CHRONIC VERUBECESTAT TREATMENT SUPPRESSES AMYLOID ACCUMULATION IN AGED 5XFAD MICE BUT FAILS TO IMPROVE COGNITIVE OUTCOMES

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MODEL-AD
Model Organism Development & Evaluation for Late-Onset Alzheimer's Disease

The Jackson Laboratory — Leading the search for tomorrow's cures

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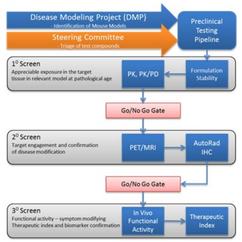
Introduction

With a major goal for improving preclinical to clinical translation in predicting therapeutic potential of novel compounds for Alzheimer's disease (AD), the Preclinical Testing Core (PTC) of the MODEL-AD consortium established a drug testing pipeline for unbiased assessments of therapeutic agents. For the present studies, we evaluated the effects of prophylactic treatment with the well characterized BACE1 inhibitor verubecestat (VER) in male and female 5XFAD mice to confirm and extend previously reported preclinical and clinical findings as part of our efforts to validate the PTC pipeline.



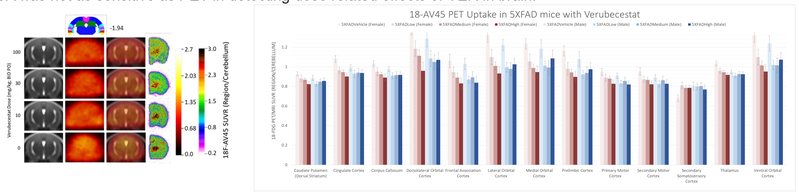
The PTC pipeline includes a primary screen to determine drug formulation, stability, and *in vivo* pharmacokinetics (PK) in models at disease-relevant ages. A secondary screen evaluates disease modifying activity utilizing non-invasive PET/MRI as a pharmacodynamic (PD) readout matched to known disease pathology in the mouse model. The tertiary screen includes functional assays that assess the compounds ability to normalize a disease-related phenotype in cognitive and functional assessments. This screening resource is available to researchers via the STOP-AD portal: ***stopadportal.synapse.org/***

Methods

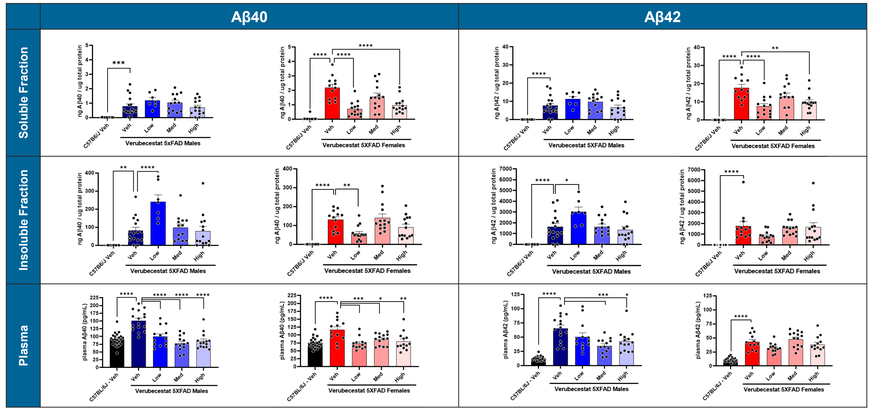
- Adult male and female 5XFAD (B6.Cg-Tg(APPSwFlLon,PSEN1*M146L*L286V)6799Vas/Mmjax; JAX#34848) mice (n=12-16 per sex/dose/genotype) on a congenic C57BL/6J background were used for all studies.
- Verubecestat trifluoroacetate (VER) was synthesized by Sellecckchem.com, analyzed by LC/MS/MS, and milled into LabDiet® 5LG4 (irradiated; TestDiet®, St. Louis, MO, USA). VER was administered *ad libitum* in chow; formulated at 60, 180, and 600 parts per million (ppm) to achieve daily dosages of 10, 30, and 100 mg/kg/day (presented as Low, Med, and High, respectively); based on average mouse weight of 30 grams and estimated daily ad libitum consumption. Pilot PK studies were conducted in 6 month aged male and female 5XFAD mice to support PK/PD modeling, and these data were used to inform the dosing regimen for long term pharmacodynamics studies. Pilot PK/PD data are reported *in Sukoff Rizzo et al 2020* and demonstrated rapid clearance in 6 month aged male and female 5XFAD mice following oral gavage warranting an alternative dosing strategy in ad libitum chow to avoid multiple oral gavage dosing daily to maintain exposure levels. All PK samples were sent to the IU CPAC for analysis. In vivo behavior and brain PK/PD were conducted at JAX and repeated at PITT. All ELISAs for both in vivo studies at JAX and PITT were conducted at PITT. In vivo PET/MR and autoradiography were conducted at IU.
- Behavioral tests were performed in order of open field activity, rotatod, spontaneous alternation, and frailty during the final weeks of the 3 month dosing period (*see Sukoff Rizzo et al 2018 for behavioral methods*). Terminal CSF, plasma, and brain tissue were collected, and soluble and insoluble fractions from hemi brain homogenates (minus cerebellum) were prepared by diethanolamine (DTT) and formic acid extraction, respectively. Aβ40 and 42 were analyzed using the MesoScale Diagnostics vPLEX Aβ Peptide Panel 1 (4G8) #K15199E with brain normalized for total protein.
- All PET scanning (15 min/ea.) was performed on the IndyPET3 scanner and post mortem brains were extracted and frozen for autoradiography (Autorad). MRIs were acquired (10 min/ea.) on a Siemens 3T Prisma scanner outfitted with a 4 channel phased array head coil. PET/MRI images were co-registered to Paxinos-Franklin atlas and 27 average brain (56 total for left and right) regions were extracted. For Autoradiography, frozen brains were sectioned at 20 um in sextuplet at 3 bregma targets, exposed on phosphor plates, scanned and manually segmented for 16 brain regions.
- All technicians were individually validated to run each behavioral assay and remained blinded to dose and genotype during execution of experiments and throughout data collection and analysis. No data were excluded based on any mathematical determination. Detailed protocols and raw data are available at www.adknowledgeportal.synapse.org.

Effects of Prophylactic Treatment with Verubecestat on Aβ Measures in male and female 5XFAD mice

Chronic treatment of Verubecestat (VER) via ad libitum diet beginning at 3 months of age prior to peak plaque deposition resulted in attenuated Aβ levels which were region- and dose-dependent as measured by 18F-AV-45 PET. As expected, measurement of Aβ levels via ELISA revealed expected increases in vehicle treated 5XFAD relative to vehicle treated C57BL/6J, however the ELISA was not as sensitive as PET in detecting dose related effects of VER in brain.



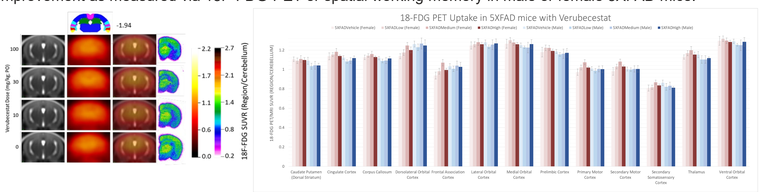
Representative images for 18F-AV45 PET/MRI and autoradiography of n=5 randomly male and female 5XFAD mice. All images are presented as SUVR to the cerebellum. Representative bregma image panel presented as average MRI (left), PET (center-left), Fused (center-right), and Autoradiography (right) as a function of chronic VER dosing (top to bottom). Quantitative analysis of 18F-AV45 PET/MRI uptake in male and female 5XFAD mice as a function of VER dose. Data presented as means ±1 SEM, and analyzed with a 2-way ANOVA, with sex and treatment as factors. As predicted, VER produced dose-dependent reductions in 18F-AV45 uptake which was regionally dependent (14 additional regions not presented did not show dose related effects).



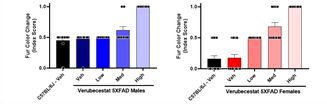
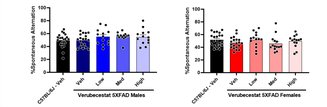
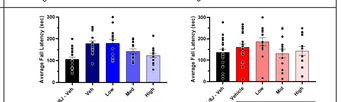
Measurements of Aβ40 and Aβ42 taken from soluble (top panel) and insoluble fractions (middle panel) from hemi brain tissue homogenates, and plasma (bottom panel). Data presented as means with SEM and individual data points plotted. Vehicle treated 5xFAD compared to age- and sex-matched vehicle treated C57BL6/J controls. Effects of verubecestat treatment in 5XFAD mice analyzed by one way ANOVA versus 5XFAD vehicle control (*=p<0.05, **=p<0.01, ***=p<0.005, ****=p<0.001). The Aβ plasma effects of VER are consistent with Villarreal et al 2017. Analysis of CSF measurements are ongoing.

Verubecestat Treatment Produced Side Effects in male and female 5XFAD Mice in the Absence of Improvements in Cognitive Outcomes

Similar to the clinical trial reports (Egan et al 2019), verubecestat produced changes in hair color that were dose related and also reduced the ability of the mice to maintain their balance on the rotarod which may be analogous to the reported side effect of increased dizziness and falls in patients. There was no indication of cognitive improvement as measured via 18F-FDG-PET or spatial working memory in male or female 5XFAD mice.



Representative images for 18F-FDG PET/MRI and autoradiography of n=5 randomly selected male and female 5XFAD mice. In all cases, images are presented as SUVR to the cerebellum. Representative bregma image panel presented as average MRI (left), PET (center-left), Fused (center-right), and Autoradiography (right) as a function of chronic VER dosing (top to bottom). Quantitative analysis of 18F-FDG PET/MRI uptake in male and female 5XFAD mice as a function of VER dose. Data presented are means ± 1 SEM, and analyzed with a 2-way ANOVA, with sex and treatment as factors. VER did not alter 18F-FDG uptake at any of the doses tested.

Verubecestat Clinical Reports *(Egan et al 2019)*	Analogous Mouse Assessment *(Sukoff Rizzo et al 2018)*	Verubecestat Effects in 5XFAD Mice		
Exposure related change in hair color	Coat Color Assessment (Frailty Index)	Exposure related coat color loss		
Worsened Cognitive Outcomes	Spontaneous Alternation Spatial Working Memory	No impairment or improvement at dose range tested		
Dizziness and increased risk of falls	Rotarod motor Coordination	Increased latency to fall in 5XFAD relative to C57BL/6J is attenuated with increasing doses of Verubecestat		

Behavioral phenotypes of 5XFAD including deficits in spontaneous alternation and increase latency to maintain balance on the rotarod as well as hyperactivity have previously been reported by MODEL-AD (adknowledgeportal.synapse.org) and others. Across 2 independent cohorts, C57BL/6J controls demonstrated reproducible and consistent behaviors however the expected deficit in 5XFAD was not consistently observed. Importantly, VER did not produce an improvement nor a greater deficit in 5XFAD mice in the spontaneous alternation task at the dose range tested although other side effects were observed.

Summary & Conclusions

- The aims of the present studies were to confirm and extend previous data reported both preclinically and in the clinic for the effects of verubecestat on Aβ levels and cognitive outcomes; applying a rigorous preclinical screening strategy established by the MODEL-AD PTC. Assessment of verubecestat using the PTC strategy are consistent with clinical findings for reductions in amyloid deposition in the absence of improvement in cognition, and with reported side effects, as was observed in patients. These data may provide insight into the lack of efficacy of verubecestat in the clinic, as well as the limited utility of 5XFAD mice for executing preclinical translational studies including their individual variability of plaque deposition and inconsistent behavioral phenotype.
- This preclinical screening pipeline is an available NIA funded resource for the AD research community. Researchers can nominate their compounds for testing in mouse models being created and characterized by MODEL-AD, through the STOP-AD Portal: *stopadportal.synapse.org/*. Compounds nominated will be best matched to a mouse model of LOAD based on its mechanism of action.

Acknowledgements

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For further information, please see

MODEL AD: www.modelad.org **STOP-AD**: https://stopadportal.synapse.org/

AD Knowledge portal: https://adknowledgeportal.synapse.org/