



OLIGOMERIX, Inc.

TARGETING TAU OLIGOMERS FOR DRUG DISCOVERY FOR ALZHEIMER'S DISEASE

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Abstract

OLIGOMERIX is uniquely focused on soluble tau oligomers as its target for drug discovery. Recent work is validating tau as a target for Alzheimer's disease (AD). Tau oligomer accumulation in brain specimens from AD patients and mouse models closely correlates with disease progression. OLIGOMERIX has shown that tau oligomers tend to progressively accumulate in cerebrospinal fluid (CSF) of AD patients and that extracellular tau may impair memory by disrupting synaptic function. High throughput assays are being used to screen compound libraries to discover small molecules that disrupt tau oligomers and that prevent their formation. Secondary assays are being used to validate hits by demonstrating activity against tau oligomers found in CSF of AD patients, and in a model memory assay in mouse hippocampal slices. Validated hits are tested *in vivo* in AD animal models. There is an urgent need for biomarkers that are specifically useful in diagnosing, stratifying, or monitoring the progression or regression of AD. They are also needed for the identification of new drug therapies to treat AD and to monitor the therapeutic efficacy of different medications during treatment. Novel biomarker assays are being developed for use in drug discovery that also have potential diagnostic applications for AD.

Validation of Tau Oligomer Target

AD Targets

There is a growing consensus that tau is a valid target for the development of therapeutics for AD and other tauopathies and that tau oligomers are the relevant toxic species (1, 2, 3). Plaques and tangles are now thought to be part of a protective mechanism that sequester neurotoxic oligomeric forms of A β and tau proteins into inert structures (3, 4). Mutations in the gene for tau protein cause a devastating dementia known as frontotemporal lobar degeneration (FTLD), demonstrating that abnormal forms of tau are sufficient for neurodegeneration causing memory loss and other neurological deficits (3). Tau is essential to A β neurotoxicity and memory deficits (3, 5). Tau oligomer accumulation in the brain correlates with disease progression in AD models (1).

Characterization of Tau Oligomers

Characterization of Tau Oligomers using TEM

Transmission electron microscopy (TEM) shows granular aggregates or fibrils of tau incubated with different conditions

Characterization of Tau Oligomers using SE-HPLC

HPLC Size exclusion chromatography of tau oligomers and tau monomer (top) and its disruption using small molecules (bottom)

Assay Validation

Validation of Tau Oligomer ELISA Inhibition using Tool Compounds

Relative % Oligomer

Compound	Relative % Oligomer
Daunorubicin	~80
Tannic acid	~30

Daunorubicin, an anthracycline chemotherapeutic, disrupts tau protein aggregates and inhibits their formation (6). ELISA methods described in (7)

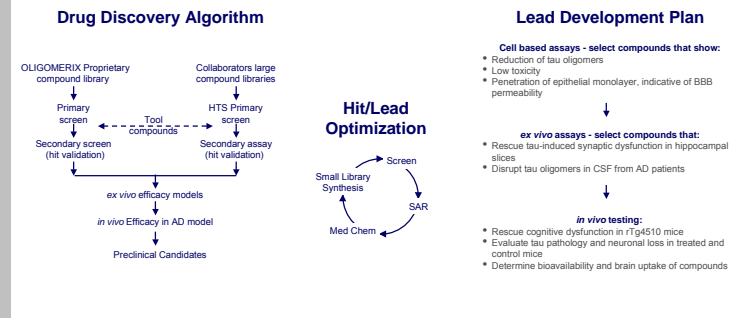
* Tannic acid, a plant polyphenol, destabilizes A β fibrils and inhibits their formation and extension (8)

Validation of SDS-PAGE Assay Using Tool Compound

Silver-stained gel of SDS resistant, reducing agent resistant, heat-stable tau oligomers (lanes 3 - 6)

* Daunorubicin inhibited the formation of tau oligomers (lane 10). PAGE methods described in (9)

Discovery - Compound Testing Algorithms



Compound Screening

Tau Oligomer ELISA

Tau Rate of Aggregation Assay

Tau Fibrillation Assay

Dose Dependent Effect on the Rate of Aggregation

Validation of TO-019

Representative Hits

Compound #	MW (Daltons)	Inhibition of Oligomer Formation (IC50)	Assay
TO-014	270	500 nM	SDS PAGE
TO-017	302	500 nM	SDS PAGE
TO-018	368	50 nM	ELISA
TO-019	379	1 μM	ELISA
TO-020	563	50 μM	SDS PAGE

Preclinical Development

Pre-clinical Development Study Design

rTg(tauP301L)4510 mice - conditional mouse model for tauopathy (10)

Loss of motor input induces degeneration of dorsal corticospinal tracts (10)

* Run in parallel

Biomarker Assays

Tau Oligomer Specific ELISA

The accumulation of tau oligomers in human CSF (11) was shown to correlate with disease stage in AD patients

Tau-A β 42 Specific ELISA

Tau-A β 42 complex was shown to be elevated in CSF from AD patients and predictive of disease state

An arbitrary cutoff set to 181 yields a preliminary assay sensitivity of 90% and specificity of 100%

	Calculated Positive	Calculated Negative
True Negative	9	1
True Positive	0	3

Conclusion

- Tau oligomers are a valid, novel molecular target for drug discovery for AD and other tauopathies
- Significant advancement has been demonstrated in the establishment of screening assays for drug discovery and development using the tau oligomer target
- The activity of TO-019 in disrupting tau oligomers from CSF validates this hit generated by our primary screening assays, provides additional validation data for our tau *in vitro* platform for drug discovery, and provides preliminary justification for advancing this compound to lead status
- AD specific biomarker assays show promise both for drug development and as potential diagnostic indicators for AD

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- Human CSF samples were provided by the Brain Bank at Columbia University

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