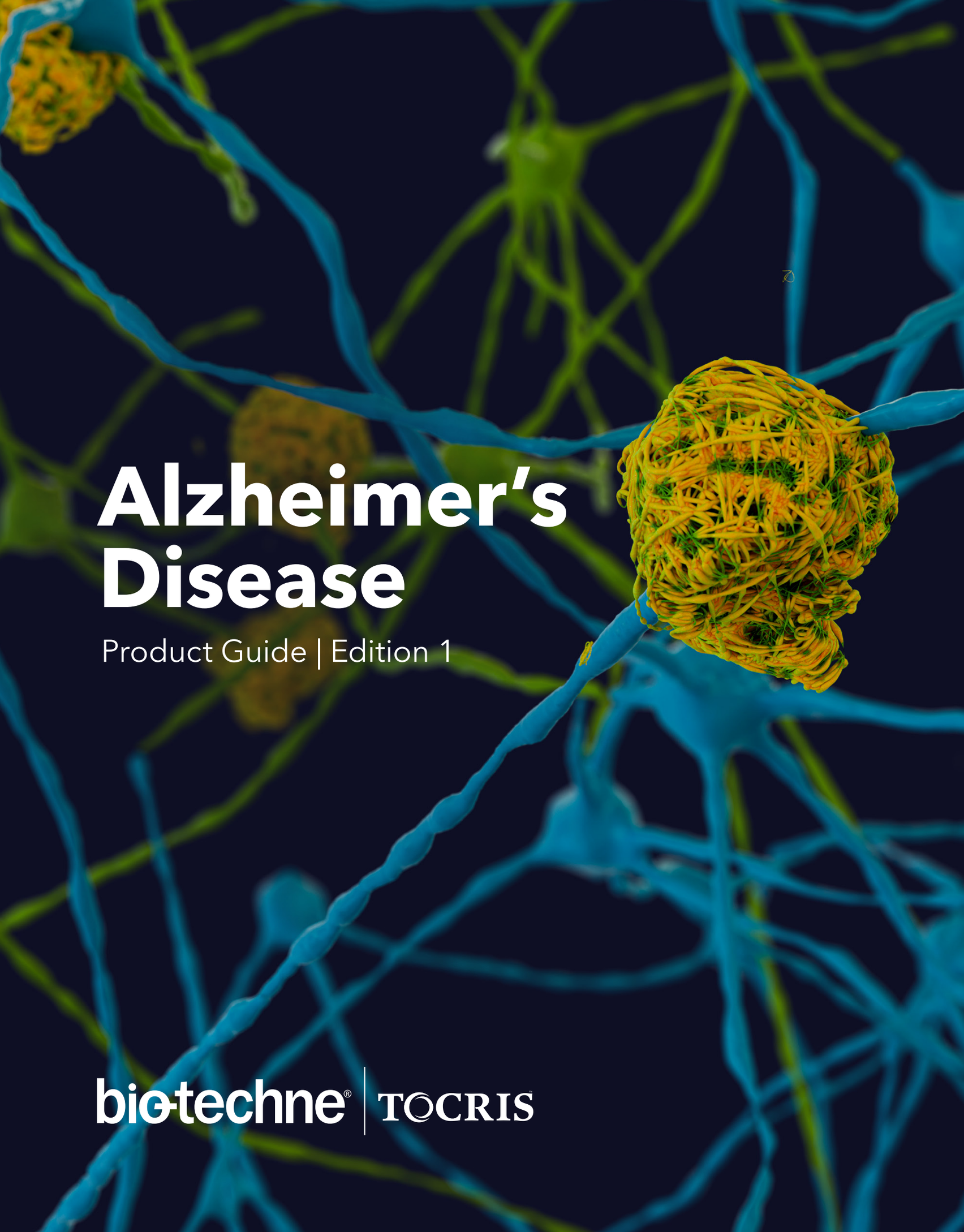

Alzheimer's Disease

Product Guide | Edition 1

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Alzheimer's Disease

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by a loss of cognitive function. Neurodegeneration in the neocortex and hippocampus – regions of the brain involved in higher functions such as sensory perception, language and memory – lead to symptoms often initially associated with dementia. Indeed, AD is the most common neurodegenerative disease, accounting for 60-80% of all dementias. AD-afflicted brains exhibit an overall decrease in size (**FIGURE 1**), and a reduction in glucose uptake that is indicative of decreased neuronal activity. Symptoms include short-term memory loss, confusion, and irritability, which progress to long-term memory deficits and withdrawal from social interactions, followed by a loss of higher cognitive function and finally, death.

The disease was first described as presenile dementia in 1906 by German psychiatrist Alois Alzheimer. Alzheimer observed a patient with a progressive loss of cognitive function and noticed a peculiar substance in the cortex of the patient's brain post-mortem. Subsequently it was discovered that these extracellular deposits, known as senile plaques, were composed of aggregated peptide called amyloid beta (Aβ). Although more than a century has passed since this discovery, there is still no cure for AD. The vast majority of current therapeutic strategies, called cognitive enhancers, act only to alleviate AD-associated symptoms. With the number of people living with AD expected to grow to 13.8 million by 2050 in the US alone, there is an ever-increasing demand for the discovery of disease-modifying drugs, in the hope that progression of AD can be slowed or prevented.

The molecular mechanisms underpinning AD are not well defined. However, emerging evidence suggests a multifaceted dysregulation of cellular function that impacts mitochondrial health, protein homeostasis (proteostasis), protein clearance, inflammatory response and neurotransmission. The primary trigger of this pathology remains obscure, although there are several major hypotheses.

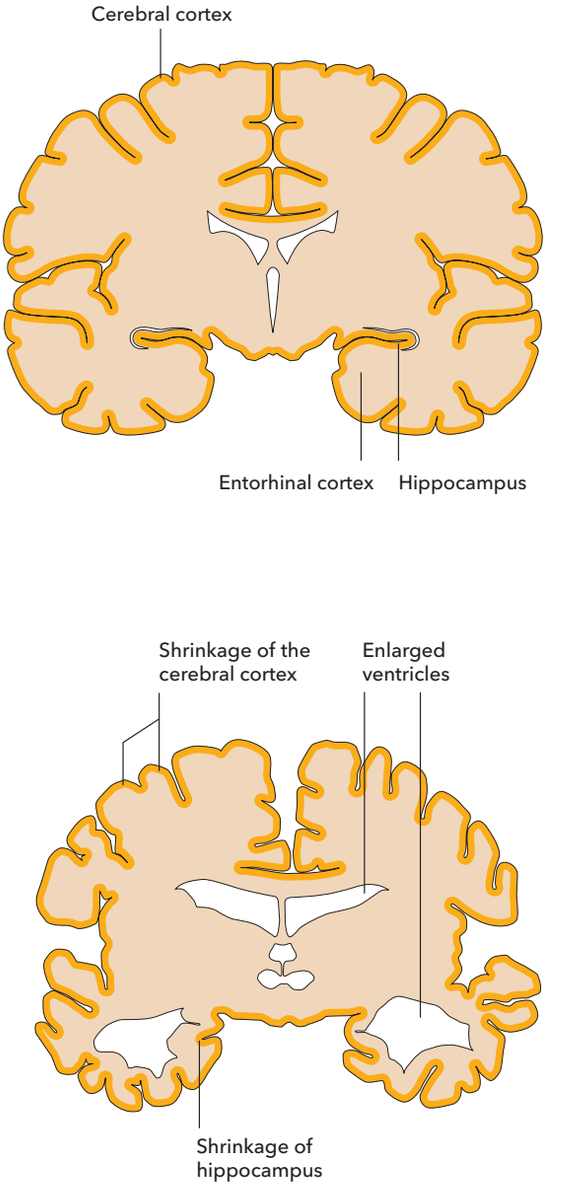


FIGURE 1: The top image shows a normal healthy brain; the lower image is a brain with advanced Alzheimer's disease. The Alzheimer's brain exhibits extreme shrinkage of the cerebral cortex (involved in language and emotion), severely enlarged ventricles and shrinkage of the hippocampus (involved in memory).

The Amyloid Cascade Hypothesis

The basis of the amyloid hypothesis, and one of the key pathological hallmarks of AD, is a progressive appearance of amyloid plaques in the brain. Amyloid plaques form when Aβ peptides aggregate, a natural phenomenon that happens even in the healthy aging brain. However, the Amyloid Cascade Hypothesis suggests that an imbalance arises in the production and clearance of Aβ peptides in AD. This results in an accumulation of pathogenic Aβ peptide species (Aβ42), which first cluster into oligomers and then into insoluble deposits. Each of these Aβ forms have been shown to trigger cellular stress, with the Aβ oligomers widely considered the most neurotoxic. At early phases of disease progression, neurons first suffer from dysregulated neurotransmission that leads to synaptic loss, and then neuronal death.

Secretases

Aβ peptide is produced through proteolysis of a larger transmembrane protein called amyloid precursor protein (APP). Three enzymatic proteases cleave APP: α-secretase, β-secretase (BACE-1), and γ-secretase, and there are two potential outcomes that result from APP cleavage. One process, non-amyloidogenic processing, generates a non-toxic peptide called p3 via cleavage of APP by α-secretase and γ-secretase. The other process, amyloidogenic processing, is directed by the cleavage of APP by β-secretase and γ-secretase and leads to the production of Aβ peptide species of variable amino-acid lengths. The Aβ fragment of 42 amino acids, known as Aβ42, shows a high susceptibility to self-aggregation into oligomers and amyloid plaques, and is considered to be the pathogenic species in AD. Treating neuronal cultures with Aβ peptides such as Aβ1-42 (Cat. No. 1428) and Aβ1-40 (Cat. No. 1191), is useful for studying the vulnerability of neurons to Aβ species and understanding the progression of the disease.

The three proteases involved in cleavage of APP are of particular interest as they are central to the generation and modulation of the Aβ species and can be targeted by small molecules *in vitro* and *in vivo*. β-secretase has therefore been seen as an attractive target for the development of inhibitors to treat AD, as this protein functions at the first step in the pathway leading to production of Aβ. However, BACE-1 inhibitors have had limited success in clinical trials. EGCG (Cat. No. 4524) exhibits inhibition of both β-secretase and amyloid assembly and could be useful to explore the role of β-secretase further.

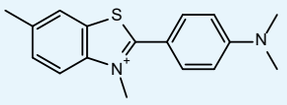
With γ-secretase acting as the final step in the production of Aβ, the development of γ-secretase inhibitors is seen to be a key goal in targeting build-up of toxic Aβ. First generation γ-secretase inhibitors had harmful side-effects due to off-target effects on Notch signaling. However, second generation γ-secretase inhibitors, developed to be Notch-sparing, have shown more favorable results. More recently, small molecule modulators of α-secretase that promote APP processing through the non-amyloidogenic pathway have shown promise, with potential candidates reaching clinical trials. EGCG has also been found to stimulate α-secretase via the PKC pathway.

BOX 1 APP processing and amyloid β

Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-Gly-Ala-Ile-Ile-Gly-Leu-Met-Val-Gly-Gly-Val-Val-Ile-Ala

Aβ1-42 (Cat. No. 1428)

Predominant amyloid β-protein fragment



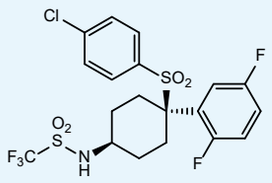
Thioflavin T (Cat. No. 7122)

Fluorescent amyloid stain, binds to the stacked β sheets of amyloid fibrils

Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-Gly-Ala-Ile-Ile-Gly-Leu-Met-Val-Gly-Gly-Val-Val

Aβ1-40 (Cat. No. 1191)

Amyloid β-protein fragment



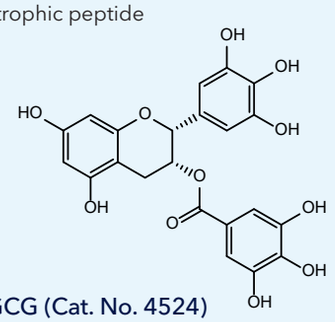
MRK 560 (Cat. No. 4000)

γ-secretase inhibitor; attenuates amyloid plaque deposition

Met-Glu-His-Phe-Pro-Gly-Pro

Semax (Cat. No. 7712)

Reduces Aβ aggregation and fibrillogenesis; neuroprotective and neurotrophic peptide



EGCG (Cat. No. 4524)

β-secretase (BACE) inhibitor; inhibits amyloid assembly

Aβ Aggregation

Targeting Aβ oligomerization and aggregation is another strategy for the prevention of Aβ plaque formation. Semax (Cat. No. 7712, see **BOX 1**) and Davunetide (Cat. No. 6779) bind Aβ1-42 to block Aβ aggregation and protect against Aβ1-42-induced neuronal cell death. Semax is active *in vivo* and increases hippocampal BDNF and TrkB expression levels. Ro 90-7501 (Cat. No. 2408) inhibits Aβ42 fibril formation, and reduces Aβ-induced neuronal toxicity, while MRK 560 (Cat. No. 4000, **BOX 1**) attenuates Aβ plaque deposition. The neuroprotective compounds CEP 1347 (Cat. No. 4924) and Colivelin (Cat. No. 3945) can protect against the neurotoxic effects of Aβ. CEP 1347 is a JNK inhibitor that protects against Aβ-induced cortical neuron apoptosis; Colivelin suppresses Aβ-induced neuronal cell death and ameliorates memory impairment in AD models *in vivo*.

Fluorescent probes and dyes that enable monitoring of Aβ aggregation have been invaluable for discovery of small molecules and peptide aggregation inhibitors, common examples include: Thioflavin T (Cat. No. 7122, **BOX 1**) a fluorescent dye that exhibits increased fluorescence intensity on binding to amyloid fibrils; K 114 (Cat. No. 3144), a fluorescent dye that detects Aβ, α-synuclein and tau *in situ*; CRANAD 2 (Cat. No. 4803) a near-infrared probe that undergoes a fluorescence intensity increase upon interacting with Aβ aggregates; and Methoxy-X04 (Cat. No. 4920), a probe that allows the detection of plaques, tangles and cerebrovascular amyloid *in situ*.

Despite the promise of the Amyloid Cascade Hypothesis, there have been notable failings in amyloid-directed therapies in the clinic. Aduhelm, an Aβ targeted antibody has been the only amyloid-targeted therapy to receive FDA approval over the last 20 years of research – the decision to approve it was controversial and concerns remain over its efficacy.

Mitochondrial Dysfunction

Mitochondrial dysfunction is implicated in Aβ-induced neuronal toxicity in AD. Overproduction of mitochondrial reactive oxygen species (ROS) and increased oxidative stress is evident in the brains of AD patients. The generation of mitochondrial ROS can be studied in live cells using dyes such as Mito-HE (Cat. No. 7641) and MitoPY1 (Cat. No. 4428, see **BOX 2**), fluorescent indicators of mitochondrial superoxide and hydrogen peroxide, respectively. Disruption in energy metabolism, enzyme function and the mitochondrial membrane permeability transition pore (MPTP) all lead to mitochondrial dysfunction. TRO 19622 (Cat. No. 2906, see **BOX 2**) is a useful tool to manipulate the activity of respiratory chain complexes and explore the impact in AD models.

A common feature in AD is the impaired ability to clear damaged mitochondria from the cell, a process called mitophagy, which further increases stress in the cell and leads to cell death (**FIGURE 2**). It is thought that mitochondrial dysfunction, especially when it leads to compromised energy production, precedes the accumulation of plaques. Mitochondrial dysfunction may play an early role in the pathogenesis of AD, as part of a pathogenic feedback loop with Aβ and tau.

Oxidative Stress

Oxidative stress is apparent in the early stages of neurodegenerative disease and is suggested to occur before the appearance of neurofibrillary tangles in AD. Some adverse effects of Aβ appear to be mediated by free radical formation and a resultant oxidative imbalance. Therefore, the free radical-scavenging properties of antioxidants such as vitamin E, L-ascorbic acid (Vitamin C, Cat. No. 4055, **BOX 2**), and Melatonin (Cat. No. 3550) may be beneficial in inhibiting this toxic effect of Aβ. The antioxidant Coenzyme Q10 (Cat. No. 3003) has been shown to preserve mitochondrial membrane potential during oxidative stress and protects neuronal cells by attenuating Aβ overproduction and deposition of intracellular Aβ plaques.

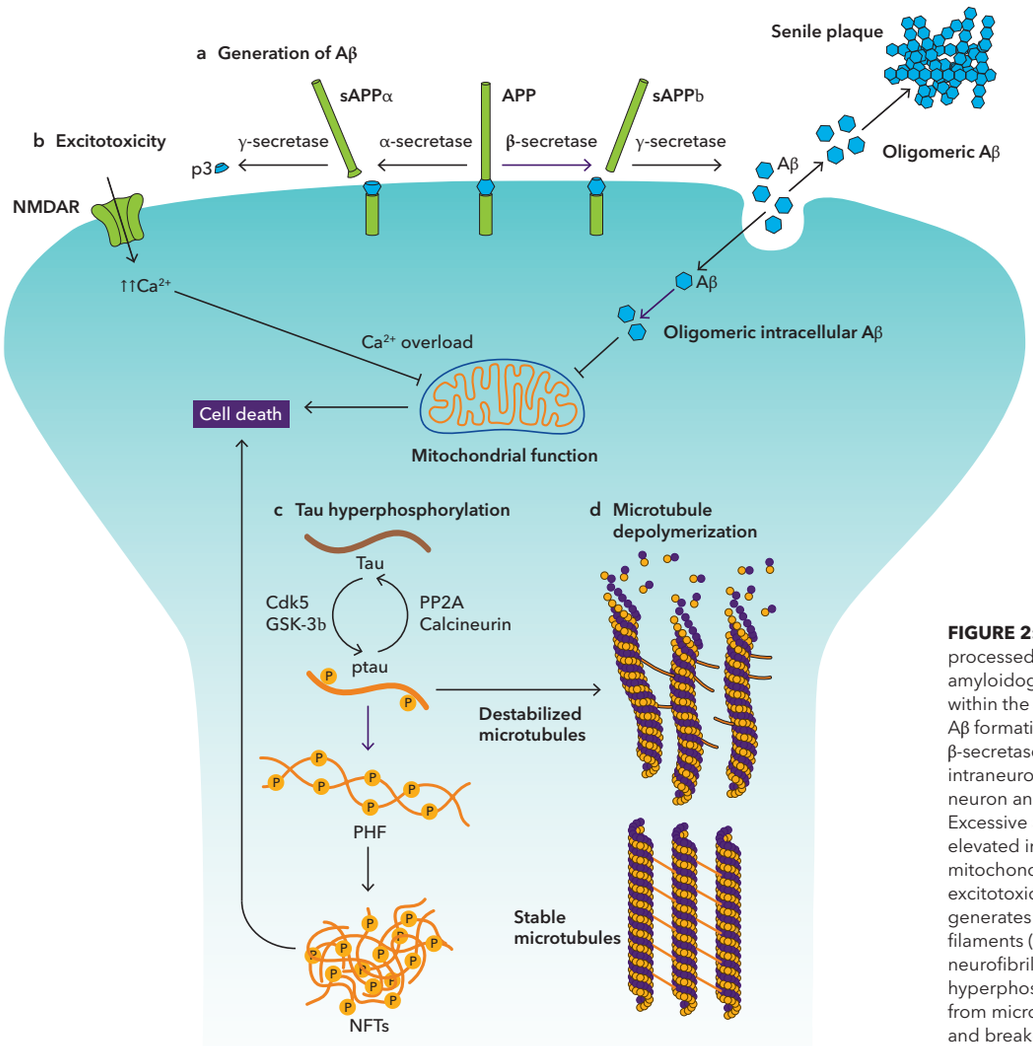


FIGURE 2: (a) Amyloid precursor protein (APP) is processed by two pathways. In the non-amyloidogenic pathway: α-secretase cleaves APP within the amyloid beta (Aβ) domain, preventing Aβ formation. In the amyloidogenic pathway: β-secretase and γ-secretase release Aβ intraneuronally, before it is exported from the neuron and aggregates to form senile plaques. **(b)** Excessive activation of NMDA receptors leads to elevated intracellular calcium, which overloads the mitochondria, leading to cell death by excitotoxicity. **(c)** Hyperphosphorylation of tau generates ptau, which forms paired helical filaments (PHF) and further aggregates into neurofibrillary tangles (NFTs). **(d)** In tauopathies, hyperphosphorylation of tau leads to dissociation from microtubules causing them to depolymerize and breakdown, disrupting neuronal function.

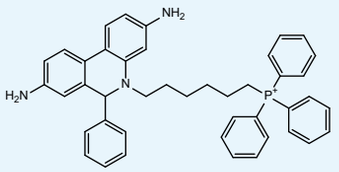
Tau Hypothesis

Deposition of neurofibrillary tangles (NFT) composed mainly of misfolded and hyperphosphorylated tau (ptau) aggregates is another major hallmark of AD. Tau is an example of a microtubule-associated protein (MAP), which is required for the stabilization of microtubules, a major component of cytoskeleton in neurons. The binding of tau to microtubules is modulated by kinases and phosphatases; phosphorylation by specific kinases promotes dissociation from microtubules; dephosphorylation by phosphatases promotes attachment. The correct balance of phosphorylation and dephosphorylation of tau is required for the optimal functioning of the neuronal cytoskeleton - the tau hypothesis suggests that in AD there is an excess of tau phosphorylation. The ptau species are described as having a "gain of toxic function", being able to sequester normal tau and other MAPs, causing destabilization of the microtubules. The deterioration of the neuronal cytoskeleton impacts numerous processes including neurotransmission, metabolic output and protein clearance (including Aβ). Ptau has the ability to spread, via seeding, between neurons and from neurons to astrocytes, propagating the damage throughout the surrounding area and implicating ptau as the primary trigger in AD pathology.

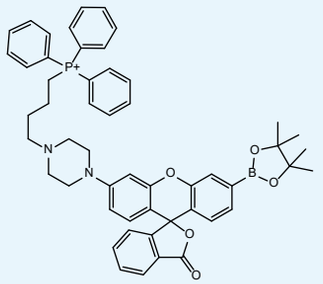
Methylene blue (Cat. No. 3213, see **BOX 3**), an inhibitor of tau aggregation, has been shown to prevent mitochondrial dysfunction and targets some of the mechanisms that are impaired in AD brains, such as aerobic respiration. A stabilized and reduced formulation of methylene blue (TRx0237) has shown efficacy in patients and is in clinical trials for Alzheimer's Disease and Frontotemporal Dementia. Immunotherapies targeting NFT are also in clinical trials - the NFT-targeted antibodies may help slow the seeding of neurotoxic ptau oligomers.

An imbalance between kinase and phosphatase activities results in the accumulation and aggregation of chronically hyperphosphorylated tau. Although the cause of this imbalance is unclear, several candidate enzymes have been identified that are likely to contribute to these events, some of which are discussed on the next page.

BOX 2 Mitochondria and antioxidants



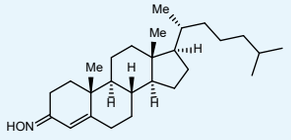
Mito-HE (Cat. No. 7641)
Fluorescent mitochondrial superoxide indicator in live cells



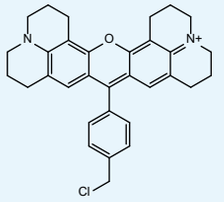
MitoPY1 (Cat. No. 4428)
Fluorescent mitochondrial hydrogen peroxide indicator

Tyr-Glu-Val-His-His-Gln-Lys-Leu-Val-Phe-Arg-Gly-Gly-Asp-Leu-Leu-Pro-Arg-Gly-Ser-NH_2

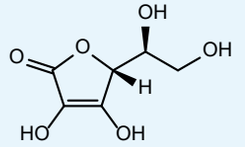
P110 (Cat. No. 6897)
Dynamin-related protein 1 (Drp1) inhibitor; cell-permeable



TRO 19622 (Cat. No. 2906)
Binds voltage-dependent anion channel (VDAC)



MitoMark Red (Cat. No. 6445)
Red fluorescent mitochondrial stain; cell permeable



L-ascorbic acid (Cat. No. 4055)
Enhances the generation of iPSCs; increases reprogramming efficiency

Kinases

The abnormal phosphorylation of tau evident in AD has driven research towards identifying the kinases involved; this may help the development of effective kinase inhibitors that block the signaling cascades that drive cellular stress responses.

Early studies demonstrated that treatment of cultured neurons with Aβ fibrils induced tau phosphorylation and that this increase was sensitive to Lithium (Cat. No. 4740), which is known to inhibit GSK-3. Inhibitors such as Indirubin-3′-oxime (Cat. No. 1813), which inhibit GSK-3β and cdk5, have been shown to diminish tau phosphorylation. SB 216763 (Cat. No. 1616, see **BOX 3**) and AZD 2858 (Cat. No. 7650) show selectivity for GSK-3, demonstrating an ability to reduce tau phosphorylation in postnatal rats and to reverse Aβ-induced tau phosphorylation. Thiazoles such as AR-A 014418 (Cat. No. 3966) compete with ATP for binding to GSK-3β and have been shown to reduce tau phosphorylation and aggregation in a mouse model of tauopathy. PT-65 (Cat.No. 7651), a GSK-3β targeted PROTAC® that induces degradation of GSK-3B, has shown efficacy in an AD mouse model. Treatment reduces GSK-3β-induced and Aβ-induced tau hyperphosphorylation, resulting in modest improvements in spatial memory tasks.

Dual-specificity tyrosine-phosphorylation-regulated kinase 1A (DYRK1A) overexpression has been suggested to be a significant factor leading to cognitive deficits in people with AD. DYRK1A may provide a link between aberrant amyloid and tau pathology in AD. DYRK inhibitors such as INDY (Cat. No. 4997) have been shown to reverse abnormal tau-phosphorylation; proINDY (Cat. No. 4998), a prodrug of INDY, displays effectiveness *in vivo*. These results suggest that DYRK1A is a promising target for AD. Protein kinase R (PKR) and PKR-like ER kinase (PERK) are commonly referred to as the "stress kinases" and their chronic induction is implicated in AD. C16 (Cat. No. 5382, **BOX 3**), a PKR inhibitor, decreases Aβ42-induced inflammatory cytokine release and apoptosis in neuronal cultures *in vivo*, and reduced neuroinflammation and neuronal loss in an acute excitotoxic rat model. PERK (also known as EIF2AK3) is an eIF2α kinase that inhibits protein translation. Global protein translation is blocked as part of the integrated stress response (ISR). Both the PERK inhibitor GSK 2606414 (Cat. No. 5107, **BOX 3**) and ISR inhibitor trans-ISRIB (Cat. No. 5284) have neuroprotective effects and reduce neuroinflammation in *in vivo* models.

Upregulation of CK1 mRNA is evident in brain samples from subjects with AD. CK1 inhibitors such as D 4476 (Cat. No. 2902) cause a significant and dose-dependent reduction in Aβ40 and Aβ42 production *in vivo*. Additionally (*R*)-DRF053 (Cat. No. 3610), a dual cdk/CK1 inhibitor, has demonstrated an ability to inhibit Aβ production.

Phosphatases

Protein phosphatases catalyze the removal of phosphate groups from target proteins. Consequently, they are targets of interest with regard to the high levels of tau phosphorylation observed in AD. The activity of the serine/threonine protein phosphates PP1, PP2A and PP5 have been shown to be decreased in AD brains. A significant amount of research has focused on PP2A, the phosphatase thought to be mainly responsible for ptau dephosphorylation. Strategies to target dysregulation of protein phosphatase activity using compounds such as Ceramide (Cat. No. 0744), a serine/threonine protein phosphatase activator, have been demonstrated to promote PP2A activity. Memantine (Cat. No. 0773, see **BOX 4**) has also been reported to elevate hippocampal PP2A activity and decrease tau phosphorylation both in cells and in rat brain slices by blocking the interaction of PP2A with I2, an inhibitor binding protein. Okadaic acid (Cat. No. 1136, **BOX 3**) and Calyculin A (Cat. No. 1336) are protein phosphatase inhibitors that have been suggested to have an additional role in AD, as they are able to stimulate secretion of APP.

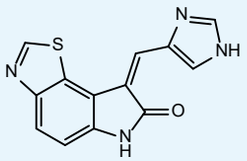
O-GlcNAcase

Post-translation modifications have been observed on tau, including reversible glycosylation of specific serines and threonines. O-GlcNAc transferase (OGT) catalyzes the addition of O-GlcNAc to tau, and O-GlcNAcase (OGA) catalyzes removal. The O-glycosylated form of tau appears to be less prone to hyperphosphorylation, which may be due to the overlap of phosphorylation and glycosylation modification sites and, as a result, has reduced self-aggregation properties. In AD models, the use of selective O-GlcNAcase inhibitor Thiamet G (Cat. No. 4390) resulted in decreased tau phosphorylation and neurodegeneration *in vivo*. This finding led to a subsequent surge of interest in the development of brain penetrant OGA inhibitors that favor the accumulation of O-glycosylated tau rather than ptau species. Several OGA inhibitors have reached early stages of clinical trials.

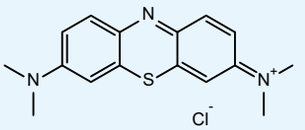
Microtubules

As a result of the generation of hyperphosphorylated tau, accumulation of NFT and microtubule instability leads to the decline of neuronal function (**FIGURE 2**). Microtubule stabilizing agents such as Taxol (Cat. No. 1097) have been used to prevent the breakdown in microtubules associated with tau hyperphosphorylation. Anthraquinones, such as the chemotherapeutics Daunorubicin (Cat. No. 1467) and Doxorubicin (Cat. No. 2252), have been identified as inhibitors of tau aggregation.

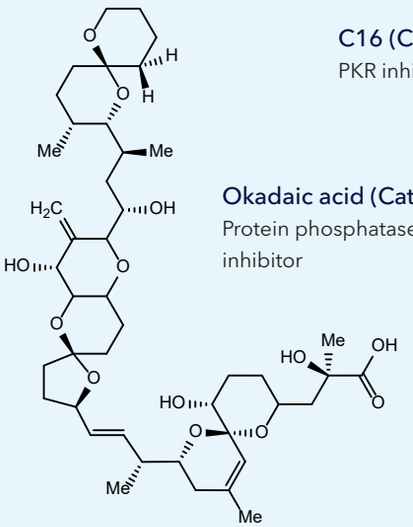
BOX 3 Tau processing



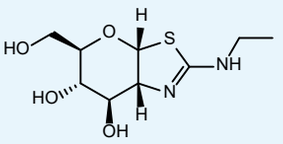
C16 (Cat. No. 5382)
PKR inhibitor



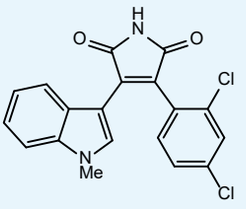
Methylene blue (Cat. No. 3213)
Biological stain; neuroprotective



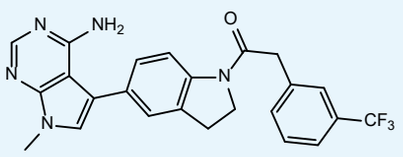
Okadaic acid (Cat. No. 1136)
Protein phosphatase 1 and 2A inhibitor



Thiamet G (Cat. No. 4390)
Potent O-GlcNAcase inhibitor



SB 216763 (Cat. No. 1616)
Potent, selective GSK-3 inhibitor



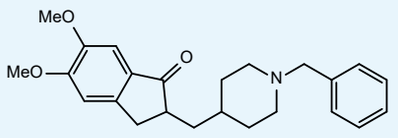
GSK 2606414 (Cat. No. 5107)
Potent and selective PERK inhibitor; orally bioavailable

An alternative strategy to reduce the amount of tau in neurons involves enhancing degradation of phosphorylated tau, for example, by inhibiting heat shock protein 90 (Hsp90) using the geldanamycin derivative 17-AAG (Cat. No. 1515). This reduces the burden of phosphorylated tau in affected brain regions. Interestingly, 17-AAG shows preferential affinity for complexes associated with misfolded proteins, suggesting that 17-AAG may not interfere with other physiologically important Hsp90-client protein interactions. An innovative method of selective degradation of target proteins is through the use of heterobifunctional small molecule protein degraders known as PROTAC® molecules. PROTACs that target tau have been reported; optimization of these degraders to enable selective targeting of ptau alongside physiologically usable pharmacokinetics *in vivo* (i.e. high brain penetration) is underway.

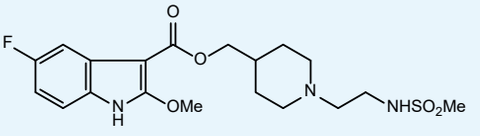
Cholinergic Hypothesis

The cholinergic system is a key modulator of excitatory amino acid (EAA) neurotransmission. Deficits in EAA neurotransmission are associated with a decline in learning and memory, which led researchers to postulate that the cholinergic system may play a role in the cognitive decline evident in AD. The cholinergic hypothesis of AD is one of the earliest theories; it proposes that AD is caused by deficits in the enzymes responsible for the synthesis of acetylcholine (ACh). Drugs that inhibit cholinesterases, the enzymes responsible for the breakdown of ACh in the synaptic cleft, display efficacy in delaying the symptoms of AD and received FDA and European approval, of which Tacrine was the first. This class of drug has continued to be dominant in AD treatment, with second generation cholinesterase inhibitors, including Donepezil (Cat. No. 4385, **BOX 4**) Rivastigmine (Cat. No. 4440) and Galanthamine (Cat. No. 0686), receiving regulatory approval. The second generation inhibitors showed improvements in terms of potency and pharmacokinetics (longer acting). Galanthamine's dual activity as both an allosteric potentiator of nAChRs and as an anticholinesterase has potentiated the cognitive enhancement observed compared to the other cholinesterase inhibitors. Nonetheless, for AD sufferers, these drugs largely offer only symptomatic relief without modifying the course of the disease.

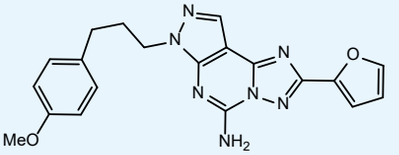
BOX 4 Neurotransmission



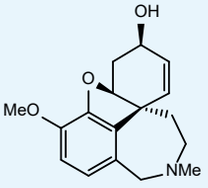
Donepezil (Cat. No. 4385)
Potent AChE inhibitor



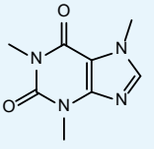
GR 125487 (Cat. No. 1658)
Potent and selective
5-HT_4 antagonist; active *in vivo*



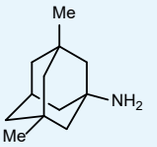
SCH 442416 (Cat. No. 2463)
Very selective, high affinity
A_{2A} antagonist



Galanthamine (Cat. No. 0686)
Cholinesterase inhibitor



Caffeine (Cat. No. 2793)
A_1 and A_{2B} antagonist; CNS stimulant



Memantine (Cat. No. 0773)
NMDA antagonist; acts at ion channel site

Theory of Excitotoxicity

Glutamate-mediated neurotoxicity is a common theme in neurodegenerative diseases such as Parkinson's and Huntington's disease, and has also been implicated in the pathogenesis of AD. Both ptau and Aβ have been implicated in dysregulating glutamatergic neurotransmission, potentially through the excessive activation of NMDA receptors. The outcome being chronic excitotoxicity that damages synapses and ultimately leads to their loss. The degree of synaptic loss is highly correlated with the severity of cognitive deficits seen in patients with early AD. Attempts to develop drugs that block the action of glutamate were unsuccessful to begin with, since these receptors are also required for normal brain function. It was a major breakthrough when Memantine (Cat. No. 0773; **BOX 4**), a selective NMDA antagonist, was discovered to have beneficial effects in AD, blocking excessive glutamate excitotoxicity that leads to cell death without affecting normal glutamate signaling. Memantine achieved FDA and European approval for treatment of moderate to severe AD in 2002/3.

Emerging Targets

5-HT Receptors

Extensive serotonergic denervation has been observed in the AD brain and the involvement of 5-HT in cognition (especially learning and memory), behavior and affective function has made its receptors an attractive target. Positive results have been noted in animal models of memory using the high affinity 5-HT_6 antagonist BGC 20-761 (Cat. No. 3326). 5-HT_4 receptors have been suggested to play a role in the regulation of Aβ and have demonstrated efficacy as cognitive enhancers in preclinical AD; therefore inhibitors such as GR 113808 (Cat. No. 1322) and GR 125487 (Cat. No. 1658, **BOX 4**), which selectively target the 5-HT_4 receptor, may be useful in characterizing its role in AD. A novel 5-HT_4 receptor partial agonist has completed Phase I clinical trials.

Adenosine Receptors

Adenosine receptors have received some attention as both a therapeutic target and a potential biomarker in AD. They were first noted in relation to AD and other cognitive disorders, as a result of epidemiologic evidence showing that regular consumption of Caffeine (Cat. No. 2793, **BOX 4**), a non-selective adenosine receptor antagonist, helped protect against the cognitive decline typically associated with aging. Although the full picture of how caffeine exerts these pro-cognitive effects remains incomplete, there is strong evidence that one mode of action is through downregulation of A_{2A} receptor expression. The A_{2A} receptor subtype is highly expressed by microglia and astrocytes and is believed to have a regulatory role in microglia activation and hence neuroinflammation. A_{2A} receptor expression and function is enhanced in AD, both in the CNS and in blood platelets. The changes in platelet expression may enable A_{2A} receptor levels to be used as a biomarker. A_{2A} receptor antagonists, such as SCH 442416 (Cat. No. 2463) and Istradefylline (Cat. No. 5147) may be useful research tools to explore this potential therapeutic avenue in AD.

Cell Therapies

Cell replacement therapy in neurodegenerative disorders has shown some promise in Parkinson's Disease (PD). Clinical trials are using human embryonic stem cell (hESC)-derived dopaminergic neuron progenitors to replace the focal loss of midbrain neurons in PD. The same approach is more of a challenge in AD, due to the widespread loss of neurons. For cell replacement to be successful, the cells would need to migrate to different areas within the brain and differentiate into functional neuronal subtypes that establish connectivity with existing neurons; this is even more of a challenge when there is widespread degeneration. There are early phase clinical trials using mesenchymal stem cells (MSC) to treat mild-to-moderate AD. MSCs are transplanted into the brain and, as a result of native signaling factors in the local environment, differentiate along a neuronal lineage *in vivo*. The findings of these trials are eagerly awaited.

Gene Therapies

Large cohort genome wide association studies show evidence of a strong hereditary component to AD risk (amounting to >90% in the case of early-onset AD). These studies show that individuals carrying specific risk variants in specific genes (i.e. APP, PSEN1/2 and APOE4) are significantly more likely to develop AD, compared to non-carriers. Gene therapies act to address this by either directly correcting the mutant gene sequence using strategies such as CRISPR-based editing systems, or by compensating for the risk variant by introducing exogenous "protective" genes. The latter strategy is being trialled in AD patients homozygous for APOE ε4 which has been shown to dysregulate cholesterol transport and impair myelination. Apolipoprotein E (ApoE) has several variants with carriers of ApoE ε4 at risk of AD, and ApoE ε2 carriers resistant to AD. Using an AAV viral vector, AD patients homozygous for ApoE ε4 have the protective ApoE ε2 variant delivered to the CNS tissue in an attempt to compensate for the actions of ApoE ε4. This same viral vector strategy has also been developed to deliver the neurotrophic factor BDNF (Cat. No. 2837) and has shown promise in preclinical models.

Future Directions

Current treatments for AD can alleviate the symptoms of the disease but do not modify its natural progression; effective disease-modifying approaches are the principal focus for AD research. Despite the prominence of several theories on the pathogenesis of AD, there are aspects of each theory that cannot fully explain the degenerative effects of AD. Pathological targets such as the amyloidogenic secretases, Aβ aggregation, kinases and phosphatases regulating tau phosphorylation and fibrillation, as well as O-GlcNAcases, are primary targets for further research. In time, it is hoped that new targets will be revealed that could lead to additional options for AD treatment and help to slow disease progression.

List of Acronyms

Acronym	Definition
AAV	Adeno-associated virus
Aβ	Amyloid beta
ACh	Acetylcholine
AD	Alzheimer's disease
ApoE	Apolipoprotein E
APP	Amyloid precursor protein
BDNF	Brain-derived neurotrophic factor
CK1	Casein kinase 1
EAA	Excitatory amino acid
FDA	Food and Drug Administration
hESC	Human embryonic stem cell
ISR	Integrated stress response
JNK	c-Jun N-terminal kinase
MAP	Microtubule-associated protein
MSC	Mesenchymal stem cells
NFT	Neurofibrillary tangles
OGA	O-GlcNAcase
OGT	O-GlcNAc transferase
PERK	PKR-like ER kinase
PKR	Protein kinase R
ptau	Hyperphosphorylated tau
ROS	Reactive Oxygen Species

Products

	Cat. No.	Product Name	Primary Action
5-HT Receptors	3326	BGC 20-761	High affinity 5-HT_6 antagonist
	1322	GR 113808	Potent and selective 5-HT_4 antagonist
	1658	GR 125487	Potent and selective 5-HT_4 antagonist; active *in vivo*
Adenosine Receptors	2793	Caffeine	A_1 and A_{2B} antagonist. CNS stimulant
	2463	SCH 442416	Very selective, high affinity A_{2A} antagonist
	5417	Istradefylline	Potent and selective A_{2A} antagonist
Acetylcholine Muscarinic Receptors	1067	Oxotremorine M	Muscarinic agonist
	3569	Xanomeline oxalate	Functionally selective M_1 agonist
	1425	(S)-(+)-Dimethindene maleate	Selective M_2 antagonist
	1671	PD 102807	Selective M_4 antagonist
	2507	J 104129 fumarate	Potent and selective M_3 antagonist
	4404	VU 0365114	Positive allosteric modulator of M_5 receptors
Acetylcholine Nicotinic Receptors	0789	(±)-Anatoxin A fumarate	nAChR agonist
	3328	Desformylflustrabromine hydrochloride	Positive allosteric modulator of α4β2 nAChRs; also muscle-type nAChR inhibitor
	3546	(-)-Nicotine ditartrate	Prototypical nAChR agonist
	2843	Mecamylamine hydrochloride	Non-competitive nAChR antagonist
	2349	Dihydro-β-erythroidine hydrobromide	α4β2, muscle type and *Torpedo* nAChR antagonist
	2809	Acetylcholine chloride	Endogenous neurotransmitter
	2810	Carbamoylcholine chloride	Non-selective cholinergic agonist
	1340	α-Conotoxin MII	Potent and selective α3β2 and β3 nAChR antagonist
AMPA Receptors	0190	CNQX	Potent and selective non-NMDA iGluR antagonist
	0189	DNQX	Selective non-NMDA iGluR antagonist
	0188	L-Quisqualic acid	AMPA agonist; also group I mGlu agonist
Amyloid Fluorescent Probes	7122	Thioflavin T	Fluorescent amyloid stain, binds to the stacked β sheets of amyloid fibrils
	3144	K 114	Amyloid fibril-specific fluorescent dye
	4803	CRANAD 2	Near-infrared probe that detects Aβ40 aggregates
	4920	Methoxy-X04	Fluorescent amyloid β detector; brain penetrant
Amyloid Proteins	1428	Aβ1-42	Predominant amyloid β-protein fragment
	1191	Aβ1-40	Amyloid β-protein fragment
	7712	Semax	Reduces Aβ aggregation and fibrillogenesis; neuroprotective and neurotrophic peptide
	6779	Davunetide	Highly potent active component of ADNP; prevents amyloid β aggregation; active *in vivo*
	2408	Ro 90-7501	Inhibitor of Aβ42 fibril formation

	Cat. No.	Product Name	Primary Action
Antioxidants	4055	L-Ascorbic acid	Enhances the generation of iPSCs; increases reprogramming efficiency
	3550	Melatonin	Endogenous hormone; agonist at MT_1 and MT_2
	1418	Resveratrol	Cyclooxygenase inhibitor
	5619	N-Acetylcysteine amide	Glutathione (GSH) precursor; neuroprotective
	6002	Trolox	Antifade reagent; antioxidant vitamin E derivative
Beta Secretases	4524	EGCG	β-secretase (BACE) inhibitor; inhibits amyloid assembly
	7081	LY 2886721 Hydrochloride	Potent and selective β-secretase (BACE) inhibitor
Caged Nicotinic Compounds	6700	PA Nic	Caged nicotine; photoactivated by one-photon and two-photon excitation
	6706	DPNB-ABT 594	Caged ABT 594 (Cat. No. 6576); suitable for one- or two-photon photolysis
Cholinesterases	4385	Donepezil	Potent AChE inhibitor
	4440	Rivastigmine	Dual AChE and BChE inhibitor
	0686	Galanthamine	Cholinesterase inhibitor; also allosteric potentiator at neuronal nAChRs
Gamma Secretases	2627	L-685,458	Potent and selective γ-secretase inhibitor
	2634	DAPT	γ-secretase inhibitor; induces neuronal differentiation; blocks Notch signaling
	4489	DBZ	γ-secretase inhibitor; inhibits Notch pathway
	6476	Compound E	γ-secretase inhibitor; induces neuronal differentiation
	4000	MRK 500	γ-secretase inhibitor; attenuates amyloid plaque deposition
Kinases	4740	Lithium	Mood stabilizer; inhibits GSK-3 *in vivo*
	4924	CEP 1347	Inhibitor of JNK signaling
	3945	Colivelin	STAT3 activator; also protects against β-amyloid neurotoxicity
	1813	Indirunib-3′-oxime	GSK-3β inhibitor; also inhibits cdks and other protein kinases
	1616	SB 216763	Potent, selective GSK-3 inhibitor
	7650	AZD 2858	Highly potent GSK-3 inhibitor; inhibits tau phosphorylation
	3966	AR-A 014418	Selective GSK-3 inhibitor
	4997	INDY	DYRK1A/B inhibitor
	4998	proINDY	DYRK1A/B inhibitor; prodrug of INDY (Cat. No. 4997)
	5382	C16	PKR inhibitor
	5107	GSK 2606414	Potent and selective PERK inhibitor; orally bioavailable
	5284	trans-ISRIB	Integrated stress response (ISR) inhibitor
	2902	D 4476	Selective CK1 inhibitor. Also inhibits TGF-βRI
	3610	(R)-DRF053	Potent CK1 inhibitor; also inhibits cyclin-dependent kinases
Lysosomes	7538	NBD-PE	Single tail (16:0) phospholipid derivative with a fluorophore (NBD) on the head for labeling lysosomal lipid bodies
	7314	Pepstatin A Janelia Fluor® 526	Fluorogenic green-emitting lysosome tracker and stain

	Cat. No.	Product Name	Primary Action
Microtubules	1097	Taxol	Promotes assembly and inhibits disassembly of microtubules
	1467	Daunorubicin	DNA topoisomerase II inhibitor
	2252	Doxorubicin	Antitumor antibiotic agent. Inhibits DNA topoisomerase II
	1515	17-AAG	Selective Hsp90 inhibitor
	6266	Taxol Janelia Fluor® 646	Red fluorescent taxol derivative; probe for microtubule staining
Mitochondria	7641	Mito-HE	Fluorescent mitochondrial superoxide indicator in live cells
	4428	MitoPY1	Fluorescent mitochondrial hydrogen peroxide indicator
	2906	TRO 19622	Binds voltage-dependent anion channel (VDAC)
	6445	MitoMark Red I	Red fluorescent mitochondrial stain; cell permeable
	6897	P110	Dynamin-related protein 1 (Drp1) inhibitor; cell-permeable
	7700	MitoBrilliant™ 646	Universal red fluorescent mitochondrial stain for both live and fixed cells
	7693	MitoBrilliant™ Live 549	Orange fluorescent mitochondrial stain for live cells, $\Delta\psi m$ dependent
	7417	MitoBrilliant™ Live 646	Red fluorescent mitochondrial stain for live cells, $\Delta\psi m$ dependent
NMDA Receptors	0773	Memantine	NMDA antagonist; acts at ion channel site
	0114	NMDA	Selective NMDA agonist
	0312	(RS)-(Tetrazol-5-yl)glycine	Highly potent NMDA agonist
	0106	D-AP5	Potent and selective NMDA receptor antagonist; more active form of DL-AP5
	0924	(+)-MK 801 maleate	Non-competitive NMDA antagonist; acts at ion channel site
O-GlcNAcase	4390	Thiamet G	Potent O-GlcNAcase inhibitor
Phosphatases	0744	Ceramide	Ser/Thr protein phosphatase activator
	0773	Memantine	NMDA antagonist; acts at ion channel site
	1136	Okadaic acid	Protein phosphatase 1 and 2A inhibitor
	1336	Calyculin A	Protein phosphatase 1 and 2A inhibitor
RAGE	7701	RAGE 229	ctRAGE-DIAPH1 interaction antagonist; reduces inflammatory signaling in diabetic mice
	6237	FPS ZM1	High affinity antagonist of RAGE
	6259	RAGE antagonist peptide	RAGE antagonist
Sigma Receptors	0883	BD 1063 dihydrochloride	Selective σ_1 ligand, putative antagonist
	0545	Ifenprodil hemitartrate	Non-competitive NMDA antagonist; also σ ligand
	7630	Pridopidine	Highly selective σ_1 agonist
	7156	WQ 1	Potent and selective σ_1 receptor antagonist; active *in vivo*
Tau	3213	Methylene blue	Inhibits tau filament formation; biological stain; also neuroprotective and antimalarial
TrkB Receptor	2837	BDNF	Activates TrkB and p75 receptors
	5062	Cyclotraxin B	TrkB receptor antagonist
	6037	LM22B 10	TrkB and TrkC agonist; brain penetrant

Further Reading

Please refer to the list of recommended papers for more information.

Abbott (2022) Could drugs prevent Alzheimer's? These trials aim to find out. *Nature* **603** 216

Bond *et al* (2020) The Integrated Stress Response and Phosphorylated Eukaryotic Initiation Factor 2α in Neurodegeneration. *J. Neuropathol. Exp. Neurol*. **79** 123

Cummings *et al* (2021) Alzheimer's disease drug development pipeline: 2021. *Alzheimers Dement. (NY)* **7** e12179

Gupta & Weaver (2022) Alzheimer's: The ABCDE Paradigm. ACS Chem. *Neurosci*. **13** 1355

Inuzuka *et al* (2022) PROTAC technology for the treatment of Alzheimer's disease: advances and perspectives. *Acta. Mater. Med*. **1** 24

Kargbo (2020) PROTAC Compounds Targeting α-Synuclein Protein for Treating Neurogenerative Disorders: Alzheimer's and Parkinson's Diseases. *ACS Med. Chem. Lett*. **16** 1086

Kondo *et al* (2017) iPSC-Based Compound Screening and *In vivo* Trials Identify a Synergistic Anti-amyloid β Combination for Alzheimer's Disease. *Cell Rep*. **21** 2304

Lu *et al* (2018) Discovery of a Keap1-dependent peptide PROTAC to knockdown Tau by ubiquitination-proteasome degradation pathway. *Eur. J. Med. Chem*. **146** 251

Mandal *et al* (2021) AD Hypotheses and Suggested Clinical Trials. *ACS Chem. Neurosci*. **12** 3968

Mielke *et al* (2022) Performance of plasma phosphorylated tau 181 and 217 in the community. *Nat. Med*. **28** 1398

Milà-Alomà *et al* (2022) Plasma p-tau231 and p-tau217 as state markers of amyloid-β pathology in preclinical Alzheimer's disease. *Nat. Med*. **28** 1797

Miller *et al* (2022) Somatic genomic changes in single Alzheimer's disease neurons. *Nature* **604** 714

Oliver & Reddy (2019) Small molecules as therapeutic drugs for Alzheimer's disease. *Mol. Cell. Neurosci*. **96** 47

Palmqvist *et al* (2021) Prediction of future Alzheimer's disease dementia using plasma phospho-tau combined with other accessible measures. *Nat. Med*. **27** 1034

Piller (2022) Blots on a field? *Science 3*. **77** 358

Potjewyd *et al* (2022) AD Informer Set: Chemical tools to facilitate Alzheimer's disease drug discovery. *Alzheimers Dement. (NY)* **8** e12246

Reinitz *et al* (2022) Inhibiting USP16 rescues stem cell aging and memory in an Alzheimer's model. *Elife* **11** e66037

Serrano-Pozo *et al* (2021) APOE and Alzheimer's disease: advances in genetics, pathophysiology, and therapeutic approaches. *Lancet Neurol*. **20** 68

Soriano-Castell *et al* (2021) The search for anti-oxytotic/ferroptotic compounds in the plant world. *Br. J. Pharmacol*. **178** 3611

Targa Dias Anastacio *et al* (2022) Neuronal hyperexcitability in Alzheimer's disease: what are the drivers behind this aberrant phenotype? *Transl. Psychiatry* **12** 257

Thomas *et al* (2022) Excessive local host-graft connectivity in aging and amyloid-loaded brain. *Sci. Adv*. **8** eabg9287

VandeVrede *et al* (2020) Targeting tau: Clinical trials and novel therapeutic approaches. *Neurosci. Lett*. **731** 134919

Zhao *et al* (2022) Targeting Necroptosis as a Promising Therapy for Alzheimer's Disease. *ACS Chem. Neurosci*. **13** 1697

Consistent Reagents for Consistent Cultures

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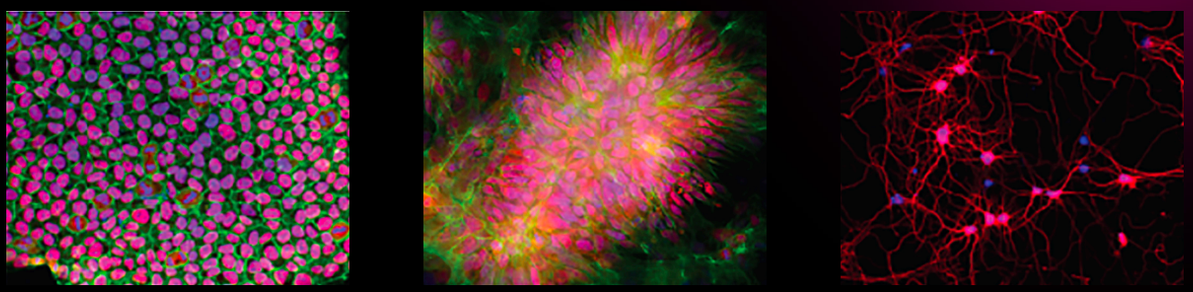
Neurons Cultured from Induced Pluripotent Cells Using Bio-techne Reagents



Human induced pluripotent stem cells (HiPSCs) maintained in ExCellerate™ iPSC Expansion Medium (Catalog # CCM036) differentiate into neurons using the StemXVivo™ Neural Progenitor Differentiation Kit (Catalog # SC035) or using research-grade or GMP-grade proteins, small molecules, and supplements: GMP SB 431542 (Catalog # 1614 or TB1614-GMP), Recombinant Human Noggin (Catalog # 6057-NG or 6057-GMP), Recombinant Human FGF basic (Catalog # 233-FB or 233-GMP), N-2 MAX Media Supplement (Catalog # AR009 or AR016), and N21-MAX Media Supplement (Catalog # AR008). Cells were stained with antibodies to Oct-3/4, E-Cadherin, Pax6, Actin, and Tuj plus DAPI.

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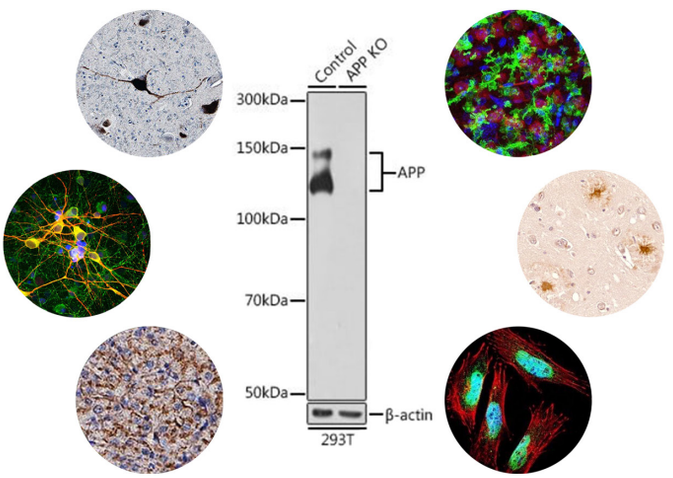

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