Therapeutic efficacy of a small molecule inhibitor targeting tau self-association in mouse models of tauopathy

J. Moe[1], P. Lopez[1], H. Jimenez-Bravar[2], L. Adrien[2], J. Eun[2], A. Wolin[2], J. Koppel[2], P. Davies[2], E. Davidowitz[1].

[1]Oligomerix, Inc. - White Plains, NY (United States), [2]The Litwin-Zucker Research Center For The Study Of Alzheimer's Disease, The Feinstein Institute For Medical Research, Northwell Health - Manhasset, NY (United States)

Oligomerix, Inc.

Background

The premise of this program is that tau oligomers are the acutely toxic species of tau and that their reduction will modify the course of AD. We have shown that tau oligomers cause disruption of neuronal signaling and inhibit the formation of memory in mice (Fá et al., 2016), and that certain forms of tau oligomers are toxic when applied to cultured neurons (Tian et al., 2013). The discovery of small molecule inhibitors was performed with assays targeting tau self-association, the initial step in the tau aggregation cascade. This program is highly differentiated in that it targets full-length, non-mutated tau, whereas other tau aggregation inhibitor programs have largely focused on inhibiting formation and or dissociating large and relatively inert fibrils which could generate toxic tau oligomers. Preventive efficacy studies were performed in htau (Davidowitz et al., 2020) and JNPL3 mice that demonstrated that the lead compound reduced self-association of soluble tau and inhibited formation of insoluble tau aggregates.

Objectives & Methods

The goal of this program is to discover and develop small molecule therapeutics targeting tau self-association for the treatment of AD and ADRD. Measurements of therapeutic efficacy include reduction of insoluble and hyperphosphorylated tau that has already accumulated and inhibition of the continued progression of tau pathology, as well as amelioration of behavioral deficits. Therapeutic studies were independently performed in male htau and female JNPL3 transgenic mice. Mice were aged to 7 months (baseline) and treated for 5 months. Each study had 4 groups including baseline (n=20), vehicle (n=25), and two treatment groups (n=25, each) that were administered 40 or 80 mg/kg dose of lead compound formulated in feed. The htau baseline group was tested for working memory performance with the Barnes maze and the JNPL3 baseline group had open field behavior and Rotarod performance testing prior to sacrifice at 7 months; the vehicle and treatment groups had behavioral testing performed at 7 and 12 months. Samples of brain were taken for biochemical analysis of levels of tau and phosphorylated tau, as well as levels and phosphorylation of insoluble, aggregated tau. Immunocytochemical examination was performed with 4 tau antibodies (MC1, PHF1, CP13 and RZ3), as well as with Iba1 and GFAP for microgliosis and astrocytosis, respectively, as time permitted under restricted access related to the pandemic.

Differentiation through Early Action

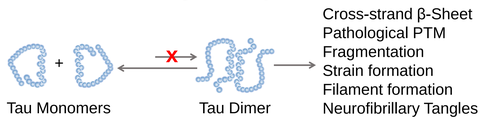


Figure 1. Schematic of tau self-association target for screening small molecule inhibitors.

- **Oligomerix lead inhibits tau self-association early in the aggregation cascade**
- Primary and confirmatory screening assays use full length tau without mutations
- Cell based assays with and without tauopathy mutations
- **Compound activity confirmed in vivo in two models of tauopathy**
- Competitors target fibril formation using aggregation facilitators

Benefits of targeting tau self-association

- By targeting the aggregation pathway upstream with a small molecule we have considerable advantages over competitors' downstream target approach including better in vivo access to target, lower treatment costs for patients and ease of administration.
- Our upstream approach is highly differentiated and complementary to competitors' immunotherapeutic approaches.

Preventive and Therapeutic htau Study Designs

Mouse model

- htau mice express all six human tau isoforms without mutations, no murine tau
- Male htau mice were used for both studies

Length of treatment

- Preventive study: 4 months from 3 to 7 months of age
- Therapeutic study: 5 months from 7 to 12 months of age

Route of administration

- Oral, compound was milled into feed
- The lots of feed were coded to blind the treatment groups

Doses

- Preventive study: vehicle feed, 10 and 40 mg compound/kg mouse (n = 26-29/grp)
- Therapeutic study: vehicle feed, 40 and 80 mg compound/kg mouse (n=25/grp)

Primary endpoint

- Reduction of insoluble tau aggregates with statistical significance

Secondary endpoints

- Dose-dependent reduction of insoluble tau aggregates
- Reduction of phosphorylated tau, soluble tau, markers of inflammation
- Amelioration of behavioral phenotypes in therapeutic studies of aged mice

Comparison of Preventive and Therapeutic Studies in Male htau Mice

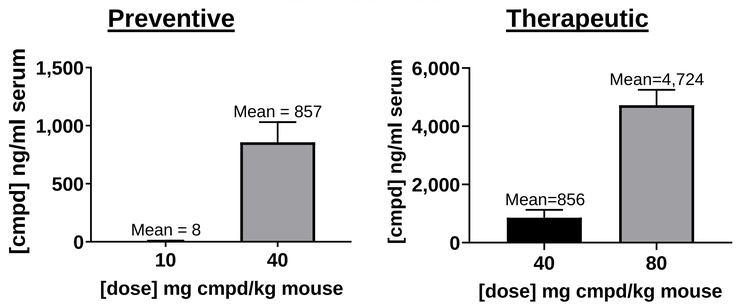


Figure 2. Compound Exposure in Serum. Serum was prepared from the mice that were bled after sacrifice and levels of the lead compound were determined by LC-MS/MS. In the preventive study, the exposure was over 100-fold greater in the 40 mg/kg treatment group relative to the 10 mg/kg group. In the therapeutic study, doubling of the dose in feed caused a 5.5-fold increase in serum levels.

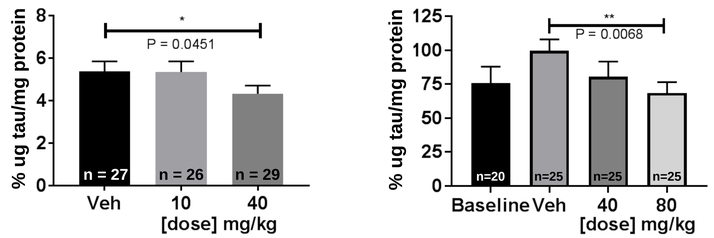


Figure 3. Sarkosyl insoluble tau P-Ser 202 in the cortex. Sarkosyl-insoluble tau and heat stable tau fractions were prepared as described (Forest et al., 2013). A sandwich ELISA using mAb CP13 specific for tau P-Ser 202 for capture and pan-tau mAb DA9-HRP for reporter was used to determine the levels of insoluble tau phosphorylated at this site. Levels of insoluble tau were normalized to soluble tau in the heat stable fractions and shown as a percentage.

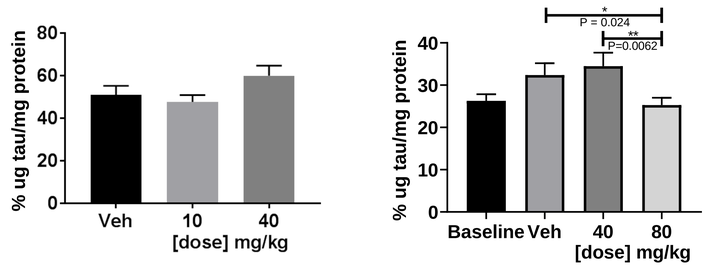


Figure 4. Heat stable tau P-Ser 396/404 in the forebrain. Heat stable tau from the forebrain was evaluated by ELISA using mAb PHF1 specific for tau P-Ser 396/404 for capture and pan-tau mAb DA9-HRP for reporter. Total tau levels were determined using pan-tau mAb DA31 for capture. Levels of phosphorylated tau were normalized to total tau and shown as a percentage.

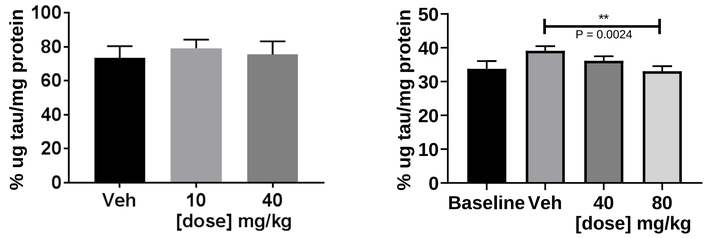
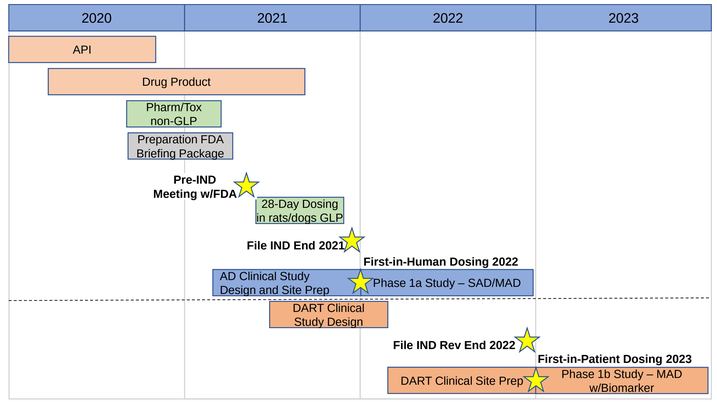


Figure 5. Heat stable tau P-Ser 396/404 in the hippocampus. Similar methods and results were found with the heat stable fractions from hippocampi as with the forebrain.

Clinical Program Planning

- Our core clinical program focuses on demonstrating the safety and efficacy of our lead compound in Alzheimer's Disease
- Our mechanism of action, early in the tau aggregation cascade, suggests that our compounds may be useful in other neurodegenerative diseases where abnormally aggregated tau is an important part of the pathophysiology.
- We have begun to explore the utility of our compound in these other tauopathies in a program that we call DART (Discovering Additional Research Opportunities in Tauopathy).
- These orphan diseases offer the opportunity to potentially demonstrate target engagement for our lead compound as early as phase Ib and may form the substrate for early product approvals through accelerated regulatory pathways.
- Together the core AD program, along with the second indication, defined through our DART program, represent significant medical and commercial potential for our compounds.

Clinical Development Timeline



Conclusion

- The ELISA evaluation of specimens from the therapeutic study in htau was completed.
- There was a 5.5-fold increase in exposure of the compound in serum between the 40 and 80 mg/kg treatment groups to about 4,702 ng/ml.
- Five months of treatment from 7 to 12 months of age caused a decrease in insoluble tau P-Ser 202 and a decrease in heat stable tau P-Ser 396/404 in the forebrain and hippocampus to baseline levels.
- Further analyses of behavioral data and specimens from htau and JNPL3 studies are in progress.
- API (GMP) completed and pre-formulation and preclinical safety studies in progress.

References

Davidowitz EJ, Krishnamurthy P, Lopez P, Jimenez H, Adrien L, Davies P and Moe JG. In vivo validation of a small molecule inhibitor of tau self-association in htau mice. J Alzheimers Dis. 2020;73(1):147-161.

Fá M, Puzzo D, Piacentini R, Staniszewski A, Zhang H, Baltrons MA, Li Puma DD, Chatterjee I, Li J, Saeed F, Berman HL, Ripoli C, Gulisano W, Gonzalez J, Tian H, Costa JA, Lopez P, Davidowitz E, Yu WH, Haroutunian V, Brown LM, Palmeri A, Sigurdsson EM, Duff KE, Teich AF, Honig LS, Sierks M, Moe JG, D'Adamio L, Grassi C, Kanaan NM, Fraser PE, Arancio O. (2016). Extracellular Tau Oligomers Produce An Immediate Impairment of LTP and Memory. Sci Rep 6, 19393.

Forest SK, Acker CM, d'Abramo C, Davies P (2013) Methods for measuring tau pathology in transgenic mouse models. J Alzheimers Dis 33, 463-471.

Tian H, Davidowitz E, Lopez P, Emadi S, Moe J, Sierks, M (2013) Trimeric tau Is toxic to human neuronal cells at low nanomolar concentrations. Int J Cell Biol 2013, Article ID 260787. https://doi.org/10.1155/2013/260787.

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