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Review - Part of the Special Issue: Alzheimer's Disease - Amyloid, Tau and Beyond

Tau-aggregation inhibitor therapy for Alzheimer's disease

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ABSTRACT

Many trials of drugs aimed at preventing or clearing β-amyloid pathology have failed to demonstrate efficacy in recent years and further trials continue with drugs aimed at the same targets and mechanisms.

The Alzheimer neurofibrillary tangle is composed of tau and the core of its constituent filaments are made of a truncated fragment from the repeat domain of tau. This truncated tau can catalyse the conversion of normal soluble tau into aggregated oligomeric and fibrillar tau which, in turn, can spread to neighbouring neurons. Tau aggregation is not a late-life process and onset of Braak stage 1 peaks in people in their late 40s or early 50s. Tau aggregation pathology at Braak stage 1 or beyond affects 50% of the population over the age of 45.

The initiation of tau aggregation requires its binding to a non-specific substrate to expose a high affinity tau–tau binding domain and it is self-propagating thereafter. The initiating substrate complex is most likely formed as a consequence of a progressive loss of endosomal–lysosomal processing of neuronal proteins, particularly of membrane proteins from mitochondria. Mutations in the APP/presenilin membrane complex may simply add to the age-related endosomal–lysosomal processing failure, bringing forward, but not directly causing, the tau aggregation cascade in carriers.

Methylthioninium chloride (MTC), the first identified tau aggregation inhibitor (TAI), offers an alternative to the amyloid approach. Phase 3 trials are underway with a novel stabilized reduced form of methylthioninium (LMTX) that has improved tolerability and absorption.

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1. The β-amyloid consensus in Alzheimer's disease

Variations of the β-amyloid theory of Alzheimer's disease (AD) have commanded a remarkable degree of academic consensus in the field for the last 20 years. This consensus has directed an estimated spend of $15 billion in the search for a disease-modifying treatment for a disease of vast societal cost. However, some 19 drugs have failed to demonstrate efficacy in randomised clinical trials or their development has been halted [1,2]. These drugs have different mechanisms of action, but share a proposed effect in reducing amyloid pathology (Table 1). These drugs have been sub-classified into those that (a) modulate processing of β-amyloid protein precursor (APP), e.g. via α-, β- and γ-secretases; (b) are small molecule inhibitors of amyloid aggregation or accumulation; or (c) enhance clearance of amyloid via active or passive immunotherapeutic approaches. In all cases, the failure of the drugs is not dependent on the mechanism of action. Furthermore, ongoing trials have similar targets to those that have already proved unsuccessful on several occasions. The results of a human post-mortem study demonstrated clearance of β-amyloid deposits in the brains of subjects actively immunised with Aβ42 peptide (AN-1792), but strikingly showed that this treatment had no impact on either clinical disease progression or progression of tau aggregation pathology [3]. Failures of solanezumab and bapineuzumab alone mark 5 large phase 3 trial failures for drugs that had suggested efficacy in phase 2 based on technical (i.e. reduction in CSF β-amyloid), but not clinical readouts. Without considering phase 1 studies, a total of nearly 15,000 subjects have been involved in these failed trials to date.

It is surprising that this record of failure has not really led to a reconsideration of the fundamental assumptions of the theory. Whereas it used to be held that β-amyloid deposition was central to the pathophysiology and pathogenesis of AD at any stage, the record of failure in disease of mild or moderate severity has led only to a repositioning of the same claims to earlier preclinical stages of the disease. Mild and moderate disease is now assumed to be too late for therapeutic intervention. The prevailing conjecture now is that treatment has to be initiated in the decades before disease appears, e.g. the Dominantly Inherited Alzheimer Network (DIAN) trial and the Anti-Amyloid in Asymptomatic Alzheimer's disease (A4) trial [4], where investigators will test β-amyloid-clearing drugs in older people considered to be in the pre-symptomatic stage of Alzheimer's. In the AD field, it appears that theory has the ability to triumph over clinical trial data.

And yet pharmaceutical development cannot survive indefinitely this prevailing dissociation between theoretical consensus and failure of clinical efficacy. The two must come into alignment eventually, because the direction of pharmaceutical research must align ultimately with the profit vector. Profitability requires clinical efficacy and competitiveness. A drug has to work better at a lower cost in the clinic relative to its competitors in order to survive. Clinical drug development is at least 2 orders of magnitude more expensive than academic research and cannot afford to be lead only by conjecture. In AD, a single clinical development programme will cost on the order of $500 million. While opinion leaders may hold sway over the grant funding agencies for a time, no company can withstand losses on this scale for long. Investors have lost so much money backing the β-amyloid consensus that a new investor consensus has emerged – AD is too hard. Some companies, such as Sanofi-Aventis [17], badly burned by their β-amyloid losses, have chosen to walk away from AD and even the entire neuroscience space altogether.

The only hope on the horizon for the amyloid-based approach for treating AD is solanezumab. Although this failed in two large phase 3 trials reported in 2012, some efficacy was seen from the combined data [4,18]. The planned size of the repeat study required by the FDA is 2100 subjects. The study therefore has the power to detect an effect size of −1.25 ADAS-cog units at 18 months, which is merely half the effect size over six months for the cholinesterase inhibitors currently available in the market (−2.7 ADAS-cog units) [19]. The aim of this commentary is to argue an alternative to the β-amyloid consensus. For the whole period of the β-amyloid hegemony there has been an entirely plausible alternative, namely the Tau-theory of AD. It now appears extraordinary in hindsight that so little research and clinical development money has been spent on this alternative.

2. The tau aggregation pathology of AD

2.1. "Alzheimer's disease"

What Alzheimer discovered, and why the disease has his name, was the neurofibrillary tangle [20]. One would be forgiven, given the pre-eminence assigned to β-amyloid, for thinking that the disease should have been called "Blocq and Marinesco's disease", given their discovery of plaques [21]. Alzheimer dismissed plaques as having no explanatory significance in accounting for the early onset dementia case he reported. The key point was that large numbers of plaques (i.e. β-amyloid plaques) can occur in the course of normal ageing without any evidence of clinical dementia. The field seems to have remembered only the name, but forgot Alzheimer's discovery.

We confirmed this at the biochemical level, showing that there was a 76% overlap in levels of β-amyloid, between AD cases at the most advanced stages and normal elderly controls [22]. The same result is now available using PET imaging markers which also detect deposits of insoluble β-amyloid. The levels of β-amyloid do not appear to discriminate between normal ageing and AD. The only emerging use of β-amyloid imaging appears to be prediction of susceptibility to progression in individuals with mild cognitive impairment (MCI) [23–25]. Whether this is primary, or whether this depends on the concomitant tau aggregation pathology also present in the neocortex, remains to be determined when data for tau-based PET imaging ligands become available.

Whereas it was the insoluble species of β-amyloid that were thought to be toxic earlier, exactly the same claims are now made for their more soluble oligomeric precursors. It is unlikely that this would change the fundamentals, since the insoluble aggregates and the soluble oligomers must be in equilibrium, such that high levels of insoluble aggregates could only occur in the presence of high concentrations of their precursors. Otherwise, on-off kinetics would favour spontaneous disaggregation in the absence of covalent stabilisation. If β-amyloid load were to be the main driver of cognitive impairment then, even if the toxic agent were an oligomer, it remains difficult to understand how normal cognitive function could be sustained in normal individuals with levels of β-amyloid comparable to those seen in advanced stages of AD.

2.2. The composition of Alzheimer's neurofibrillary tangles

The neurofibrillary tangle comprises a dense whorl of fibres occupying the entire perinuclear cytoplasm of cortical pyramidal cells and other large neurons in the brainstem (nucleus basalis of Meynert and locus coeruleus). These fibres were termed Paired Helical Filaments (PHFs) by Kidd [26]. Structurally, the PHF is a de-novo polymer of C-shaped subunits forming a left-handed helical ribbon with a periodicity of ~70 nm [27]. Neurofibrillary tangles can be labelled in situ with antibodies against a variety of neuronal proteins, including vimentin, actin, ubiquitin, MAP2 and β-amyloid. In crude preparations, PHFs can be labelled with antibodies against MAP2, neurofilament, ubiquitin and tau [28–38]. It was only when we succeeded in isolating a short 12-kD

Table 1
Randomised clinical trials for AD for interventions targeted to different aspects of β-amyloid.[a]

Drug	Company, sponsor	Trial phase	Trial outcome (duration; number of AD subjects)	Mechanism	Clinical trial/ reference
1. Modulation of APP processing					
Tarenflurbil/R-Flurbiprofen/ Flurizan™	Myriad Pharmaceuticals Inc.	3	Failed (18mo; 1649)/halted	Amyloid-lowering agent (γ-secretase modulator)	[5]
Avagacestat/BMS-708163	Bristol Myers Squibb	2	Failed (6mo; 209)/halted	Aβ clearance (GSI)	NCT00810147, NCT00890890 [6]
Semagacestat/LY-450139	Eli Lilly	3	Failed (18mo; ~2600)/halted	Aβ clearance (BSI)	NCT00594568, NCT00762411 (IDENTITY, IDENTITY2) [7]
Lipitor/atorvastatin	Pfizer	3	Failed (16mo; 640)	Cholesterol-lowering; amyloid-lowering; HMG-CoA reductase inhibitor	LEADe, NCT00024531 [8]
Avandia/rosiglitazone	Glaxo Smith Kline	3	Failed (6mo; 553)	BSI; PPARγ activator	NCT00428090
Actos/pioglitazone/AD-4833	Takeda/Zinfandel	2/3	P2 failed; P3 in MCI (410 [5800 enrolment])	BSI; PPARγ activator	NCT01931566
MK-8931	Merck	3	18 mo, 1900 AD 18 mo 1500 prodromal AD	BSI	NCT01739348 (EPOCH) NCT01953601
Huperzine A	Neuro-Hitech/Shandong Luye Pharmaceutical	2	12mo, 150/6mo/390; halted	APP processing	NCT00083590/ NCT01282169 [9]
Posiphen®	QR Pharma Inc.	1	1mo, 120; halted	Inhibitor of Aβ toxicity/AChEI	NCT01072812 [10]
Begacestat/GSI-953	Pfizer	2	Halted	GSI	NCT00959881 [11]
PF-3084014	Pfizer	2	Halted	GSI	
NIC5-15/D-pinitol	Humanetics Corp	2	7wk; 15	GSI	NCT00470418
Bryostatin-1	Blanchette (BNRI)	2	4wk, 9	Increased α-secretase activity	NTC00606164
Etazolate/ETH-0202	ExonHit Therapeutics	2	3mo, 159	Increased α-secretase activity; $GABA_A$ receptor	NTC00880412
EVP-6124	EnVivo Pharmaceuticals	2	6mo, 409	Nicotine α7-receptor agonist in Aβ toxicity	NTC01073228
Dimebon®/latrepirdine	Medivation/Pfizer	3	Failed (6mo, 598; 12mo, 1003), halted	Several, with possible action on amyloid	NCT00675623 (CONNECTION), NCT00829374 (CONCERT); [12]
2. Small molecule amyloid aggregation/deposition inhibition					
Alzhemed™/tramiprosate/ homotaurine	Bellus Health Inc./ Neurochem Inc.	2/3	Failed (18mo; 950 US, 930 EU), halted	Aβ antagonist; glycosaminoglycan mimetic	NTC00088673 (US/Can), NTC00217763 (EU) [13]
ELND005/scyllo-inositol	Elan/Transition Therapeutics	2	Failed (18mo, 353)	Amyloid-lowering agent	NCT00568776 [14]
Clioquinol	Prana Biotechnology	2	Halted	Chelator; metal-dependent Aβ aggregation inhibitor	
PBT-2	Prana Biotechnology	2	3mo, 80	Chelator; metal-dependent Aβ aggregation inhibitor	NCT00471211
3. Immunotherapeutic clearance of amyloid from brain by active or passive immunisations					
Gammagard/IVIg	Baxter	2/3	P2 Failed (6mo, 58); P3 Failed (18mo; 390)/halted	Non-specific, passive (natural antibodies)	NCT00818662
Bapineuzumab/AAB-001	J&J/Elan/Pfizer	3	Failed (18mo, 1121 [ApoE4+], 1331 [ApoE4−]), halted	Passive (N-terminal Aβ epitope)	NCT00575055, NTC00574132
ACC-001	J&J/Elan/Pfizer	2	24mo; 86; halted	Active (N-terminal Aβ)	NCT00479557
AN-1792 (with QS-21 adjuvant)	Janssen/Pfizer	2	Failed (300, early termination)/ halted	Active (Aβ42)	NCT00021723 [15]
Solanezumab/LY-2062430	Eli Lilly	3	Failed (18mo; 1332); ongoing (18mo; 2100); A4 (1000) and DIAN trial (24mo; ~100)	Passive (central domain epitope; binds soluble Aβ)	NCT00905372, NCT00904683, NCT01900665 (EXPEDITION 1, 2 and 3); NCT01760005
Crenezumab/MABT5102A	Genentech	2	18mo, 450 (with OLE for 400 to 24mo)	Passive IgG4 (oligomeric, fibrillar and soluble Aβ)	NCT01343966, NCT01723826
Gantenerumab/RO-4909832	Hoffmann-LaRoche	3	Prodromal (770) and DIAN trials (24mo; ~100)	Passive (N-terminal plus central domain epitope of Aβ and oligomers and fibrils)	NCT01224106, NCT01760005 [16]

[a] The results for randomised clinical trials (RCTs) for drugs that have reached phase 2 or 3 and where the proposed mechanism of action includes an effect on Aβ. Trial outcome is indicated by failure to demonstrate efficacy and instances where the drug development programme has been halted. Trials for 19 drugs have either failed or been halted. Some phase 2 trials are included where only safety and tolerability outcomes have been addressed, rather than efficacy. Such studies are of short duration and with limited enrolment. ClinicalTrials.gov identifiers are given for trials. Numbers of subjects for ongoing studies indicates prospective enrolment. References include both mechanism of action studies or results of randomised RCTs. Results of most recent trials are often only available as company press releases and these have been used to update the data in the review by Mangialasche *et al.* [1] GSI, γ-secretase inhibitor; BSI, β-secretase inhibitor; OLE, open-label extension.

protein fragment from highly enriched preparations of proteolytically stable core PHFs that it was possible to establish unequivocally that a short segment of tau protein from the repeat region of the molecule is an integral structural constituent of the PHF.

A common misconception, which has entered the literature since the papers by Lee et al. and Goedert et al., is that PHFs are composed "almost entirely of hyperphosphorylated tau protein" [39,40]. The further finding that hyperphosphorylation of tau protein leads to a 20-fold inhibition of tau–tubulin binding affinity has led to a widely held view that abnormal phosphorylation of tau protein plays a critical role in the pathogenesis of neurofibrillary degeneration. The idea is that the balance between kinases and phosphatases is disturbed in AD, leading tau protein to become detached from microtubules, and secondarily to aggregate. In this scenario, a tau-based therapeutic approach would target a kinase particularly responsible for a pattern of phosphorylation causing reduced microtubule stability.

2.3. Failure of phase 2 trials in progressive supranuclear palsy (PSP) and likely non-role for abnormal tau phosphorylation

Two phase 2 trials of adequate size have been conducted targeting kinase GSK 3β or interfering with tau phosphorylation. However both failed to demonstrate any effect on cognitive decline in Progressive Supranuclear Palsy (PSP), a disease associated with prominent tau aggregation pathology (so-called "tauopathy"). Noscira tested the GSK 3β inhibitor tideglusib, but found no efficacy in PSP (NCT01049399; 12mo 146 subjects) [41]. Allon Therapeutics Inc. announced in December 2012 that davunetide (AL-108) failed to show efficacy for PSP in a phase 2 trial (NCT01110720; 18mo; 313 subjects). Participants showed no benefit on either of the primary outcome measures or exploratory endpoints and further development in the drug was halted. Davunetide is a neuroprotective octapeptide that was claimed to target tau pathology. It blocks tau hyperphosphorylation in mice and may stabilise microtubules [42].

There are sound theoretical reasons to have predicted these failures. Although PHFs isolated without protease digestion can be immunolabelled by tau antibodies directed against phosphorylation-dependent epitopes located in the N-terminal half of the molecule, this immunoreactivity is lost after proteolytic removal of the fuzzy coat [43,44]. The fuzzy coat consists of the lengthy N-terminal portions of tau molecules that cover the surface of the filaments and are readily sensitive to proteolytic digestion. Such digestion leaves intact the proteolytically stable core structure comprising the left-handed helical ribbon of repeated C-shaped subunits. In other words, the fuzzy coat comprising phosphorylated tau does not contribute to the structural core of the PHF. It is possible to deduce the relative contributions of tau protein to the structural core and the fuzzy coat. Since the mean molecular mass of the protease-resistant core of the PHF is ~65 kDa/nm [44], and since the only tau fragments isolated from the core of the PHF are restricted to the repeat domain with a predicted mass of ~10 kD, there must be 6 or 7 tandem-repeat fragments per nm to account for the observed mass of ~65 kD/nm (if tau is the only constituent). If these tau molecules were N-terminally intact in fuzzy PHFs, the predicted mass of the PHF would be ~210 kD/nm, since the additional N-terminal mass is ~23 kD per tau molecule [6.5 × (10 + 23) = 210]. This would add an additional 145 kD/nm to the fuzzy coat. However, the majority of PHFs isolated from the brain without proteases have a mass of only 80–95 kD/nm and the maximum measured mass is 110 kD/nm. This implies that only 1 in 7 of the tau molecules making up the PHF is N-terminally intact, the remainder being truncated and restricted to the repeat domain of the molecule. The alternative is that there is another non-tau molecule which contributes to the core of the PHF. We have shown that the latter is not the case, and that tau protein indeed accounts for at least 93% of the protein content of the PHF [45]. Indeed biochemical studies which set out to quantify the amount of PHF-tau which is phosphorylated showed the figure to be less than 5% [46,47], in line with the structural mass data. This is not to say that full-length tau cannot aggregate in vitro [100], simply that this aggregation is not relevant to the formation of PHFs in AD.

Furthermore, it is extremely unlikely that hyperphosphorylation of tau plays a critical role in aggregation of tau protein through the repeat domain. A detailed analysis of the properties of this binding interaction showed that hyperphosphorylation of tau is uniformly inhibitory to tau–tau binding both in the solid and aqueous phases, by a factor of 10–50-fold [45]. Indeed, the degree of inhibition is comparable for the tau–tau and tau–tubulin binding interactions. The inhibitory effect appears to be largely conformational, in that it is entirely reversed when tau is bound to a solid-phase substrate. In this configuration, a binding site is made available in the repeat domain which is at least 20-fold (unphosphorylated tau) and as much as 40-fold (hyperphosphorylated tau) more favourable than the tau–tubulin binding interaction. There is therefore no need to invoke phosphorylation as a mechanism to explain the redistribution of the tau protein pool from microtubule-bound to PHF-bound that is a characteristic feature of AD [48]. Rather, the inherent binding affinity at the tau–tau site in the repeat domain is sufficient of itself to explain the extensive transfer of tau protein into the aggregated phase and corresponding loss of microtubule function. In terms of pharmaceutical development, it is difficult to see how a kinase-inhibitor would be expected to have any efficacy in AD, since the net effect of such a drug would be to enhance rather than inhibit tau aggregation. It has also been shown by other groups that phosphorylation of tau is itself inhibitory to its aggregation [49] and not required for the propagation of the tau fibrils [50]. The small quantity of phosphorylated tau found as a surface coating on the structural core of the PHF may simply represent a secondary stage of tau sequestration that is non-critical to either the oligomerisation or polymersation of tau.

2.4. Truncated tau and its propagation

Of much greater interest was the discovery that the repeat domain tau fragment originally isolated from the core of the PHF has prion-like properties in vitro [51]. Using a relatively simple assay in which the core tau fragment of the PHF was adsorbed to a solid phase, we found that binding of full-length tau locked the repeat domain of the bound molecule into a proteolytically stable configuration which reproduced a characteristic C-terminal truncation at position Glu-391 seen both in early pathological oligomers in the brain and within the core of the native PHF [51]. Surprisingly, when the bound complex was taken through repeated cycles of digestion with proteases and re-incubation of full-length tau, there was elimination of N-terminal tau immunoreactivity, and a progressive build-up of immunoreactivity associated with the truncated repeat-domain fragment of the PHF core. Thus, the repeat domain of tau is able to catalyse and propagate the conversion of normal soluble tau into accumulations of the aggregated and truncated oligomeric form (Fig. 1).

If this process were restricted only to affected neurons, tau protein aggregation would be damaging but self-limiting. However, it has recently emerged that proteolytically stable tau oligomers are able to propagate between neurons and initiate the cascade in previously healthy neighbouring neurons [52–54]. Transneuronal movement of proteins and aggregates has been documented in vivo for several neurodegenerative disorders in which the aggregating pathological proteins are tau, amyloid, synuclein, prion protein and polyglutamine proteins. Further elucidation of

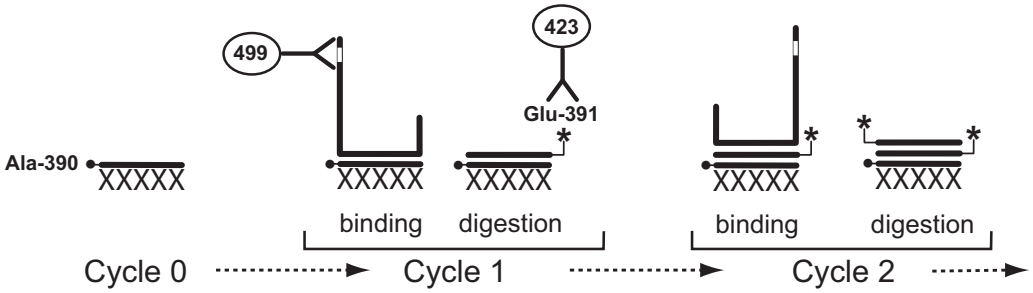

Fig. 1. Tau propagation in vitro. The core-PHF is composed of a tau protein fragment of nearly 100 amino acids in length. The tau is C-terminally truncated at Glu-391, revealing an epitope recognised by the monoclonal antibody, mAb 423. An in vitro assay was developed using tau truncated at Ala-390 bound to solid phase and allowing full-length tau to bind. Cycles of proteolytic removal of N- and C-termini of tau followed by binding of further tau showed that the stepwise of capture of tau is an autocatalytic process in which there is progressive accumulation of tau de novo truncated at Glu-391. The conformation of protein in tau oligomers provides a high affinity substrate for further tau capture [51].

the mechanism by which the specific proteins or their aggregates bind to and enter cells may explain the differential selectivity of neurons affected in the different clinical diseases [55]. Whatever the mechanism of spread, the tau pathology of AD can be understood as a self-propagating "prionosis". Once the cascade has been initiated in any given neuron, it cannot be arrested by cytosolic proteases, because the resulting oligomers are inherently stable to such proteases. However, the process does not stay circumscribed. Oligomers are transported by cytoplasmic flow to nerve terminals, where they damage synapses, are released, and proceed to initiate the same cascade in neighbouring neurons. This also provides a basis for the spread of pathology along neural networks that could account for the spread of tau aggregation pathology documented in the Braak staging system [56].

3. The epidemiology of tau aggregation pathology

The pattern of spread of the tau aggregation pathology in the human brain is highly characteristic and stereotyped. In the cortex, it begins in layer II of entorhinal cortex. From here, the pathology spreads via the perforant pathway to hippocampus. Projections from the hippocampus return to layer IV of the entorhinal cortex and also to other limbic structures. From here, the pathology spreads into isocortex, initially into temporal and parietal lobes, and eventually into frontal and occipital neocortex. This pattern of progression and spread forms the basis of the 6-stage Braak staging system for neurofibrillary degeneration in AD [56]. Braak has also provided a corresponding staging for β-amyloid deposition, with three levels of amyloid deposits: no deposits and three levels with increasing amyloid (stages A–C). This has been compared with tau staging for 2661 consecutive autopsy cases of subjects between the ages of 25 and 95 years [57], and it is clear from this that tau aggregation precedes β-amyloid deposits by about 30 years, confirming earlier reports showing the same thing [48,58].

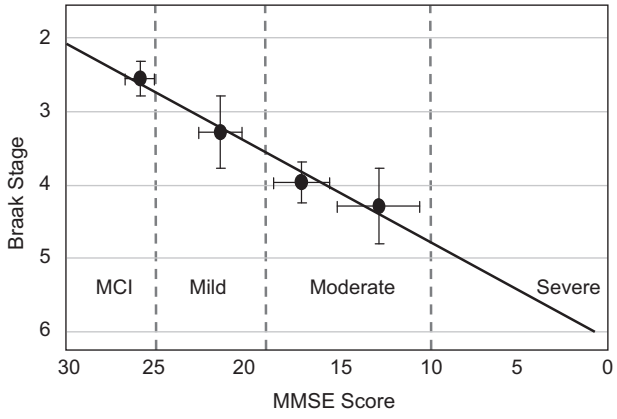

Fig. 2. Braak staging correlation with cognitive decline measured by mini-mental state examination (MMSE). Results from a prospective clinico-pathological study [48,66].

Several studies have confirmed the correlation between Braak stage and cognitive decline measured by a number of cognitive scales, the most commonly used in clinical practice being the Mini Mental State Examination, MMSE [59–62]. The MMSE takes about 15 minutes to administer and measures cognitive decline on a 30-point scale. MMSE scores for minimal cognitive impairment are in the in the range 30–25. Mild/moderate/severe grades of dementia correspond approximately to the ranges 25–20, 20–10, and <10, respectively. In our epidemiological study based on repeated sampling of an original population in primary care, where MMSE scores were measured 12–18 months prior to death, we were able to define the clinical versus Braak stage trajectory (Fig. 2). It is surprising that for the earliest detected stages of minimal cognitive decline typically detected in clinical practice, tau aggregation pathology has already advanced to stages 2–3. Braak stage has also been shown to correlate with progression of functional scan defects measured by PET and SPECT [63–65].

The time-course of disease progression can be calculated from a seminal paper from the Braak group which provides data from 847 post mortems with 17 cases per year of life from ages 45–95 [67]. The data set comes from routine autopsies, and has not been selected for presence of cognitive impairment. From this data set, we have used a Kaplan–Meier survival analysis to calculate the survival probabilities for transitions from Braak stage 0 → 1 or beyond, Braak stage 1 → 2 or beyond, Braak stage 2 → 3 or beyond and Braak stage 3 → 4 or beyond. These probabilities are shown in Fig. 3A.

As can be seen, there is no sense in which the tau aggregation pathology can be considered a late phenomenon, as is often assumed by supporters of the β-amyloid theory. Indeed, Duyckaerts compared the age for appearance of tau pathology at stage 1 and the age for appearance of β-amyloid pathology at stage A, and found that in general β-amyloid pathology appears some 30 years after the onset of tau aggregation pathology [58]. We found the same thing in the epidemiological population we studied, with β-amyloid plaques only increasing over the normal ageing background at Braak stage 4 or beyond. By contrast, aggregation of tau protein could be measured biochemically in the neocortex from Braak stage 2 onwards. As can be seen from Fig. 3, the time between Braak stages is roughly 10 years.

We have applied the Braak transition probabilities by age (shown in Fig. 3A) to estimate the number of affected persons in the US by age (Fig. 3B), using WHO data for the US 2010 population. We calculate that for the population over the age of 45, there is a 50% probability of having some degree of tau pathology in the brain. This can be divided as follows: 25% at Braak stage 1, 10% at

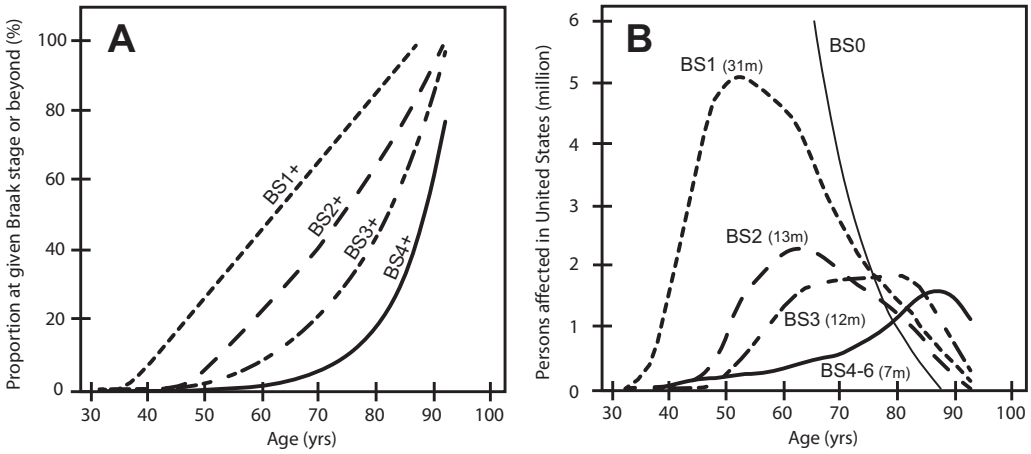

Fig. 3. (A) Age-specific probability of Braak stage (BS) transitions, calculated by the authors using a simple Kaplan–Meier survival analysis of data from 847 post-mortems aged 45–95, with ~17 cases per year of life [67]. (B) Number of persons at a given Braak stage in US population at 2010 with numbers affected for each stage, in millions, calculated from the survival probabilities shown in (A).

Braak stage 2, 10% at Braak stage 3, and 5% at Braak stage 4 or beyond. The age profile of the affected population in the US is shown in Fig. 3B. We estimate that there are approximately 64 million people in the US affected with some degree of tau aggregation pathology in their brains: 31 million at Braak stage 1, 13 million at Braak stage 2, 12 million at Braak stage 3, and 7 million at Braak stage 4 or beyond. It is only the latter figure which is typically captured by prevalence estimates of AD in the US (e.g., [68]). The projected figures for all affected persons in the US are 88 million in 2030 and 105 million in 2050.

Applying the same methodology to European data, the affected population is currently estimated to be 170 million, increasing to 208 million in 2030 and 223 million in 2050. The figures for Asia are truly staggering. We estimate that across all of Asia (including China, India, Indonesia and Japan), there are at present 520 million persons affected, with 227 million at Braak stages 2 or beyond. By 2030, the total figure is expected to increase to 889 million by 2030, with 428 million at Braak stages 2 or beyond. By 2050, the total figure is expected to increase to 1.2 billion, with 665 million at Braak stages 2 or beyond.

The tauopathy of AD does not wait till late life to make its appearance. The peak age for Braak stage 1 is 55, but it can appear as early as 38 years. Braak has suggested that the process may well begin in the 20s [69]. For those who convert to Braak stage 2, the transition can occur as early as 48, but the peak age for Braak stage 2 is the mid-60s. Based on the cross-sectional estimates of the population data, it appears that only half of those at Braak stage 1 progress to Braak stage 2. However, the estimated population at Braak stage 2 is equivalent to that at Braak stage 3, but shifted in age by about 10 years. This suggests that Braak stage 1 is a state of risk, from which it is possible not to progress, with about a 50% probability. However, once Braak stage 2 has been reached, there is very little chance of escape from further progression. The worrying feature of this stage is that it precedes the appearance of deficits which are typically picked up in clinical practice. It should be recalled that these figures reflect degrees of spread of an endogenously generated infectious process throughout the brain. Viewed in these terms, any degree of tau aggregation pathology is dangerous, but particularly so for Braak stage 2, which is entirely preclinical in the absence of concomitant vascular or other pathology.

4. Inhibition of tau aggregation for treatment and prevention of AD

A critical feature that distinguishes the repeat domain fragment isolated from the core of the PHF from the normal repeat domain of tau is that it is phase shifted with respect to the normal repeats. The overall length of the repeat domain is exactly 3 repeats in length, but the positioning of the alternating tubulin-binding segments and the intervening linker segments is reversed [45]. The repeat domain in the PHF core is therefore subject to quite precise structural constraints that distinguish the tau–tau binding interaction from the tau–tubulin binding interaction. This has important pharmaceutical implications, in that it suggests that it should be possible to distinguish between the two binding interactions with potential aggregation inhibitors. This is obviously critical, since an inhibitor of tau aggregation would be of little therapeutic use if it also impaired the normal tau–tubulin binding interaction. We showed that this pharmacological discrimination is indeed feasible for compounds based on the diaminophenothiazine scaffold that we first identified as tau-aggregation inhibitors [51]. With thionine (thioninium chloride), for example, the Ki of inhibition of tau–tau binding based on a solid-phase tau–tau binding interaction was found to be 98 nM. In a similar solid-phase assay measure tau–tubulin binding, the calculated Ki was 7.9 mM, an 8000-fold difference. In a cell-based model of inducible tau aggregation through the repeat domain, the Ki was nearly identical (100 nM) and, for a closely related compound (methylthioninium chloride, MTC), the Ki was 123 nM [70]. An even more potent variant has been identified (dimethyl-methylthioninium chloride) with a cell-based Ki of 4 nM. Therefore, compounds of this class serve as exemplars of highly potent and selective inhibitors of pathological binding through the repeat domain.

In the case of MTC, it has been argued by Crowe and colleagues [71] that it has a potentially broad pharmacology, including inhibition of microtubule assembly. It is possible to calculate from their data that the concentration required for ~50% diminution of microtubule assembly is 50 μM MTC. By contrast, we have determined the IC_{50} for dissolution of PHFs isolated from AD brain to be 0.15 μM, a 280-fold difference. We estimate the brain levels of the active methylthioninium (MT) moiety in brain after oral dosing of MTC 60 mg three times per day is in the range 0.2–0.4 μM. This concentration would therefore be about the minimum required to achieve clinical inhibition of tau aggregation in the human brain. Assuming linear scaling, the dose required to achieve inhibition of microtubule assembly with MTC, would be about 50 g MTC per day. This dose exceeds the LD_{50} for MTC in a range of species. Similar considerations apply to other proposed effects of MTC. For example, it has been claimed that MTC could potentially reduce endogenous production of tau protein [72]. However, the EC_{50} for this effect is 10 μM, which would require a human clinical dose of 9 g of MTC per day, a dose that could not

safely be administered even as a single dose in humans, let alone chronically. Another claim has been that MTC might potentially exert a therapeutic effect via Hsp70 ATP-ase inhibition [73], thereby affecting tau phosphorylation. However, the EC_{50} for this effect is 83 μM, which would require a theoretical dose in humans of 75 g MTC per day to achieve relevant concentration in the brain. Congdon et al. and O'Leary et al. have reported that MTC increases proteasomal and autophagic degradation of tau in vitro [74,75]. However, the claimed brain concentration of MT (~250 μM) achieved by dosing 20 mg/kg/day [74] suggests problems with assay methodology for measuring MT in brain tissues. There are similar concerns over O'Leary et al. who quote brain concentrations on the order of 470 μM after oral dosing [75]. From the radioactive MTC studies that we have conducted, we are able to conclude categorically that such concentrations are entirely implausible.

Other effects reported in vitro which are also clinically irrelevant are: acetylcholinesterase inhibition (1 μM [76]), nitric oxide synthase inhibition (5 μM [77]), oxidation of cysteine residues in the tau repeat domain preventing formation of disulphide bridges (2–30 μM [101], inhibition of β-amyloid aggregation (2.3–12.4 μM [78,79]), monoamine oxidase B inhibition (5.5 μM [80]), glutamatergic inhibition (5–50 μM [81]), noradrenaline uptake inhibition (50 μM [82]), guanylate cyclase inhibition (60 μM [77]). The only non-tau activities of MTC which are of potential clinical relevance considering realistic clinical doses and corresponding brain levels are: enhancement of mitochondrial β-oxidation (0.3 μM [83]) and inhibition of monoamine oxidase A (0.16 μM [80]). A further activity, which has potential relevance for the treatment of frontotemporal dementia (FTD), is inhibition of aggregation of TDP-43 (0.05 μM [84]). The latter is of interest, since the pathology of FTD typically involves aggregation of either tau protein or TDP-43 in roughly equal proportions of cases (i.e. approximately 45% each) [85].

5. Implications of potential efficacy of TAI therapy in AD

The feasibility of using a tau aggregation inhibitor (TAI) for AD is now being confirmed in a global phase 3 programme. Previously, in a large phase 2 study in 321 subjects, MTC was found to stabilise the progression of AD over 50 weeks in both mild and moderate AD; the overall effect size for the dose of 138 mg/MT per day delivered as MTC dose was −6.8 ADAS-cog units versus a decline of 7.8 units in the placebo/comparator arm, using a mixed effects analysis with slope-wise imputation for missing data [86]. MTC was chosen for this study because of if its long history of prior clinical use, and evidence of efficacy in a psychiatric context [87–89]. A stable, reduced version of methylthioninium (leucomethylthioninium with a suitable counter-ion, LMTX) has been developed which has better tolerability and absorption than MTC and can be administered orally twice daily. LMTX is the active agent in three parallel phase 3 studies in AD and frontotemporal dementia now ongoing in 250 centres in 22 countries world-wide, including 140 centres in the US. At the time of writing, the AD trials have already recruited just under half their target numbers, and first readout should be available in early 2016.

Should the efficacy of TAI therapy in mild/moderate AD seen clinically in the Phase 2 study be confirmed in these phase 3 studies, one could ask what implications this would have for the β-amyloid theory, and the potential future for β-amyloid therapy. There are two fundamental pillars of the prevailing β-amyloid consensus: (1) that in a small number of cases, genetic mutations in the amyloid precursor protein lead to early onset AD; (2) that all cases of AD have evidence of β-amyloid deposition. As discussed earlier, this consensus has withstood the numerous failures of the theory's predictions at many different levels, from transgenic animal models, clinico-pathological correlation, and ultimately in clinical trial failures. It may be possible, however, to envisage a different role for abnormal processing of APP which is contributory, but not fundamentally causative or rate-limiting.

5.1. Initiators of tau aggregation

As discussed above, the epidemiology of tau aggregation pathology indicates a process which becomes extraordinarily widespread as human populations age. It is extremely unlikely that such a widespread phenomenon could be explained by any pattern of APP or related genetic mutations. It is more likely that biological concomitants of ageing per se are critical determining factors. In our studies that first led to isolation of a tau protein fragment from highly enriched preparations of proteolytically stable PHFs, we were surprised to find a small family of other proteins which copurified in detergent-resistant tau-bound complexes. All of these derive from mitochondria (porin, core protein 2 of complex III and ATP-synthase subunit 9 [45]), and have been found to accumulate in the cytosol in the course of normal ageing as the lipofuscin deposits found in long-lived, non-dividing, high-activity cells such as neurons and myocardial cells.

A key factor triggering tau aggregation is binding to a non-specific substrate which exposes a high affinity tau–tau binding domain in the repeat region which then has the ability to propagate itself once it has been initiated. For example, the inhibitory (i.e. protective) effects of phosphorylation on the tau–tau binding interaction can be abrogated by its prior adsorption to a non-specific substrate, e.g. polyanionic substrates, such as heparin or RNA have been shown to promote tau aggregation in vitro [90–92], and by products of mitochondrial clearance [93]. Lipofuscin deposits, comprised of undigested products of mitochondrial turnover, could provide the primary substrate needed to initiate the tau aggregation cascade. Such a scenario would then locate the initiation of tau aggregation within a very widespread framework of age-related dysfunction. A commonly held understanding of this dysfunction is a progressive age-related loss of efficiency of the endosomal–lysosomal pathway which is needed to process a range of proteins, including membrane-bound proteins and mitochondria [94,95].

In this theoretical framework, the primary driver for the initiation of the tau aggregation cascade would be progressive failure of endosomal–lysosomal processing, i.e. autophagy. This loss, combined with the triggering of tau aggregation, would have two consequences, illustrated schematically in Fig. 4. The first is that endosomal–lysosomal processing is, in effect, the only pathway available for clearance of proteolytically stable tau oligomers once these have begun to accumulate. The oligomers are inherently resistant to cytosolic proteases once formed. However, their accumulation would only add to the load placed on an already failing system and would cause further failure/overload of the endosomal–lysosomal processing pathway. We have previously shown that one of the early pathological features of tau aggregation, namely the appearance of granulovacuolar degeneration, is in fact derived from the endosomal–lysosomal system full of tau oligomers truncated at the hallmark Glu-391 position [98]. In other words, a phase in the tau aggregation pathway is in effect a tau-lysosomal storage disease. The second consequence is that as tau oligomers continue to be formed in the cytosol, but fail to be cleared by endosomal–lysosomal pathway, they become the seeds for further autocatalytic propagation of the tau aggregation cascade.

The action of TAIs of the MT type is not only to inhibit for formation of new oligomers, but more importantly to release soluble tau from oligomers and PHFs in a monomeric form which is susceptible to proteases [51]. Thus, aggregated forms of tau, which can otherwise be cleared only inefficiently via the endosomal–lysosomal pathway due to proteolytic stability, have available

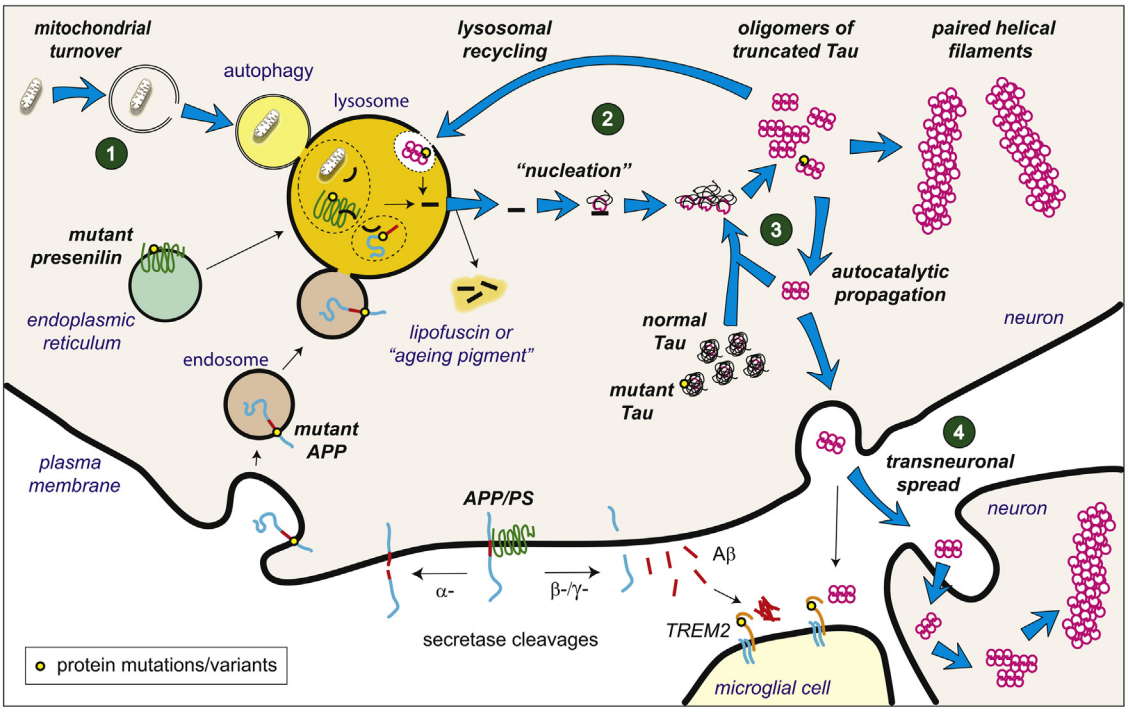

Fig. 4. The fate of tau protein in the endosomal–lysosomal pathway. (1) Proteins derived from mitochondrial turnover and other membrane proteins feed into the lysosomal pathway. This pathway becomes defective in later life, leading to release of partially digested/aggregated mitochondrial degradation products which accumulate in the cytosol as lipofuscin. These deposits are the most likely substrate for initial seeding or nucleation of tau aggregation. (2) Nucleation of tau generates oligomeric tau aggregates, capturing normal tau (or mutant tau in the case of FTD) in the process. Tau oligomers can only be cleared via the endosomal–lysosomal processing pathway, as they are inherently resistant to cytosolic proteases. These contribute to further congestion and dysfunction in lysosomal processing. (3) Tau aggregation propagates itself by autocatalytic binding of tau and ultimate formation of tau fibrils or PHFs. (4) Proteolytically stable tau aggregates are able to spread to neighbouring neurons by exocytosis/endocytosis or via cellular nanotubes. This leads to autocatalytic propagation of the tau aggregatation cascade in interconnecting neurons. Various mutations of APP and presenilin, being membrane proteins and requiring processing via the already congested endosomal–lysosomal pathway, may bring forward the timing of critical failure leading to escape of aggregated mitochondrial degradation products and triggering tau aggregation. Such mutations would not be directly causative of tau aggregation in the absence of endogenous age-related failure of the pathway. APP, β-amyloid protein precursor; PS, presenilin; TREM2, triggering receptor expressed on myeloid cells 2 protein [96,97].

more efficient proteolytic and proteasomal clearance pathways in the presence of TAIs. This provides direct relief both to kinetic trapping of aggregated tau, but more importantly blocks autocatalytic propagation of the process by destroying the tau oligomer seeds which catalyse the cascade.

5.2. Role of β-amyloid in tau aggregation

What of the role of APP/β-amyloid and presenilin proteins in this model? According to this model, APP turnover, and in particular defective APP/presenilin turnover resulting from pathogenic mutations, would simply contribute to the progressive failure of the endosomal–lysosomal processing, since as membrane-bound complexes, they are obligate users of this pathway. Pathogenic mutations would simply bring forward the timing of critical failure in the pathway. This kind of understanding would provide explanations for two otherwise paradoxical features of β-amyloid accumulation. On the upstream side, mutations in the APP/presenilin complexes (in those rare individuals with these mutations) would simply add to the age-related failure of endosomal–lysosomal processing, bringing forward the age at which there is critical triggering of the tau aggregation cascade (Fig. 5). In this way, such mutations would appear to "cause" early onset AD, or to "potentiate" the toxicity tau aggregation [102]. However, what is missing in the pure APP/presenilin causal hypothesis is the ageing component. In other words, the mutations alone, in the absence of age-related loss of endosomal–lysosomal processing efficiency, would not be causative. The second paradoxical feature of β-amyloid accumulation is that it increases substantially only after the onset of tau aggregation [48,58,69]. This is difficult to explain if abnormal processing of APP/presenilin is conceived as directly causative of the tau aggregation cascade. However, if the critical link is failure of endosomal–lysosomal processing, then extracellular accumulation of β-amyloid would simply represent another manifestation of endosomal–lysosomal failure mediated by the postulated tau-lysosomal storage disease.

A scenario such as that outlined would then provide a basis for understanding the following features of AD: (1) presence of β-amyloid deposits in the AD brain, (2) the potential upstream role of mutant APP/presenilin in bringing forward the age of onset of AD, (3) the potential downstream accumulation of β-amyloid deposits after the onset of tau aggregation. It would also provide a way of understanding both the potential "causative" role of APP/presenilin dysmetabolism and also the failure of therapeutic approaches targeting any aspect of this supposed causative pathway. The latter is explained simply by the data showing that neither the accumulation nor the clearance of amyloid impacts directly on cognitive decline in humans. Having more or less amyloid does not seem to make humans any more or less demented [3,22].

5.3. Implications for β-amyloid intervention trials

As to the currently ongoing preventative study in the Dominantly Inherited Alzheimer's Disease Network (DIAN) trial [4], the foregoing analysis predicts that an intervention critically targeting the lysosomal processing of the aberrant APP/presenilin complex could delay, but not ultimately prevent,

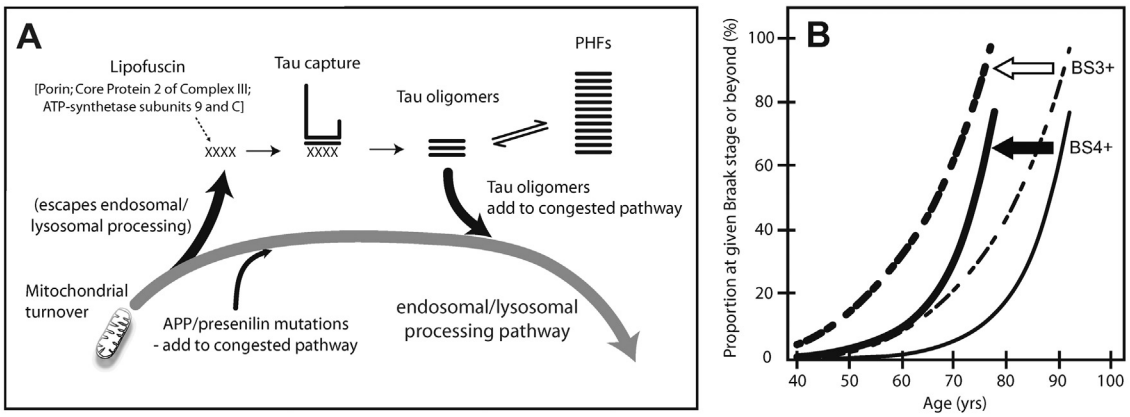

Fig. 5. Involvement of the endosomal–lysosomal pathway in removal of aggregated proteins. Congestion of the clearance pathway associated with progressive age-related failure of normal mitochondrial turnover leads to release of products of failed clearance which become seeds for triggering tau aggregation. The resulting tau oligomers add to congestion in the pathway and themselves catalyse further tau aggregation. Abnormal amyloid processing simply adds to the endogenous load on endosomal processing, and brings forward the time of critical failure (A). The effect of abnormal amyloid processing resulting from genetic mutations simply brings forward the timing of the population risk curve for initiation of the tau aggregation pathway that would have in any case occurred in the absence of such mutations (as depicted in B). Although mutations in APP and the presenilin proteins can cause a left-shift of the population risk curve and lead to early-onset AD, it does not follow that preventing these abnormalities will affect the age-related drivers of the tau aggregation cascade. The tau aggregation cascade proceeds by an autocatalytic process of binding and proteolysis of tau, initiated by its capture by products of failed mitochondrial clearance resulting from age-related failure of endosomal–lysosomal processing (A).

the onset of AD. It is not clear however that any of the interventions currently being tested do intervene in this manner. As for the Anti-Amyloid in Asymptomatic Alzheimer's Disease (A4) trial [4], the expectation would be that there is no greater likelihood of efficacy than the failures already documented in mild/moderate AD.

Such efficacy as has been shown for β-amyloid intervention, for example in the solanezumab trials, is thought to be based on sequestering β-amyloid in the peripheral circulation by binding to circulating antibodies delivered by regular infusions. This presumably alters the on-off kinetics for formation of β-amyloid oligomers/polymers within neurons in the brain, thereby reducing the load on endosomal–lysosomal processing and thereby indirectly lowering the rate of accumulation of tau aggregates. However, more direct inhibition of tau aggregation via a TAI provides a much more efficient way to achieve the same result by releasing tau from oligomers and PHFs, and permitting clearance by much more efficient proteases and proteasomal clearance pathways. Comparing the available results with those from our phase 2 trial of TAI therapy, the disease-modifying effect of solanezumab appears to be modest.

The optimal time for seeing the disease-modifying effect for either drug in mild AD is between 40 weeks and 80 weeks. This is because decline typically seen in clinical trials in subjects with mild AD are minimal for the first 6–9 months. It is unlikely that there is a real difference in rate of decline between weeks 0–40 versus weeks 40–80. Rather this initial failure to decline is thought to be linked to the availability of cognitive reserve [99], i.e. the ability of subjects to call on alternative cognitive strategies to help in their responses to typical cognitive instruments such as ADAS-cog.

β-Amyloid sequestration in mild AD using solanezumab produced a reduction in the rate of decline between week 40 and week 80 of 22% (±16%), or a reduction from 6.7 to 5.2 ADAS-cog units of decline per annum (an effect size of 1.5 ADAS-cog units at 80 weeks, as against 2.7 ADAS-cog units for *cholinesterase* inhibitors at 26 weeks [19]). In other words, those receiving active treatment continued to decline, but at a rate equivalent to 78% of the expected decline. By comparison, the effect seen in our phase 2 study represented an 87% (±30%) reduction in the rate of disease progression over 12 months in mild/moderate AD, i.e. those receiving treatment of 138 mg MT per day progressed at a rate equivalent to 13% of expected decline. It appears unlikely that therapy targeting β-amyloid will be able to arrest progression altogether, based both on the solanezumab data and the earlier data from Holmes et al. [3]. As for TAI therapy, it remains to be seen whether complete arrest of progression can be achieved at a higher therapeutic dose than those tested to date. Exactly the same argument as advanced for the β-amyloid approach, namely that earlier intervention is likely to have greater potential efficacy in slowing disease progression, can be advanced for TAI therapy. As tau aggregation begins about 20 years before clinical symptoms appear, there is ample scope for early preventative intervention in the tau aggregation pathway, preventing the prion-like spread of the pathology out of medial temporal lobe structures at Braak stages 1 or 2.

6. Conclusion

A recent meeting hosted by the New York Academy of Sciences had the title: "A Truce in the BAP-tist/Tau-ist War?" A truce only needs to be called when one side no longer sees any hope of outright victory. The extraordinary history of repeated clinical trial failures at phases 2 and 3 based on the β-amyloid hypothesis does suggest a need for βAP-tists to find a way out of an untenable situation. For long-term Tau-ists such as the authors, it is early days in the campaign, as we are only conducting the very first tau-based phase 3 clinical trial. It would be understandable that we would see no need for a truce at this stage. As we have sketched out in this paper, the actual role of altered processing of APP may be much less significant than previously assumed. If this is borne out in clinical trials, then the terms of any truce are unlikely to prove acceptable to long-term βAP-tists. The long debate about tau vs β-amyloid, which in effect began already in Alzheimer's time, will ultimately be resolved where it began, in the clinic. The long and extremely expensive diversion into the β-amyloid theory may ultimately fall by the wayside, and ordinary clinical practice, particularly in developing countries, will be shaped by the simple principles of efficacy and cost. How it came about that 20 years of research endeavour came to be dominated by a theory which was fundamentally flawed from the outset will be a matter for the historians of medicine to explain.

Conflict of interest

CMW is Chairman, CRH is Chief Scientific Officer and JMDS is Head Chemist of TauRx Therapeutics Ltd.

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