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REVIEW ARTICLE

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Targeting α7 nicotinic acetylcholine receptors and their protein interactions in Alzheimer's disease drug development

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Abstract

The decades-old cholinergic hypothesis of Alzheimer's disease (AD) led to clinical testing and FDA approval of acetylcholinesterase inhibitor drugs. Subsequently, the α7 nicotinic acetylcholine receptor (α7nAChR) was proposed as a new drug target for enhancing cholinergic neurotransmission. Nearly simultaneously, soluble amyloid $\beta_{1\text{-}42}$ ($A\beta_{42}$) was shown to bind α7nAChR with picomolar affinity to activate kinases that hyperphosphorylate tau, the precursor to tau-containing tangles. Multiple biopharmaceutical companies explored α7nAChR as a drug target for AD, mostly to enhance neurotransmission. Directly targeting α7nAChR proved to be a drug development challenge. The ultra-high-affinity interaction between $A\beta_{42}$ and α7nAChR posed a significant hurdle for direct competition in the AD brain. The receptor rapidly desensitizes, undermining efficacy of agonists. Drug discovery approaches therefore included partial agonists and allosteric modulators of α7nAChR. After substantial effort, numerous drug candidates were abandoned due to lack of efficacy or drug-related toxicities. As alternatives, proteins interacting with α7nAChR were sought. In 2016, a novel nAChR regulator was identified, but no drug candidates have emerged from this effort. In 2012, the interaction of filamin A with α7nAChR was shown to be critical to $A\beta_{42}$'s toxic signaling via α7nAChR, presenting a new drug target. The novel drug candidate simufilam disrupts the filamin A–α7nAChR interaction, reduces $A\beta_{42}$'s high-affinity binding to α7nAChR, and suppresses $A\beta_{42}$'s toxic signaling. Early clinical trials of simufilam showed improvements in experimental CSF biomarkers and indications of cognitive improvement in mild AD patients at 1 year. Simufilam is currently in phase 3 clinical trials as a disease-modifying treatment for AD.

KEYWORDS

amyloid β_{42}, filamin A, nicotinic acetylcholine receptors, tau

1 | INTRODUCTION

This historical review describes the identification of α7nAChR as a potential target for AD drug development from two vantage points, each with differing approaches thought to be therapeutically beneficial: one would enhance normal neurotransmission via this receptor, and the other would seek to block a pathogenic pathway through this receptor. These goals were not necessarily mutually exclusive, though drug development almost exclusively focused on the initial goal of enhancing cholinergic neurotransmission more effectively than acetylcholinesterase inhibitors. Dozens of agents targeting α7nAChR were explored. The more prominent α7nAChR-targeting drug development efforts are summarized, as well as a novel small molecule compound (currently in phase 3 clinical trials)

that targets an α7nAChR-interacting protein to disrupt the pathogenic pathway.

2 | SELECTION OF α7nAChR AS A DRUG TARGET FOR AD AND FIRST DRUG CANDIDATE

The α7nAChR has been investigated as a drug target for AD for approximately two decades. The initial cholinergic hypothesis of AD was based on the loss of nicotine and acetylcholine binding sites in AD postmortem brain tissue (Nordberg & Winblad, 1986), along with cognitive deficits produced by cholinergic antagonists such as scopolamine, administered to women in the early 1900s so they would not recall the experience of childbirth (Cartwright, 2019) (and used very recently as a "stress test" to identify AD [Alber et al., 2020]). Cholinergic lesions in animals were used to model AD (Smith, 1988). This understanding led to the development of acetylcholinesterase inhibitors to prolong the activity of acetylcholine. The symptomatic benefit for AD patients, however, is generally lost over time due to desensitization of the receptor. Although muscarinic acetylcholine receptors are much more numerous, several laboratories demonstrated dramatic losses in nicotinic but not muscarinic acetylcholine receptors in AD postmortem brain tissue (Nordberg & Winblad, 1986; Sugaya et al., 1990; Whitehouse et al., 1986), prompting a drug development focus on nicotinic receptors. Nicotinic agonists fell into two groups, the high affinity α4β2 receptor agonists and the lower affinity α7 receptor agonists, both of which enhanced cognition in animals. The lower affinity α7 receptors became appealing targets because they remain as AD progresses (unlike α4β2 receptors), are highly permeable to calcium and showed neuroprotective effects against β-amyloid—if applied first (Kem, 2000) (likely due to β-amyloid's ultra-tight binding to the receptor, described below).

The first α7nAChR partial agonist appears to have been GTS-21 (GTS for Gainesville and Taiho Scientists), synthesized by the University of Florida in collaboration with Taiho Pharmaceutical (Kem, 2000). This drug candidate enhanced cognitive behavior in animals, showed a possible cognitive benefit in healthy human volunteers, and was neuroprotective in vitro against both β-amyloid and nerve growth factor depletion (Kem, 2000). A startup company (CoMentis) later conducted a randomized trial to compare four doses of GTS-21 (25, 50, 75, and 150 mg t.i.d.) against placebo over 28 days in probable AD (NCT00414622). The study failed to show any treatment benefit. Although GTS-21 also showed no cognitive benefit in schizophrenia, it improved negative symptoms (NCT00100165) (Freedman et al., 2008).

3 | IDENTIFICATION OF α7nAChR AS MEDIATOR OF TOXIC SIGNALING BY SOLUBLE $A\beta_{42}$

In the same year that the GTS-21 publication suggested that agonists or partial agonists of α7nAChR may enhance cognition in Alzheimer's disease, Wang identified α7nAChR as an ultra-high-affinity target of extracellular soluble $A\beta_{42}$, binding at picomolar affinity (Wang, Lee, D'andrea et al., 2000; Wang, Lee, Davis, et al., 2000). This interaction was later shown to be slightly subpicomolar using membranes from cultured neuronal cells and postmortem human cortex (Wang et al., 2012). This was the first demonstration of an ultra-high-affinity target of $A\beta_{42}$, and no others have since been identified. Binding targets of soluble $A\beta_{42}$ identified later include PrP^C, the cellular prion protein, which $A\beta_{42}$ binds at 50–100 nM affinity to suppress Long-Term Potentiation (LTP) in brain slice cultures (Laurén et al., 2009) and neuroligin-1 (Dinamarca et al., 2011), also a nanomolar interaction. At the same time as the identification of the high-affinity $A\beta_{42}$–α7nAChR interaction, soluble $A\beta_{42}$ was gaining attention as more likely than insoluble amyloid plaque to initiate pathology that impairs brain function in AD. Compared to the abundance of plaques, soluble $A\beta_{42}$ was shown to be more highly correlated with cognitive impairment and synaptic deficits (Näslund, 2000).

Wang and others studying the high-affinity $A\beta_{42}$–α7nAChR interaction proposed that it *initiates* amyloid plaque formation: $A\beta_{42}$ binding to α7nAChR leads to internalization of the $A\beta_{42}$-α7nAChR complex by endocytosis, accumulation within the lysosome, disruption of neuronal function, cell death, and eventual amyloid plaques (D'Andrea et al., 2001; Nagele et al., 2002). The numbers of α7nAChR-expressing neurons with prominent intraneuronal $A\beta_{42}$ aggregates are closely correlated with the extent of amyloid plaque in AD brain tissue (D'Andrea & Nagele, 2006; D'Andrea et al., 2001; Nagele et al., 2002). Additionally, the presence of neuron-derived nuclear remnants in the dense core of amyloid plaques, the susceptibility of cholinergic neurons to cell death in AD, and the dramatic reduction of intraneuronal $A\beta_{42}$ load (co-localized with α7nAChR) by blocking endocytosis all support the hypothesis that amyloid plaques are the lysis remnants of $A\beta_{42}$-filled, degenerated neurons. Finally, nicotine treatment of AD transgenic mice reduced insoluble amyloid plaque, implicating α7nAChR in its formation (Hellström-Lindahl et al., 2004).

Soon after the publication of the high-affinity $A\beta_{42}$–α7nAChR interaction, Dineley showed that it activates the MAP kinase cascade ending in ERK2 activation (Dineley et al., 2002). Dineley further showed that α7nAChR was upregulated in AD mouse models, that ERK2 was downregulated in aged mice, and that CREB, the downstream signaling target of ERK2, was hypo-phosphorylated, impairing ERK2-mediated LTP. These findings together suggested disruption of hippocampal signal transduction by $A\beta_{42}$ binding to α7nAChR, implicating the $A\beta_{42}$–α7nAChR interaction in AD pathogenesis.

Follow-on research by Wang published in 2003 showed that activation of ERK2 and JNK1 by the ultra-high-affinity binding of $A\beta_{42}$ to α7nAChR also hyperphosphorylated tau (Wang et al., 2003). Hyperphosphorylation of tau leads to its aggregation, eventually forming neurofibrillary tangles, the second pathological hallmark in AD. The discovery of this pathogenic signaling pathway mechanistically linked amyloid and tau pathologies in AD. Importantly, even before neurofibrillary tangle formation, hyperphosphorylation of tau

disrupts its normal function of stabilizing microtubules, needed for intraneuronal protein transport (Alonso et al., 1994). This toxic signaling pathway of $A\beta_{42}$ via α7nAChR has been confirmed by multiple laboratories under conditions that maintain $A\beta_{42}$ as soluble monomers or small oligomers (Dineley et al., 2002; Nagele et al., 2002; Wang, Lee, D'andrea et al., 2000).

Tau hyperphosphorylation, together with the disruption of LTP, by the $A\beta_{42}$–α7nAChR interaction suggested that blocking or disrupting this pathogenic cascade of soluble $A\beta_{42}$ would perhaps slow the progression of AD. The obvious hurdle for drug development was that full antagonism of α7nAChR might be grossly detrimental to hippocampal neurotransmission and cognition. Ironically, deletion of α7nAChR in a mouse model of AD was shown to improve cognition, protect against loss of synaptic markers, reduce gliosis and improve LTP (Dziewczapolski et al., 2009). The authors suggested that disrupting α7nAChR function would be therapeutic in AD. However, conflicting research showed that loss of α7nAChR in an AD mouse model enhanced Aβ oligomer accumulation and exacerbated cognitive decline and septohippocampal pathology (Hernandez et al., 2010).

It is unclear whether the numerous drug candidates targeting α7nAChR of the early 2000s aimed to disrupt this pathogenic pathway or merely to stimulate α7nAChR without the rapid desensitization known to occur following administration of full agonists or acetylcholinesterase inhibitors. The likely primary goal was achieving a longer lasting and improved efficacy over acetylcholinesterase inhibitors, which were the only approved treatments for AD until memantine in 2003, and which remain the only approved drugs targeting acetylcholine neurotransmission in AD today. Disrupting the pathogenic pathway may have been viewed as an added benefit, if considered at all. Or, the ultra-high-affinity binding of $A\beta_{42}$ monomers or small oligomers for this receptor may not have been considered significant, simply because directly competing with this nearly irreversible interaction would pose an extremely high hurdle for any direct agonist. If the $A\beta_{42}$–α7nAChR interaction was contemplated, it was likely assumed that enough receptors remained unbound by $A\beta_{42}$ and available to a drug if treatment starts early.

4 | PARTIAL AGONISTS OF α7nAChR

Other review articles have more comprehensively reviewed α7nAChR agonists and modulators (Bertrand et al., 2015; Vallés et al., 2014; Yang et al., 2017) as well as the contrasting physiological and pathological roles of amyloid interacting with α7nAChR (Ma & Qian, 2019). This review will summarize the more prominent compounds intended to treat AD that progressed to clinical trials and some reasons for their discontinuation, when known (Table 1). Because the primary goal was cognitive enhancement and not disease modification, these clinical trials were typically of short duration.

4.1 | Encenicline (EVP-6124)

The α7nAChR partial agonist to advance the furthest in clinical development for AD was encenicline (EVP-6124), a small molecule originally developed by Bayer Healthcare and licensed in 2004 to Forum Pharmaceuticals (previously EnVivo). The mechanistic goal was to "tickle" the α7nAChR receptor and not to "hammer" it (personal communication), using a partial agonist instead of a full agonist, to avoid triggering rapid desensitization of α7nAChR. The stated therapeutic goal was symptomatic treatment, not disease-modification (Deardorff et al., 2015). Like many α7nAChR partial agonists, it was considered for both AD and schizophrenia, the latter due to cognitive deficits in this disease. A 6-month phase 2 trial in mild-to-moderate AD patients showed significant improvements on cognitive and functional measures compared to placebo (NCT01073228) (Deardorff et al., 2015); phase 1 (NCT01556763) (Preskorn et al., 2014) and phase 2 (NCT00968851) trials in schizophrenia were also promising. However, in 2015, FDA halted two 6-month phase 3 trials in mild-to-moderate AD patients due to serious gastrointestinal side effects in a small number of patients (NCT01969123, NCT01969136). Two fully enrolled phase 3 studies in schizophrenia failed to meet primary endpoints (NCT01714661, NCT01716975), and no further studies in AD were conducted with this compound.

4.2 | SSR180711

SSR180711 was a compound synthesized by Sanofi-Aventis shown to increase acetylcholine levels by in vivo microdialysis and to enhance LTP in rat and mouse hippocampal slices (Biton et al., 2007). The effects of three doses of SSR180711 were evaluated in a placebo-controlled phase 2 clinical trial in mild AD patients over 4 weeks (NCT00602680). This study was terminated in 2008 due to an insufficient benefit versus risk, per clinicaltrials.gov.

4.3 | AZD0328

AZD0328 was an AstraZeneca compound that improved operant responding acquisition and novel object recognition in mice (Sydserff et al., 2009). However, in a 14-day phase 2a clinical study in 100 patients with schizophrenia, AZD0328 did not show a statistically significant improvement in cognition or any secondary endpoints (NCT00669903). Although a phase 1 pharmacokinetic study in healthy elderly subjects was conducted in 2008 (NCT00687141), the compound was not tested in AD. In 2019, AstraZeneca discontinued development of this drug in schizophrenia and in AD. In 2021, a clinical trial sponsored by King's College London was planned for MCI in Parkinson's Disease, but the study was withdrawn due to COVID delays, per clinicaltrials.gov (NCT04810104).

TABLE 1 Development summary of drug candidates targeting α7nAChR.

Drug candidate	Class	Latest clinical phase	Trial description	Result/reason discontinued	Clinicaltrials registration	Most relevant publication
ABT-126	Partial agonist	2	AD 24 weeks	No significant effect	NCT01527916	Gault et al. (2016)
ABT-126	Partial agonist	2	Schizophrenia	Trend on negative symptoms	NCT01678755	Haig et al. (2016)
APN1125	Partial agonist	1/2	Schizophrenia	Halted for business reasons	NCT02724917	None found
AQW051	Partial agonist	2	Mild cognitive impairment	Terminated; unknown reason	NCT00582855	Feuerbach et al. (2015)
AQW051	Partial agonist	2	Schizophrenia	Not reported for this study	NCT01730768	Barch et al. (2016)
AQW051	Partial agonist	2	L-DOPA-induced dyskinesia	No significant improvements	NCT01474421	Trenkwalder et al. (2016)
AVL-3288	Type I PAM	1	Schizophrenia	No efficacy indication	NCT02978599	Kantrowitz et al. (2020)
AVL-3288	Type I PAM	1	First-in-human, healthy adults	Suggestion of cognitive enhancement	NCT01851603	Gee et al. (2017)
AZD0328	Partial agonist	1	PK in healthy elderly	AstraZeneca decision	NCT00687141	Sydserff et al. (2009)
AZD0328	Partial agonist	2	Schizophrenia	Unlikely to meet target product profile	NCT00669903	Sydserff et al. (2009)
AZD0328	Partial agonist	2	Parkinson's disease with MCI	Not conducted due to COVID delays	NCT04810104	Sydserff et al. (2009)
BMS-933043	Partial agonist	1	PK in Healthy adults	Unknown	NCT01605994	King et al. (2017)
EVP-6124	Partial agonist	3	AD 6 months	FDA hold due to GI side effects	NCT01969123, NCT01969136	Deardorff et al. (2015)
EVP-6124	Partial agonist	3	Schizophrenia	Did not meet endpoints	NCT01714661, NCT01716975	Preskorn et al. (2014)
GTS-21	Partial agonist	2	Probable AD 28 days	No treatment benefit	NCT00414622	Kem (2000)
GTS-21	Partial agonist	2	Schizophrenia	Improved negative symptoms	NCT01400477	Freedman et al. (2008)
MEM3454	Partial agonist	2	AD 24 weeks	Not reported	NCT00884507	Memory Pharmaceuticals (2007)
MEM3454	Partial agonist	2	Schizophrenia	No cognitive improvement	NCT00604760	Umbricht et al. (2014)
SSR180711	Partial agonist	2	Mild AD 4 weeks	Terminated (insufficient benefit/risk)	NCT00602680	Biton et al. (2007)
TC-5619	Full agonist	1	PK in healthy elderly and AD	Unknown	NCT01254448	Mazurov et al. (2012)
TC-5619	Full agonist	2	ADHD	Not reported	NCT01472991	None found
TC-5619	Full agonist	2	Schizophrenia	No benefit for negative or cognitive symptoms	NCT01488929	Walling et al. (2016)

4.4 | ABT-126

A phase 2a clinical trial of ABT-126, an Abbvie/Abbott α7nAChR partial agonist, showed an improvement in the high dose (25 mg) arm on ADAS-Cog of 1.19 ± 0.9 points in 274 mild-to-moderate AD patients over 12 weeks that trended towards significance versus placebo ($p = .095$) (NCT00948909) (Gault et al., 2015). An exposure-response analysis from this study suggested a higher dose range. A subsequent placebo-controlled phase 2b 24-week trial using 25, 50, and 75 mg doses in 438 mild-to-moderate AD patients showed no significant improvement for these three doses of ABT-126 (NCT01527916) (Gault et al., 2016). A phase 2 trial in schizophrenia showed no significant effects on cognition, but a trend ($p = .059$) toward a significant effect on negative symptoms (apathy and flat affect) (NCT01678755) (Haig et al., 2016). No phase 3 studies were initiated.

4.5 | MEM3454/RG3487/RO5313534

In 2002, Roche licensed MEM3454 from Memory Pharmaceuticals, a startup company. The goal for MEM3454 was symptomatic treatment by promoting acetylcholine neurotransmission. In 2007, the startup claimed positive results of a phase 2a study for MEM3454 in 80 patients with mild-to-moderate AD with three daily doses of MEM3454 (NCT00454870) (Memory Pharmaceuticals, 2007). Using the drug name RO5313534, Roche completed a 6-month phase 2 study in 389 subjects with mild-to-moderate AD in 2010 comparing three doses of RO5313534 to placebo added to donepezil (NCT00884507), but results were not reported. No further studies in AD were conducted. MEM3454 did not improve cognitive deficits in a trial in schizophrenia (NCT00604760) (Umbricht et al., 2014). Roche and the startup subsequently dropped development of MEM3454.

4.6 | AQW051

In 2007, Novartis evaluated compound AQW051 in a randomized controlled 4-week phase 2 study in 54 subjects with mild AD or amnestic mild cognitive impairment (NCT00582855). Clinicaltrials.gov lists the study as terminated, but no reason is given. After an inconclusive fMRI study in schizophrenia (NCT00825539) (Barch et al., 2016), Novartis assessed cognitive effects of AQW051 in a larger study in schizophrenia (NCT01730768), but results are not reported. In 2013, Novartis completed a 74-patient study of AQW051 in L-Dopa-induced dyskinesia that showed no significant improvements (NCT01474421) (Trenkwalder et al., 2016). Further development of AQW051 was discontinued.

4.7 | Other α7nAChR partial agonists

Other partial agonists of α7nAChR include APN1125 (CoMentis); JN403 (Novartis) (Feuerbach et al., 2007); BMS933043 (Bristol Meyer Squib) (King et al., 2017); tropisetron (Novartis), a 5-HT3 receptor antagonist used as an antiemetic that is also able to "prime" or sensitize α7nAChR (Callahan et al., 2017); A-582941 (Abbott) (Tietje et al., 2008); and S 24795 (Servier), both a partial agonist and allosteric modulator (see below). Although BMS933043 completed phase 1 (NCT01605994) and a phase 1 of APN1125 (NCT02724917) was halted for business reasons, no others entered clinical trials.

5 | FULL AGONISTS OF α7NACHR

Although several full agonists were investigated, only TC-5619 entered clinical trials. PNU-282987 (Pfizer) is a full agonist used widely in research but never developed due to potential toxicity of excessively high calcium influx (Ng et al., 2007).

5.1 | Bradanicline (TC-5619)

In 2000, R. J. Reynolds, the tobacco giant, spun off Targacept as an independent company to develop a series of novel nicotinic drugs that Reynolds had discovered. Glaxo and AstraZeneca collaborated with Targacept on the development of these drugs, including TC-5619. TC-5619 was clinically tested in a 603-patient study in schizophrenia (NCT01003379) (Walling et al., 2016), a 250-patient study in ADHD (NCT01472991), and in a phase 1 study in healthy elderly and AD subjects (NCT01254448). The drug failed in these indications, and by 2014, Targacept halted development efforts with TC-5619 and all its nicotinic drug candidates.

6 | POSITIVE ALLOSTERIC MODULATORS (PAMS) OF α7NACHR

It is unclear why fewer PAMs advanced to clinical trials compared to α7nAChR partial agonists. Allosteric modulators do not compete directly at the orthosteric ligand site and therefore would not compete with $A\beta_{42}$'s tight binding to this receptor.

6.1 | S 24795

Servier's S 24795, initially designed as a partial agonist, was also shown to act as an allosteric modulator. S 24795 reduced the $A\beta_{42}$–α7nAChR interaction, and this reduction was not diminished by the α7nAChR antagonist methyllycaconitine at concentrations that largely blocked S 24795's agonist activity (Wang et al., 2010). Because the agonist activity of S 24795 was not essential for reducing the $A\beta_{42}$–α7nAChR interaction, this activity was assumed to be mediated by a second, allosteric binding site for S 24795. We know of no other PAMs that were intended to or were found to disrupt $A\beta_{42}$'s pathogenic pathway through α7nAChR. Despite encouraging preclinical efficacy data, S 24795 was not tested

clinically, due to findings in preclinical safety studies (personal communication).

6.2 | AVL-3288

AVL-3288, developed by UC Irvine, appears to be the only PAM of α7nAChR to have entered clinical trials. It is a type 1 PAM, meaning it enhances the peak current following agonist stimulation without affecting desensitization of the receptor. The first-in-human study in 12 healthy volunteers (NCT01851603) suggested potential cognitive enhancement (Gee et al., 2017). AVL-3288 was assessed in a 3-period cross-over phase 1 study in 24 schizophrenia patients that showed nonsignificant worsening (NCT02978599) (Kantrowitz et al., 2020). No further studies are reported.

6.3 | Others

Other investigational PAMs of α7nAChR include type 1 PAMs NS1738 (NeuroSearch) (Timmermann et al., 2007), LY-2087101 (Lilly) (Young et al., 2008) and BNC375 (Binomics/Merck) (Wang et al., 2020). Type 2 PAMs, which delay desensitization and reactivate desensitized receptors, include PNU-120596 (Pfizer), widely used in research (Szabo et al., 2014), JNJ-1930942 (Janssen) (Dinklo et al., 2011), GAT-107 (Lilly) and A-867744 (Abbvie) (Malysz et al., 2009). These compounds showed promise in preclinical evaluations but were never tested in clinical trials.

7 | SEARCHING FOR INTERACTING PROTEINS

Given the difficulties of directly targeting α7nAChR either to augment its neurotransmission or to disrupt the toxic signaling of $A\beta_{42}$ (or both), some groups searched for critical interacting proteins in the receptor complex. Such searches needed to be conducted with functioning receptors in cell culture or in organotypic brain slice cultures.

7.1 | NACHO

An in vitro genomic screening effort by Janssen Pharmaceuticals did not use cells that naturally express α7nAChR (such as SK-N-MC, SH-SY5Y, or PC12 cells). Instead, Janssen used HEK cells transfected with α7nAChR along with one of 3880 genes from a cDNA library of nearly all transmembrane and secreted proteins (Gu et al., 2016). The doubly transfected cells were subjected to a high-throughput functional assay to screen for molecules that potentiate a weak calcium influx evoked by acetylcholine plus an α7nAChR agonist in this transfected cell line. This work identified a transmembrane protein that profoundly augmented the weak acetylcholine + agonist response in this system, which the authors named novel AChR regulator, or NACHO (perhaps without due awareness to cultural appropriation). The authors concluded that co-transfection of NACHO with α7nAChR solves challenges of reconstituting functional α7nAChR transfected into cell lines to be used in drug discovery efforts. Additionally, the authors suggested that NACHO itself, as a chaperone for α7nAChR, may represent "a valuable drug target" (Gu et al., 2016). This group later showed that NACHO knockout mice displayed impaired cell membrane expression of all types of nicotinic receptors and impaired cognitive behavior (Matta et al., 2017). Despite additional work identifying components of α7nAChR required for NACHO assembly of α7nAChR and NACHO's other interacting proteins in the endoplasmic reticulum (Kweon et al., 2020), no drug candidate is publicly known to have emerged from NACHO. Although normal signaling and neurotransmission of α7nAChR is impaired in AD, no studies suggest that the assembly of α7nAChR is disrupted in AD or other disorders. In fact, α7nAChR protein density is upregulated in human brain samples from AD patients and in AD animal models (Bednar et al., 2002; Dineley et al., 2002).

7.2 | Filamin A

Four years before the publication of NACHO, the researcher who initially discovered the ultra-tight binding of $A\beta_{42}$ to α7nAChR and the consequent tau hyperphosphorylation published another critical discovery regarding this toxic cascade. Wang observed that a large protein co-immunoprecipitates together with α7nAChR and $A\beta_{42}$ (when $A\beta_{42}$ binds α7nAChR), which he initially believed to be an aggregate. He identified it as the scaffolding protein filamin A (FLNA) and showed that FLNA interacts with α7nAChR in brain slice cultures incubated with $A\beta_{42}$ and in a mouse model of AD (Wang et al., 2012). Importantly, this aberrant FLNA–α7nAChR interaction is critical to both the femtomolar binding affinity of $A\beta_{42}$ for α7nAChR and to the consequent pathogenic signaling (Wang et al., 2012). The importance of this scaffolding protein–receptor interaction to the toxic amyloid signaling was evidenced by its disruption using simufilam and other proprietary filamin A-binding compounds. The marked levels of FLNA linkage to α7nAChR in the AD mouse model and in AD postmortem brain tissue were significantly reduced by simufilam treatment of the mice or ex vivo simufilam incubation of the postmortem brain tissue (Wang et al., 2012, 2017). Our understanding of the mechanism of action of simufilam is depicted in Figure 1.

The FLNA–α7nAChR linkage can be induced in age-matched healthy control brain tissue incubated ex vivo with $A\beta_{42}$, along with tau hyperphosphorylation at S202, T181, and T231 (Wang et al., 2012). Co-incubation with simufilam blocks the $A\beta_{42}$-induced FLNA–α7nAChR linkage and tau hyperphosphorylation. A decoy pentapeptide of FLNA corresponding to the simufilam binding site was used in excess concentrations to block the effects of simufilam, illustrating that the drug effects were mediated predominantly through its binding to FLNA. By binding FLNA to disrupt the FLNA–α7nAChR interaction, simufilam

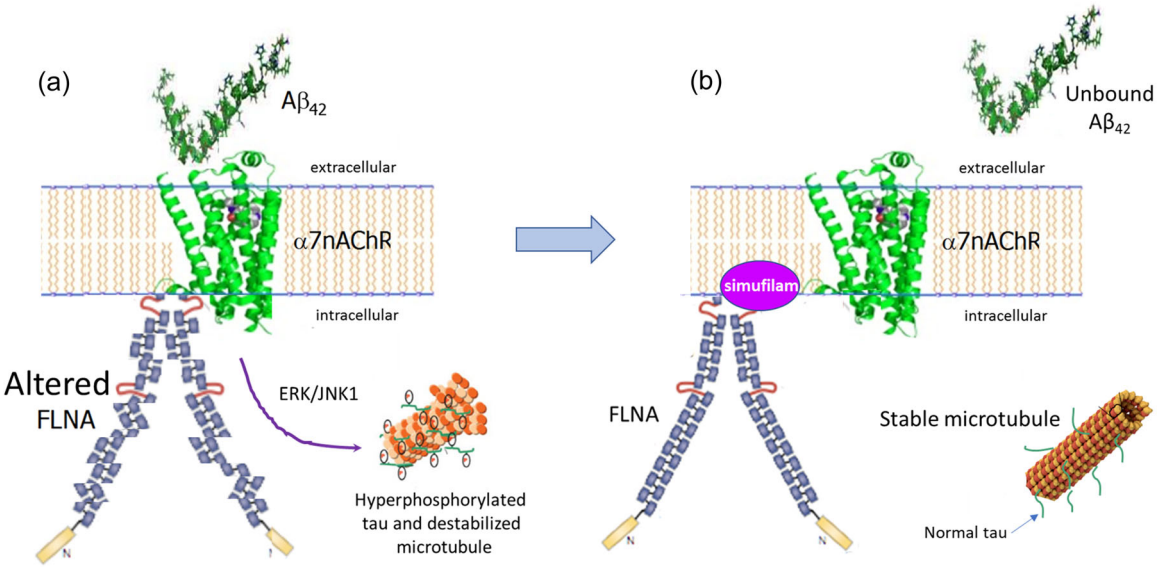

FIGURE 1 Proposed Mechanism of action of simufilam. (a) In the AD brain, $A\beta_{42}$ binds membrane-bound α7nAChR on neurons and recruits FLNA to the receptor. $A\beta_{42}$ can bind as a monomer as depicted or as small oligomers. The recruitment of FLNA tightens the whole complex through the membrane so that the conformation of FLNA is altered and $A\beta_{42}$ binds with ultra-high affinity, continuing to pile onto α7nAChR (Nagele et al., 2002; Wang et al., 2012; Wang, Lee, D'andrea et al., 2000). FLNA's altered conformation is suggested by a shift in isoelectric focusing point (Wang et al., 2017) and insolubility (Aumont et al., 2022). The $A\beta_{42}$–α7nAChR–FLNA complex activates kinases ERK and JNK1 to hyperphosphorylate tau (Wang et al., 2012, 2017), rendering tau incapable of stabilizing microtubules. Unstable microtubules can no longer support intracellular transport of proteins, leading to neuronal dysfunction and degeneration of the neuron. (b) Simufilam binds FLNA in the 24th repeat, near its dimerization domain, which is normally situated near or linked to the cell membrane. We propose that the altered conformation of FLNA in the AD brain exposes simufilam's binding site, as simufilam binds to altered FLNA 100-fold more tightly than to native FLNA (Wang et al., 2017). Simufilam binding disrupts the FLNA–α7nAChR linkage and dramatically reduces the $A\beta_{42}$ binding affinity for α7nAChR so that $A\beta_{42}$ is lifted off the receptor, evidenced by reduced $A\beta_{42}$–α7nAChR levels following simufilam treatment (Wang et al., 2012, 2017, 2020). The hyperphosphorylation of tau by ERK and JNK1 is stopped, and tau remains functional and stabilizes microtubules. AD, Alzheimer's disease; FLNA, filamin A.

indirectly reduces the high femtomolar binding affinity of $A\beta_{42}$ for α7nAChR: $A\beta_{42}$'s ultra-high binding affinity was reduced 1000-fold in postmortem control brain tissue and 10,000-fold in SK-N-MC cells (Wang et al., 2012). Additionally, in an AD mouse model and in AD postmortem brain tissue, simufilam treatment reduced levels of $A\beta_{42}$ complexed with α7nAChR by 60% (Wang et al., 2012). A downstream benefit of disrupting the toxic signaling pathway of soluble $A\beta_{42}$ via α7nAChR is improved receptor function of α7nAChRs, NMDA receptors, critical for learning and memory, and insulin receptors, important for cell survival and health (Wang et al., 2012, 2017). The improved receptor function of these key neuronal receptors suggests cognitive enhancement potential of simufilam. Triple transgenic AD mice treated orally with simufilam for 2 months displayed improved cognitive behavior (Wang et al., 2017).

Additional details of the mechanism of action of simufilam were provided by isoelectric focusing. FLNA has an altered isoelectric focusing point in AD transgenic mice, in AD postmortem brain tissue, and in control postmortem brain tissue incubated with $A\beta_{42}$ (Wang et al., 2017). The shift in isoelectric focusing point reflects a change in overall pH of the protein, likely caused by a change in conformation. Treatment with simufilam largely restores FLNA to its native isoelectric focusing point. Complete dephosphorylation by alkaline phosphatase showed that the shift in isoelectric focusing point in AD versus control, and the shift back following simufilam treatment, are not due to changes in phosphorylation but are likely conformational, coincident with the disease-associated interaction with α7nAChR. Simufilam binds FLNA in postmortem AD brain tissue more tightly than in control brain tissue (Wang et al., 2017), suggesting a conformational difference in AD that exposes the binding site of simufilam on FLNA.

Complementing the conformational change of FLNA in AD implied by the shift in isoelectric focusing point, another group has recently shown higher levels of insoluble versus soluble FLNA in prodromal AD and AD dementia compared to healthy control and preclinical AD (Aumont et al., 2022). Although these data again indicate an AD-related change in FLNA, exactly how and when FLNA changes in AD is yet to be elucidated. Relatedly, simufilam was shown by a third-party to reduce seizure frequency and alleviate neuronal abnormalities in a mouse model of a type of epilepsy that overexpresses FLNA (both the model and the human disease) (Zhang et al., 2020). Because simufilam did not affect FLNA levels in the mouse model, we hypothesize that FLNA's overexpression might induce abnormalities that are then reversed by simufilam.

8 | CLINICAL DEVELOPMENT OF SIMUFILAM

Simufilam is currently in two large phase 3 clinical trials in mild-to-moderate AD patients (NCT04994483, NCT05026177). Prior studies include a first-in-patient clinical trial of simufilam that measured pharmacokinetics and changes in exploratory CSF biomarkers after 28-day twice-daily treatment in 13 mild-to-moderate AD patients (NCT03748706) (Wang et al., 2020). CSF biomarkers total tau, P-tau181, neurofilament light chain, neurogranin, YKL-40, IL-6, IL-1β, and TNF-α were significantly reduced from baseline after 28-day oral treatment. $A\beta_{42}$ increased significantly in plasma but did not reach significance in CSF. To replicate these biomarker effects, a randomized placebo-controlled trial of 64 mild-to-moderate AD patients assessed a panel of exploratory CSF biomarkers and two exploratory cognitive endpoints (NCT04079803). Research-use-only biomarkers of AD pathology, neurodegeneration, neuroinflammation and blood brain barrier compromise were significantly reduced from baseline in both dose arms versus placebo, with $A\beta_{42}$ showing a trend towards significance with multiplicity adjustments. Mean improvements (not significant) in total errors were seen in both dose arms in a test of spatial working memory and in a sensitivity analysis of episodic memory. Following this phase 2 study, a 12-month, open-label safety study was conducted in over 200 mild-to-moderate AD patients (NCT04388254). The full analysis set of mild AD patients ($n = 133$) showed a 0.73-point mean improvement from baseline in ADAS-Cog11 at month 12, contrasting with a 4-point decline in historical placebo groups or observational studies in mild AD (Ito et al., 2010).

9 | CONCLUSIONS

Considerable effort has been devoted to drug development around α7nAChR for AD, from partial agonists and allosteric modulators to exploring interacting proteins. Two very different goals have been considered. The first and simplest was to enhance neurotransmission through α7nAChR while minimizing the desensitization that occurs following chronic treatment with agonists or acetylcholinesterase inhibitors, which prolong the availability of the natural ligand acetylcholine. It is perhaps not surprising that the second goal, that is, disrupting the toxic signaling of $A\beta_{42}$ via this receptor, appears not to have been in the forefront of drug developers' minds, if only because of its near impossibility due to the femtomolar binding affinity of $A\beta_{42}$ for α7nAChR. This femtomolar binding might also be one reason so many α7nAChR partial agonists or PAMs were discontinued, particularly if preclinical studies sought activity at the receptor without using AD models. Yet, even with $A\beta_{42}$'s ultra-tight binding, acetylcholinesterase inhibitors provide some short-lived cognitive enhancement in AD, which might not be expected if soluble amyloid substantially blocks normal neurotransmission via this subtype of nicotinic receptor. Exploring the larger receptor complex of interacting proteins was logical, either to enhance α7nAChR's normal function or to disrupt α7nAChR's role in soluble amyloid's signaling that leads to hyperphosphorylation of tau. One interacting protein critical to this pathogenic cascade was identified as FLNA. The oral drug candidate simufilam binds an altered conformation of FLNA to disrupt its interaction with α7nAChR and therefore also the pathogenic signaling of soluble $A\beta_{42}$. Ongoing phase 3 clinical trials of simufilam will determine whether this approach can benefit cognition and slow AD progression.

CONFLICT OF INTEREST STATEMENT

L. H. B. is an employee and shareholder of Cassava Sciences, and simufilam is a proprietary drug candidate of this company. H. Y. W. is a consultant and shareholder of Cassava Sciences. Both L. H. B. and H. Y. W. are inventors on simufilam patents. The remaining author declares no conflict of interest.

DATA AVAILABILITY STATEMENT

Research data are not shared.

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