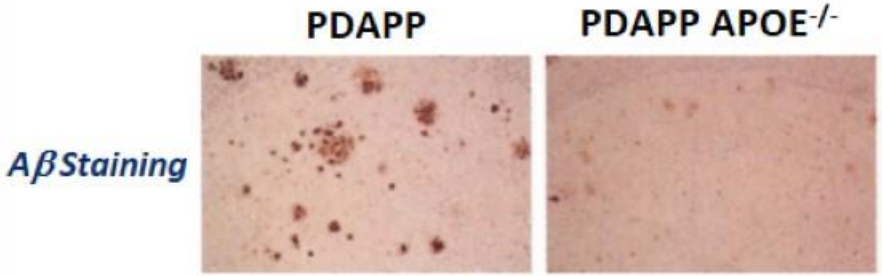
APOE and Alzheimer's Disease Pathology



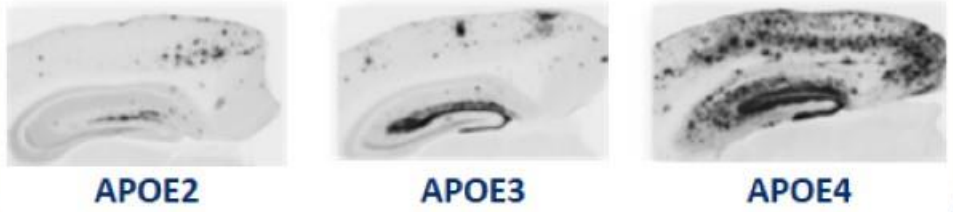
APOE Localizes to Aβ Plaques



APOE Knockout Reduces Plaque Burden



Isoform Dependent Increase in Aβ Plaques



Wisniewski T, Neurosci Lett (1991); Bales, et al. Nature (1997); Castellano, et al. Sci. Trans Med (2011)

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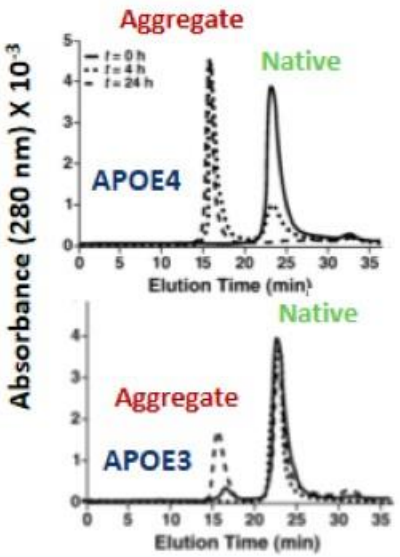
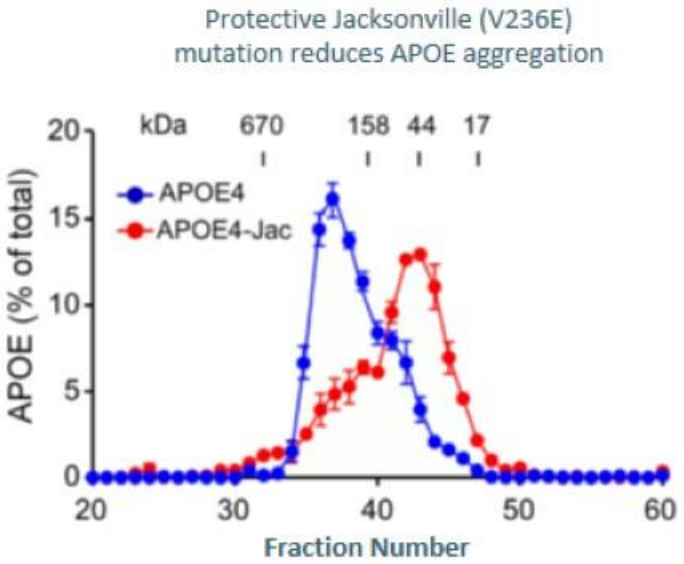
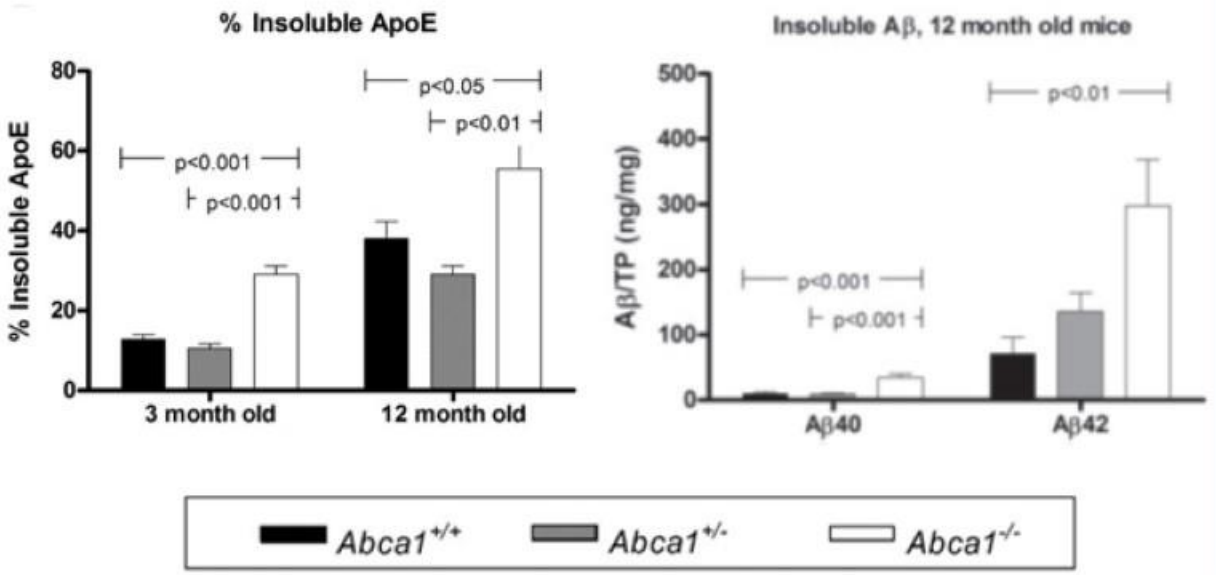
Aggregation and Lipidation States of APOE Associated with Disease Pathology

APOE4 Prone to Aggregate; Stabilizing Mutations Protect Against AD





ABCA1 Deletion Promotes APOE and Amyloid Aggregation



Hatters, et al. *JMB* (2006); Liu C, et al. *STM* (2021); Wahrle, et al. *JBC* (2005)

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Growing Evidence of APOE4's Role in Multiple Aspects of AD Pathology

Increases Aβ Plaque Formation	ALZHEIMER'S DISEASE Human apoE Isoforms Differentially Regulate Brain Amyloid-β Peptide Clearance
Promotes Tau Spreading	ALZHEIMER'S DISEASE Network Tau spreading is vulnerable to the expression gradients of *APOE* and glutamatergic-related genes
Disrupts BBB & Neurovascular Unit	Article ***APOE4* leads to blood–brain barrier dysfunction predicting cognitive decline**
Drives Microglia-Mediated Inflammation	ARTICLE Microglia drive APOE-dependent neurodegeneration in a tauopathy mouse model

Targeting APOE4 May Have Multiple Benefits

Identification of Anti-APOE Antibody HAE-4

Washington University in St. Louis

David M. Holtzman, MD
Scientific Director, Hope Center for Neurological Disorders Dept. of Neurology

The Journal of Clinical Investigation RESEARCH ARTICLE

Targeting of nonlipidated, aggregated apoE with antibodies inhibits amyloid accumulation

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[1]Department of Neurology, Hope Center for Neurological Disorders, Charles F. and Joanne Knight Alzheimer's Disease Research Center, Washington University School of Medicine, St. Louis, Missouri, USA. [2]Denali Therapeutics Inc., South San Francisco, California, USA. [3]Department of Medicine, Duke University, Durham, North Carolina, USA. [4]MassGeneral Institute for Neurodegenerative Disease, Massachusetts General Hospital and Harvard Medical School, Charlestown, Massachusetts, USA.

The apolipoprotein E *E4* allele of the *APOE* gene is the strongest genetic factor for late-onset Alzheimer disease (LOAD). There is compelling evidence that apoE influences Alzheimer disease (AD) in large part by affecting amyloid β (Aβ) aggregation and clearance; however, the molecular mechanism underlying these findings remains largely unknown. Herein, we tested whether anti–human apoE antibodies can decrease Aβ pathology in mice producing both human Aβ and apoE4, and investigated the mechanism underlying these effects. We utilized APPPS1-21 mice crossed to apoE4-knockin mice expressing human apoE4 (APPPS1-21/APOE4). We discovered an anti–human apoE antibody, anti–human apoE 4 (HAE-4), that specifically recognizes human apoE4 and apoE3 and preferentially binds nonlipidated, aggregated apoE over the lipidated apoE found in circulation. HAE-4 also binds to apoE in amyloid plaques in unfixed brain sections and in living APPPS1-21/APOE4 mice. When delivered centrally or by peripheral injection, HAE-4 reduced Aβ deposition in APPPS1-21/APOE4 mice. Using adeno-associated virus to express 2 different full-length anti–apoE antibodies in the brain, we found that HAE antibodies decreased amyloid accumulation, which was dependent on Fcγ receptor function. These data support the hypothesis that a primary mechanism for apoE-mediated plaque formation may be a result of apoE aggregation, as preferentially targeting apoE aggregates with therapeutic antibodies reduces Aβ pathology and may represent a selective approach to treat AD.

HAE-4 Properties

- Preferentially binds to APOE3 and APOE4
- Does not bind to lipidated APOE in serum
- Increased binding to aggregated APOE
- Recognizes APOE4 associated with plaques
- Removes plaques comparable to Aβ mAb in an Fc dependent manner

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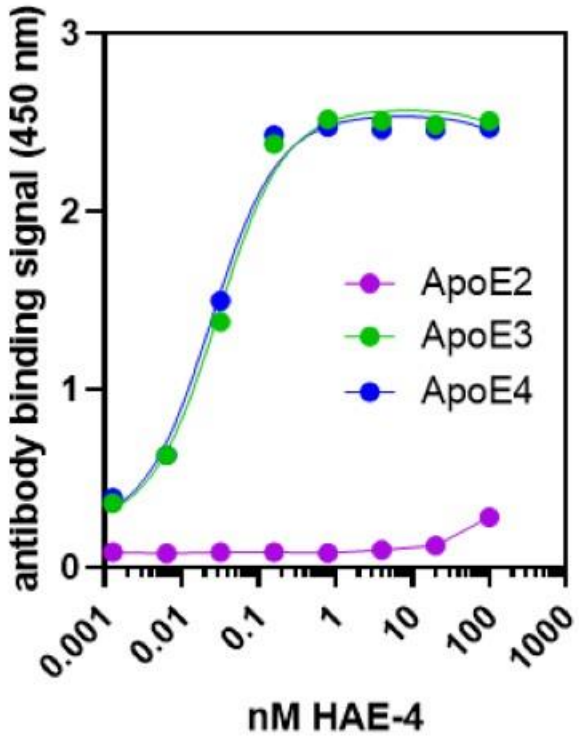
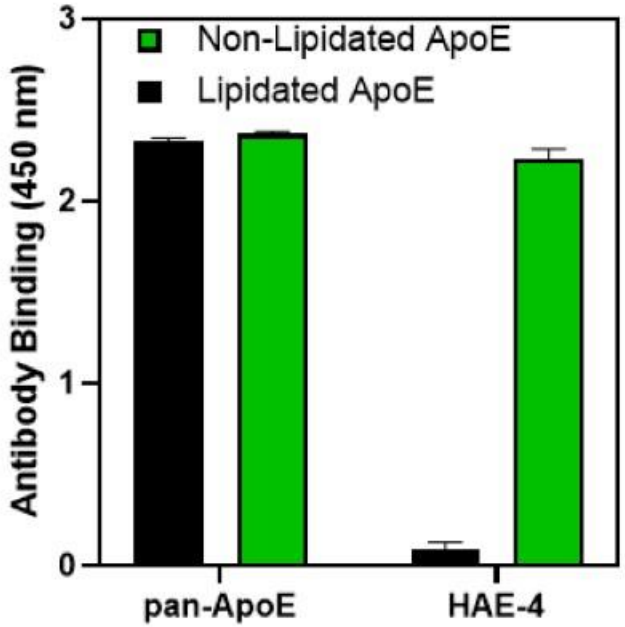
Preferentially Binds Non-Lipidated APOE3/4 & Demonstrates Standard PK Profile

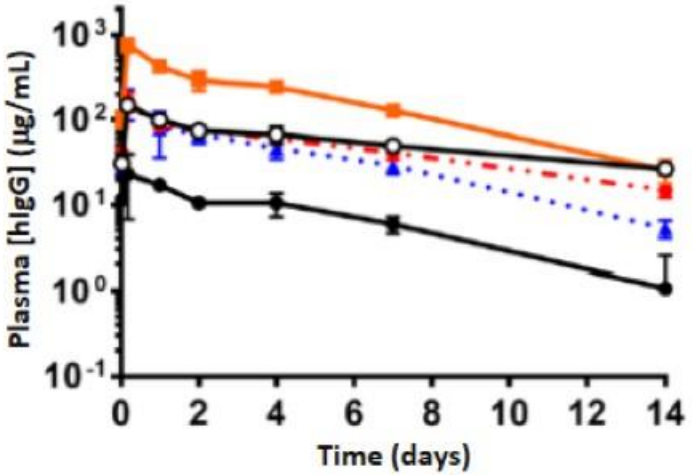
Preferentially Binds APOE3/4



Does Not Bind Lipidated APOE



Demonstrates Standard PK Profile



APOE4 Knock In Mice

- HAE-4 – 2mg/kg
- HAE-4 – 10mg/kg
- HAE-4 – 50mg/kg
- Control – 10mg/kg

APOE4 Knock Out Mice

- HAE-4 – 10mg/kg

Liao et al. *JCI* (2018)

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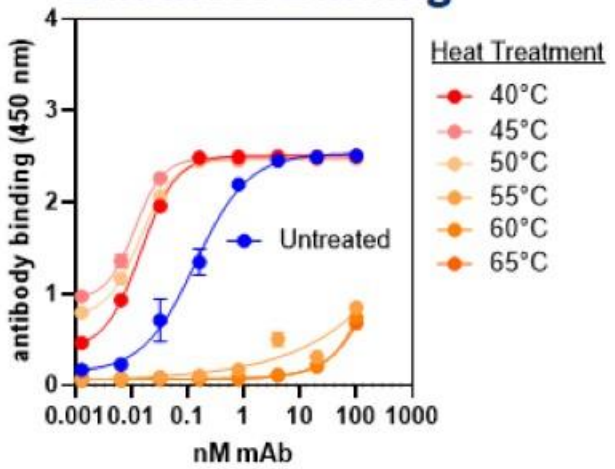
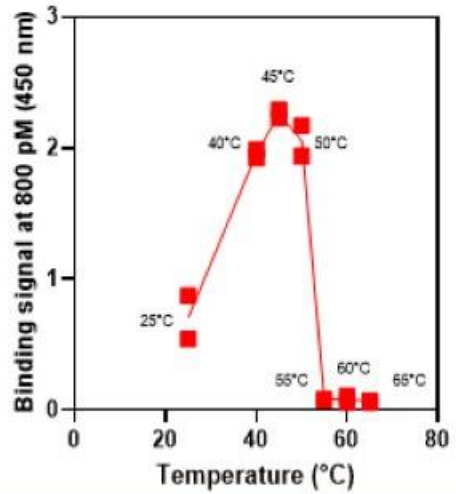
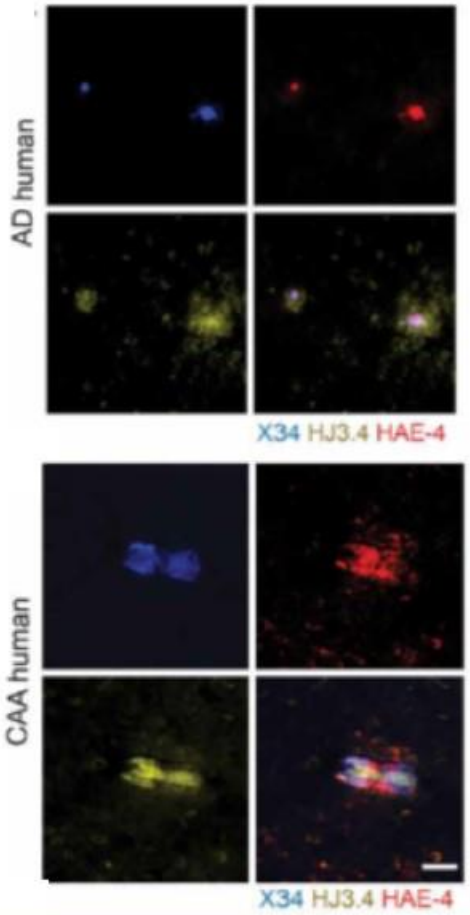
Preferential Staining and Localization to Plaque-Associated APOE4

APOE4 Aggregation Enhances Binding

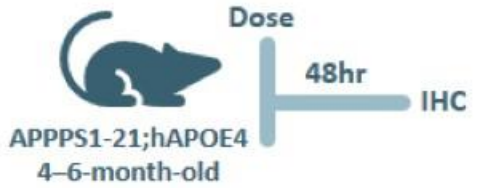




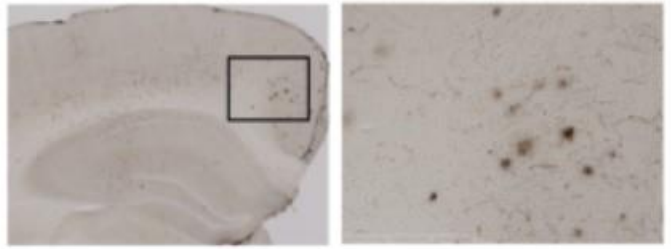
Colocalization with Amyloid Plaques in Humans



Crosses BBB & Localize to Plaques



Chimeric-HAE-4 (1 dose, 50 mg/kg)



Control IgG, 50 mg/kg

Liao et al. JCI. 2018; Xiong et al. *Sci. Trans. Med.* 2021

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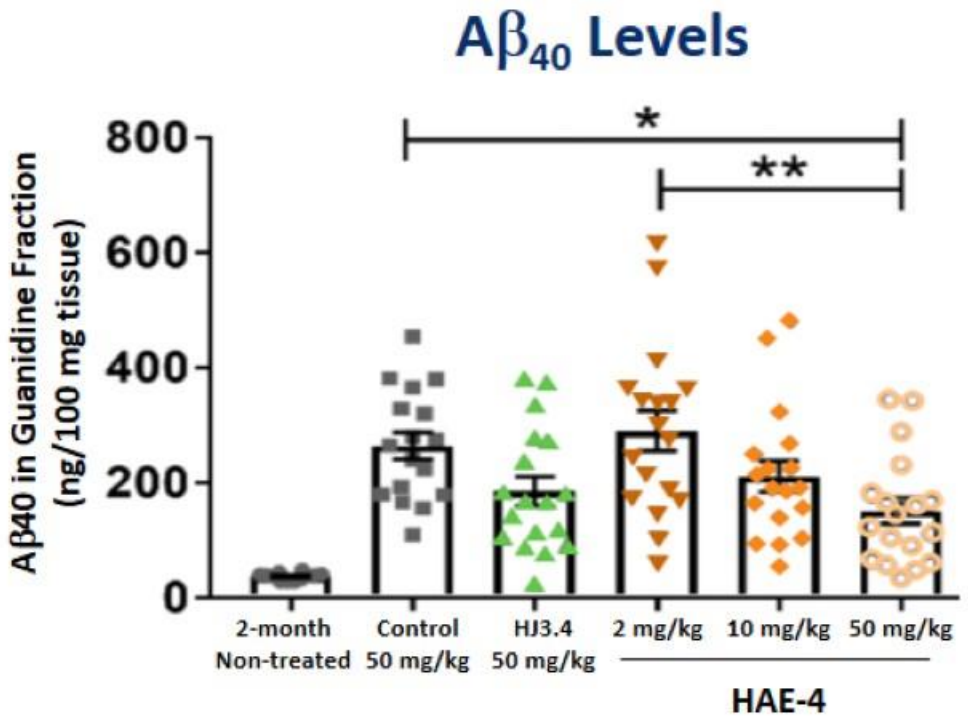
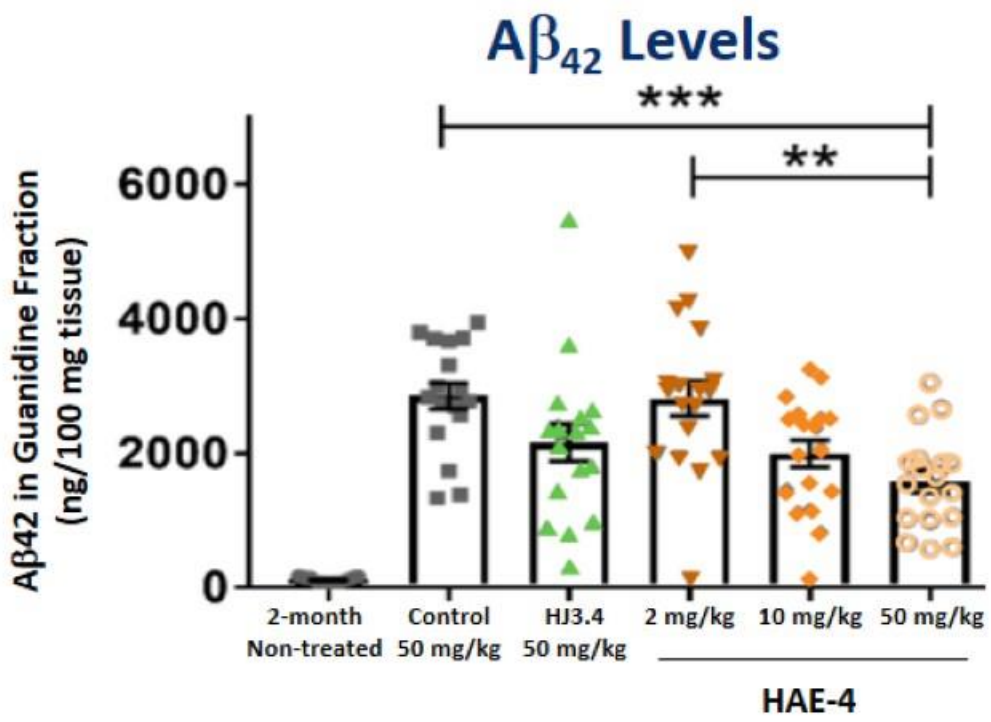
Dose Dependent Reduction of Amyloid Comparable to Targeting Aβ





HJ3.4 – Antibody against N-terminal $A\beta_{1-13}$

Liao, et al. *JCI* (2018)

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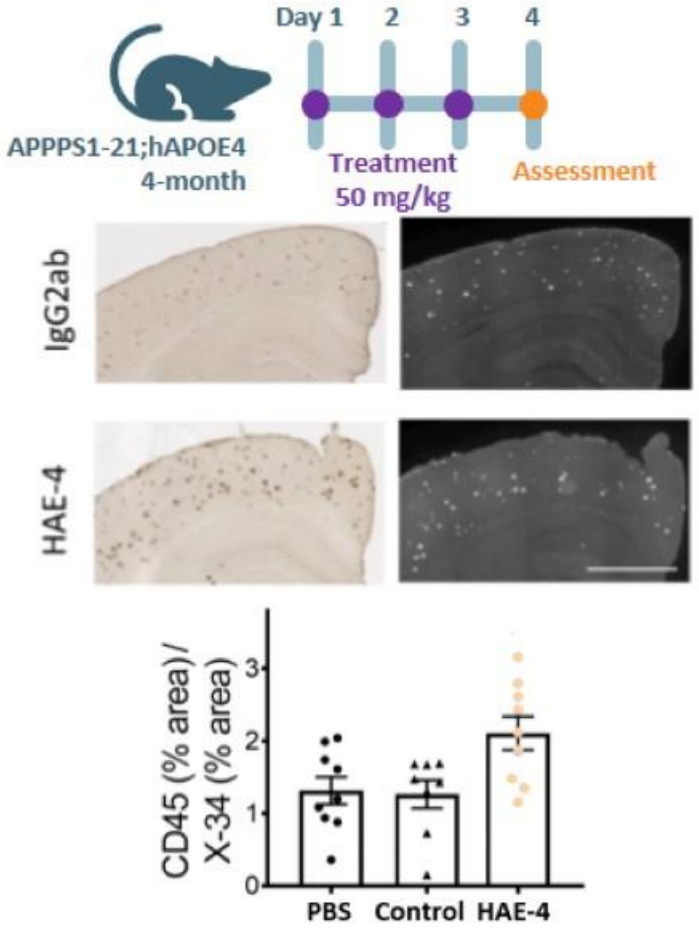
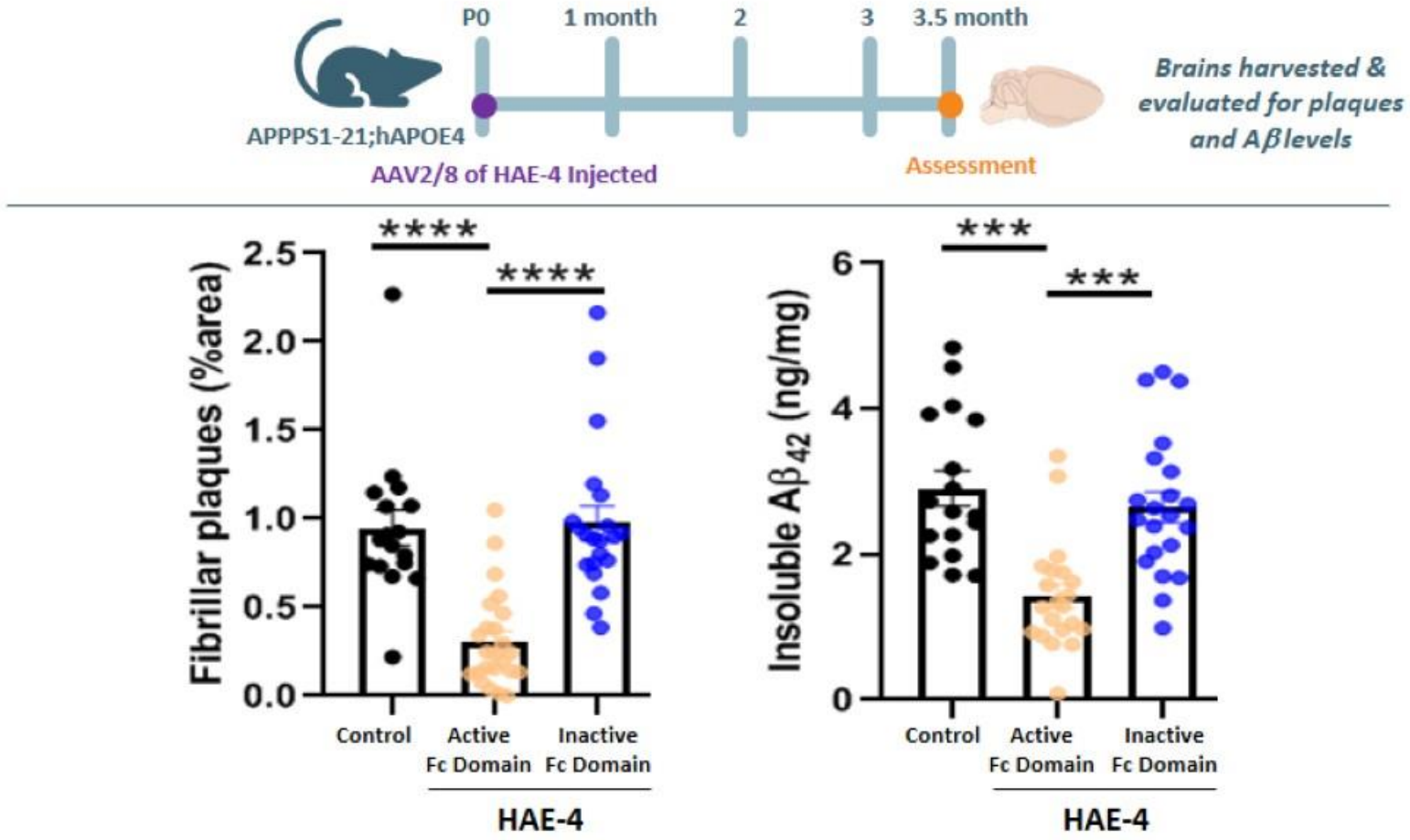
Clearance of Plaques is Mediated by Microglial Activation

HAE-4 Recruits Microglia to Plaques



Fc Function Required for HAE-4 Activity



Liao, et al. *JCI* (2018)

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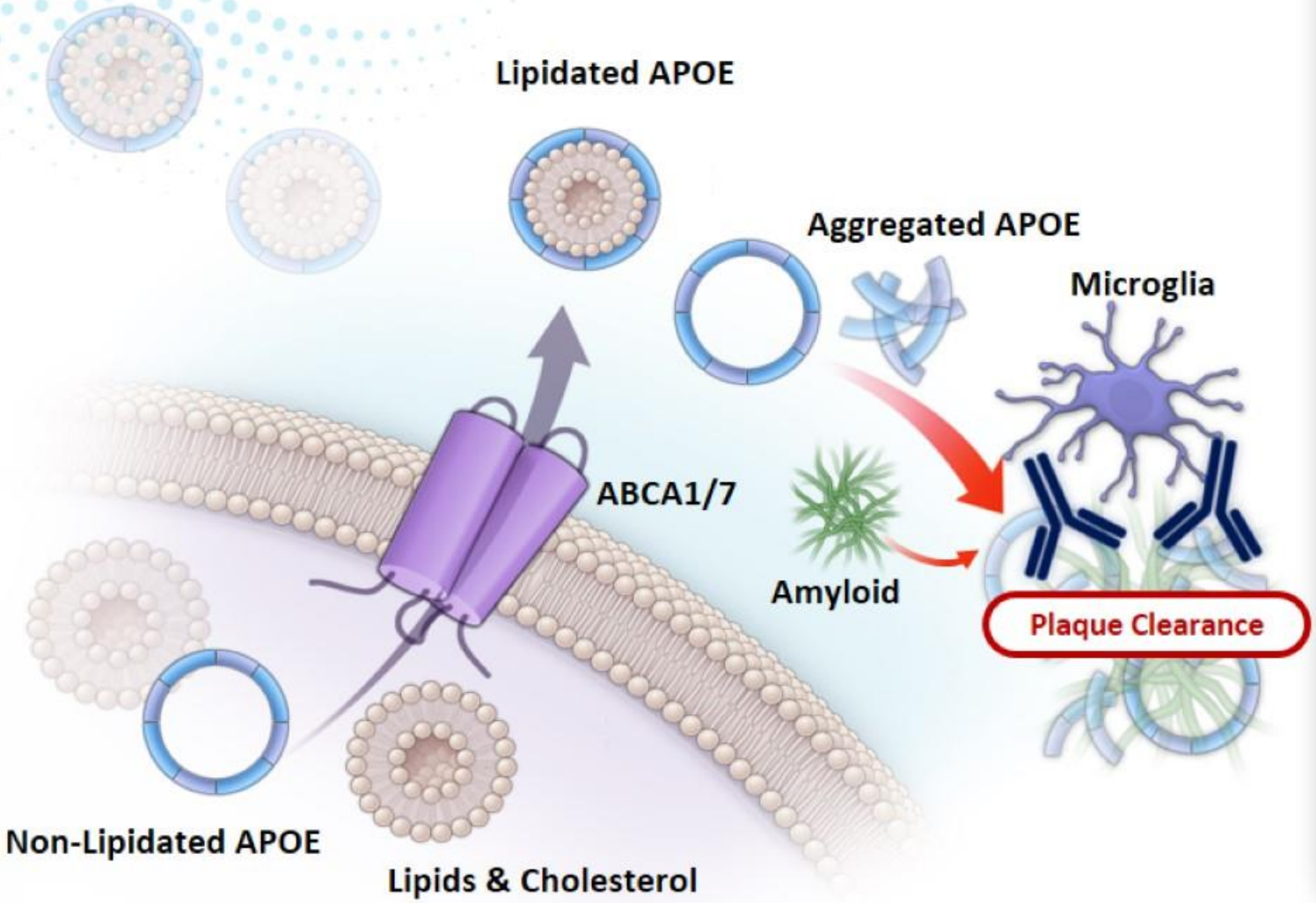
HAE-4 MOA in Alzheimer's Disease



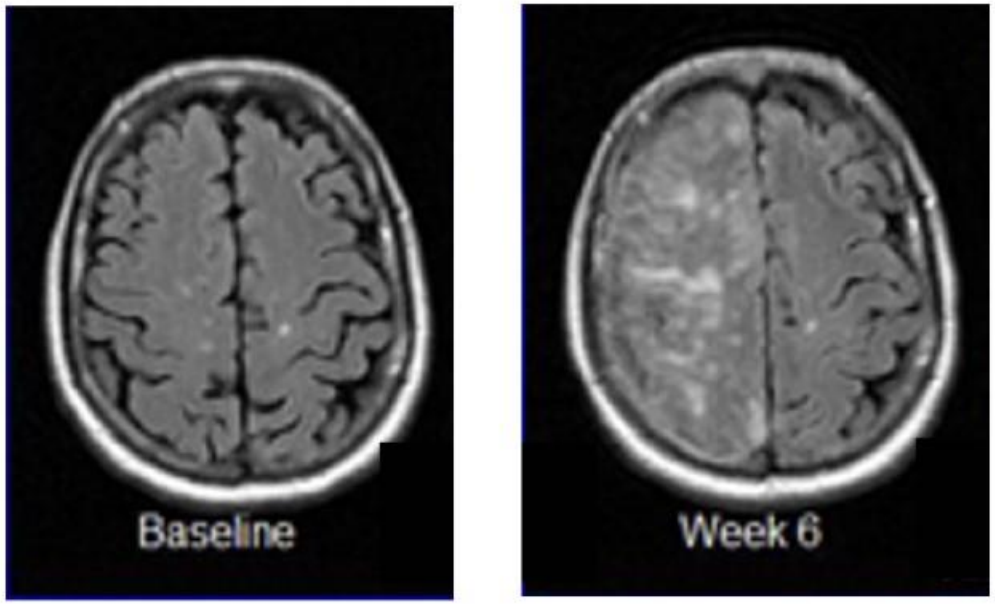
HAE-4 Summary

- Recognizes poorly lipidated form of APOE, putatively a core component of fibrillar plaques
- Reduces parenchymal plaque deposition
- Recruits microglia to amyloid plaques
- Effector function required for plaque reduction

Differentiation from current Aβ therapies?

ARIA: A Significant Concern for Current Aβ Therapies

Example of ARIA-E[1] Observed with Aβ Antibody[2]



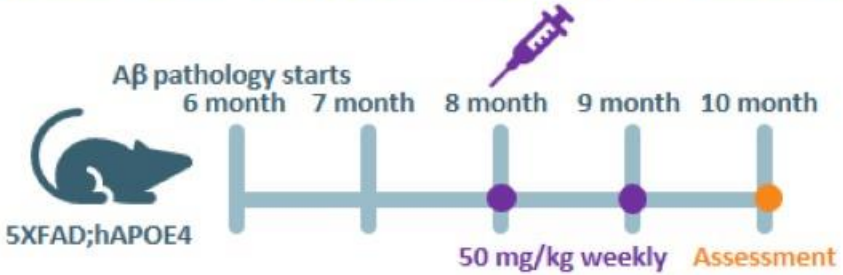
APOE ε4/4 carrier in 1.0 mg/kg in Phase 2 trial of Bapineuzumab

	ARIA-E	ARIA-H
Aducanumab (Emerge & Engage) Biogen	Total: **25.6% – 35.7%** ε4 carriers: **29.8% - 42.5%**	Total: **15.5% - 18.6%**
Lecanemab (Clarity AD) Eisai	Total: **12.6%** ε4 carriers: **15.8%** **(32.6% for homozygous)**	Total: **17.3%** ε4 carriers: **19.7%**
Donanemab (Trailblazer) Lilly	Total: **24%** ε4 carriers: **27%**	Total: **31.4%** ε4 carriers: **37%**

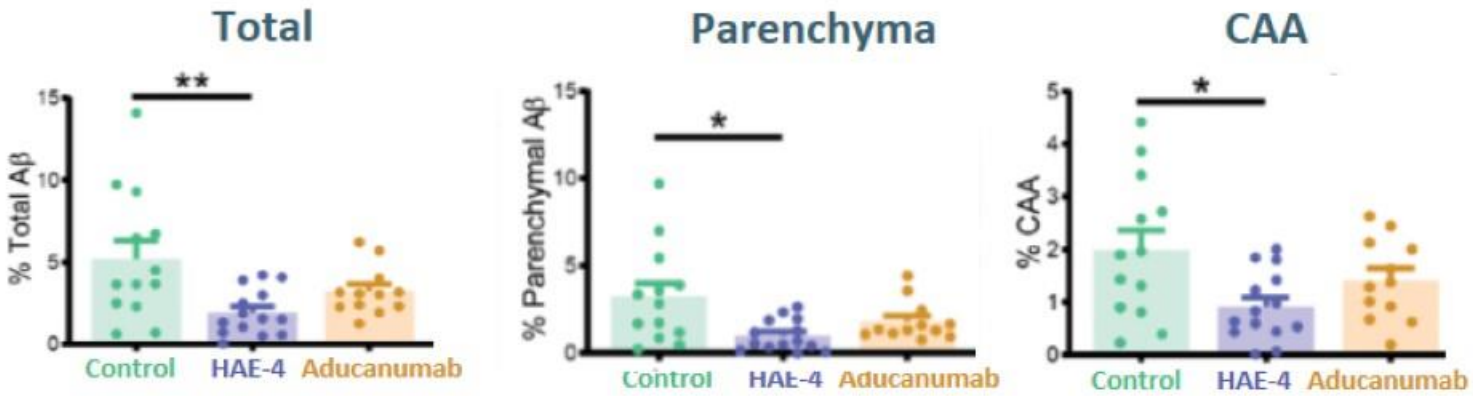
1. Amyloid Related Imaging Abnormalities with Cerebral Edema (ARIA-E) or hemorrhages (ARIA-H)
2. modified image from https://www.alzforum.org/news/conference-coverage/paris-renamed-aria-vasogenic-edema-common-anti-amyloid-therapy

Superior Reduction of Vascular Amyloid With Reduced Microhemorrhages

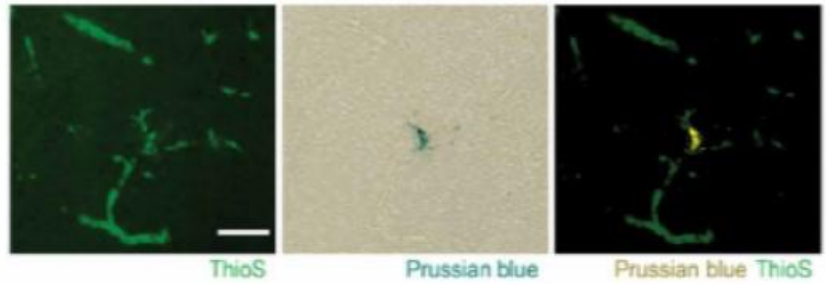
Comparable or Superior Activity to Aducanumab

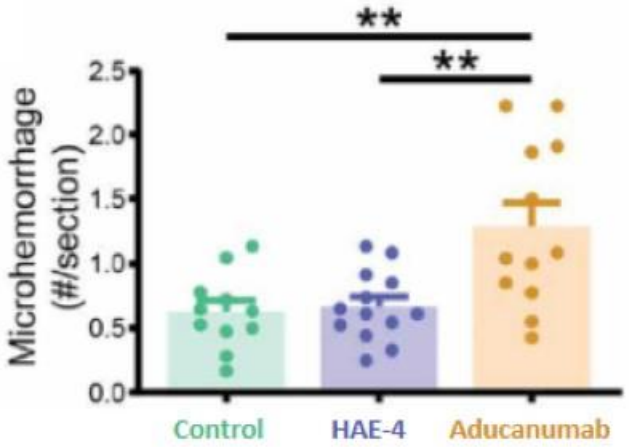


Aβ Staining



Lack of Treatment-Induced Microhemorrhages





Suggests HAE-4 Will Have A Better Safety Profile – Why?

Xiong, et al. *STM* (2021)

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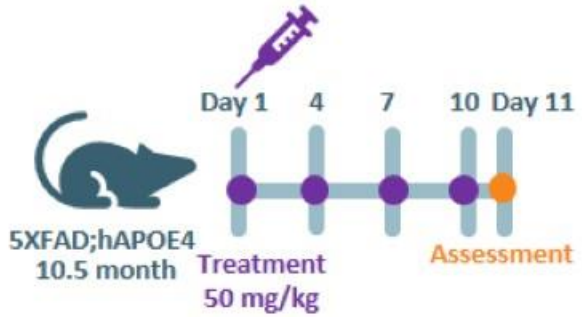
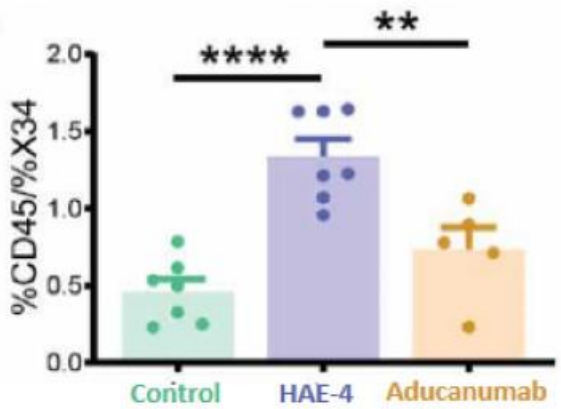
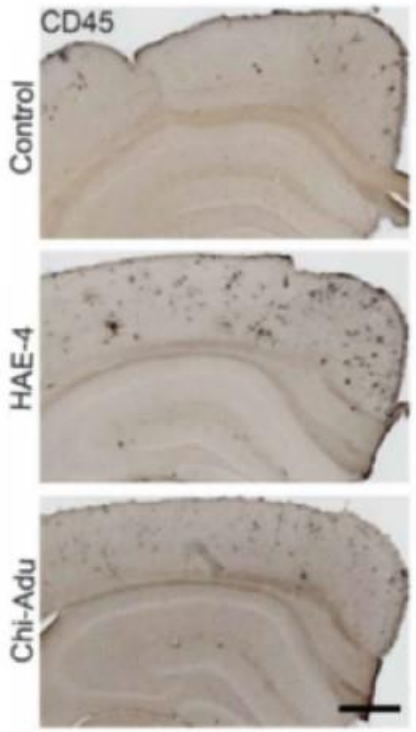
Acute Treatment with HAE-4 Results in Transient Increase in Microglial Activation

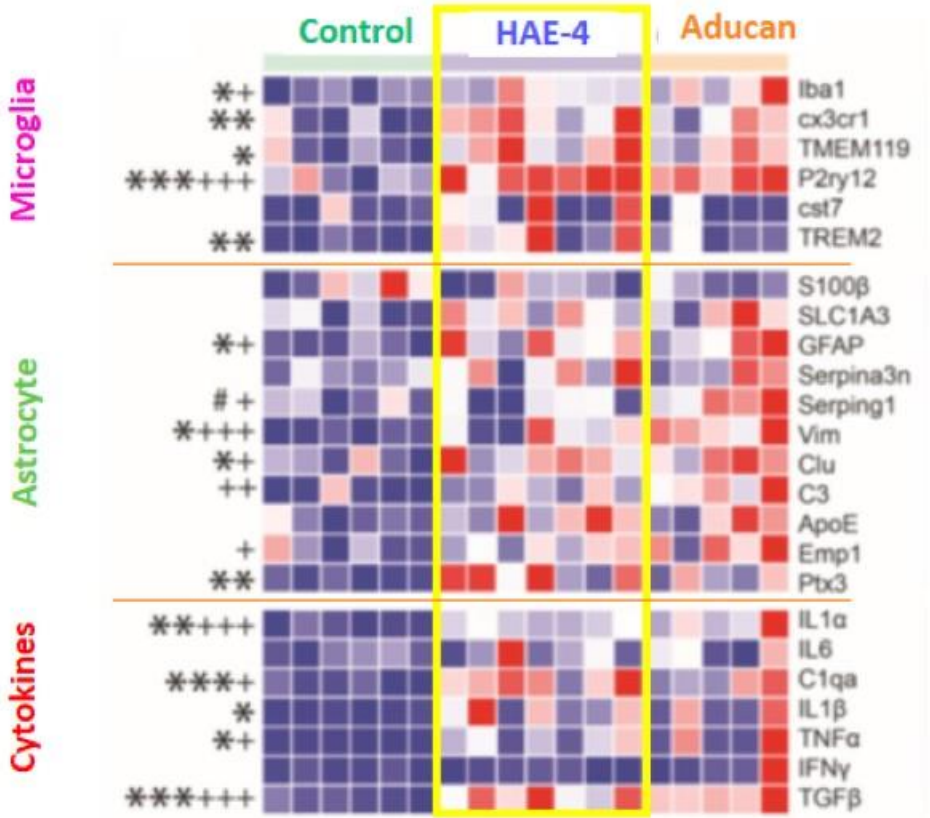
Increased Recruitment of Microglia to Plaques







Acute Increase in Inflammatory Markers



Xiong, et al. *STM* (2021)

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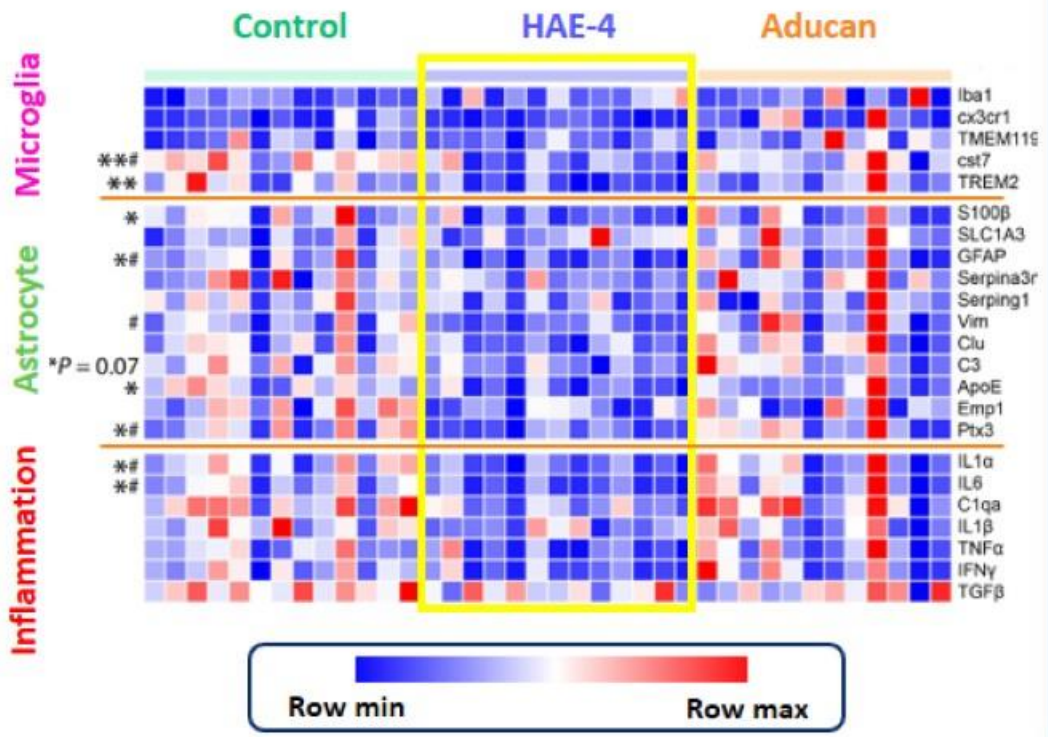
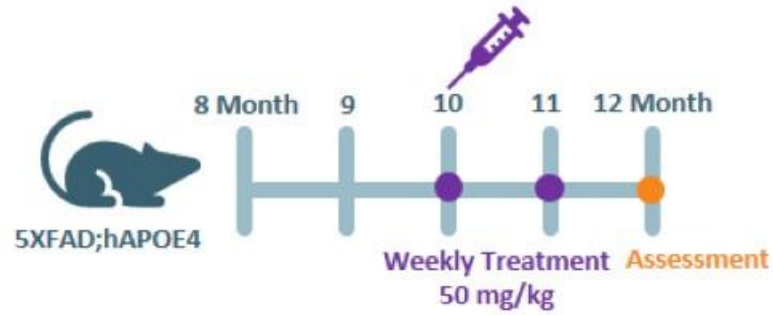
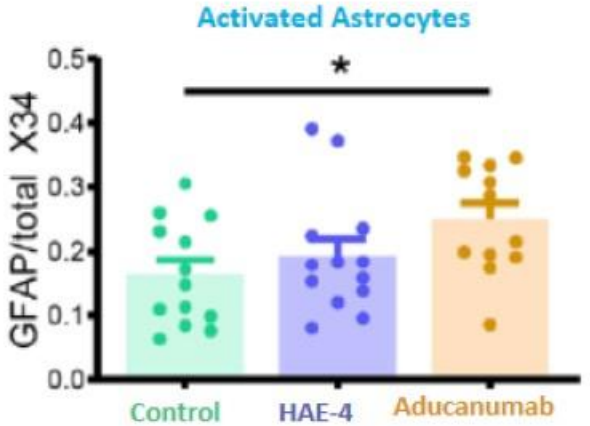
Chronic HAE-4 Treatment Leads to Reduction in Neuroinflammation

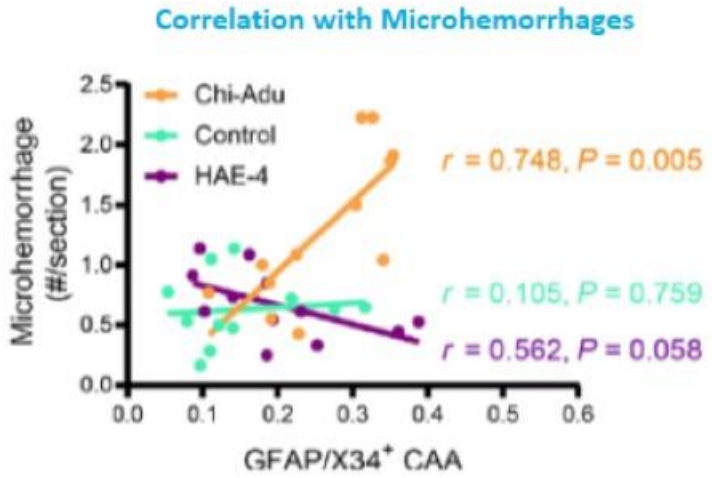
Reduction in Inflammatory State Following Chronic HAE-4 Treatment



Aducanumab Resulted in Activation of Astrocytes Correlated with Microhemorrhages







Xiong, et al. *STM* (2021)

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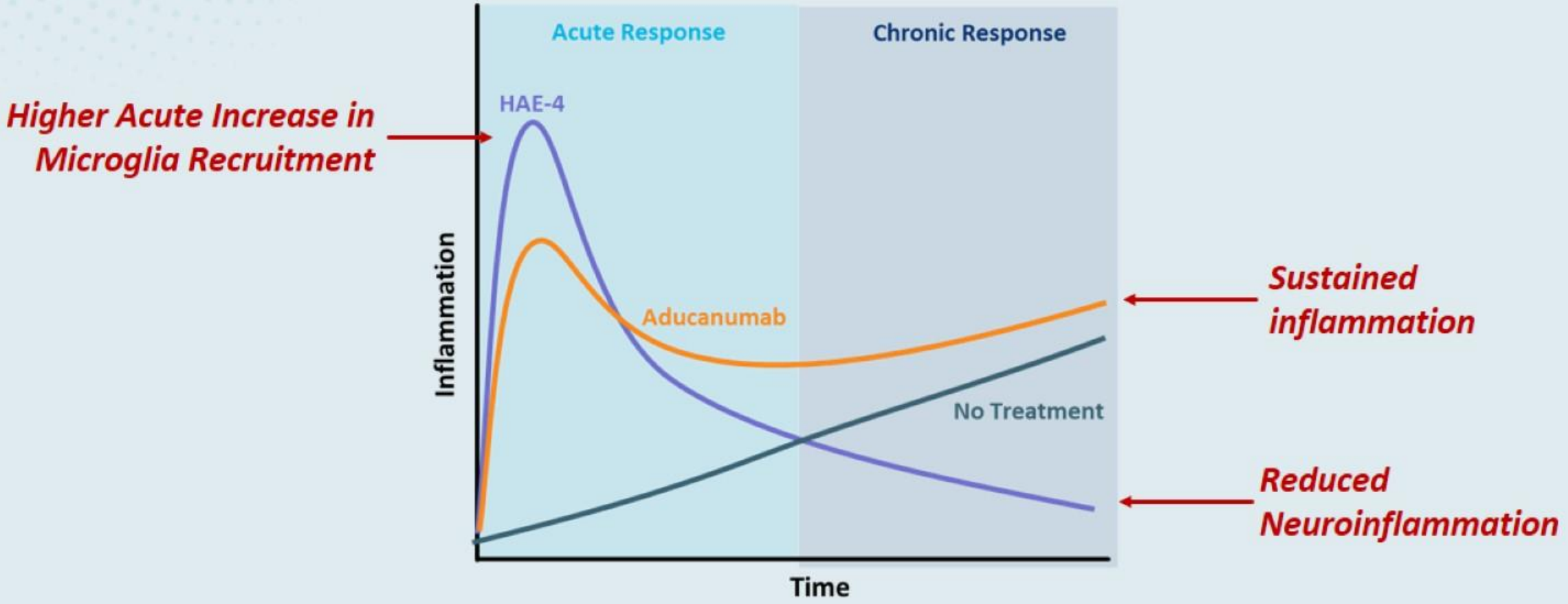
A Different Neuroimmune Response May Explain Reduced Vascular Toxicity



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Overview & Continued Progress

NC181

First in Class Therapeutic
Removes Plaques & Prevents Neuroinflammation

SUMMARY

- ✓ Removes plaques in parenchyma and vasculature
- ✓ Lack of treatment-induced microhemorrhages
- ✓ Improves cerebrovascular function
- ✓ Humanization complete with cell line development on-going
- ✓ Cyno PK study complete; no impact on plasma or CSF APOE
- ✓ Identification of potential biomarkers to enable development

ONGOING & NEXT STEPS

- Master cell bank testing
- GLP Tox
- File IND