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PERSPECTIVE

Evidence for a novel neuronal mechanism driving Alzheimer's disease, upstream of amyloid

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Abstract

This perspective offers an alternative to the amyloid hypothesis in the etiology of Alzheimer's disease (AD). We review evidence for a novel signaling mechanism based on a little-known peptide, T14. T14 could drive neurodegeneration as an aberrantly activated process of plasticity selective to interconnecting subcortical nuclei, the isodendritic core, where cell loss starts at the pre-symptomatic stages of the disease. Each of these cell groups has the capacity to form T14, which can stimulate production of p-Tau and β-amyloid, suggestive of an upstream driver of neurodegeneration. Moreover, results in an animal AD model show that antagonism of T14 with a cyclated variant, NBP14, prevents formation of β-amyloid, and restores cognitive function to that of wild-type counterparts. Any diagnostic and/or therapeutic strategy based on T14-NBP14 awaits validation in clinical trials. However, an understanding of this novel signaling system could bring much-needed fresh insights into the progression of cell loss underlying AD.

KEYWORDS
Alzheimer's disease, biomarkers, isodendritic core neurons, T14 peptide, therapy

Highlights

- The possible primary mechanism of neurodegeneration upstream of amyloid.
- Primary involvement of selectively vulnerable subcortical nuclei, isodendritic core.
- Bioactive peptide T14 trophic in development but toxic in context of mature brain.
- Potential for early-stage biomarker to detect Alzheimer's disease.
- Effective therapeutic halting neurodegeneration, validated already in 5XFAD mice.

1 | INTRODUCTION

Alzheimer's disease (AD) has been the subject of intense research for decades, primarily with a focus on the β-amyloid hypothesis, in which β-amyloid accumulation initiates a cascade of neurodegeneration leading to the characteristic symptoms of memory loss, cognitive impairment and confusion. The presence of this marker is a major factor in the formal diagnosis of AD (https://www.nia.nih.gov/health/alzheimers-disease-diagnostic-guidelines). However, the β-amyloid pathological cascade itself, and closely associated changes, are recognized as highly

complex, involving multiple mechanisms including tau phosphorylation, defective mitochondrial function, ionic imbalances, dysfunctional autophagy, synaptic loss, neuroinflammation and impaired brain energy metabolism.[1]

This Perspective presents an alternative approach where we discuss a possible mechanism upstream of this multifactorial, but familiar, scenario and consider the properties of the interconnecting subcortical cell groups that have been increasingly recognized as the first to degenerate at the start of AD.[2,3] Identification of the features that differentiate these neurons from the rest of the brain could provide valuable insight into the basic process underlying AD, and point the way to an eventual means of preventing the β-amyloid cascade from being initiated.

2 | SELECTIVE NEURONAL VULNERABILITY IN NEURODEGENERATION

The mechanisms underlying neuronal vulnerability in neurodegenerative disease are likely to include several genetic, molecular, or developmental factors that, until now, have been explored mainly in relation to cortical areas.[4] However, a growing weight of evidence shows early involvement of certain subcortical brain regions in AD due to their selective vulnerability to tau pathology.[3,5] These subcortical nuclei form the so-called isodendritic core (IC), comprising an extensive interconnected group of neurons located within the brainstem and basal forebrain, which have a common and distinct phylogenetic origin, the basal plate.[2,6,7] IC cells share characteristic properties that distinguish them from other neurons as early as 4 weeks of gestation.[2] During the embryonic stage of brain development, brain cells located in the alar plate specialize early, losing their plasticity.[7] In contrast, the IC cells, derived from the basal plate, retain their capacity to respond to neurotrophic factors.[7] Since IC nuclei give rise to a widespread network of neuromodulatory pathways they are also described as global neurons[7] (Figure 1); they regulate crucial physiological processes such as arousal and sleep-wake cycles. Interestingly, subsequent studies have shown that before cognitive decline is apparent there are neuropsychiatric or sleep impairments with degeneration occurring at one of the IC sites, the locus coeruleus (LC).[8] This link is particularly telling considering that AD has a long preclinical phase in which subcortical degeneration may be increasingly present, causing a variety of sentinel symptoms.[5,9] The IC is affected in a wide spectrum of neurodegenerative disorders: in the case of AD, the noradrenergic LC is one of the nuclei more severely affected, showing profound neuronal loss from early stages despite an absence of β-amyloid but correlated instead with tau-pathology.[3,9]

Braak et al., (2011)[10] identified a pre-tangle stage consisting of an accumulation of hyperphosphorylated tau (p-tau) in the LC, while β-amyloid is absent, with neuronal loss occurring caudally at this site[10]; a similar occurrence of tau pathology again without any β-amyloid has been reported elsewhere in the brainstem.[3] Data from animal studies validate these findings by showing that a chemical lesion of the LC in the APP transgenic mouse model induces AD neuropathologi-

RESEARCH IN CONTEXT

1. **Systematic review**: The authors reviewed the literature using traditional (e.g., PubMed) sources and meeting abstracts and presentations. We explored the nuclei that are primarily vulnerable to Alzheimer neurodegeneration at the earliest stage of the disease, before amyloid is present.
2. **Interpretation**: We develop a hypothesis, consistent with the literature, that Alzheimer's results from inappropriate activation of an erstwhile developmental mechanism specific to the vulnerable cells and driven by a bioactive peptide.
3. **Future directions**: If the peptide T14 is indeed the pivotal driver of the neurodegenerative process, then it offers a novel means of early-stage detection, prior to the onset of cognitive symptoms, and upstream of amyloid. Moreover, direct interception of the T14 process would result in a more effective therapeutic with the potential for halting cell loss. If such treatment were given in the presymptomatic window, there is a realistic prospect that cognitive impairments would never present.

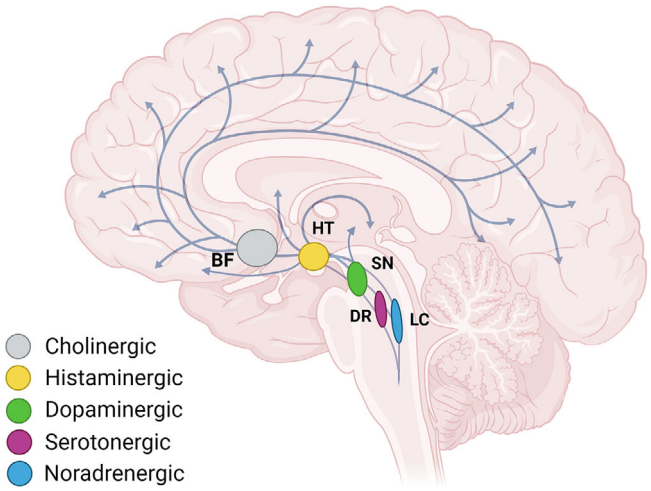

FIGURE 1 Schematic representation of the main areas comprising the IC. The nuclei comprising the IC are heterogeneous with regard to transmitter systems. However, a common feature is their expression of acetylcholinesterase irrespective of the presence of acetylcholine.[13] Note that all areas are interconnected and project distantly to the cortex. In Alzheimer's disease, BF, LC, DR, and HT show tau-related degeneration. In Parkinson's Disease, alfa-synuclein predominantly affects SN, LC, and DR. BF, basal forebrain; DR, dorsal raphe; HT, hypothalamus; IC, isodendritic core; LC, locus coeruleus; SN, substantia nigra; Created by BioRender.com.

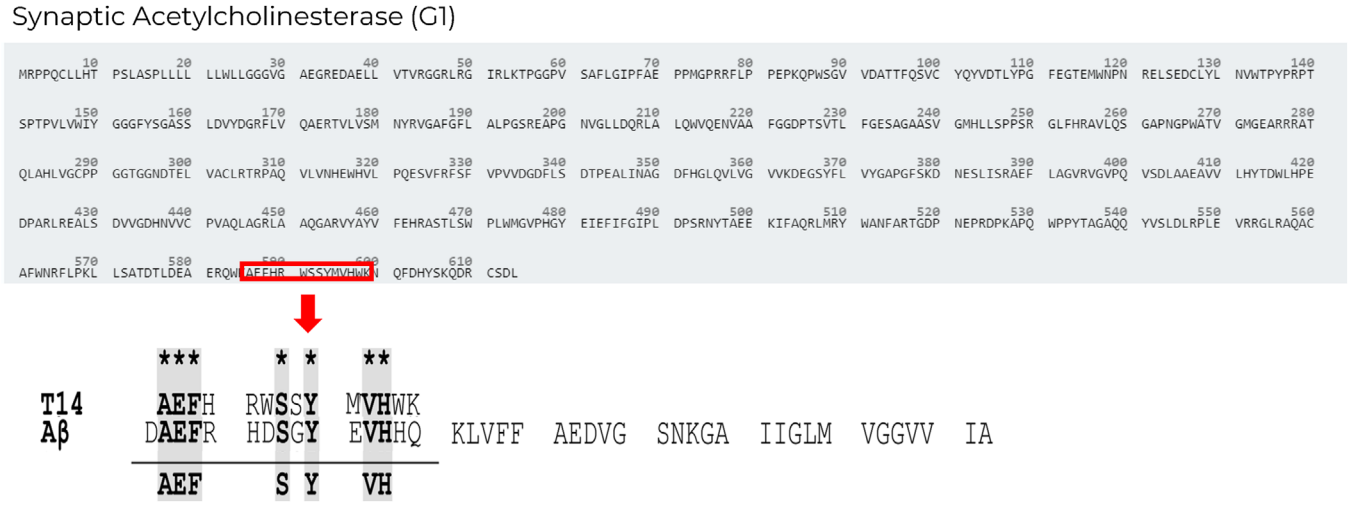

FIGURE 2 T14, C-terminal peptide of acetylcholinesterase. The synaptic variant of acetylcholinesterase contains the T14 peptide in its C-terminal, AEFHRWSSYMVHWK. As shown in the figure, T14 has a 50% homology with the corresponding 14 amino acid sequence of β-amyloid (asterisks and graey shading).

cal changes before β-amyloid plaque deposition.[11] Similarly, infusion of pseudo-hyperphosphorylated human tau into the LC of rats, results in a spread of pre-tangle tau to other IC nuclei (e.g., dorsal raphe) and eventually to cortical areas.[12] Studies on individuals with mutations in genes causing autosomal-dominant AD provide an excellent opportunity to investigate preclinical changes in vivo: in such cases, the decline of LC integrity is independent of β-amyloid, is negatively correlated with cortical damage and memory loss, and starts up to 12 years before the clinical onset.[8] There is a persuasive case therefore to search for any agent upstream of amyloid in the degenerative process, that features as a signaling molecule in the IC.

3 | THE T14 SIGNALING PATHWAY

The nuclei comprising the IC are heterogeneous with regard to transmitter systems. However, a common feature is the expression of acetylcholinesterase (AChE) irrespective of the presence of acetylcholine (ACh),[13] its principal substrate, and independent of any cholinergic function. Non-cholinergic roles have been recognized for AChE for many years.[14]

AChE, acting in a non-cholinergic capacity has been implicated in developmental processes; it is transiently expressed in the developing nervous system, in particular during neuronal proliferation, migration, and axonal outgrowth.[15] AChE can operate as a neurotrophic signaling molecule via activation of calcium influx into neurons.[13] This particular non-enzymatic action of AChE has been attributed to a 14-mer peptide, T14, which is cleaved from the carboxy-terminus of the AChE variant most common in the brain and shares a striking sequence homology with β-amyloid (Figure 2).

In the brain, AChE exists as a monomeric form (G1) that can oligomerize into tetrameric forms (G4), anchored to the plasma membrane. The C-terminus containing the T14 sequence is the region that includes the residues that build the disulphide bonds necessary for oligomerization of G1 into the G4. Consequently, when T14 is cleaved from the parent molecule, AChE cannot oligomerize and the monomer G1 predominates.[13] Thus, the presence of G1, which is more abundant than G4 in the early embryonic brain, can be regarded as an indirect index of free T14. This cleavage product is a bioactive agent, triggering calcium influx and hence promoting cell growth[16] through activation of the mTOR pathway (Figure 3A): indeed, T14 and mTOR correlate highly in the human brain.[17]

The calcium influx induced by T14, while beneficial in development, may be excitotoxic in aging, when tolerance to calcium is markedly reduced.[13] In AD there is a high level of the G1 form of AChE, as in the embryonic and early postnatal brain.[18] The increment in G1 may be due to a failure of the oligomerization process of the tetrameric forms as a result of proteolytic cleavage at its C-terminus.[13] An increase in free T14 is indeed associated with the progression of AD.[13] A consequent inappropriate promotion of calcium influx within the adult central nervous system (CNS), may now trigger AD pathology, with T14 acting as a signaling molecule (Figure 3A). The notion that the neurodegeneration seen in AD may be an aberrantly activated process of plasticity, reflecting phenomena seen during development, is not new.[19] Here we are suggesting that T14 is the key molecule driving such a process. The recapitulation in AD of the profile of G1/G4 found in the human embryo,[20] where the level of G1 is increased, suggests that a common process may be operating, with the rise in G1 indicative of increased levels of free T14.

Although caution must be exercised in extrapolating from in vitro and animal preparations to the clinic, further evidence for T14 in perpetuating AD pathology has been found in PC12 cells: the peptide triggers an increase in phosphorylated GSK3, the major tau kinase, and in p-tau,[13] as well as a reduction in the membrane bound β-amyloid precursor protein (APP) and an increase in β-amyloid release which may result from an increment in the proteolytic processing of APP

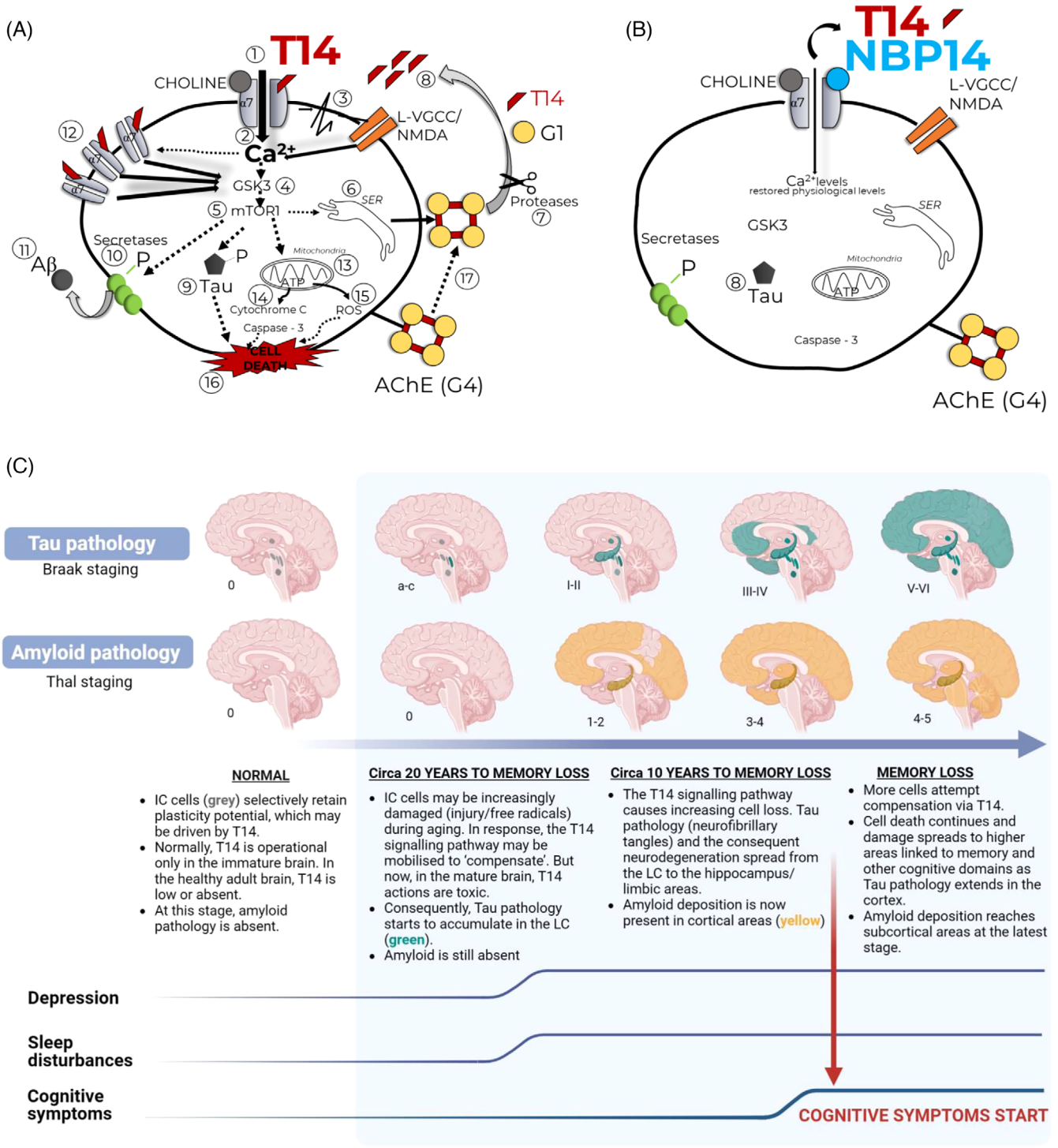

FIGURE 3 Overview of the processes by which T14 may drive Alzheimer's disease[13,44]: (A) The T14 signaling pathway: (1) T14 binds to neurons that express the $\alpha 7$ nicotinic receptor, enhancing (2) calcium influx causing (3) depolarization and activating voltage sensitive calcium channels. (4) Aberrantly raised calcium triggers GSK-3 activation resulting in (5) mTOR1 activation that triggers (6) AChE release from intracellular storage, for example, dendritic smooth endoplasmic reticulum into extracellular space; subsequently (7) proteases, for example, IDE56 cleave T14 from AChE. (8) T14 diffuses into extra-synaptic space to act on $\alpha 7$ receptors, perpetuating the cycle in neighboring cells. Lacking the T14-containing disulfide bonds, G1 monomers are unable to oligomerize to G4 (AChE), accounting for their dominance in development and increased levels in AD. mTOR1 triggers (9) Tau phosphorylation and (10) cleavage of β-amyloid from APP, (10) release of β-amyloid, enhancing the toxicity of T14.[44] (12) The T14-induced calcium gradually causes proliferation of target surface $\alpha 7$ receptors, further enhancing T14 actions. Potentiated calcium influx leads to (13) calcium uptake into mitochondria and decreased ATP synthesis, causing electron leakage, triggering (14) cytochrome C release, followed by caspase-3 activation, increasing (15) free radical and (16) cell death. Consequent (17) membrane disintegration

(Figure 3A). This effect of T14 can also be seen in ex vivo rat brain slices[13] and is consistent with the finding that its parent molecule, AChE, can modulate the expression of presenilin 1 (PS1), the catalytic component of gamma-secretase increasing PS1 at both the protein and transcript levels.[21] The increases in β-amyloid in the extracellular domain and in cytosolic p-tau may further enhance the expression of AChE[22] and, hence, probably the production of more T14. In AD, β-amyloid and T14 could develop a synergistic action that prolongs further cell death accompanied by increased release of AChE.[13]

The target for T14 is an allosteric site on the α7 nicotinic receptor,[13] where it operates exclusively,[23,24] one of the most powerful calcium ionophores in the brain, even compared with the NMDA receptor[25] (Figure 3A). The α7 receptor is co-expressed with AChE during development, including in brain regions devoid of ACh, where choline, derived from the diet, could act as an alternative primary ligand.[26]

The calcium influx enhanced by activation at the allosteric site of the α7 receptor, to which T14 binds,[13,24,27] induces an increase in AChE release,[13] providing a target for further proteolytic cleavage and hence increased free T14.[13] T14 has been shown to upregulate the expression of this receptor, leading to even more calcium influx.[13] Maintenance of this positive feedback results in excitotoxicity and progressive cell death[13] (Figure 3A).

The α7 nicotinic receptor has long been implicated in AD,[28] where it is upregulated in the cholinergic basal nucleus neurons[29] and astrocytes.[30] A transgenic AD mouse model that expresses high β-amyloid levels displays upregulation of this receptor.[31] β-Amyloid can bind to the α7 receptor, with actions on PC12 cells comparable to, though less potent than, T14.[13]

There is persuasive evidence that T14 acts exclusively via the α7 nicotinic receptor.[13,24,27] The peptide is effective in oocytes transfected with the α7 receptor,[13] and α-bungarotoxin, a well established α7 blocker, prevents the actions of T14 in SH-SY5Y cells[32] and organotypic hippocampal cultures.[13] Furthermore, T30, a peptide which includes T14, attenuates evoked neuronal responses in the substantia nigra and basal forebrain,[27] areas which are rich in α7 receptors, but not in the striatum, where α7 receptors are absent despite expression of other cholinergic receptors.[33] Most compelling of all however, is the more recent finding that overexpression of α7 receptors in PC12 cells promotes an enhanced calcium influx when compared with the wild-type PC12 cells[24]; in contrast, the $\alpha7_{345\text{-}348A}$ mutation effectively abolishes the T14-triggered responses. The close relationship between T14 and the α7 receptor was further evidenced in the more physiological preparation of the ex vivo rat brain, where T30 increases α7 receptor mRNA, and also in human brain *post mortem*, where levels of T14 and α7 nAChR exhibit a strong correlation, reflecting the progression of neurodegeneration. Taken together these data would make it hard to account for T14 binding to any other receptor, and thus interception at this binding site would make a very attractive and remarkably specific therapeutic strategy.[24]

The continued increase in T14 in AD would act on the increasing number of α7 nicotinic receptors[13] to generate yet more β-amyloid. Thus, T14 could promote a vicious cycle of pathological events, resulting in increased production of the familiar AD hallmarks, β-amyloid and p-tau (Figure 3A).

4 | T14 IN THE AD BRAIN

The significance of T14 in AD is further suggested by the increase in the peptide found in various areas of the AD brain,[13] as well as in a mouse model of AD.[34] The 5XFAD transgenic mouse model of AD expresses human *APP* and *PSEN1* transgenes with a total of five AD-linked mutations and displays an AD-like pathology involving rapid-onset of β-amyloid.[35] These mice display T14 immunoreactivity in hippocampal neurons and adjacent immunoreactive plaques, particularly in the subiculum,[13] where a high incidence of β-amyloid has been previously reported[35]; the immunoreactivity is scarcely detectable in the transgene negative controls.[13] In contrast, T14 is present in the dopamine neurons of the substantia nigra pars compacta in wild-type mice,[27] indicating the potential of IC neurons to drive AD pathology. T14 immunoreactivity is detected in the pars compacta in early-stage AD, increasing with Braak staging.[27] These immunohistochemical findings further indicate that the IC cell groups comprise a functionally distinct set of primarily vulnerable nuclei in which the T14 system retains the potential to be mobilized into adulthood. Against this background, we now explore further the significance of T14 for driving AD pathogenesis, with increased β-amyloid and p-tau as downstream consequences (Figure 3C).

In advanced AD, T14 levels are doubled in comparison with age-matched controls in the midbrain.[13] In the hippocampus, the level of T14 is significantly elevated at late Braak stages compared with early stages; there is even an increase in T14 between presymptomatic Braak 0-I and Braak II stages.[13] At later stages, T14 immunoreactivity is detected in neurons in the immediate vicinity of T14-immunoreactive plaques, as in the 5XFAD mouse model.[13] A close correspondence between the level of T14 immunoreactivity and the Braak stage is also

makes previously membrane-bound AChE vulnerable to further protease degradation, leading to increased T14 availability for diffusion to neighboring neurons via the neuropil characteristic of basal plate-derived neurons, facilitating volume transmission and perpetuating the T14 process. (B) NBP14, a cyclated variant of T14 that antagonizes its binding,[13] intercepts the chain of events activated by T14 as illustrated in panel A. (C) Illustrates a four-stage proposal aligned with Tau[10] and amyloid staging[52] about how the T14 process interacts with predisposing risk factors such as free radical attack and β-amyloid and p-tau production and how the symptoms of cognitive impairment may take up to 20 years to become apparent. Note that T14 toxicity would first trigger the deposition of Tau pathology in the LC and other IC nuclei leading to mood and sleep disturbances in early Braak stages (I-II). Conversely, cognitive symptoms would start in more advanced stages (Braak III-IV) once Tau has extended to the entorhinal and temporoparietal cortices. In parallel, T14 and Tau would promote and perpetuate β-amyloid deposition within the cortices along the disease course. (A) and (B) are based on Garcia-Rates et al.[44]; (C) was created by BioRender.com. APP, β-amyloid precursor protein.

seen in the substantia nigra, where the signal within neurons increases in density as the number of neurons decreases with disease progression, indicating intraneuronal accumulation of T14 at the later Braak stages.[27]

5 | EVENTUAL CLINICAL APPLICATIONS BASED ON THE T14 SYSTEM

The data from human tissue and the mouse model of AD suggest that T14 could serve as a biomarker reflecting the severity of AD pathology, including at the pre-symptomatic stage. An increase (24% increment) has been found in *post mortem* CSF in AD cases compared with age-matched controls.[13] This observation suggests that T14 CSF levels could serve as a biomarker reflecting AD etiology. Furthermore, nasal secretions may be informative given that a significant proportion of their content has been shown to derive from CSF in non-human animals[36]; these secretions have already been used in a lateral flow format to detect β-amyloid changes in AD.[37] It should be noted that age-dependent levels of T14 have been detected at the epidermis,[38] a site at which pathological aberrations have been associated with neurodegenerative disease including AD.[39] Since Parkinson's disease has been detected by a change in chemical composition of sebum,[40] sebum may offer a further matrix for diagnostic detection of T14 in AD. The prospect of a lateral flow test to detect T14 as a pre-symptomatic biomarker based on nasal secretions, sebum, or perhaps saliva (which is known to contain AChE,[41]) is worth pursuing.

Might blockade of T14 lead to successful therapy? There is currently no truly effective treatment for AD. AChE inhibitors (donezepil, rivastigmine) and an antagonist of NMDA receptors (memantine) are the therapeutic agents employed most often to improve the functional and cognitive deficits of AD patients. Lecanemab, an anti-β-amyloid monoclonal antibody, is the latest in the line of therapies; it can slow progression of the cognitive deficit early after diagnosis by 27%, albeit with significant side effects and for only a limited period of time.[42] The recent approval of lecanemab has given clinicians the first disease-modifying drug for patients with mild cognitive impairment or early AD.[4] While lecanemab has been shown to be effective in removing amyloid, there is still much to learn about how this impacts a patient's daily life. More recently, two Phase 3 trials of gantenerumab proved effective in reducing the amyloid burden, but were not associated with any slowing of cognitive decline.[43] Some clinicians do not feel that the clinical benefits outweigh the potential side effects associated with amyloid antibody medication, including the development of amyloid related imaging abnormalities (ARIA). AD is a complex disease, and amyloid is likely a downstream component of AD rather than the primary initiating factor.

Hence, alternative therapeutic strategies are needed that could target the process of degeneration and, ideally, halt cell loss at the pre-symptomatic stages. Antagonism of T14 emerges as an attractive therapeutic strategy. Accordingly, an anti-T14 antibody (Ab-19) has been tested in vitro and shown to inactivate selectively the free peptide and block its effects.[13] An alternative approach would be to intercept the binding of T14 at the level of its highly selective receptor; consequently, a cyclic form of the linear T14, NBP14, has been developed and validated in several preparations[13] (Figure 3B). In *post mortem* human AD brain tissue, NBP14 displaces the binding of T14 to the allosteric site on α7 nicotinic receptors with a clear dose-response.[13] This competitive displacement has also been demonstrated in ex vivo rat brain slices.[13] At the α7 receptor, NBP14 displaces T14 more effectively than galantamine,[44] an established therapy for AD with limited efficacy.[45] A possible explanation for the relatively poor therapeutic outcome with galantamine and other α7 antagonists is that the key target site is likely to be occupied by endogenous T14. NBP14, as the cyclated version of T14, can more readily displace the endogenous linear counterpart, thereby abolishing its deleterious effects including induction of β-amyloid, and tau phosphorylation.[13,44]

NBP14, given intranasally twice weekly for up to 14 weeks, has neuroprotective effects in vivo in the 5XFAD mouse model.[13] By 6 weeks of treatment, appearance of intracellular β-amyloid in the hippocampus and frontal cortex is significantly lower than in vehicle-treated controls. After 14 weeks, when extracellular β-amyloid plaques become apparent in the basal forebrain in the vehicle-treated group, the NBP14-treated mice express much lower levels of this marker.[13] By this later stage, the NBP14 treatment results in a significant improvement in cognitive performance to the level of wild-type counterparts, with an increased recognition index for a new object compared with the vehicle-treated group.[13] These neuroprotective actions of NBP14 in the AD animal model suggest this cyclic form of T14 could inspire a novel therapeutic strategy for AD (Figure 3B) as well as variants that may be even more potent.[46]

6 | A THREE STAGE DESCRIPTION OF ALZHEIMER'S DISEASE

Although the actions of T14 can be demonstrated at the level of the single cell, the question remains, as posed at the outset, as to why the T14-rich IC neurons are selectively vulnerable, and why this vulnerability to neurodegeneration is particularly conspicuous with aging. As pointed out previously, an interesting key difference is that the IC basal plate derived cells retain the potential for plasticity, showing a response to neurotrophic agents that is not seen in the alar plate derived cell populations.[7] This persistent capacity for plasticity may provide a clue as to why such cells can succumb to a degenerative process, possibly in response to reactivation of an erstwhile developmental process (Figure 3C).

Calcium influx is a crucial factor in neuroplasticity; in excess, however, intracellular calcium will be sequestered in the mitochondria where it compromises oxidative phosphorylation, causing a leakage of electrons and the formation of free radicals, leading to membrane disintegration and finally cell death through excitotoxicity.[47] There are various factors determining whether calcium influx is ultimately beneficial or detrimental. Tipping the balance from trophic to toxic depends not only on the amount,[48] which is related to the duration of influx,[13]

but also on the age; tolerance to calcium can change dramatically by a factor of three in cell cultures within just a week.[49]

Andres-Benito et al.[50] have shown that LC neurons are particularly susceptible to oxidative damage and altered mitochondria early on in AD. Complex alteration of several metabolic pathways occurs in the LC accompanying neurofibrillary tangle formation at early and middle asymptomatic stages.[50] This situation would be exacerbated in the aging brain where the decline in scavenging mechanisms[51] would enhance the risk of free radical damage. Such damage could trigger the mobilization of T14 and a vicious cycle of toxicity that results in neurodegeneration.

In summary, IC cells are differentially vulnerable in AD due to their sensitivity to neurotrophic factors reflecting their embryological mandate. This vulnerability may become clinically significant with aging given the capacity of all the IC cell groups, irrespective of their principal neurotransmitter, to produce T14 from AChE.

7 | CONCLUSIONS AND FUTURE DIRECTIONS

This Perspective explores findings that offer a promising upstream complement to the familiar amyloid hypothesis. The peptide T14 is gaining wider recognition for its actions and expression: it can enhance production of β-amyloid in vitro and ex vivo, and is a conspicuous feature of the brain in clinical cases of AD and in a widely established animal model of AD. Moreover, antagonism of T14 with the cyclated variant NBP14 suppresses the toxic actions of β-amyloid in vitro and in vivo and prevents its production in the brain. We conclude that T14 may act as a pivotal driver of the neurodegenerative cascades in AD and, in highlighting its actions, this perspective provides new insights into its etiology, diagnosis, and potential treatment. Further work is needed to evaluate T14 as a biomarker in humans by investigating its profile in a readily accessible fluid such as nasal secretions or sebum. In parallel, initiation of Phase 1 human clinical trials using NBP14 as a therapeutic intervention may lead to a drug that halts further cell death. A pre-symptomatic biomarker combined with an intervention targeted at T14 to prevent the appearance of cognitive impairment would be a novel and attractive approach toward the goal of ensuring that future generations can look forward not only to improved physical health, but also to a clear-minded old age.

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CONFLICT OF INTEREST STATEMENT

Sara Garcia Ratés is an employee of Neuro-Bio Ltd. María-Salud García-Ayllón and Neus Falgàs have no conflict of interest. Sharon A. Brangman has an institutional grant from the National Institute of Aging. She is a consultant for Biogen, Genentech and Eisai. She is part of the speaker's bureau at Biogen. She is in the advisory board of DSMB- National Institute of Aging- Escitalopram for Agitation in Alzheimer's Disease Research Group. Finally, she is on the committees of Alzheimer's National Registry for Treatment and Diagnostics Leadership Team and the National Committee for Quality Assurance, Committee on Performance Measurement. ME is a shareholder in CytOx group. Clive W. Coen holds shares in Neuro-Bio. Susan Adele Greenfield is the founder and CEO of Neuro-Bio Limited and holds shares in the Company. Author disclosures are available in the supporting information.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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