Profiling the immune response to anti-Aβ immunotherapy

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1. Abstract

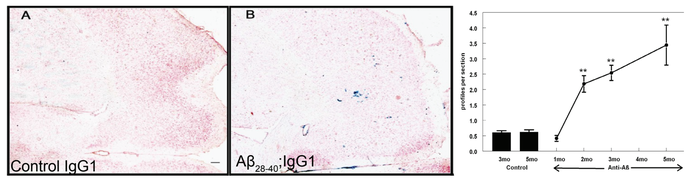
Background:
Amyloid-related imaging abnormalities (ARIA) is a frequent and problematic adverse event of anti-Aβ immunotherapy. With ongoing clinical use of lecanemab or donanemab, identifying the underlying causes of ARIA is critical. Microglia have been implicated in Aβ plaque dynamics and clearance, thus we hypothesize that neuroinflammatory processes contribute to cerebrovascular dysfunction underlying ARIA. Preclinical work has shown increased matrix metalloproteinase (MMP) activity after chronic anti-Aβ immunotherapy. We additionally hypothesize that MMP inhibition will reduce cerebrovascular dysfunction and ARIA after chronic anti-Aβ administration.

Methods:
We utilized the hABSAA mouse model (Swedish, Arctic, and Austrian mutations) and administered 3D6 (anti-Aβ) or isotype control IgG and for MMP inhibition we injected a small molecule MMP inhibitor, Marimastat, or vehicle saline. We performed the two treatment regimens for 3mo starting at 17mo. To evaluate microhemorrhages longitudinally, we performed monthly T2* MRI. Additionally, we did postmortem Prussian blue staining for a more sensitive analysis of microhemorrhages. Further, we assessed Aβ plaque localization (diffuse, dense, CAA) to examine the effects of the anti-Aβ immunotherapy and changes to CAA burden. We evaluated cellular substates, transcriptional programs, and intercellular communication (GO/KEGG, CellChat) by single cell sequencing (scSeq), coupled with immunohistochemistry for microglia morphology (cell area, branching, abundance).

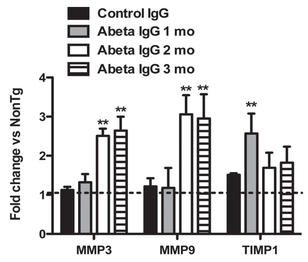
Results:
Preliminary assessments show increased microhemorrhages with 3D6 immunotherapy detected by MRI and Prussian blue. scSeq revealed fewer disease-associated microglia (DAM stage 2) and more homeostatic microglia after 3D6 immunotherapy, suggesting a reversal of microglia activation. Early analysis did not suggest an effect of MMP inhibition on microhemorrhages or microglial substate abundance, however analyses are ongoing.

Conclusions:
This work identifies the effects chronic anti-Aβ immunotherapy at a cellular and molecular level to associate microglial involvement with cerebrovascular dysfunction resulting in ARIA.

2. Background and Justification

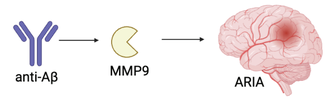


In 2004, Wilcock et al. showed that there were more microbleeds with continued anti-Aβ treatment, increasing drastically at 2mo.

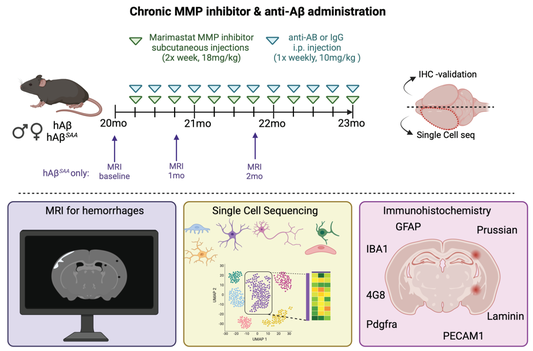


In 2011, Wilcock et al. showed that several MMPs increased in expression after 2mo of anti-Aβ treatment.

This discovery led to the hypothesis that MMP dysregulation may be contributing to anti-Aβ induced vascular dysfunction.



3. Experimental Design and Survival



Interventions used:

anti-Aβ = 3D6

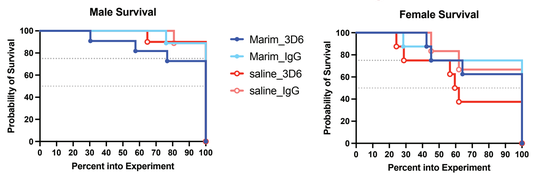
3D6 is the murinized form of anti-Aβ drug bapineuzumab and targets residues 1-5 of the Aβ protein.

MMP inhibitor: Marimastat



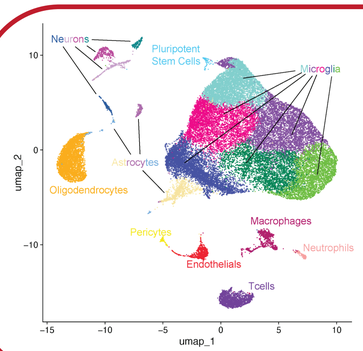
Marimastat is a broad spectrum inhibitor of MMP-1, MMP-3, MMP-7, MMP-9, and MMP-14. Our injection paradigm successfully showed Marimastat in the brain (data not shown).

Attrition rates are higher in females than in males



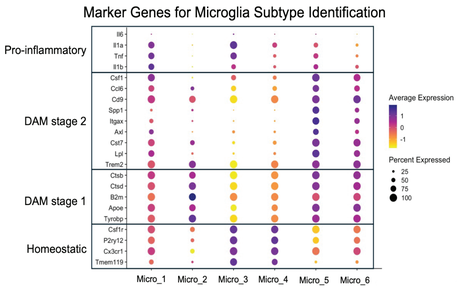
Unfortunately, over the course of the experiment, female mice on 3D6 showed reduced health and therefore had higher attrition rates. Due to this, **the following data being shown is only from male mice.**

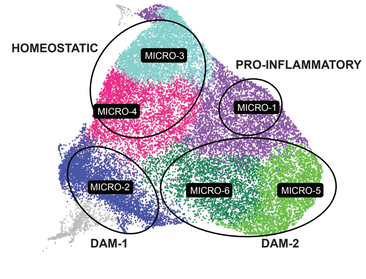
4. 3D6 treatment: fewer DAM, more homeo microglia

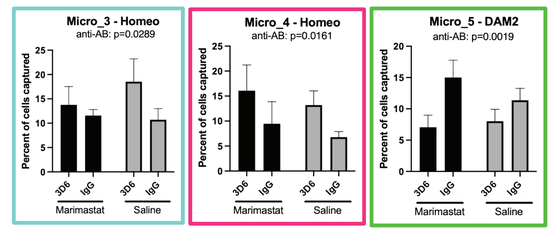


Various cell types were identified by single cell sequencing analysis (left).

Microglia clusters were subdivided and average expression of multiple genes aided in the ideniti-fication of substates (highlighted genes shown - right).





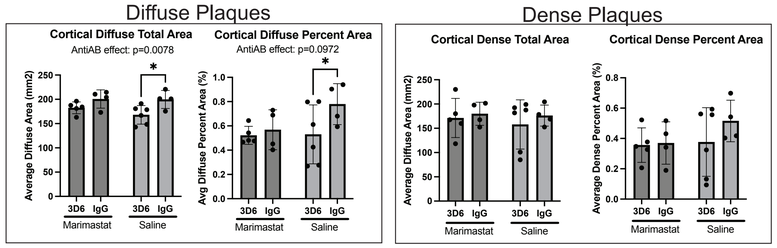


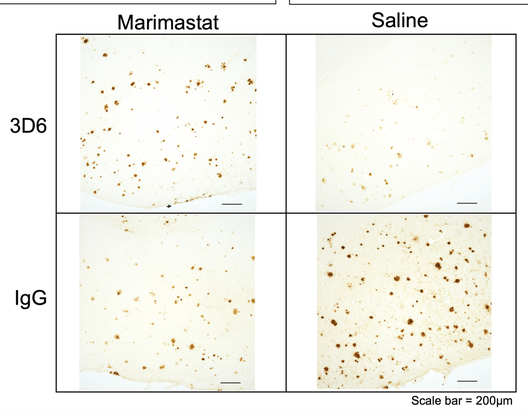
Homeo in 3D6 | Homeo in 3D6 | DAM2 in 3D6

Regardless of Marimastat or Saline treatment, we saw a reduced number of DAM2 microglia and increased number of homeostatic microglia due to 3D6 treatment.This suggests a potential return to non-active state after 3mo of 3D6 treatment.

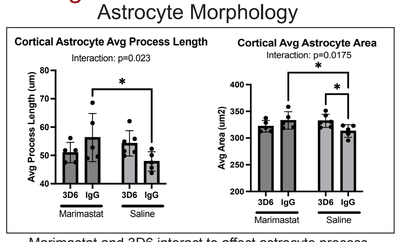
5. 3D6 and Marimastat differentially affect plaque burden, neuroinflammation, and microhemmorhages

4A. 3D6 reduces diffuse plaque burden but not dense plaques

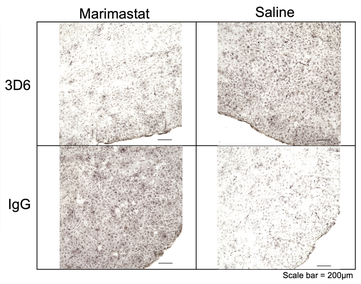


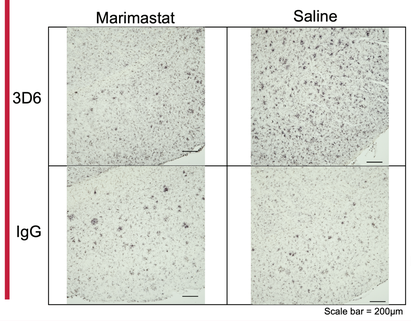


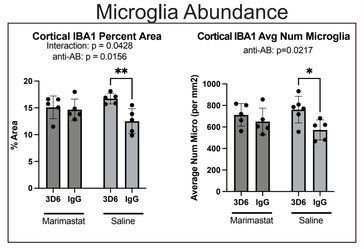
4B. Astrocyte morphology is affected by combination therapy, microglia abundance increased with 3D6, abated with Marimastat



Marimastat and 3D6 interact to affect astrocyte process length and overall area. With Saline treatment alone, 3D6 increased process length and area, while with Marimastat there was an inital increase that was diminished with 3D6 combination treatment.

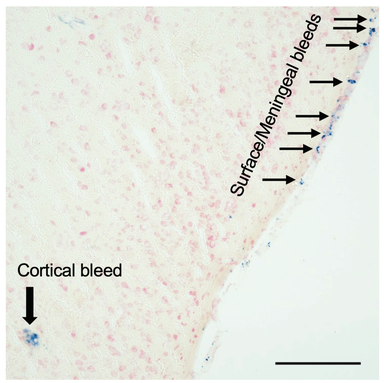




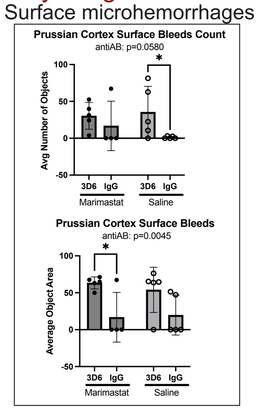


Marimastat did not appear to greatly affect microglia abundance (area or number). However, with saline treatment, 3D6 treatment increased the percent area and number of microglia.

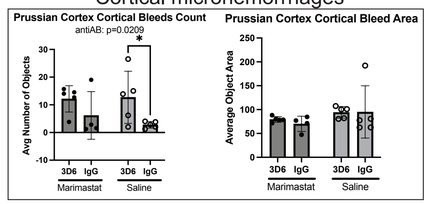
4C. Microhemorrhages increased with 3D6, Marimastat does not substantially mitigate



Surface microhemorrhages



Cortical microhemorrhages



Prussian bleed counts were increased in 3D6 treated mice along both the meningies (surface) and deeper cortical layers.

Preliminary analysis showed that Marimastat did not appear abate this effect.

- Wilcock, D. M., et al. (2004). "Passive immunotherapy against Aβ in aged APP-transgenic mice reverses cognitive deficits and depletes parenchymal amyloid deposits in spite of increased vascular amyloid and microhemorrhage." Journal of Neuroinflammation 1(1): 24.
- Wilcock, D. M., et al. (2011). "Activation of matrix metalloproteinases following anti-Abeta immunotherapy; implications for microhemorrhage occurrence." J Neuroinflammation 8: 115.
- Foley, K. E. and D. M. Wilcock (2022). "Vascular Considerations for Amyloid Immunotherapy." Current Neurology and Neuroscience Reports

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