Tau-directed treatment approaches in Alzheimer’s disease
How far have we come?

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EADC Symposium,
26. Alzheimer Europe Conference, Copenhagen
31.10.-2.11.2016
Stages of the Pathologic Process in Alzheimer Disease: Age Categories From 1 to 100 Years

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J Neuropathol Exp Neurol
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D stages 1a, 1b, I-II  
E stages III-IV  
F stages V-VI

no pathology  
Tau pathology  
Amyloid pathology
Human Microtubule-Associated Protein Tau – a complex protein

**Six human brain tau isoforms**

Alternative mRNA splicing of E2 (red), E3 (green), and E10 (yellow) gives rise to six tau isoforms.

 Constitutively spliced exons (E1, E4, E5, E7, E9, E11, E12, and E13) are shown in blue.

The repeats (R1–R4) are shown, with three isoforms having four repeats each (4R) and three isoforms having three repeats each (3R).

Highly conserved region, necessary for microtubule-binding
Post-translational modifications of tau protein

• Functional interactions of tau are highly regulated at the post-translational level by protein phosphorylation and a large variety of other modifications (O-glycosylation, glycation, unquietination, SUMOylation, nitration, methylation and acetylation), prolyl isomerisation and truncation.

numerous phosphorylation sites exist on tau protein

• The combination of alternative splicing with site-specific posttranslational modifications of tau gives rise to an enormous heterogeneity of individual tau molecules

A pathological pathway leads from soluble to insoluble / filamentous tau:
This ordered assembly causes disease and is a gain-of-toxic function. It involves
the transition from an intrinsically disordered monomer to a highly structured filament.

Short tau fibrils constitute the major species of seed-competent pathological tau in the brain.

Goedert M. Alzheimer’s & Dementia 12 (2016) 1040-1050
Main hypothesis
1. Antibody will enter the brain
2. bind to a pathological species, which leads to subsequent clearance and prevent the spread of pathology.
3. Two complementary hypotheses:
   - **extracellular tau** or tau associated with the synaptic membrane, which may be detrimental to neuronal function and/or
   - **intracellular tau**: Ab’s are taken up by neurons, promote tau clearance by disassembling aggregates by lysosomal enzymes

Mechanism of Tau involvement in Neurodegeneration – unresolved questions

Question 1:
Does tau pathology come upstream or downstream to Aβ pathology?
Several lines of evidence now suggest an interplay between tau and Aβ in both directions, i.e. Aβ can drive tau pathology and tau can drive Aβ pathology.

Question 2:
How does tau pathology spread from one molecule to the next (intracellularly)?
Current findings suggest a model of progression of tau pathology through the brain in a prion-like manner of self propagation.

Question 3:
How does (intracellular) tau pathology spread out through the brain (transcellularly)?
Fibrillary tau aggregates but not monomeric tau can be taken up by cells and subsequently induce fibrillization of intracellular tau.
a. trans-synaptically in anatomically connected brain areas.
b. via non-synaptic propagation, e.g. by ectosomal and exosomal release mechanism

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Clinical trials program with TRx 0237 (LMTX™)

- **TRx 0237 (LMTX™)** is a second-generation tau protein aggregation inhibitor for the treatment of Alzheimer's disease (AD) and frontotemporal dementia. It is a replacement formulation for Rember®, the first proprietary formulation of methylthioninium chloride (MTC).
- Both TRx 0237 and Rember are purified forms of Methylene Blue.

- **Mechanism of action:** To prevent tau aggregation or dissolve existing aggregates to interfere with downstream pathological consequences of aberrant tau in Alzheimer's and other neurodegenerative diseases
Clinical trials program with TRx 0237 (LMTX™) in Alzheimer’s disease

• A 4-week Phase 2 safety study of 250 mg/day of TRx0237 in patients with mild to moderate Alzheimer's disease began in September 2012 but was terminated in April 2013 for administrative reasons.

• A Phase 3 study compares a single 200 mg/day dose to placebo in a planned 700 patients with a diagnosis of either all-cause mild dementia or Alzheimer's disease (MMSE >20).
  – more than 90 sites in North America and Europe.
  – As primary outcomes, this trial uses standard cognitive (ADAS-Cog 11) and clinical (ADCS-CGIC) batteries, as well as temporal lobe brain metabolism as measured by FDG-PET and safety parameters.

• The 2nd Phase 3 trial compares 150 and 250 mg/day of TRx0237 to placebo in 891 patients with mild to moderate Alzheimer's disease (MMSE >13).
  – 91 sites in North America, Australia, Europe, and Asia.
  – It uses clinical (ADCS-CGIC), cognitive (ADAS-Cog 11), and safety measures as primary outcomes.
  – Treatment duration was 15 months
Results of the phase III clinical trial with TRx 0237 (LMTX™) in AD

- The drug missed its co-primary endpoints of slowing cognitive and functional decline in mild to moderate Alzheimer’s disease, as measured by the ADAS-cog and ADCS-ADL battery
  - Approx. 30% drop-out
  - Both standard treatment (Memantine or AChE-I) and no standard treatment were allowed
  - The plc group had 5mg LMTX for blinding reasons (urine coloration)
  - Adverse events: 25% diarrhea, 10% dysuria (pain)
- In a pre-specified subgroup analysis, 136 subjects (15%) of the 891 patients in the trial not on standard therapy, but in the active groups with LMTX™ had a slower cognitive and functional decline with less brain atrophy
  - However, they were not compared to an adequate control group
  - Also, this was a secondary analysis;

Indirect approaches to Tau aggregation: inhibition of $O$-GlcNACase (OGA)

- Merck Sharpe & Dohme / Alectos (MK-8719)
- small-molecule-based indirect attack on tau. Because tangle formation depends on the hyperphosphorylation of tau, the strategy is to control the post-translational processing of tau with the sugar tag $O$-linked $N$-acetylglucosamine ($O$-GlcNAc). $O$-GlcNAc is physiologically added to tau at the same residues at which hyperphosphorylation occurs. By inhibiting protein $O$-GlcNAcase (OGA), the enzyme that cleaves $O$-GlcNAc from post-translationally modified proteins, prevent the hyperphosphorylation of tau and thereby reduce the development of tangles.
- Phase I trial of the OGA inhibitor (MK-8719) completed in healthy subjects.

Anti-Tau antibodies for AD

- 4 classes of Antibodies: (i) N-terminal; (ii) hyperphosphorylation; (iii) conformational; and (iv) truncation-specific

- **AC Immune / Genentech**: RO7105705; mouse monoclonal antibody against phospho-serine 409 of tau; humanization of two antibodies hACI-36-2B6-Ab1 and hACI-36-3A8-Ab1; Phase I in mild to moderate AD and in healthy volunteers

- **Axon Neuroscience**: DC8E8; mouse antibody against tau peptide 294–305(AADVac1), qualified in a transgenic rat model expressing truncated human tau and humanized. No phase I, yet.

- **Biogen / Panima Neurosciences**: tau autoantibodies from older healthy individuals with no sign of degenerative tauopathy (B cell clones), no phase I, yet.

- **C2N Diagnostics / AbbVie**: ABBVie-8E12, mouse antibody against tau peptide 25–30. In P301S transgenic mice reduced levels of hyperphosphorylated tau. Phase I trial in PSP.

- **iPerian / BMS**: antibodies IPN001 and IPN002 recognize an N-terminal epitope (residues 9–18); In P301L mice partial reversal of progressive motor deficits; Phase I trial in healthy volunteers
Active tau vaccines in clinical trials: ACI-35

- **ACI-35**: developed by AC Immune AG with Janssen Pharmaceuticals.

- **Vaccine**: 16-amino acid tetra-palmitoylated phospho-tau peptide (tau393-408[pS396, pS404]) in liposomes of di-myristoylphospho-tidylcholine (DMPC), di-myristoylphosphatidylglycerol (DMPG), cholesterol, and the adjuvant monophosphoryl lipid A (MPLA).

- **Transgenic P301L mice**: rapid immune response against the immunogen in wild type and transgenic P301L mice, resulting in a mild reduction of hyperphosphorylated (64 kDa) tau and tau pathology by immunohistochemical characterization. No adverse inflammatory response, suggesting a good safety profile for human studies.

- **Clinical trials (ISRCTN13033912)**: Phase Ib w/ plc; completed, (end 2015, 1 Finland + 3 UK) in patients with mild to moderate Alzheimer's disease: injection 2, 3 or 5 times over 6 months, booster injection after 6 or 16 months. Outcome: safety and immunogenicity (antibody titre response against pTau), 5x MRI + 2x CSF + clinical measures.
Active tau vaccines in clinical trials: AADvac1

- **AADvac1**: first anti-tau vaccine developed by Axon Neuroscience (Bra-tislava, Slovak Republic).

- **Vaccine**: a tau peptide fragment, (294KDNIKHVPGGGS305), linked to keyhole limpet hemocyanin (KLH) through an N-terminal cysteine, and administered with an Alhydrogel aluminum adjuvant. Designed to target misfolded tau in AD.

- **Transgenic tau rats**: reduced tau pathology and associated behavioral deficits.

**Tau peptide vaccine (AADvac01) induces antibody titres specific to the tau peptide**

294KCNIKHVPGGGS305

This regulatory domain is essential for pathological tau-tau interaction in AD

*Kontsekova et al. Alzheimer Res & Therapy 2014: 6;44*
Active tau vaccines in clinical trials: AADvac1

• **Clinical trial phase Ib**: (Austria 2013-2015) 12-week RCT placebo-controlled with 12-week open-label extension (NCT 2012-003916-29) in 30 patients with mild-moderate AD.

• Randomization: 4:1 3 s.c. doses of AADvac01 (n=24; 67.7 years of age, MMSE 20.7, 58% Apo E4, 22 patients on standard treatment) or plc (n=6; 68.5 years of age, MMSE 20.3, 67% Apo E4, 5 patients on standard treatment) in monthly intervals. Open-label extension with all patients on active treatment.

• 2 patients withdrawn due to SAE (viral infection/epilepsy, multimorbidity/ troponin T elevation), 5 patients with SAE. 50% local injection site reaction.

• No safety signals in clinical (e.g. allergy), lab or MRI.

• AACvac01 was highly immunogenic = 29 patients (mean Ab IgG titre 1:31415 against peptide 108

• Antibodies raised by AACvac01 targeted pathological Tau isolated from human AD brain

*Novak P et al. (2016) Lancet Neurol in press*
Active tau vaccines in clinical trials: AADvac1

- **Phase II clinical trial:** 24 Months Safety and Efficacy Study of AADvac1 in Patients With Mild Alzheimer's Disease (ADAMANT)
- 185 subjects, 6 countries (approx. 20 sites): 60% on AADvac1 s.c. 40% on placebo s.c., started in Sep 2016

- Safety / tolerability: AEs, vital signs, ECG, lab, MRI, physical and neurological examination, Columbia Suicide Severity Rating Scale (C-SSRS) and Patient Diary
- Secondary Outcomes: CDR - Sum of Boxes, Cogstate International Shopping List Task
Conclusions I

• Tau-directed therapeutic approaches are a rapidly evolving field in translational Alzheimer‘s research.
  – The mechanisms of pathological tau assembly appear the most rational treatment targets at present.
  – Controversy about intracellular versus extracellular treatment targets

• The molecular biology and pathophysiology of Tau protein is even more complex and diverse than amyloid pathology.
  – Widespread physiological functions (axonal transport, „synaptic break“, neuronal cell cycle)
  – Cascade of pathological tau assembly in the human brain

• At last 14 compounds have been developed which target tau aggregation and aim at removing tau seeds by active and passive immunotherapy.
Conclusions II

• At least 6 compounds are in phase I – III of clinical development
• TRx 0237 (LMTX™), a second-generation tau protein aggregation inhibitor, has been shown to be ineffective in a phase III clinical trial.
  – The results of a second comparable clinical trial are planned to be presented in December 2016
• AADvac1, a 13-amino acid tau peptide fragment, is the first anti-tau vaccine in clinical testing.
  – Phase Ib results showed a good safety profile
  – Phase II clinical trial (185 patients w/ mild-moderate Alzheimer dementia) is recruiting
• ACI-35, a 16-amino acid phospho-tau peptide, is the second anti-tau vaccine in clinical testing
  – Phase Ib results have been completed, but not reported, yet.