Lilly

Answers That Matter.

CRACK IT

Alzheimer's Research UK

Defeating Dementia

Challenge:UnTangle

Presenters: Mike O'Neill and Hugh Nuthall

Lilly Research Centre, Eli Lilly & Co. Ltd., Windlesham UK

Sponsors:
NC3Rs, Alzheimer's Research UK, Lilly and Janssen

NC 3Rs National Centre for the Replacement Refinement & Reduction of Animals in Research

Lilly

Challenge: UnTangle

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.

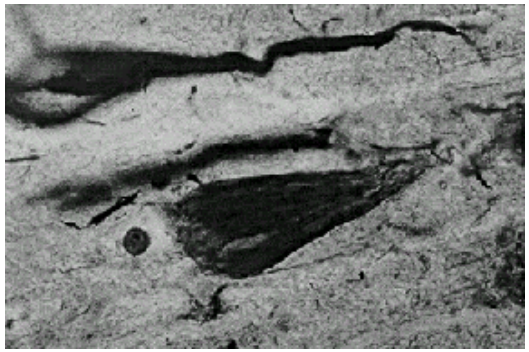
Key deliverables

An assay which:

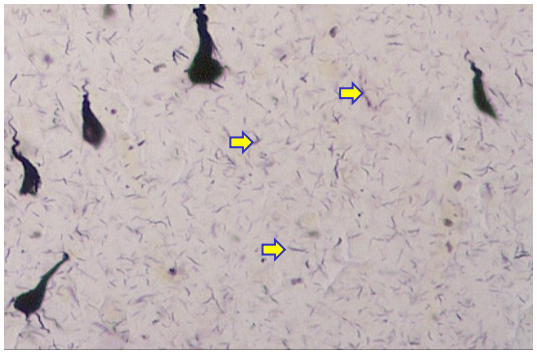
- Models tau aggregation, seeding and the related formation of insoluble hyper-phosphorylated tau with spread/transmission between cells
- Links to network dysfunction and neurotoxicity
- Provides the ability to research and predict the efficacy and unexpected pharmacological effects of novel compounds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

Neurofibrillary (tau) pathology is a defining feature of Alzheimer's Disease and related tauopathies

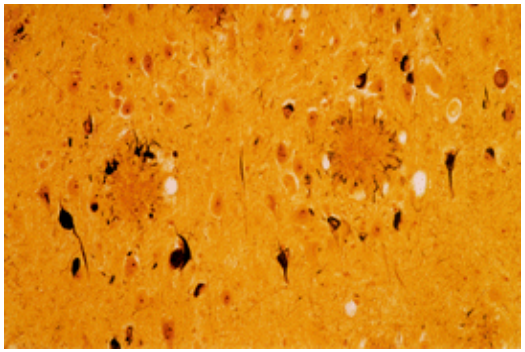
Neurofibrillary Tangles (NFT)



Neuropil Threads (NT)

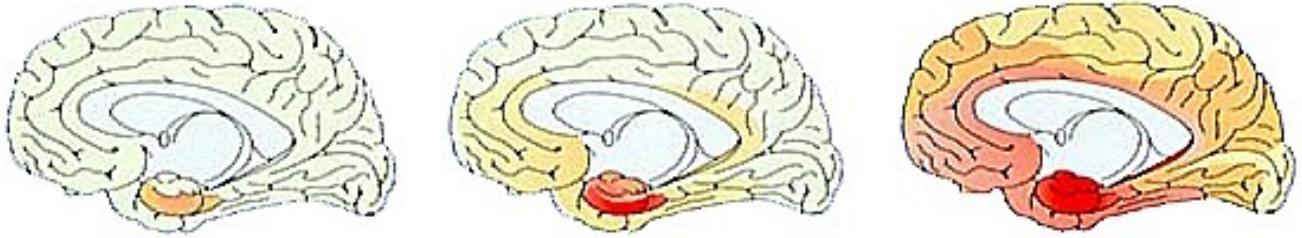


Tau positive neuritic plaques



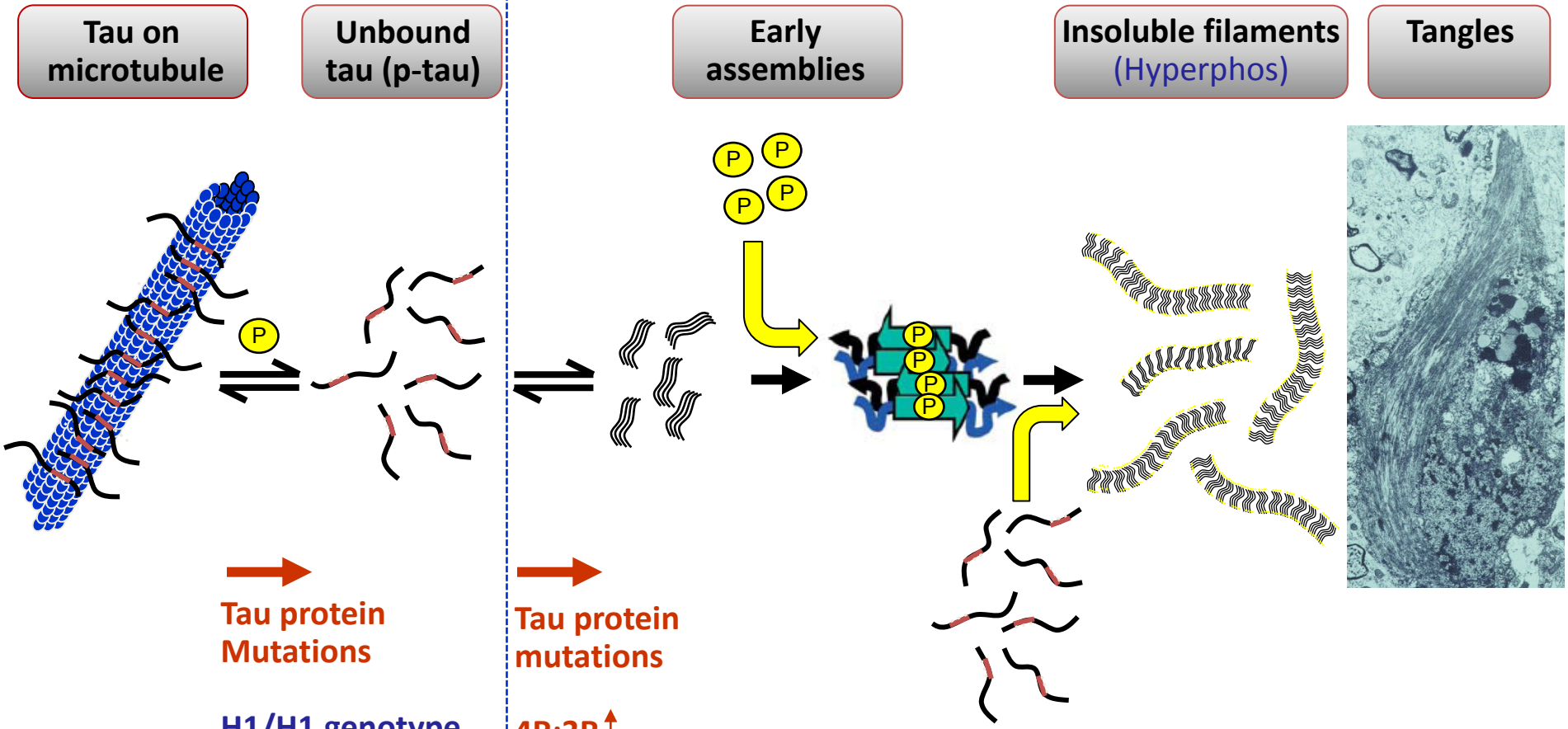
Clinically silent → **Incipient AD** → **Fully developed AD**

transentorhinal stages Braak Stage I-II | **limbic stages III-IV** | **neocortical stages V-VI**

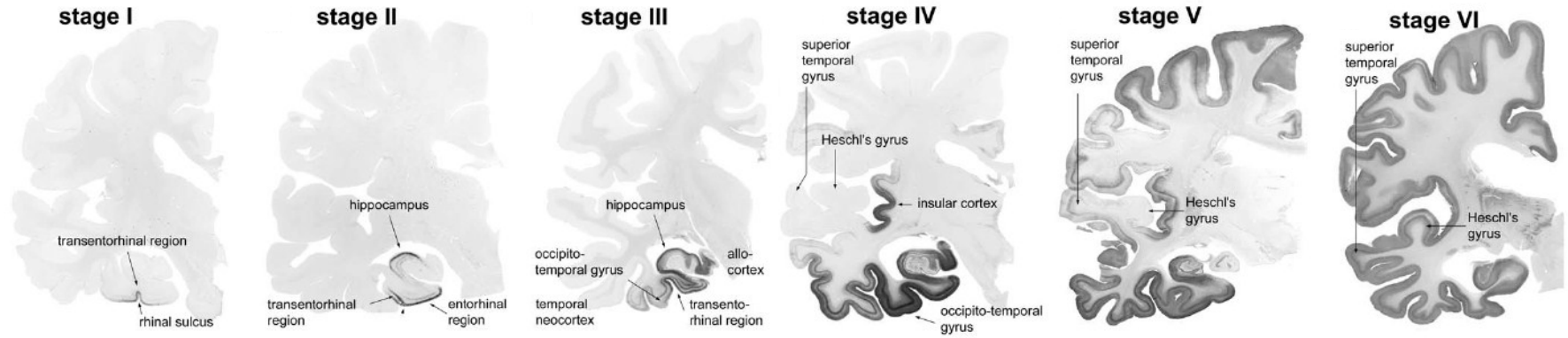


- Tau pathology (density and distribution) correlates with cognitive decline and neurodegeneration in Alzheimer's Disease

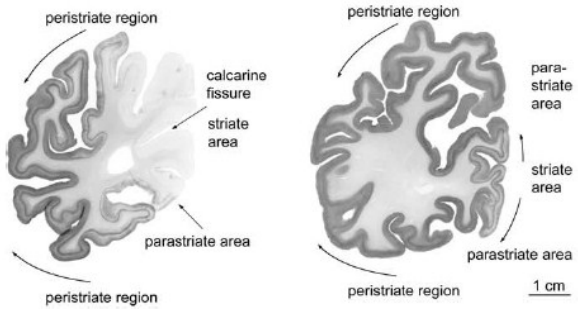
FTDP-17 and PSP genetics implicate tau misfolding/aggregation in the mechanism of neurodegeneration



Alzheimer's Disease: Spread of tau pathology with disease progression



Transentorhinal region → ***Entorhinal region*** → ***Hippocampus*** → ***Medial temporal gyrus*** → ***Superior temporal gyrus*** → ***Neocortex (primary)***



Peristriate region → ***Parastriate area***

Stereotypical progression/spread of tau pathology in AD

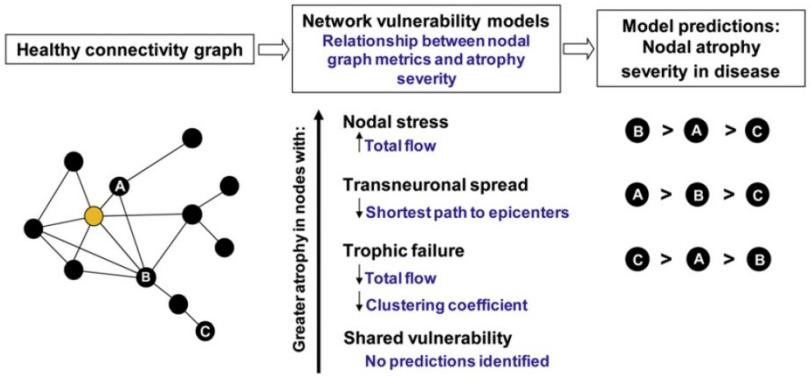
Network connectivity and spread of pathology in human neurodegenerative disease

Neuron
Article

Predicting Regional Neurodegeneration from the Healthy Brain Functional Connectome

Juan Zhou,[1,2] Efstathios D. Gennatas,[1] Joel H. Kramer,[1] Bruce L. Miller,[1] and William W. Seeley[1,*]

- Used task-free fMRI to derive the intrinsic connectivity patterns seeded by brain regions vulnerable to various neurodegenerative diseases
- Specific regions emerged as critical network "epicenters". Regions with higher total connectional flow and, more consistently, shorter functional paths to the epicenters, showed greater disease-related vulnerability
- These findings best fit a transneuronal spread model of network-based vulnerability

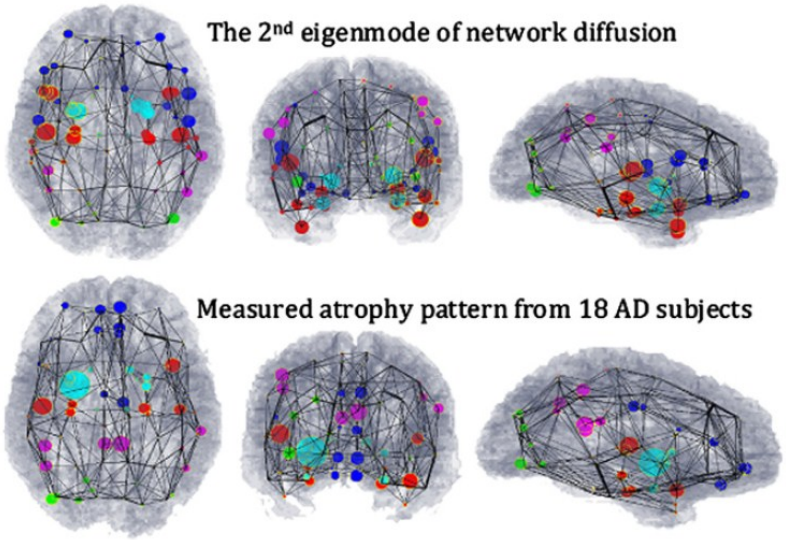


Neuron
Article

A Network Diffusion Model of Disease Progression in Dementia

Ashish Raj,[1,*] Amy Kuceyeski,[1] and Michael Weiner[2]

- Mathematically modeled trans-synaptic transmission of the brain's connectivity network from tractography of brain MRIs
- Subsequent graph theoretic analysis = testable, predictive model of dementia
- Predict spatially distinct "persistent modes" which recapitulate known patterns of dementia and match recent reports of selectively vulnerable dissociated brain networks



Zhou et al., Neuron 73, 1216-1227 (2012); Raj et al., Neuron 73, 1204-1215 (2012)

Transmission and spreading of tau pathology
- in vivo models

nature cell biology

Transmission and spreading of tauopathy in transgenic mouse brain

Florence Clavaguera[1], Tristan Bolmont[2], R. Anthony Crowther[3], Dorothee Abramowski[4], Stephan Fr
Alphonse Probst[1], Graham Fraser[3], Anna K. Stalder[5], Martin Beibel[4], Matthias Staufenbiel[4], Mathias
Michel Goedert[3,6,7] and Markus Tolnay[1,6,7]

1024 • The Journal of Neuroscience, January 16, 2013 • 33(3):1024–1037

Neurobiology of Disease

Synthetic Tau Fibrils Mediate Transmission of Neurofibrillary Tangles in a Transgenic Mouse Model of Alzheimer's-Like Tauopathy

Michiyo Iba, Jing L. Guo, Jennifer D. McBride, Bin Zhang, John Q. Trojanowski, and Virginia M.-Y. Lee
Center for Neurodegenerative Disease Research, Institute on Aging, Department of Pathology and Laboratory, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104

Neuron
Article

Propagation of Tau Pathology in a Model of Early Alzheimer's Disease

Alix de Calignon,[1,2,8] Manuela Polydoro,[1,8] Marc Suárez-Calvet,[1,3] Christopher William,[1] David H. Adamowicz,[1] Kathy J. Kopeikina,[1,4] Rose Pitstick,[5] Naruhiko Sahara,[6] Karen H. Ashe,[7] George A. Carlson,[5] Tara L. Spires-Jones,[1] and Bradley T. Hyman[1,*]
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[5]McLaughlin Research Institute. Great Falls, MT 59405, USA
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[8]These authors contributed equally to this work

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PLoS one

Trans-Synaptic Spread of Tau Pathology *In Vivo*

Li Liu[1], Valerie Drouet[1], Jessica W. Wu[1], Menno P. Witter[2], Scott A. Small[3], Catherine Clelland[1], Karen Duff[1,4]*

1 Department of Pathology and Cell Biology, Taub Institute for Alzheimer's Disease Research, Columbia University, New York, New York, United States of America, **2** Kavli Institute for Systems Neuroscience and Centre for the Biology of Memory, Norwegian University of Science and Technology, Trondheim, Norway, **3** Department of Neurology, Taub Institute for Alzheimer's Disease Research, Columbia University, New York, New York, United States of America, **4** Department of Psychiatry, New York State Psychiatric Institute, New York, New York, United States of America

Example of an *in vivo* propagation model with injection of brain extracts

nature cell biology

Transmission and spreading of tauopathy in transgenic mouse brain

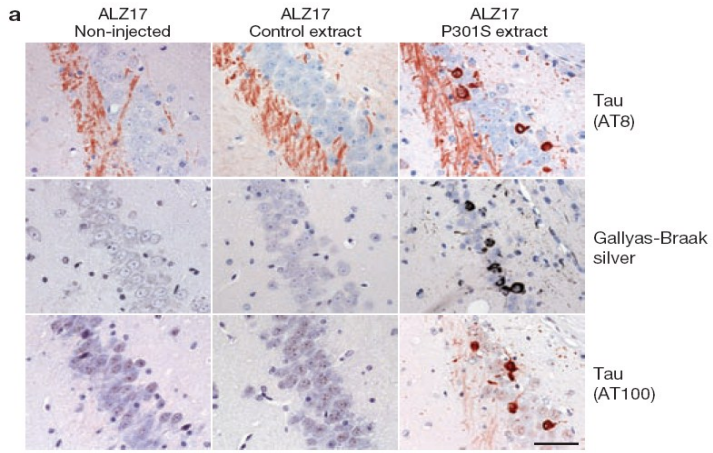
Florence Clavaguera[1], Tristan Bolmont[2], R. Anthony Crowther[3], Dorothee Abramowski[4], Stephan Frank[1], Alphonse Probst[1], Graham Fraser[3], Anna K. Stalder[5], Martin Beibel[4], Matthias Staufenbiel[4], Mathias Jucker[2], Michel Goedert[3,6,7] and Markus Tolnay[1,6,7]

6 month old
Robust tau pathology
Brains homogenised

3 month old
No tau pathology
Homogenate infused

6 - 15 months
Distribution of tau pathology?

1. ***Seed*** *– Brain extracts from old P301S mice containing pathological tau*
2. ***Transmission*** *– Induction of tau pathology in ALZ17 mice*
3. ***Spread*** *– Tau pathology anterior, posterior and contra-lateral to infusion site*



Reason for challenge and 3R's 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:

• *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.

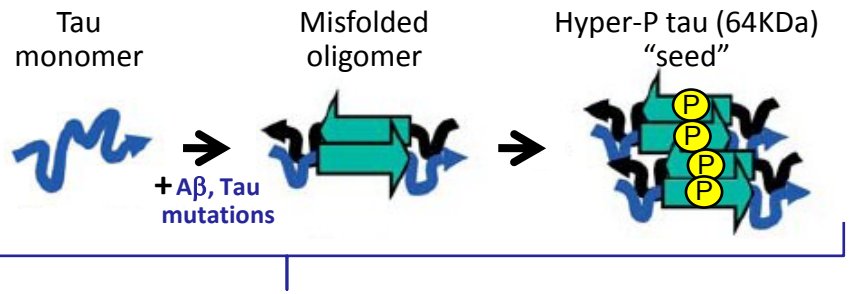
•*Variability in levels of tau expression.* Some transgenic lines express varying levels of tau and/or only a certain portion of mice are suitable for drug testing (e.g. only 25% of tg4510 mice are bigenic). Therefore, large breeding programmes are needed to generate sufficient numbers of mice for each study.

• *In vivo tau propagation models:* In addition to the general concerns above many of the literature models are labour intensive and some take a long time (12-18 months) to develop a network spread of tau pathology.

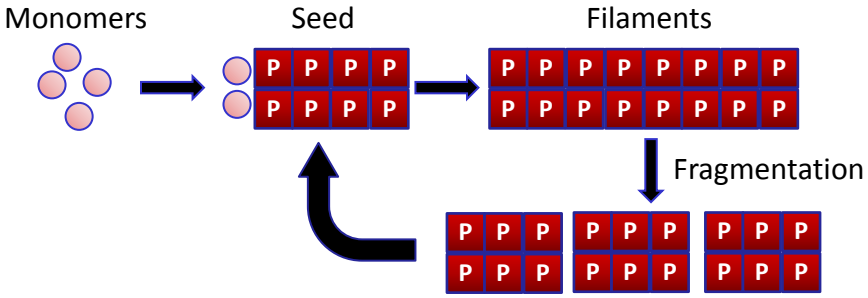
A human cell-based assay would reduce the number of transgenic animals used to investigate tau pathology, allow basic mechanistic studies (tau species involved, mechanisms of transfer and spread) to be carried out rapidly and allow assessment of target interventions. This would have a huge impact and improve the research and development of new treatments in this area.

Tau pathogenesis – *Initiation, transmission and spreading*

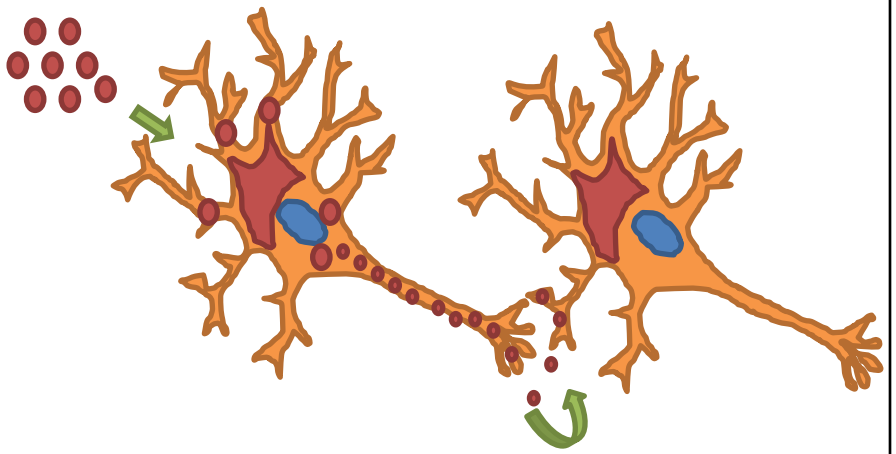
1. Initial stochastic misfolding/aggregation events
(determines type of tauopathy - location, morphology)



2. Autocatalytic intraneuronal propagation of tau filaments



3. Release of tau seeds leads to trans-neuronal spreading of tau pathology



Tau propagation in cultured cells seeded with pre-formed tau fibrils

Propagation of Tau Misfolding from the Outside to the Inside of a Cell*

Received for publication, November 19, 2008, and in revised form, March 2, 2009 Published, JBC Papers in Press, March 11, 2009, DOI 10.1074/jbc.M808759200

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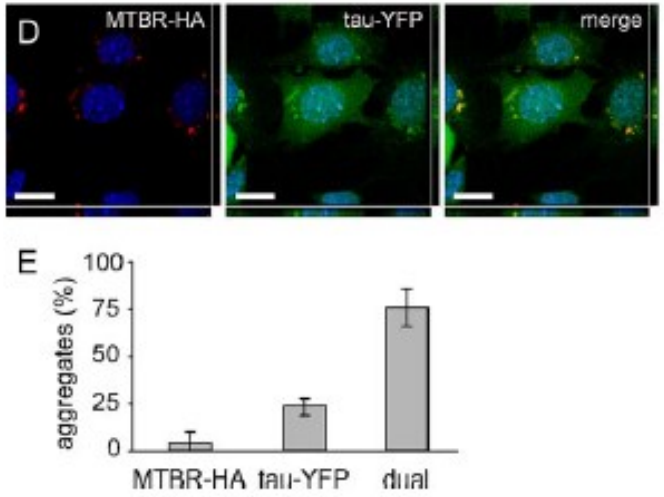

Seeding of Normal Tau by Pathological Tau Conformers Drives Pathogenesis of Alzheimer-like Tangles*[S]

Received for publication, December 4, 2010, and in revised form, March 3, 2011 Published, JBC Papers in Press, March 3, 2011, DOI 10.1074/jbc.M110.209296

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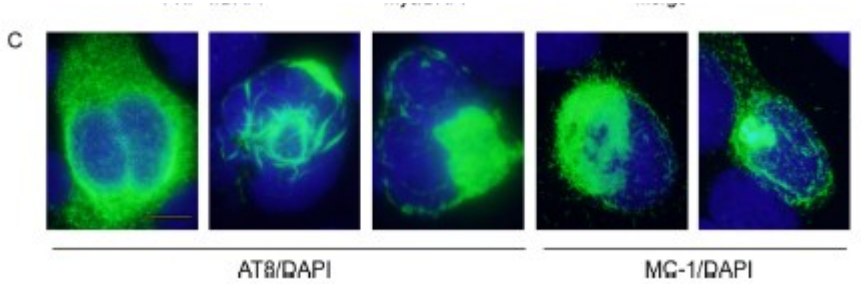
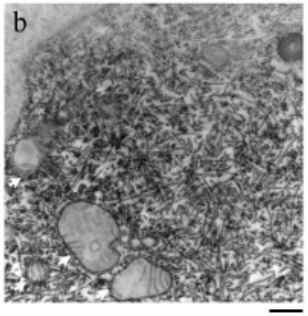
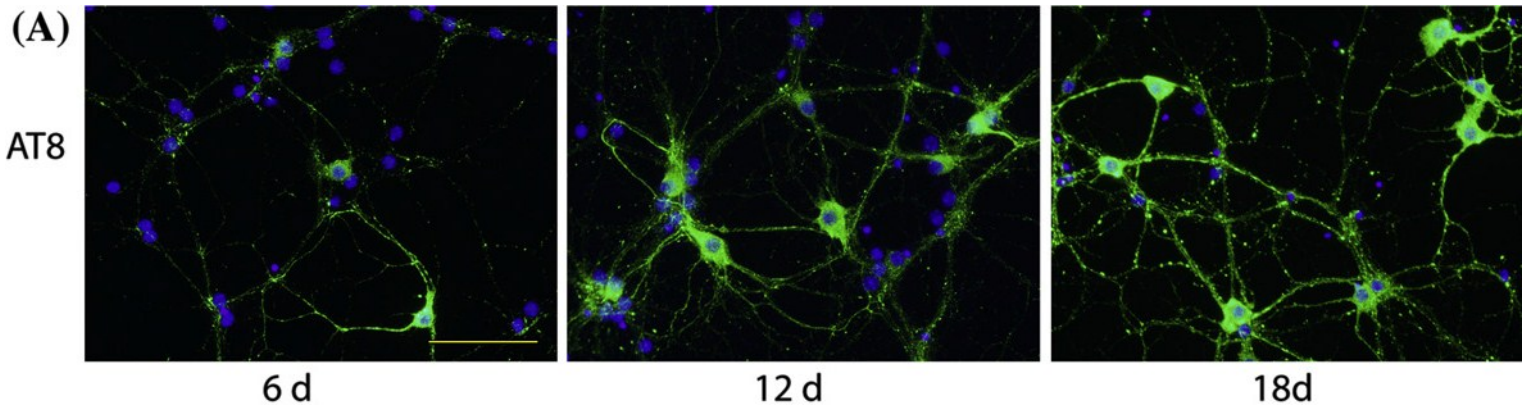
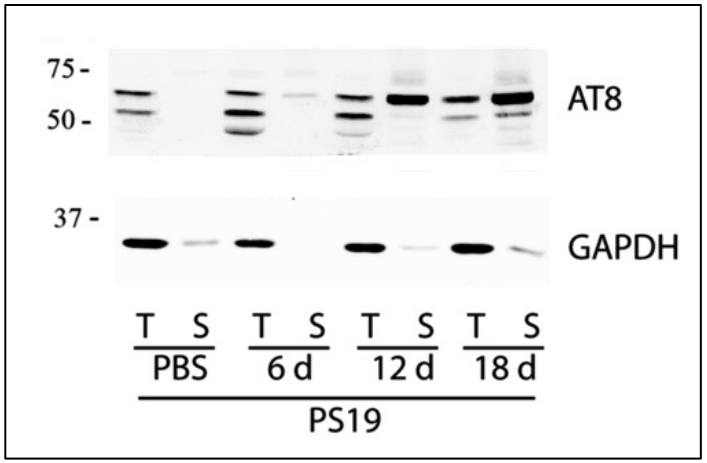




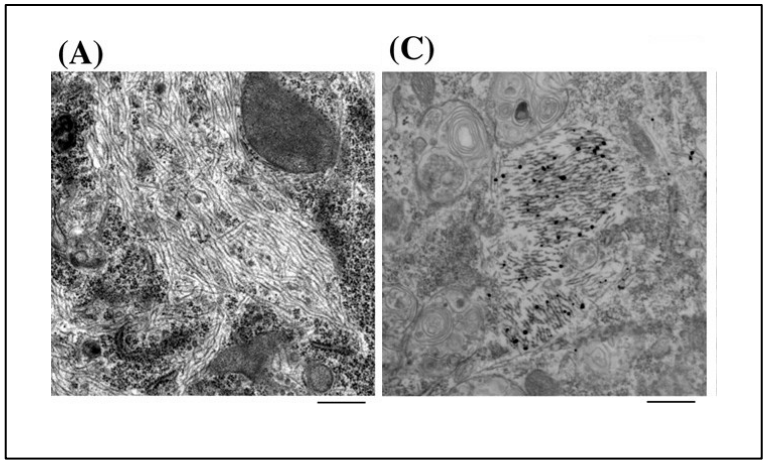
Tau propagation in primary neuronal cells from PS19 mice (P301S, 1N4R) seeded with pre-formed fibrils (K18-P301L)

Time-dependent increase in pff-induced tau pathology





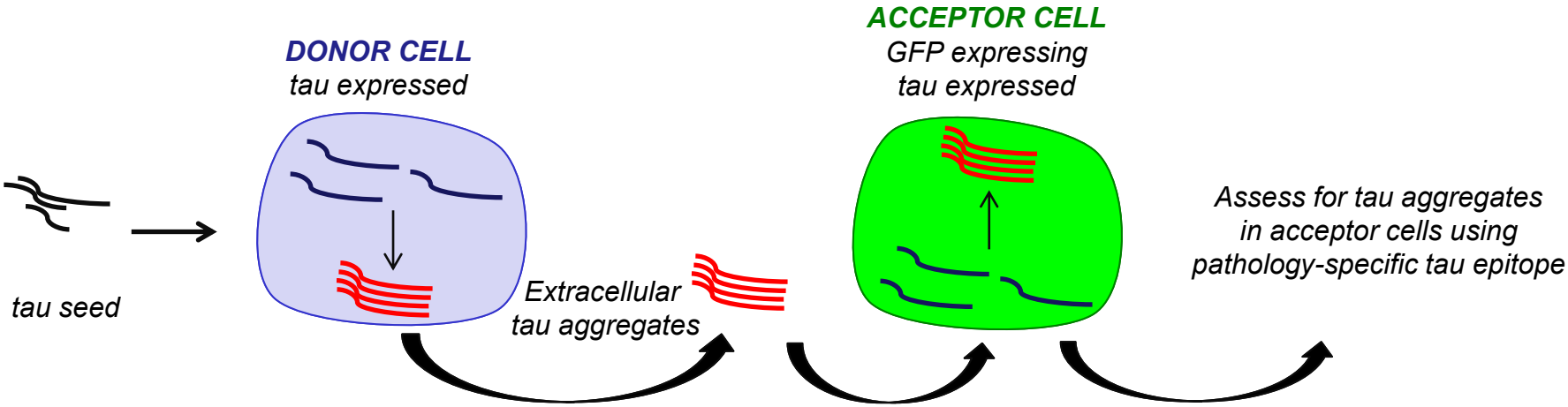
Time-dependent increase in hyper-phosphorylated, insoluble tau

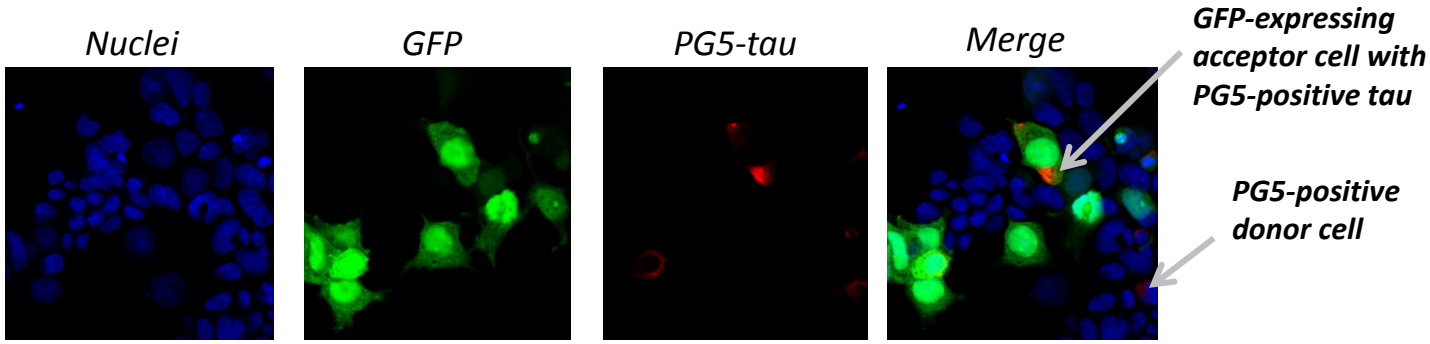


EM reveals cytoplasmic filaments in neurons treated with pffs

Guo and Lee, FEBS Letts 2013

Transfer of tau aggregates from donor to acceptor cells demonstrated using co-culture experiments





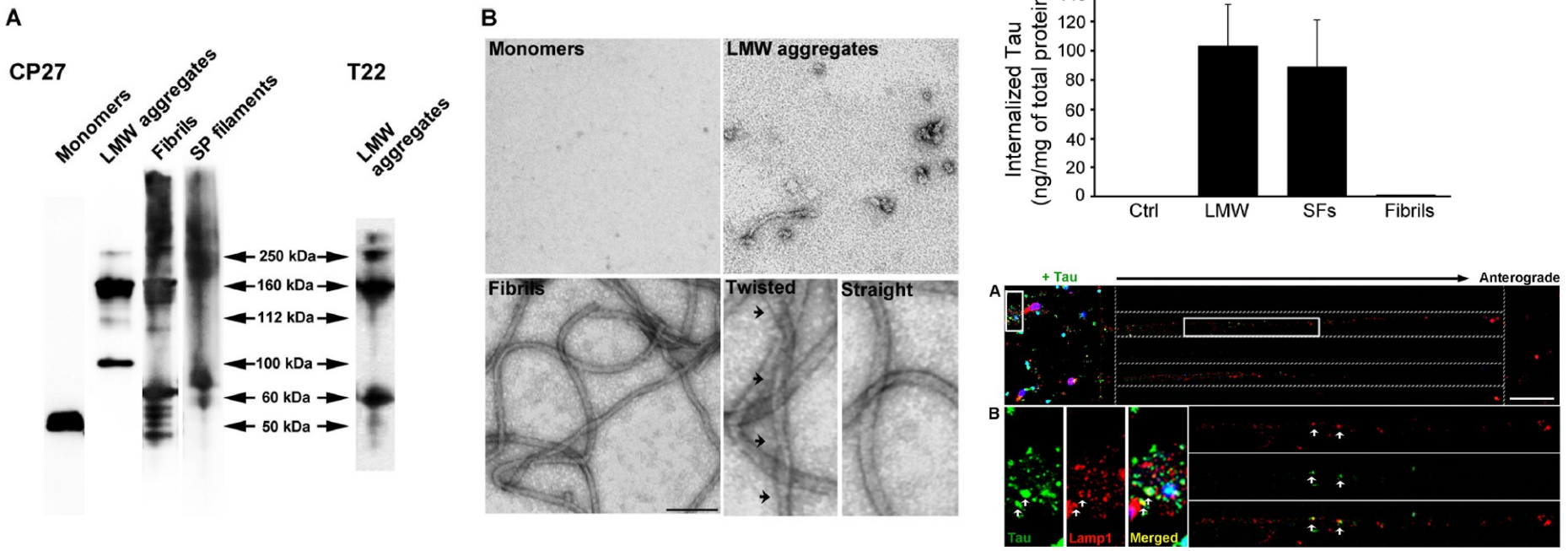
Co-culture experiments - transfer of aggregates from donor to acceptor cells

Various techniques (microfluidic chambers) can be used to show transport of tau species in vitro

Small Misfolded Tau Species Are Internalized via Bulk Endocytosis and Anterogradely and Retrogradely Transported in Neurons*

Received for publication, June 22, 2012, and in revised form, November 21, 2012 Published, JBC Papers in Press, November 27, 2012, DOI 10.1074/jbc.M112.394528

Jessica W. Wu‡, Mathieu Herman‡, Li Liu‡, Sabrina Simoes‡, Christopher M. Acker§, Helen Figueroa‡, Joshua I. Steinberg‡, Martin Margittai¶, Rakez Kayed‖, Chiara Zurzolo**, Gilbert Di Paolo‡, and Karen E. Duff‡,‡‡1



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

Key Deliverables

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 and Capabilities of the Assay and model system(s)

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 reliably measure:

- Neuron-neuron transmission of aberrantly folded tau 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 within the scope of commercial use by the pharmaceutical industry

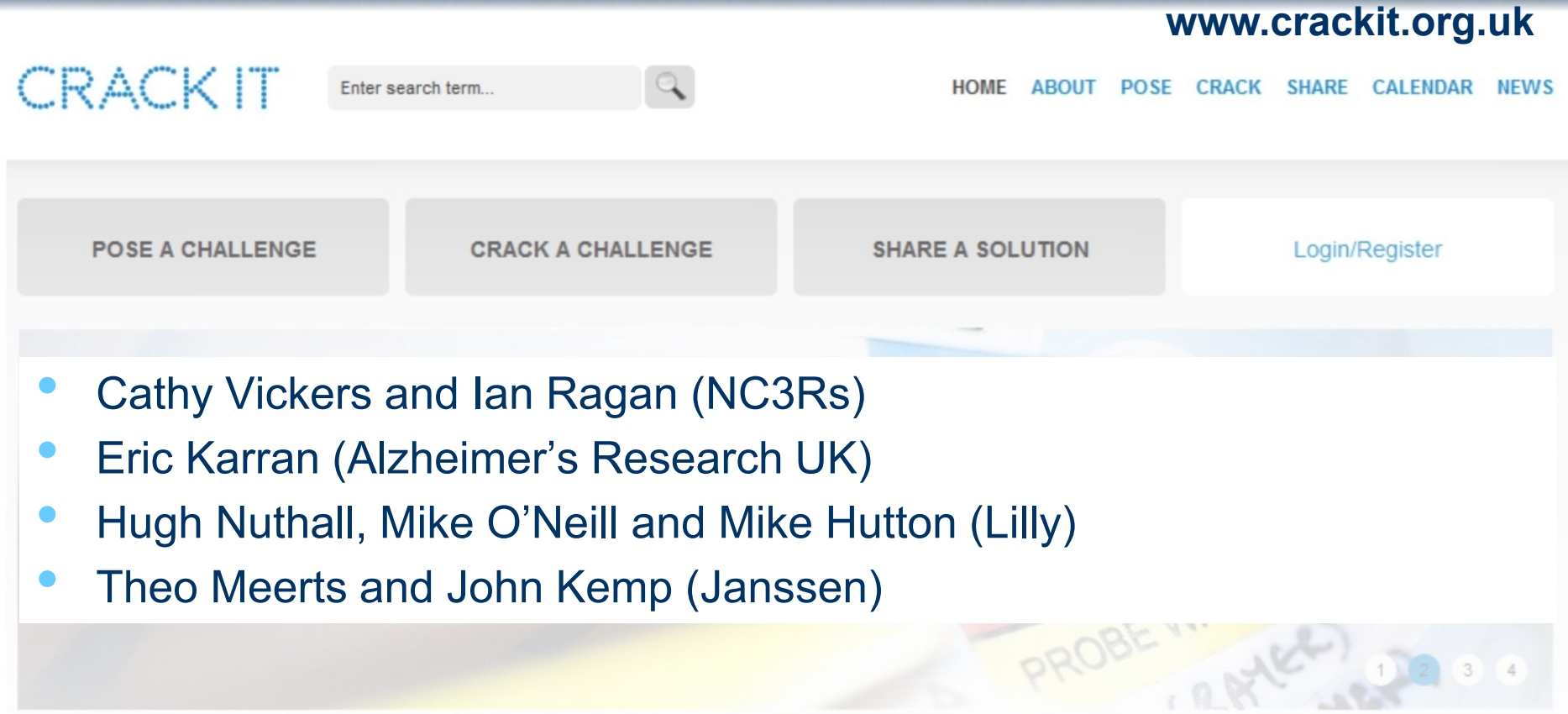
Budget and Timing

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

Acknowledgements



- Cathy Vickers and Ian Ragan (NC3Rs)
- Eric Karran (Alzheimer's Research UK)
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- Theo Meerts and John Kemp (Janssen)

NC 3Rs National Centre for the Replacement Refinement & Reduction of Animals in Research

Alzheimer's Research UK Defeating Dementia

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