Pre-clinical evaluation of the exposure and safety of a small molecule inhibitor of tau self-association

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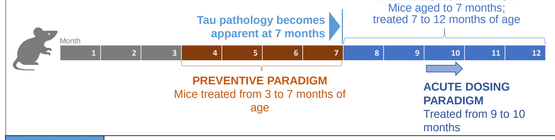
Abstract

The overall goal of this program is to develop a disease modifying therapeutic for Alzheimer's disease and related dementias characterized by the formation of tau aggregates. We have shown that tau oligomers cause disruption of neuronal signaling and inhibit the formation of memory in mice (Moe J. et al., 2010; Fá M et al., 2016). We also found that certain forms of tau oligomers are toxic when applied to cultured neurons, whereas tau monomer was not toxic at the same concentrations (Tian H et al., 2013). Tau self-association was chosen as the target for drug discovery as it is necessary for the initiation and growth of tau aggregates. A small molecule approach was used to enable access to the CNS and cells where tau is aggregating in disease, with good oral bioavailability and ease of manufacture and distribution. We have demonstrated in vivo efficacy in studies with lead compound in the human tau/htau (Davidowitz EJ et. al., 2020) and P301L tau JNPL3 mouse models of tauopathy.

The purpose of these IND enabling studies was to characterize the safety and pharmacokinetic (PK) profile of OLX07010 in established in-vitro and in-vivo pre-clinical models to inform the design of human studies. Single dose oral PK studies were performed in rodents and non-rodents. The in-vitro profiling of metabolites generated in hepatocytes from rat, dog and human was performed. In-vitro cytochrome P-450 induction and inhibition and transporter studies were performed using human liver hepatocytes and standard cell systems, respectively. 28-day GLP toxicity studies with 28-day recovery were performed in rats and dogs. In the genotoxicity program, OLX07010 was negative in the Ames test and Micronucleus Assays. In PK studies, OLX07010 demonstrated good oral bioavailability and a moderate half-life in multiple species. The drug is extensively metabolized in vitro by rat, dog, and human hepatocytes, and does not appear to be a substrate or inhibitor for human transporters. In addition, it did not inhibit cytochrome P450s, but did result in some induction. In 28-day rat and dog GLP toxicity studies, the toxicology of OLX07010 was similar in both species, as the liver was the only target organ. No adverse effects were observed, and the NOAEL was the highest dose tested in each species. Based on the above-described non-clinical pharmacokinetic and associated in vitro studies, the absence of cytochrome P450 inhibition with minimal induction, no effects on transporters, and the relatively modest toxicity seen in the 28-day GLP toxicity studies, OLX-07010 appears to be an excellent candidate for clinical development for neurodegenerative diseases.

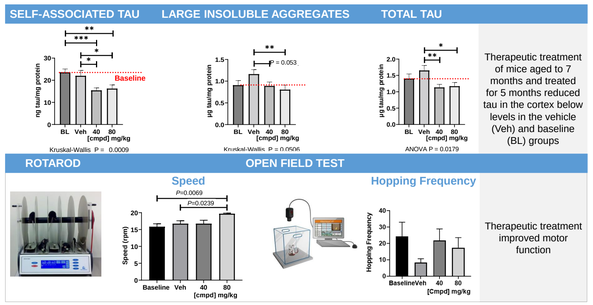
Transgenic animal model studies

Therapeutic and preventive studies were performed using male human tau (htau) and homozygous female P301L tau (JNPL3) mouse models of tauopathy



Administration	• Compound formulated into feed • Doses based on average mouse weight and average grams of food consumed daily
Study Conduct	• Independently performed • Vehicle and treatment batches of feed were blinded, provided to laboratory of Peter Davies, Ph.D. for treatment as well as collection and analysis of specimens
Primary Endpoint	• Reduction of Sarkosyl insoluble tau aggregates (with statistical significance)
Secondary Endpoints	• Dose-dependent reduction of insoluble tau aggregates • Reduction of phosphorylated tau; Reduction of soluble tau; Motor behavior

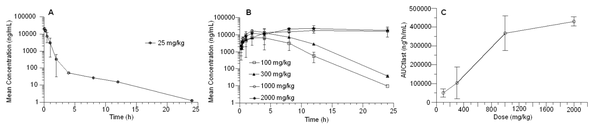
Therapeutic Treatment of Aged Transgenic Mice Reduced Tau Aggregates and Improved Motor Function



Inhibitory Activity of OLX-07010 on Tau Aggregation

- In vitro, the association of tau monomers into oligomers was inhibited in the primary and confirmatory assays, and reduced tau in neuroblastoma cells producing aggregation-prone tau protein.
- In vivo, treatment of young mice prevented the formation of tau aggregates in two different transgenic models of tauopathy, htau representing tau aggregation in Alzheimer's disease and JNPL3 modeling tau in PSP.
- Treatment of aged JNPL3 mice with pre-existing tau aggregates inhibited the progression of tau aggregation and rescued their hind limb motor impairment caused by tau aggregation.
- In vitro pharmacology studies of TO-0582 using screens of tier 1 and tier 2 panels and IC50 studies have shown minimal off-target activity.

A Single-Dose Oral PK and Bioavailability in Male SD Rats

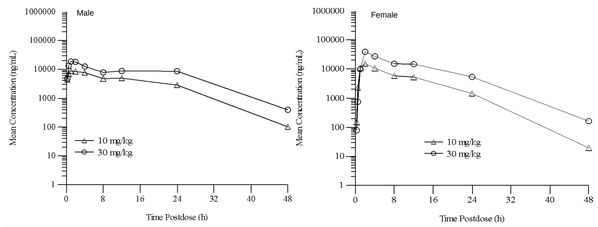


Route	Dose (mg/kg)	AUC_{tlast} (ng*h/mL)	AUC_{tlast}/D	Cmax (ng/mL)	Tmax (h)	T1/2 (h)	Vz (mL/kg)	Cl (mL/h/kg)	F (%)
Oral Gavage	100	51300 ± 22300	513 ± 223	10000 ± 2570	3 (2 - 4)	2.01 ± 0.309	NC	NC	102
	300	104000 ± 84300	346 ± 281	13000 ± 9420	4 (4 - 8)	2.19 ± 0.427	NC	NC	69
	1000	367000 ± 91900	367 ± 91.9	20100 ± 3480	18 (2 - 24)	NC	NC	NC	73
	2000	429000 ± 23500	214 ± 11.7	27600 ± 6700	18 (8 - 24)	NC	NC	NC	43

Units for AUClast/D = (ng*h/mL)/(mg/kg); Values for Tmax are presented as median; NC = Not calculable

PK study of TO-0582AA from 100 to 2,000 mg/kg in male rats (n = 4). A. PK of IV dose at 25 mg/kg. B. PK of oral doses at 100, 300, 1000 and 2000 mg/kg. C. Plot of AUCtlast vs. Dose. No toxicity was observed following single doses up to 2,000 mg/kg in the MTD phase of a dose range-finding study in rats. Half-lives could not be calculated at 1000 and 2,000 mg/kg due to persistence of peak plasma drug levels beyond the last collection time point (24 hours).

A Single-Dose Oral PK and Bioavailability in Beagle Dogs



Dose (mg/kg)	AUC_{last} (ng•hr/mL)	C_{max} (ng/mL)	$T_{1/2}$ (h)	T_{max} (hr)	F (%)
Males					
10 - Low (Oral)	188,000 ± 41,400	25,000 ± 9050	3.78 ± 0.237	1 (1-1)	47.7
30 - High (Oral)	554,000 ± 249,000	64,600 ± 27,400	4.66 ± 0.536	1 (1-4)	46.9
Females					
10 - Low (Oral)	286,000 ± 45,200	34,900 ± 8380	5.25 ± 0.701	2 (2-4)	62.8
30 - High (Oral)	593,000 ± 94,000	53,700 ± 20,400	4.64 ± 0.647	4 (1-4)	43.4

Rat and Dog DRF 14 Day Repeat Dose Studies

Study	Rat Two Week DRF Study	Dog Two Week DRF Study
Dose Level (mg/kg/day)	0, 100, 300, 1000 (n = 5 male; 5 female)	50, 150, 500 (n = 2 male; 2 female)
Clinical Signs	None	Slight tremors at 500
Body Weight	Transient at 1000	None
Food Consumption	Transient at 1000	None
Clinical Pathology: Chemistry, Hematology, Coagulation	Serum cholesterol + sorbitol dehydrogenase ↑ at 300 and 1000 APTT ↓ at 1000 (synthesized in liver)	Possibly ↑ ALT at 500 F
Necropsy / Organ Weights	↑ Liver weights at all dose levels	
Histopathology: All animals in all groups; adrenal glands, brain, heart, kidneys, liver, spleen, testes/ovaries	• Liver lesions • No histopathologic lesions in brain, dorsal root ganglia, or sciatic nerves	

Administration of OLX-07010 to rats and dogs by once daily oral gavage for 14 days was well tolerated clinically at all dose levels. In rats, effects on body weights, food consumption, liver weights, serum sorbitol dehydrogenase and cholesterol, and microscopic findings in the liver were observed at 300 and/or 1000 mg/kg/day; all findings were non-adverse.

In dogs, at 500 mg/kg/day, slight generalized tremors 1–2 hours postdosing on multiple days were observed. In addition, minimal hepatic effects were seen at all dose levels.

Genotoxicity

- **Ames Test –** negative
- **In Vitro Micronucleus Assay–** negative
- **In Vivo Micronucleus Assay (part of 28-day rat study) –** negative

Rat and Dog GLP 28 Day Repeat Dose Studies

Study	Rat Four Week GLP Study	Dog Four Week GLP Study
Dose Level (mg/kg/day) NOAEL in maroon	0, 30, 100, 300	15, 50, 150
Clinical Signs	None	None, except for tremors in one dog once at 150
Functional Observational Battery	No effects	----
Body Weight	No effects	No effects
Food Consumption	No effects	No effects
Micronuclei in Retics (Wk4)	No effects	----
Clinical Chemistry, Hematology, Coagulation	No effects	No effects
Necropsy / Organ Weights	Non-statistically sig. increase liver weights at all dose levels	Increased liver weights at mid and high dose levels
Histopathology: All tissues in all C + HD dogs, + liver in L , M and recovery (C + HD) groups	No histopathologic lesions	

Administration of OLX-07010 by once daily oral gavage to rats and dogs for 28 days was well tolerated at all doses. In rats, no treatment-related effects were seen for clinical chemistry, hematology, coagulation, or urinalysis parameters, or for gross necropsy or microscopic evaluations, and no increase micronuclei in reticulocytes. Although not statistically significant, marginally higher mean liver weights were noted in females at ≥ 100 mg/kg/day and the 300 mg/kg/day group males. Marginal increased liver weights were still seen for males, but not females, at 300 mg/kg/day at the end of the 4- week recovery period. These increased liver weights were not supported by microscopic correlates and were therefore considered nonadverse. Based on these results, the no-observed-adverse-effect level (NOAEL) was considered to be 300 mg/kg/day. This dose corresponded to mean AUC0-24hr values of 86,300 and 136,000 ng•hr/mL and mean Cmax values of 10,500 and 14,000 ng/mL for males and females, respectively, on Day 28.

In dogs, non-adverse higher liver/gallbladder weights for females at 50 and 150 mg/kg/day (and possibly 15 mg/kg/day) that were not supported by microscopic changes, and under the conditions of this study, the no-observed-adverse-effect level (NOAEL) was considered to be 150 mg/kg/day. This dose level corresponded to mean AUCtlast values of 265,000 and 291,000 hr•ng/mL and mean Cmax values of 25,100 and 31,300 ng/mL for males and females, respectively, on Day 28.

Safety Pharmacology

Cardiopulmonary Safety Pharm Study in Dogs

- Same dose levels (single administration) as the 4-week dog GLP toxicity study were tested: 0, 15, 50, and 150 mg/kg
- Cardiovascular and respiratory parameters were measured.
- The only effects were as follows:
- Very slight, but statistically significant increases in systolic, diastolic, and mean arterial blood pressure at 150 mg/kg
- Slight, statistically significant increases in pulse pressure on several occasions and decreases in body temperature at 50 and 150 mg/kg
- None of these slight changes was greater than 5%, and therefore were not considered biologically relevant or adverse.

In Vitro Effect of OLX-07010 on hERG Current (IKr)

- OLX-07010 had little effect on hERG inhibition from 0.1μM to 14μM (n=4)
- 100μM - 13.9 ± 9.1% (statistically significant)
- Based the results, an IC50 could not be calculated but was estimated to be > 100μM
- OLX-07010 had no effect on hERG current up to 14 μM, ~96 times greater than the anticipated unbound drug C_{max} in humans (0.146 μM).

Protein Binding

Using equilibrium dialysis, the binding of OLX-07010 to mouse, rat, dog, monkey and human plasma proteins as well as C57BL/6 mouse brain homogenate was evaluated at 25.0 and 5.00 μM, respectively. Based on the concentrations evaluated,

- In mouse, rat, dog, monkey and human plasma, samples containing 25.0 μM of OLX-07010 were found to be bound at 95.6%, 93.3%, 98.7%, 97.7% and 99.5%, respectively.
- In C57BL/6 mouse brain homogenate samples containing 5.00 μM of OLX-07010 was found to be bound at 85.4%.

In Vitro Transporters Studies

Assessment of OLX-07010 as a substrate of human OAT1, OAT3, OCT1, OCT2, OATP1B1, OATP1B3, MATE1, MATE2-K, BCRP, P-gp, BSEP, and MRP2 mediated transport showed it is not a substrate as defined by regulatory guidance documents for any of the human transporters.

Assessment of OLX-07010 as an inhibitor of human transporters showed:

- Statistically significant inhibition was not seen for OCT1, OATP1B3, and MATE2-K
- Statistically significant inhibition was seen for OAT1, OAT3), OCT2, OATP1B1, MATE1, BSEP, MRP2, BCRP and P-gp.
- Based on the level of inhibition, further characterization of the inhibitory effects of OLX-07010 on OAT3, OATP1B1 and BCRP was conducted.
- IC50 values were determined to be 23.9 μM for OAT3, 12.9 μM for OATP1B1. and 144 μM for BCRP.
- All three IC_{50}/C_{max} ratios are > 50, so clinical DDI would not be anticipated negating the need for such studies.

Metabolism

CYP P450 Induction Study

- In the CYP P450 induction study, hepatocytes from three human donors responded to exposure of OLX-07010.
- Relative to vehicle controls after exposure to 0.00128, 0.00640, 0.0320, 0.160, 0.800, 4.00, 20.0, and 100 μM, overall quantities of mRNA for CYP1A2, CYP2B6 and CYP3A4 changed in concentration-dependent fashion from 0.881- to 5.21-fold, 0.828- to 15.8-fold, and 0.641- to 49.0-fold, respectively, for all three donors.
- However, mean mRNA fold increases at 4.00 μM were 2.09, 1.61, & 1.89 for CYP1A2, CYP2B6, & CYP3A4, respectively, & less than 1.5 at 0.800 and 0.160 μM.
- Compared to the anticipated unbound clinical C_{max}, 4.00 μM represents a 27-fold margin of safety.
- As such, risk regarding clinical DDIs of OLX-07010 with drugs metabolized by CYP1A2, CYP2B6, and CYP3A4 would be minimal.

CYP P450 Inhibition Study

- Direct (reversible) inhibition assay
  - OLX-07010 caused >50% inhibition of 2C8, 2C9, 2C19, 2D6 and 3A4.
  - IC50 will therefore be less than 100 μM, and may require determinations
- Possible mechanism-based inhibition
  - >2-fold shift was observed for 1A2, 2C8, 2C9, 2C19 and 3A4,and may require additional studies
- Time-Dependent Inhibition
  - No results > 50%

Reaction Phenotyping of CYP450 Enzymes

- 2D6 rapidly metabolized OLX-07010 such that 28.3% remained at the end of 60 minutes.
- 3A4 metabolized OLX-07010 at a slower rate such that 82.0% remained after 60 minutes.
- Minor metabolism of OLX-07010 was observed when incubated with 2C9.
- Isoforms (1A2, 2B6, 2C8 or 2C19 were not able to significantly metabolize OLX-07010 .

Chemistry, Manufacturing and Controls

Drug Substance

Process development: The synthesis procedure of a single polymorphic form was made robust through process parameter optimization. Analytical methods were developed and qualified in support of that process, including methods for release of regulatory starting materials, in-process controls, isolated intermediate release, and final product testing.

Manufacture: NCSS (1.6 kg) and GMP (2.85 kg) batches; NCSS 5 kg scale manufacture planned this year

Stability of GMP batch: Met specifications at initial CofA, 1, 3, 6, 9, and 12 months, at both -20 °C , and 25 °C /60% RH, and through 6 months at 40 °C/ 75% RH

Reference Standards: A portion of the demonstration lot for the GMP process was set aside and fully tested to qualify it as a reference standard for use in validating test methods and for carrying out release testing. A tetra-deuterated version of OLX-07010 was successfully prepared (d4-OLX-07010).

Drug Product

Preformulation: development, completed.

Prototype manufacture: stability study through 6 months at 25 °C /60% RH and at 40°C/ 75% RH met specifications

GMP manufacture, completed: 25 and 75 mg strength capsules (5,000 each), packaged 30/bottle

Stability of DP: Met specifications at 9 months of ongoing study at 25 °C /60% RH and 40 °C/ 75% RH

Conclusion

The preclinical development program demonstrated that OLX-07010 is an excellent candidate for clinical development.

- Pharmacologic activity in mouse models
- Reasonable pharmacokinetic characteristics
- Minimal DDI potential
- Lack/minimal effects on cardiovascular, pulmonary and CNS systems
- Lack of genotoxicity
- Relatively modest findings which were not considered adverse were observed in 28-day rat and dog GLP toxicity studies
- Drug substance showed high purity and stability, drug product shows good stability
- A 6-month rat GLP toxicity study is planned to start mid 2023 to enable a phase 1b clinical study
- OLX-07010 is an excellent candidate for clinical development for treatment of neurodegenerative diseases

Long-term treatment for chronic diseases such as AD requires safe, effective, and economically feasible approaches. This small molecule, CNS drug-like lead substantially fulfills these requirements based on our preliminary results and the fact that it would not need cold-storage or expensive infusion centers for administration for ease of treatment to address the unmet global health crisis.

References

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