Evaluate the Efficacy of the Axl inhibitor Bemcentinib in the 5xFAD mouse model of Alzheimer's Disease

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Background

Alzheimer's Disease (AD) brains are characterized by the presence of phosphorylated Tau forming neurofibrillary tangles and extracellular deposits of amyloid beta (Aβ). Microglia has been proposed to be involved in Aβ plaque formation. Activated microglia produce inflammatory cytokines that contribute to a hostile neuronal environment, exacerbating AD pathogenesis.[1] The 5xFAD mouse model is a transgenic mouse model with five familial AD-linked mutations. This model develops AD neuropathological features including microglia activation, formation of Aβ -plaques, and cognitive deficits.[2]

Objective

We hypothesized that 5xFAD mice treated with Bemcentinib (BGB), an inhibitor of the autophosphorylation of AXL, will lead to the inhibition of downstream signaling pathways.[3] Therefore, we will evaluate the efficacy of inhibiting AXL as a potential therapeutic for AD

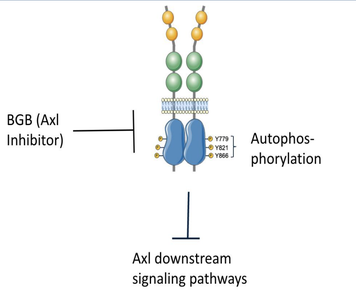

Fig 1. Inhibition of AXL. *BGB inhibits the autophosphorylation of AXL, leading to inhibition of Axl downstream signaling pathways.*

Methods

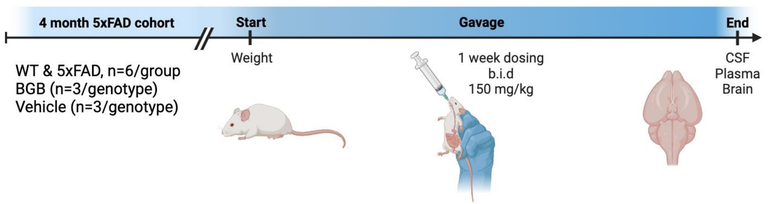

Fig 2. Experimental Design. *WT and 5xFAD mice were dosed twice a day (b.i.d.) with either BGB or Vehicle (Veh) and were perfused after a week of dosing. Their CSF, plasma, and brain were collected for posterior analysis.*

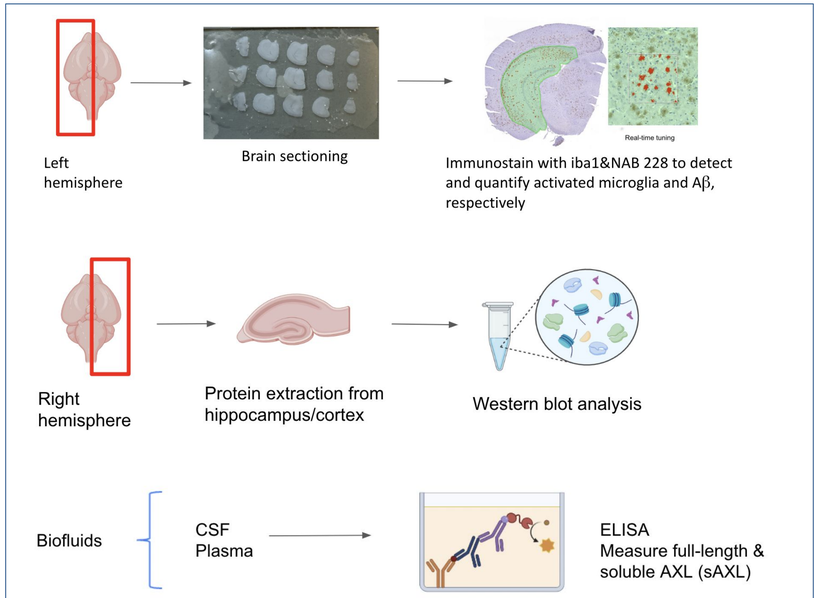

Fig 3. Workflow. *Brains from dosed mice were collected and divided into half. The left hemisphere was used for immunohistochemistry and digital quantification. The right hemisphere was used for immunoblot and ELISA analysis. The CSF and plasma were used to measure soluble Axl levels by ELISA.*

Results

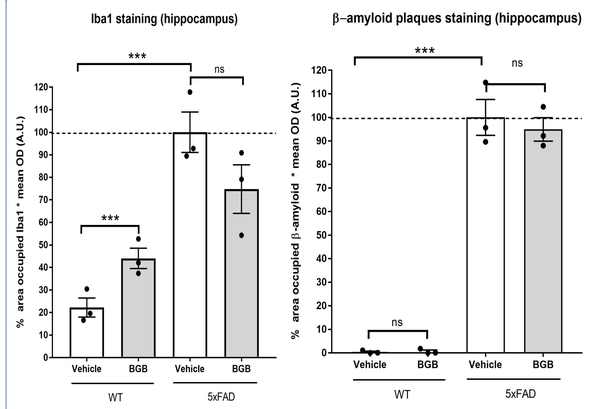

Fig 4. Iba1 & Aβ immunohistochemistry analysis in WT and 5xFAD treated mice. *Plot shows the % area occupied by Iba1 (A) & NAB 228 (B) immunoreactivities in the hippocampus of WT and 5xFAD treated with Vehicle or BGB. Bars show the mean ± SEM. Each dot represents one mouse. ANOVA was used for statistical analysis* with ns indicates $p > 0.05$, * $p \leq 0.05$, ** $p \leq 0.01$, and *** $p \leq 0.001$.

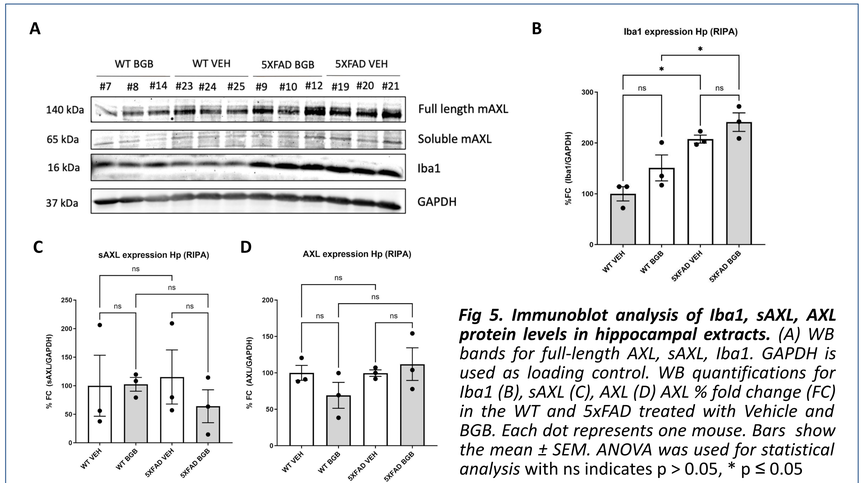

Fig 5. Immunoblot analysis of Iba1, sAXL, AXL protein levels in hippocampal extracts. *(A) WB bands for full-length AXL, sAXL, Iba1. GAPDH is used as loading control. WB quantifications for Iba1 (B), sAXL (C), AXL (D) AXL % fold change (FC) in the WT and 5xFAD treated with Vehicle and BGB. Each dot represents one mouse. Bars show the mean ± SEM. ANOVA was used for statistical analysis* with ns indicates $p > 0.05$, * $p \leq 0.05$

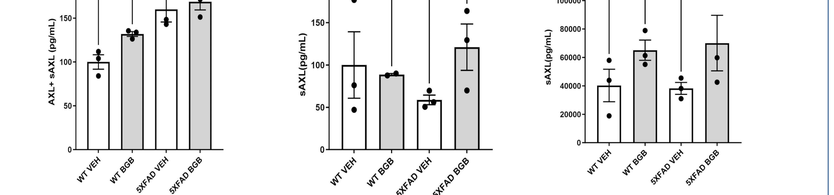

Fig 6. AXL protein levels measured in Hp, CSF and Plasma by ELISA. *Amount of sAXL+AXL (pg/ml) measured in the WT and 5xFAD treated with Vehicle or BGB measured in Hp (A), CSF (B) and plasma (C). Each dot represents one mouse. Bars show the mean ± SEM. ANOVA was used for statistical analysis* with ns indicates $p > 0.05$, ** $p \leq 0.01$

Conclusion/Future Directions

We have confirmed based on immunohistochemistry and immunoblot analysis the activation of microglia (Iba1+) in 5xFAD cohort compared to the WT cohort. However, BGB treatment does not have a significant effect in 5xFAD cohort when compared to Veh. This could be due to insufficient drug penetrance in the brain. Increasing dosing time and cohort size could potentially improve the result. In the future, we will repeat the efficacy study using the rNLS8 mouse model to target AXL signaling for ALS.[4]

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References

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