Critical Evaluation of the Pharmacokinetics of Verubecestat in Aged 5XFAD Mice

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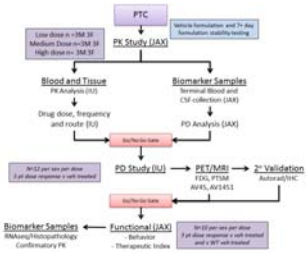
MODEL-AD
Model Organism Development & Evaluation for Late-Onset Alzheimer's Disease

INTRODUCTION

The preclinical testing core (PTC) of the Model Organism Development for Late Onset Alzheimer's Disease (MODEL-AD) consortium has established a streamlined preclinical drug screening pipeline including a primary screen to evaluate in vivo pharmacokinetics (PK) at disease-relevant ages in mouse models of Alzheimer's disease (AD) in advance of chronic prophylactic treatment for preclinical efficacy assessment.

In line with this tiered strategy, the PK profile of the BACE1 inhibitor verubecestat was initially evaluated to inform PK/PD modeling for pharmacodynamics assessments.

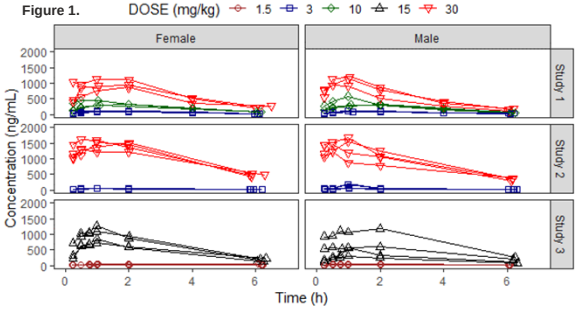


The PTC strategy includes a primary screen to determine: 1) drug formulation; 2) drug stability; and 3) *in vivo* PK and target tissue concentrations in models at disease-relevant ages. A secondary screen evaluates target engagement and disease modifying activity utilizing non-invasive PET/MRI as a pharmacodynamic (PD) readout matched to known disease pathology in the model. Compounds demonstrating positive PD effects in the secondary screen are further interrogated via a tertiary screen of functional assays that assess the compounds ability to normalize disease-related phenotypes in cognition and neurophysiological tests.

METHODS

- Verubecestat trifluoroacetate (MK-8931; synthesized by Selleckchem.com) was analyzed by LC/MS/MS to confirm dose and stability for all formulations. For chow studies, verubecestat was milled into LabDiet® 5LG4 (irradiated; TestDiet®, St. Louis, MO, USA).
- Five experiments were conducted in separate cohorts of 6 month aged male and female 5XFAD mice (B6.Cg-Tg(APPSwFlLon,PSEN1*M146L*L286V)6799Vas/Mmjax; #34848)
  1. Acute 3, 10, 30 mg/kg (10ml/kg, PO) in OH-propyl-β-cyclodextrin
  2. Sub-chronic (7 day) 3 or 30 mg/kg BID (10 ml/kg, PO) in 0.5% methylcellulose
  3. Acute 1.5 or 15 mg/kg (10ml/kg, PO) in 0.5% methylcellulose
  4. 30 mg/kg/day (180PPM) for ~ 2 wks milled in chow (pulverized), individually housed mice
  5. 30 mg/kg/day (180PPM) for ~ 2 wks milled in chow (pellets), group housed mice
- Blood samples were obtained by tail bleed, processed to plasma and stored at -20C. Verubecestat was assessed in plasma by LC/MS/MS.
- Population PK modeling was performed using NONMEM 7.3 (ICON; Hanover, MD) utilizing the plasma concentration-time profiles from the oral gavage studies. Mean PK parameters were used to simulate various ad lib dosing scenarios and compared to observed plasma concentrations

Plasma concentration-time profiles of verubecestat (ng/mL) following oral administration in 6 month aged male and female 5XFAD mice.



- Plasma concentrations of verubecestat following oral gavage administration fit a one-compartment first-order absorption model.
- Following oral gavage, verubecestat clearance was 0.24 L/h (15.7% RSE) and volume of distribution was 0.84 L (15.6% RSE), resulting in a terminal half-life of approximately 2.5 hrs (Figure 1). Verubecestat was not detected at time zero.
- Administration of verubecestat via oral gavage resulted in sexually dimorphic effects on clearance which was 23% greater in males than females (Figure 1).
- PK/PD modeling from oral gavage data indicated a requirement of dosing at least every 12 hours to minimize Cmin:Cmax (Figure 2).

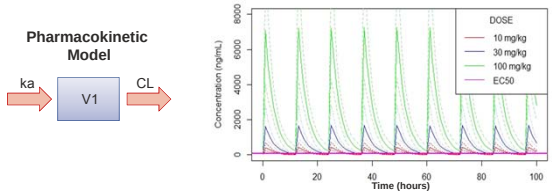


Figure 2. PK model and simulated plasma concentration-time profile of varying doses of verubecestat administered every 12 hours compared to published EC50 values for rats from *Kennedy et al Sci Trans Med.* 2016;8(363). Dashed lines represent 90%-prediction interval.

RESULTS

Plasma concentrations of verubecestat (ng/mL) following ad libitum feeding in chow (180ppm) in 6 month aged male and female 5XFAD mice

Study	Sex	2 hr post lights off	12 hr post lights off	18 hr post lights off	22 hr post lights off	2 hr post lights off (pellets)
Single Housed Powder	Male (n=5)	153.4 (126.6-176.9)	147.6 (123.2-164.2)	-	68.8 (44.4-90.4)	141.1 (115.5-165.1)
	Female (n=5)	214.0 (124.3-352.8)	205.5 (126.4-281.7)	-	99.2 (87.9-139.6)	198.5 (151.4-321.2)
Group Housed Pellets	Male (n=7)	69.4 (40.3-76.3)	96.0 (65.9-122.0)	39.1 (26.5-49.1)	39.1 (27.8-70.6)	-
	Female (n=7)	148.6 (76.3-294.5)	93.3 (70.1-111.8)	81.9 (45.1-123.8)	69.6 (33.5-115.1)	-

Data presented as median (range). A concentration of 180 parts per million (ppm) was targeted to achieve a dose of 30 mg/kg/day based on an average 30 g mouse consuming ~5 g chow per day. There was a 2-3 day inter sample collection interval with the exception of individually housed mice at the 22 hr post lights off sample which was collected on the same day after the 12 hr post lights off sample.

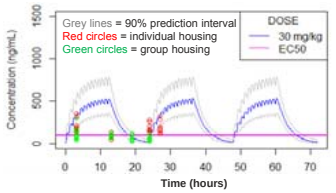


Figure 3. Simulated plasma concentration-time profile of 30 mg/kg of verubecestat (assuming 12 hours feeding (1/12th of daily dose per hour); 12 hours no feeding) compared to published EC50 values in rats *(Kennedy et al., Sci Trans Med, 2016)*. Red and green circles indicate observed data from individual-housed and group-housed mice, respectively.

- QC of chow revealed significant inter-pellet variability (31% CV) and intra-pellet variability (8-36%) with concentrations significantly lower (54±17%) than expected from 180ppm.
- Significant differences in verubecestat concentrations were observed for single vs. group housed, male vs. female, and time of day.
- Verubecestat concentrations were lower in male mice than in female mice. Inter-individual variability was greater in female mice than in male mice.
- Irrespective of whether chow was pulverized or in pellets, regardless of inter- and intra-pellet variability similar concentrations of verubecestat were observed (within individual subjects).
- PK/PD modeling supported selection of appropriate dose range (10-100 mg/kg/day) for chow formulation to cover pellet variability and sex differences in exposure levels for long term PD studies *in progress*. Consistent with previous reports, after ~ 4 weeks of chronic dosing in pelleted chow, coat color changes were observed. No other adverse events were observed.

ACKNOWLEDGEMENTS

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• MODEL AD: modelad.org • AMP-AD knowledge portal: ampadportal.org