



Title of Challenge: UnTangle

Background

One of the most active areas of research into Alzheimer's disease centres on the tau protein that forms the classic neurofibrillary tangles. Aberrant intracellular inclusions composed of hyperphosphorylated filamentous tau are not only a neuropathological hallmark of Alzheimer's disease, but are also the key pathological species in progressive supranuclear palsy and other sporadic neurodegenerative disorders, which have been collectively termed tauopathies.

The discovery that pathogenic mutations in the tau gene microtubule associated protein tau (MAPT) can cause a familial neurodegenerative tauopathy has provided compelling evidence that tau dysfunction is sufficient to cause neurodegeneration. Many recent *in vivo* studies have shown that tau aggregates have 'prion-like' properties which not only allow them to transmit or seed further tau aggregation, but also spread to neighbouring cells or functionally connected brain regions. This process is referred to as 'tau propagation' and might explain the stereotypic progression of tau pathology in the brains of Alzheimer's disease patients.

Study of the development and spread of tau pathology in animal models requires large numbers, is time consuming and expensive. Often, the mechanisms and/or potential drug targets that are identified do not translate into humans.

3Rs benefits

Thousands of transgenic mice are used per year in large pharmaceutical companies with additional breeding animals to obtain the required genotype. There are specific challenges associated with tau transgenics:

1. *Phenotypic variability*. Mice often undergo chronic treatment with a candidate drug to test for disease modifying actions. Large numbers of animals are required per treatment arm to ensure clear results. For example, Lilly have noted wide phenotypic variability in mice expressing mutant human tau (JNPL3) leading to n=25 animals per treatment arm being required for drug studies.
2. *Variability in levels of tau expression*. Some transgenic lines express varying levels of tau and/or only a certain portion of mice are suitable for drug testing (e.g. only 25% of tg4510 mice are bigenic). Therefore, large breeding programmes are needed to generate sufficient numbers of mice for each study.

A human cell-based assay would reduce the number of transgenic animals used to investigate tau pathology and also improve the research and development of new treatments in this area.

Need for collaboration

The generation of an *in vitro* assay for tau aggregation, seeding, pathology, transmission and toxicity requires expertise from the fields of neuronal culture, iPS cells, electrophysiology, and biochemistry as well as from engineering and microfluidics. Sponsors will provide access to compounds and/or antibodies with which to validate the model along with expertise in tau pathology and in-house validation of the assay.

Overall aim

To develop a physiologically relevant human stem cell-derived neuronal assay to predict the efficacy and unexpected pharmacological effects of new chemical entities and biologics targeting tau in Alzheimer's disease.

Key deliverables

An assay which:

- Models tau aggregation, seeding and the related formation of insoluble hyper-phosphorylated tau with spread/transmission between cells
- Links to network dysfunction and neurotoxicity
- Provides the ability to research and predict the efficacy and unexpected pharmacological effects of novel compounds and antibodies
- Is not already available e.g. current models of tau phosphorylation

Phase 1

- Acquisition of cells from patients, mutation-carriers and healthy subjects
- Evidence of differentiation into neurons and glia, synaptic and neuronal markers, the presence of tau isoforms and activation states
- Details regarding materials and substrates planned to assess in Phase 2
- Investigation of potential of the assay for electrophysiology
- Consideration of commercial strategy

Phase 2

Development of an assay that:

- Can express different tau species, cleavage products, conformational changes, aggregated and phosphorylated tau
- Expresses synaptic markers and/or markers of plasticity (e.g. synaptophysin, NMDA receptors, PSD95)
- Is reproducible, scalable and has potential for use in screening
- Shows defined disease phenotypes in control and disease state iPS cells

Validation of the assay through:

- High content based imaging focusing on e.g. neurite length and branching, synapse density and shape, granularity measures
- Electrophysiology to measure activity in the culture system(s)

The system should demonstrate the capability to measure reliably:

- Neuron-neuron transmission of aberrantly folded tau (the 'prion hypothesis'), including the release and endocytic re-uptake of tau
- Neuronal: microglial interaction
- Neuro-inflammatory responses to aberrantly folded tau
- Mitochondrial function
- Synaptic activity (e.g. intracellular, extracellular, network)
- System should be developed and validated in within the scope of commercial use by the pharmaceutical industry

Sponsor in-kind contributions

Phase 1

- Sponsors will initially provide advice and input on tau biology and share some in-house data (e.g. from cell and animal models)
- Purified tau sample and/or tissue from transgenic animals if required for validation (e.g. positive control for Westerns)
- Reagents (e.g. tau antibodies for validation, possibly some existing iPS cell lines)

Phase 2

- Provide compounds and/or antibodies with which to validate the model
- Provide in house assessment of the *in vitro* system(s) using automated screening approaches e.g. in 96 well mode
- Provide access to in house cell and animal data for comparison and validation that the *in vitro* model recapitulates what has been detected *in vivo*

Duration

Phase 1: six months. Phase 2: up to three years

Budget

Phase 1: up to £100K. Phase 2: up to £1 million

Sponsors

Alzheimer's Research UK, Lilly and Janssen