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Experimental modelling of Alzheimer's disease for therapeutic screening

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ABSTRACT

Neurodegenerative diseases, including Alzheimer's disease (AD), pose a significant and urgent challenge to healthcare systems worldwide. With an increasing life expectancy, these progressive age-related disorders are expected to rise exponentially. No cure currently exists for AD, and the aetiology remains poorly understood. Furthermore, AD drug development faces one of the highest failure rates. Thus, a review of the experimental modelling of the disease is crucial to understanding how the current disease models can be applied to gain useful results while also considering their limitations. Disease models include *in vitro*, *in vivo*, *ex vivo*, and *in silico* systems as well as clinical trials. These systems are important for testing potential therapeutics to advance drug development, in addition to modelling the pathology of the disease to gain a greater understanding of the cause and progression. This review will discuss the current experimental models employed for the study of AD with the aim of providing an overview of how they are used and discuss their benefits and drawbacks as model systems, as well as highlighting the potential future of the experimental modelling of AD.

1. Introduction

Experimental modelling of Alzheimer's disease (AD) has been crucial to the development of current knowledge on the pathogenesis of the disease, and in the testing of potential treatments. At present, numerous models of AD exist to simulate the pathological alterations associated with the disease in humans including cell, animal, and computational models [1]. While these experimental models continue to be useful in AD research, none are able to replicate the complete pathophysiology of AD and as a result, there has been considerable doubt cast over the reliability of the results obtained through the use of these models. Development of experimental models that better mimic the complexity of AD in humans continues. This review aims to summarise the experimental models employed for AD at present, and discuss their role in the drug development process by providing examples of therapeutics that have been studied in each model. This highlights the ways in which to best utilise these models to obtain appropriate and reliable insight into the potential of screened therapeutics while acknowledging the limitations of each model.

2. Alzheimer's disease

Alzheimer's disease is a major cause of death worldwide, with over 50 million people suffering from this debilitating neurodegenerative disease [2]. Alois Alzheimer first described the disease in 1906 and noted the characteristic senile plaques and neurofibrillary tangles in patients' brains that continue to be synonymous with the disorder today [3]. As one of the most prevalent causes of death and the most common cause of dementia, AD is accountable for a vast social and economic burden. AD is age-related, and causes increasingly incapacitating symptoms as the disease advances including significant memory loss, confusion, language disturbances, and behavioural changes. Despite its exponential prevalence in correlation with the rising global life expectancy and its devastating effects, AD remains incurable and drug development faces one of the highest failure rates in any therapeutic area. Only four drugs are clinically available for the treatment of AD in the UK and these drugs aim to mitigate symptoms only, with no disease-modifying effects. This disappointing situation did not change for almost two decades from 2003 when memantine was approved [4] (Fig. 1). Since then, only around 50 drug candidates have passed Phase 2 trials and only one has succeeded Phase 3. With so little progress in this area despite extensive research, it is crucial to review the drug discovery process for AD. At the core of the issue is the lack of understanding regarding the exact origin of AD. However, key hallmarks of the disease have been the subject of significant research, as well as the ways in which these hallmarks can be modelled for experimental therapeutic screening.

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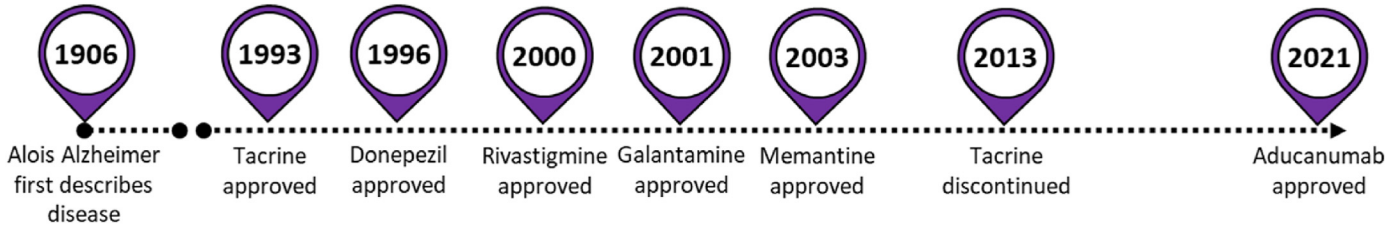

Fig. 1. Key dates in AD drug development. The years listed are correct for the US Food and Drug Administration (FDA). At present, aducanumab has not been approved by the European Medicines Agency (EMA).

3. Development of AD

Numerous hallmarks of AD have been identified since the discovery of the disease, and these have been applied as targets for the development of AD therapeutics. Amyloid plaques and neurofibrillary tangles were the initial hallmarks of the disease, and remain the major targets for AD drug development. More recently, inflammation has also emerged as a key feature of AD pathology (Fig. 2).

3.1. Amyloid hypothesis

The amyloid hypothesis has dominated AD research for the past two decades. This hypothesis postulates that the abnormal deposition of beta-amyloid (Aβ) proteins extracellularly in the brain is responsible for initiating the cascade of pathological alterations associated with AD [5]. The insoluble amyloid aggregates, generated *via* the proteolytic cleavage of amyloid precursor protein (APP), deposit around the neurons. Aβ aggregates induce neurotoxicity, however the exact relationship between amyloid deposition and the development of AD is still not fully understood. Recently, the amyloid hypothesis has faced increasing controversy due to the lack of success in clinical trials of drugs that are aimed at counteracting amyloid aggregation [6]. While the drugs are reported to reduce plaque formation *in vitro* and *in vivo*, there has been a lack of positive results in patients in terms of improving cognitive function. Within the last few years, drugs which target the soluble neurotoxic amyloid oligomers rather than the plaques have demonstrated greater clinical efficacy. Evidence suggests that the oligomeric amyloid species may play a key role in triggering AD pathology [6]. Despite the previous failure of anti-amyloid treatments, significant evidence persists to demonstrate the clear importance of amyloid aggregation in AD pathology. Human biomarker studies have shown that plaque formation precedes other AD-associated changes including hyperphosphorylated tau deposition, neuron loss, and cognitive decline [7]. Furthermore, familial AD (FAD) which is the hereditary form of the disease, responsible for a minority of AD cases, is associated with mutations in PSEN1, PSEN2, and APP genes which are all linked to the formation of abnormal amyloid plaque formation [8]. People with Down's syndrome also exhibit a genetic defect which is associated with a build-up of amyloid plaques, and consequently these individuals are at a greater risk of developing AD [9]. Carriers of the ApoE4 allele are pre-disposed to the development of the more common form of AD, sporadic or late-onset, as this allele reduces the rate of amyloid clearance in the brain which leads to a build-up of excess Aβ proteins [10]. Overall, it is clear that amyloid aggregation is a key marker of AD even at early stages in the development of the disease. Therefore, it remains an important target of AD therapeutics and a vital hallmark to replicate in experimental models of the disease.

3.2. Tau hypothesis

Hyperphosphorylated tau fibrils aggregate as intraneuronal neurofibrillary tangles in the brains of AD patients. In healthy brains, tau is a phosphoprotein that promotes the assembly of tubulin into microtubules and stabilises this structure. Normal tau is highly soluble, whereas tau oligomers formed by hyperphosphorylated tau are insoluble and can self-assemble into neurofibrillary tangles [11]. Hyperphosphorylated tau is associated with numerous neurodegenerative diseases including Pick disease, dementia pugilistica, and fronto-temporal dementia with Parkinsonism linked to chromosome 17 [12]. In such tauopathies, the

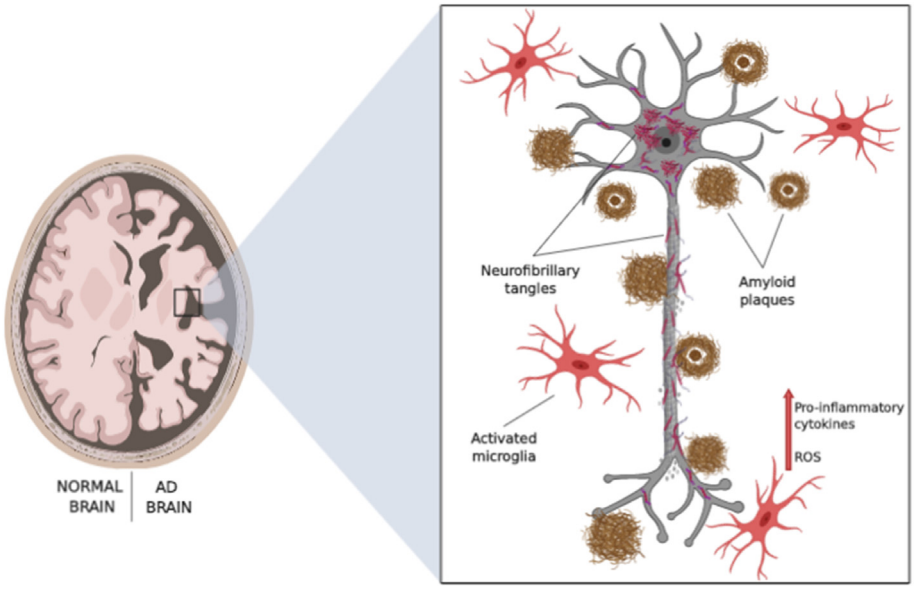

Fig. 2. Key hallmarks of AD: intraneuronal tau neurofibrillary tangles, extracellular amyloid plaques, and activated microglial cells. Activated microglia generate a rise in the production of pro-inflammatory cytokines and ROS, resulting in neuroinflammation.

presence of abnormally hyperphosphorylated tau in the neocortex is linked to dementia. The level of total tau in AD brains is four to eight-fold greater than in normal aged brains, and this rise is exclusively in the form of aberrantly hyperphosphorylated tau [13]. Tau in the form of neurofibrillary tangles does not function as typical tau proteins in healthy brains, and appears to be inert. However, hyperphosphorylated tau occurring in the cytosol and not polymerised into tangles can induce toxic effects by inhibiting the assembly of tubulin and disrupting microtubule structures. It can aggregate with normal tau into oligomers and consequently self-assemble into tangles, and it can also sequester other microtubule-associated proteins into amorphous aggregates [14]. It has been postulated that this disruption of microtubules and sequestering of microtubule-associated proteins by the cytosolic hyperphosphorylated tau is the trigger for neurodegeneration and cognitive decline, and the aggregation of hyperphosphorylated tau into neurofibrillary tangles is likely a self-defence mechanism induced by the affected neuron [15]. As a result, inhibiting aberrant tau hyperphosphorylation is a key therapeutic route for the treatment of AD. Furthermore, accurately modelling this tauopathy is important for screening potential AD drugs.

3.3. Inflammation

While the former two hallmarks of AD have been well-established since the discovery of the disease by Alois Alzheimer [3], a third key feature of AD has emerged within the last two decades [16,17]. The brains of AD patients have been found to exhibit chronic inflammation due to a sustained immune response. The presence of elevated markers of inflammation is not exclusive to AD, and is now associated with numerous neurodegenerative diseases including Parkinson's (PD), multiple sclerosis (MS), and amyotrophic lateral sclerosis (ALS) [18]. Neuroinflammation is initially caused by neuronal loss and other AD pathologies as an acute neuroprotective response, however this becomes detrimental and exacerbates the severity of the disease as the immune response persists. As depicted in Fig. 2, activated microglia disrupt the equilibrium of anti-inflammatory and pro-inflammatory signalling towards the latter and release a variety of toxic products, including numerous cytokines (e.g. interleukins, tumour necrosis factors) and reactive oxygen species (ROS) [19]. Chronic neuroinflammation is attributed to the exacerbation of amyloid and tau pathologies. Reactive microgliosis, whereby there is sustained activation of microglia as part of the inflammatory response, stimulates amyloid aggregation and chronically produces pro-inflammatory cytokines which damage neurons [20]. Cytokines, in particular interleukin-6, reportedly stimulate the hyperphosphorylation of tau by activating protein kinases (namely, CDK5) [21]. Furthermore, interleukin-1 enhances acetylcholinesterase (AChE) expression and activity which results in cholinergic dysfunction and the loss of cholinergic neurons [22]. Overall, the importance of inflammation in AD is evident and further study of its role in AD models is crucial to the development of anti-inflammatory therapeutics which have the potential to slow or delay the progression of the disease.

4. Drug discovery process

The drug discovery process for AD is a time-consuming, arduous, and costly procedure (Fig. 3). It encompasses several stages including research and development, preclinical studies, clinical trials, and a final review and approval by the regulatory body: the Food and Drug Administration (FDA) in USA, and the European Medicines Agency (EMA) in European Union. Each stage also involves numerous steps and processes to focus in on the lead that will be optimised and taken to clinical trials, from the initial vast library of compounds. Following the identification and optimisation of a lead, the compound must undergo preclinical studies including *in vitro* and *in vivo* models, as well as toxicity studies [23]. The subsequent clinical trials will be discussed further in section 12, but notably this stage poses the greatest hurdle in the drug development process with the highest cost both financially and in terms of duration. The failure rate for disease-modifying AD therapeutics in clinical trials is currently 100%, and the number of agents reaching clinical trials for the treatment of AD is around 97% lower than that for cancer [24]. This striking disparity is largely attributed to the higher success rate of cancer trials, which thereby attracts more funding and subsequently leads to the development of further therapies. Finally, following the clinical trials, successful drugs are passed to the appropriate regulatory body to be approved. This process includes the review of evidence substantiating the drug's safety and efficacy [23]. When a drug is approved, it can then be manufactured and prescribed to patients. However, the regulatory body continues to monitor the product's safety in the marketplace.

5. Natural and synthetic compounds as AD therapeutics

Traditionally, synthetic single-target therapeutics have been designed and implemented for the treatment of neurodegenerative diseases. This includes small molecule inhibitors against targets such as cholinesterases and amyloid aggregation. With the advance of computational simulations and *in silico* studies, synthetic drug design has become progressively

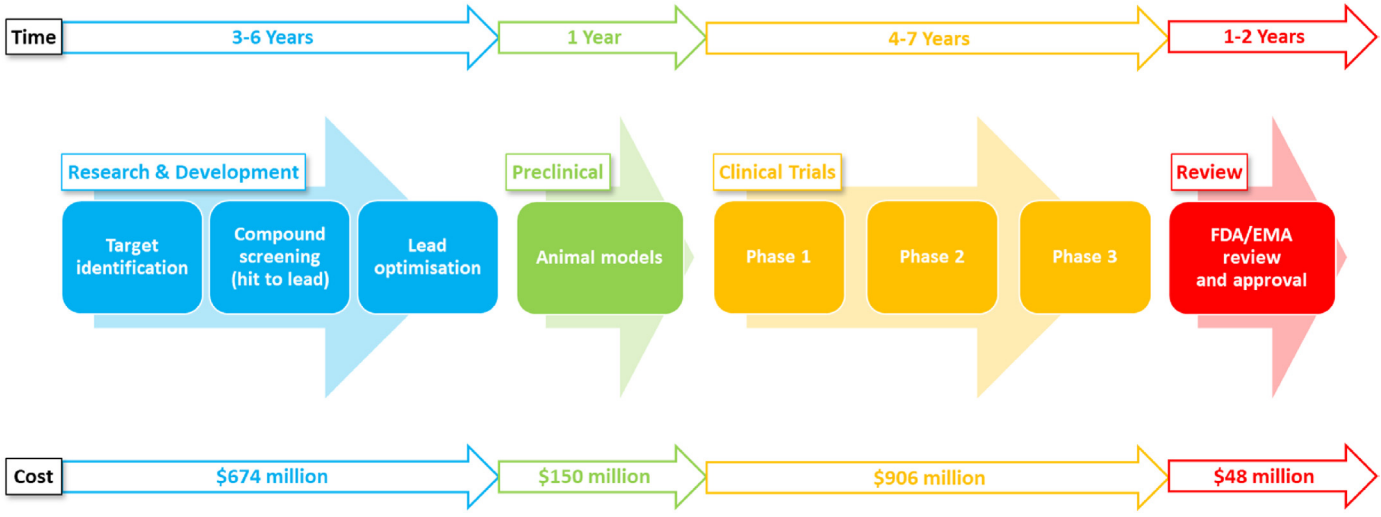

Fig. 3. Drug development process from research and development to clinical trials and final review. The typical total duration and cost associated with the process is 9–16 years and around $2 billion.

simpler. Predictions on pharmacokinetic and pharmacodynamic properties can be made rapidly and with increasing accuracy [25]. Large libraries of compounds can be narrowed down to a manageable number of structures with promising activity, which are then synthesised and evaluated. This can save immense costs and time. Since the beginning of the century, a multi-target approach to drug development has gained attention due to the lack of disease-modifying effects observed with the administration of single-target therapeutics in patients [26–29]. This approach involves the generation of synthetic hybrid compounds with the capacity to counteract multiple targets of complex diseases such as AD simultaneously. This effect is expected to slow or prevent the progression of the disease, and such multi-target agents have demonstrated promising results in experimental models. However, no therapeutics of this type for AD have passed clinical trials so far.

Semi-synthetic drugs, or synthetic drugs based on natural scaffolds, constitute the majority of clinically approved AD therapeutics: donepezil (selective AChE inhibitor), rivastigmine (non-selective cholinesterase inhibitor), and memantine (NMDA receptor antagonist) [30]. Galantamine is the exception to the other semi-synthetic approved AD drugs. It is derived from plants, specifically from the *Amaryllidaceae* family [30]. Natural products have become increasingly popular due to the widely held conception that 'natural' means safe. While there are some reports of fewer side effects, natural agents can still induce toxic effects [31]. Furthermore, the conversion of natural products into therapies faces several challenges including difficulty isolating the active agent(s), limited efficacy, and poor bioavailability. Nevertheless, animal and plant-based products have exhibited potential as therapeutics including multi-target activity and synergistic effects between active agents within an extract [31].

6. Current AD therapeutics

At present, only four drugs are clinically available for the treatment of AD in the UK (Fig. 4). Of these, three are acetylcholinesterase (AChE) inhibitors while the other is an antagonist of the *N*-methyl-D-aspartate receptor (NMDAR) [32]. AChE inhibitors, including donepezil, rivastigmine, and galantamine, are typically prescribed for mild to moderate AD cases whereas the NMDAR antagonist, memantine, is for severe cases. The AChE inhibitors have differing modes of action, but with the same core aim of preventing cognitive decline associated with the loss of cholinergic neurons. While donepezil and rivastigmine function to prevent the degradation of acetylcholine (ACh, a neurotransmitter) by inhibiting the activity of AChE, galantamine exerts a similar effect *via* an alternative mechanism by inducing increased levels of ACh through the stimulation of pre- and post-synaptic nicotinic receptors [33]. Memantine interacts with NMDARs to block the effects of glutamate, a neurotransmitter which exerts excessive stimulation on neurons causing excitotoxicity and preventing normal neurotransmission [34]. Although these drugs alleviate symptoms of AD, they are unable to slow or prevent the progression of the disease. AChE inhibitors are also associated with adverse gastrointestinal effects [33]. Therefore, the need for a disease-modifying or curative agent for this disease remains incessantly urgent.

6.1. Current AD therapeutics in clinical trials

Although numerous agents are entered into clinical trials every year, not since 2003 has there been a novel drug approved for the treatment of AD in the UK [4]. The disappointing failure rate in AD trials has brought about a shift in research focus, namely the development of drugs with alternative targets to the typical anti-amyloid agents [35]. As the amyloid hypothesis has been challenged in recent years due to a lack of positive results in human testing, the number of agents entering clinical trials targeting tau and inflammation have increased. Furthermore, combination therapies and multi-target drugs have also gained attention [35]. This approach has been driven by the fact that modulation of a single target of complex, multifactorial diseases such as AD is not sufficient to yield the desired disease-modifying efficacy. Nevertheless, anti-amyloid agents constitute the majority of AD drugs in clinical trials (Fig. 5). However, these trials are now directed at patients in early or preclinical stages of AD. A potential justification for the high failure rates of AD drugs, in particular the anti-amyloid agents, is that the patients recruited for trials were often in late-stages of the disease with symptoms so severe that any disease-modifying effects would be unlikely [36]. With the recent approval of aducanumab by the FDA (discussed further in section 11), there is renewed hope in the field particularly for agents which can target the neurotoxic, soluble oligomeric form of amyloid [6].

With the above considerations in mind, the drug development process has continued. At present, there are around 70 AD drugs in clinical trials (based on a clinicaltrials.gov search of drug trials that are currently active). There are 11 agents in Phase 1 trials, 43 in Phase 2, and 13 in Phase 3. Fig. 5 below displays the major targets of the agents in each phase of the trials. Most of the agents were small molecule therapeutics (59%), followed by antibodies (26%). The remaining drugs were combination therapeutics or DNA/RNA based (5% each), and supplement/dietary (3%) or hormones (2%).

7. Experimental models of AD

Experimental models are critical for elucidating the fundamental

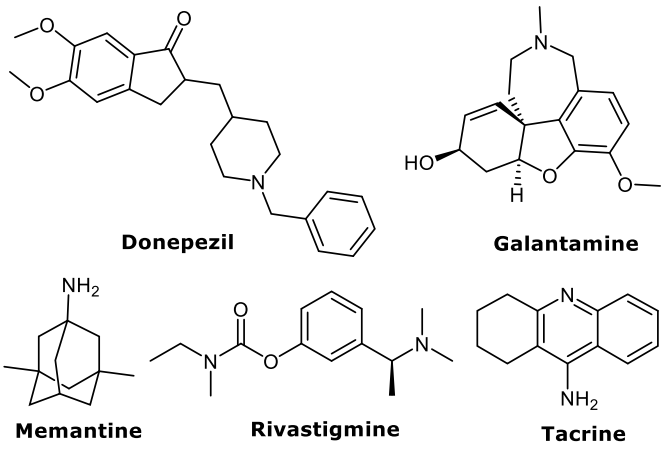

Fig. 4. Chemical structures of the four current clinically available AD therapeutics (donepezil, galantamine, memantine, rivastigmine), and the first AChE inhibitor (tacrine) which was withdrawn in 2013 due to hepatotoxicity.

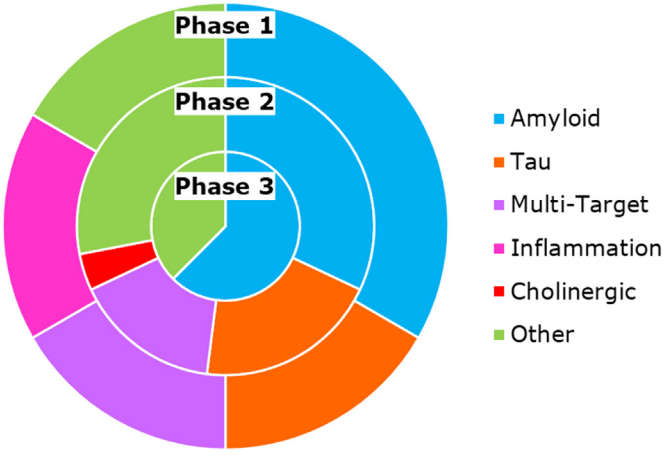

Fig. 5. Targets of AD drugs currently in clinical trials. These data were taken from clinicaltrials.gov, and the search was focused on drugs in clinical trials for Alzheimer's disease that are currently active ('active, not recruiting').

mechanisms underlying AD, as well as evaluating novel therapeutics. Typically, *in vitro* and *in vivo* models (e.g. cell and rodent models respectively) are employed prior to clinical trials on human patients [37]. *Ex vivo* models (e.g. rodent brain slices) and, more recently, *in silico* models (e.g. virtual ligand screening) have also been developed to further aid in modelling AD.

7.1. General advantages and disadvantages of current AD models

Experimental models are vital for toxicity studies prior to human trials. Currently, a minimum of 2 mammalian species are required for preclinical toxicity studies. Any toxic effects would typically be established in initial *in vitro* and *in vivo* studies, and attempts would be made to reduce these adverse effects prior to mammalian and subsequent human testing [28]. These studies provide important information about projected safe and tolerable dosage ranges, as well as the pharmacokinetic profile of the drug. As mentioned above, experimental models are also useful for deciphering AD pathology by simulating the changes observed in humans during the disease. *In vivo* models can provide information regarding the complex pathogenesis of AD and reproduce the progressive nature of the disease as seen in patients. In *in vitro* models, in-depth cellular studies can be performed to establish the mechanisms that generate the hallmarks of AD [37]. Unfortunately, none of the current experimental models can reproduce the complexity of the disease as observed in human patients. Poor translation of positive preclinical results to patient trial outcomes has been attributed to the lack of accurate disease modelling [37]. Therefore, research is ongoing to produce an experimental model that can better represent AD development. Furthermore, it is increasingly common practice to employ several AD models in preclinical studies that replicate different features of the disease to achieve a more reliable indication of the potential effects in humans. Although an accurate representation of the human condition during AD remains unavailable at present, the importance of experimental modelling is indisputable in terms of advancing current knowledge of AD development and testing novel therapies.

8. *In vitro* models of AD

In vitro models of AD allow the study of pathological changes at a cellular level (Table 1). These models have the advantage of strictly controlled environmental conditions, in addition to lower costs and simpler maintenance and handling compared to *in vivo* models [37]. Studies can also be carried out with shorter timescales, and preliminary efficacy and pharmacodynamic experiments can be performed on these cell models [38]. Although initial toxicity studies can be carried out, these models cannot provide reliable pharmacokinetic data due to their simplicity. For the purposes of this report, *in vitro* models will include 2D and 3D cell culture and induced pluripotent stem cells (iPSCs) while tissue models and primary cultures will be discussed later as *ex vivo* models.

8.1. Therapeutics tested in in vitro models of AD

PC-12 and SH-SY5Y neuron cell lines are among the most commonly employed cell models for neurodegenerative diseases. Both lines can be used in undifferentiated or differentiated forms. Using PC-12 cells, Tong

Table 1
Summary of common *in vitro* models of AD; including the pathological relevance of each model to AD, the studies that can be performed, and the advantages and disadvantages of the models.

Model		Pathological Relevance to AD	Phenotype & Assessments	Advantages	Disadvantages	Ref
2D Cell Culture	HBMEC	Barrier properties like BBB	Study drug delivery	• Inexpensive • Well-established • Simple to manipulate and analyse • Mass of comparative literature	• Not representative of real environments • Response to stimuli not reflective of actual case • Usually only one cell type; lack of interaction and contribution of different cell types • Often cancer-derived, with a multitude of genetic changes	[39]
	BCEC	Retain BBB characteristics	Study drug delivery Study Aβ effect on BBB			[40] [41]
	RBE4	Retain BBB properties Express BACE-1 and APP	Study BACE-1 activity and APP processing Study drug delivery			[42] [43]
	SH-SY5Y	Neuron model Can be differentiated into cholinergic phenotype Express tau	Study neurotoxicity Study AD mechanisms and pathways including Aβ and oxidative stress	• Easy to control environment		[44] [45]
	SK-N-MC	Cholinergic-like neuron model	Study AD mechanisms including Aβ			[46]
	PC-12	Neuron model	Study AD mechanisms and pathways including Aβ and oxidative stress			[47]
	HEK293	Express tau	Study tauopathy			[48]
	7W CHO	Express APP	Study Aβ pathway			[49]
	BV-2	Inflammation model	Study inflammatory pathways			[50]
iPSCs	Neurons, astrocytes, microglia, etc	Differentiated into different cell types	Study AD mechanisms	• Compare cell types of interest from healthy vs AD patients	• Genetic diversity between individuals • Genomic instability • Reproducibility issues • More complex, but still not entirely representative of *in vivo*	[51] [52]
3D Cell Culture	Derived from cell lines or iPSCs	Can contain multiple cell types Cellular environment may be more similar to that of organs	Study AD mechanisms	• iPSCs from AD patients better represent AD pathology • 3D conditions better reflect *in vivo* environments		[53] [54]

HBMEC – human brain microvascular endothelial cell.
BCEC – brain capillary endothelial cell.
RBE4 – rat brain endothelial cell.
SH-SY5Y – human neuroblastoma cell.
SK-N-MC – human neuroepithelioma cell.
PC-12 – rat pheochromocytoma cell.
HEK293 – human embryonic kidney cell 293.
7W CHO – 7W Chinese hamster ovary cell.
BV-2 – murine microglial cell.

et al. [55] and Yang et al. [47] tested extracts from traditional Chinese herbal medicines against $A\beta_{1-42}$-induced cell injury. However, Tong et al. [55] differentiated PC-12 cells using nerve growth factor (NGF) to induce a more neuron-like phenotype with extended neurites. Meanwhile, Yang et al. [47] employed the undifferentiated PC-12 model. Both studies tested the effects of the natural extracts on $A\beta_{1-42}$-induced cytotoxicity, in addition to LDH release and MDA. While the differentiated cells were pre-treated for 12 h with extracts followed by exposure to 100 μM $A\beta_{1-42}$, the undifferentiated cells were treated with extracts in the presence of 0.5 μM $A\beta_{1-42}$ for 24 h. A reduction in cell viability to 35% was reported in differentiated PC-12 cells, whereas a viability of 63% was reported for the undifferentiated cells at their respective stressor concentrations and conditions. In both cases, an increase of around 150% LDH leakage was reported. However, for MDA levels, an increase of around 25% was observed in undifferentiated cells while the differentiated cells only demonstrated a 10% increase. Tong et al. [55] tested the therapeutic effects of shikonin, isolated from the traditional Chinese herb *Lithospermum erythrorhizon*, which is used for wound healing and various allergic conditions. Yang et al. [47] investigated the neuroprotective effects of various phenylethanoid glycosides derived from *Herba Cistanche* – a traditional Chinese herbal medicine for treating kidney disorders. These natural products demonstrated antioxidant and anti-apoptotic properties as well as significant neuroprotective effects.

Natural products, including traditional Chinese herbal medicines, have also been tested for their potential as AD therapeutics in SH-SY5Y cells. Chang and Teng [45] tested β-asarone, a major component of *Acorus tatarinowii Schott*, in undifferentiated SH-SY5Y cells stressed with $A\beta_{25-35}$. The authors found that β-asarone was able to protect against inflammation and autophagy induced by $A\beta_{25-35}$. A similar methodology was employed by Li et al. [56] for testing a different natural product – trichostatin A, which is produced by *Streptomyces hygroscopicus*. Trichostatin A is an established reversible inhibitor of histone deacetylases, and demonstrated antioxidant and anti-autophagy activity in an undifferentiated SH-SH5Y model stressed with $A\beta_{25-35}$. Like PC-12, SH-SY5Y cells are also regularly used in a differentiated form. Krishtal et al. [57] compared the effects of $A\beta_{1-42}$ on undifferentiated and RA/BDNF-differentiated SH-SY5Y cells (retinoic acid with brain-derived neurotrophic factor). The authors reported that undifferentiated cells cannot be used as a reliable model for the toxic effects of native Aβ since they exhibited a low sensitivity to $A\beta_{1-42}$. However, only 48 h and 72 h timepoints were tested. At 48 h, the viability of undifferentiated cells decreased to 84% yet there was no significant reduction in viability at 72 h. However, this contrasted with the results seen for differentiated cells where no significant decrease was observed at 48 h, but viability was significantly reduced to 57% at 72 h. On the other hand, in a subsequent publication [58], the authors reported that the same conditions resulted in a viability of 57% at 48 h in undifferentiated cells rather than the previously reported 84%. Various inducers of differentiation have been employed for SH-SY5Y experiments. In Krishtal et al. [58], undifferentiated cells were compared to cells differentiated with N(6),2′-O-dibutyryladenosine 3':5′ cyclic monophosphate (dbcAMP), retinoic acid (RA) with brain-derived neurotrophic factor (BDNF), and RA with tetradecanoylphorbol acetate (TPA). The authors observed that dbcAMP-differentiated cells had a significantly increased susceptibility to the toxic effects of $A\beta_{1-42}$. Cells treated with RA/BDNF were also more sensitive to $A\beta_{1-42}$, but only at lower concentrations (10 μM $A\beta_{1-42}$). In contrast, RA/TPA-differentiation induced a high resistance to $A\beta_{1-42}$-induced neurotoxicity. Different differentiation-inducing agents also result in various phenotypes. For example, dbcAMP treatment induces a noradrenergic phenotype [58], and RA with TPA stimulates a dopaminergic phenotype, while RA alone induces a cholinergic phenotype and RA with BDNF further enhances the cholinergic markers [44]. Therefore, it is vital to consider the desired phenotype for studies, and whether it is more appropriate to employ an undifferentiated or differentiated cell line as part of the experimental design.

Similarly, iPSCs can be employed in an undifferentiated or differentiated form. iPSCs are artificial stem cells derived from somatic cells, and can be used to generate any specialised cell type. The major benefit of iPSCs is that comparisons can be made between healthy and diseased human patients, leading to the potential for personalised medicine. Furthermore, these comparisons can aid in the identification of disease-associated markers which can enhance current knowledge on the pathogenesis of AD while revealing novel potential therapeutic targets. Li et al. [52] generated iPSCs from cells isolated from the blood of an AD patient with a presenilin 1 mutation and from a cognitively normal individual, and they observed that both Aβ and p-Tau levels were elevated by over 2-fold in the diseased iPSC-derived neurons compared to the control. Upon treatment with the BACE-1 inhibitor, LY-2886721, the levels of Aβ and p-Tau were significantly reduced. Once isolated from the patients, iPSCs can be differentiated into the cell type of interest. Li et al. [52] differentiated their patient-derived iPSCs into cortical neurons, while Wu et al. [59] generated glutamatergic neurons. Wu et al. [59] tested the Chinese herbal medicine, *Graptopetalum paraguayense*, in their model and reported a significant reduction in extracellular Aβ by around 1.5-fold in addition to an attenuation of the hyperphosphorylation of tau proteins. Pomeshchik et al. [60] generated hippocampal spheroids from iPSC lines derived from skin fibroblasts. The authors demonstrated that this 3D system could be used to complement 2D *in vitro* studies for testing the therapeutic effects of potential drugs, while allowing the evaluation of their mechanism of action at a cellular level.

9. *In vivo* models of AD

Both transgenic and non-transgenic animal models of AD have been developed to simulate the pathological changes associated with the human disease. Most commonly, mammalian models such as mice and rats are employed for AD studies, however the use of non-mammals including *C. elegans* (*Caenorhabditis elegans*) and fruit flies (*Drosophila melanogaster*) is advantageous as they are subject to less stringent ethical standards and incur lower costs [1] (Table 2). In general, animal models allow in-depth studies into AD pathogenesis, and can reproduce the major disease hallmarks [37]. Animal models are also crucial for safety assessments of novel therapeutics as their complex systems provide a better reflection of human pharmacokinetics and therefore improved toxicity predictability, in comparison to cell models. However, the complexity of animal models results in a lack of control on experimental conditions [38]. Furthermore, transgenic models are limited in their ability to accurately reflect the human condition as sporadic AD cases are associated with age rather than genetic mutations [61]. With compounding evidence pointing to the multifactorial nature of AD, disease models with only a single, often artificial, cause are not able to reproduce the complete human pathology. Higher costs and strict ethical standards are also associated with animal models, compared to *in vitro* models.

9.1. Therapeutics tested in in vivo models of AD

C. elegans, *Drosophila* fruit flies, and zebrafish are increasingly employed as *in vivo* models due to their low costs and relative ease of maintenance. AD-like phenotypes are commonly induced in these models *via* transgenic methods, however they can also be chemically induced. Capatina et al. [71] treated zebrafish with scopolamine to stimulate memory impairment and oxidative stress. Pre-treatment with an extract of Rosmarinus officinalis reportedly reduced oxidative stress as evidenced through the analysis of oxidative stress and lipid peroxidation markers (superoxide dismutase, catalase, glutathione peroxidase, malondialdehyde). Levels of acetylcholinesterase were also found to be regulated following treatment with the extract. Spatial memory in the zebrafish was assessed using the Y-maze, with a significant improvement observed in locomotion pattern and memory in the Rosmarinus officinalis extract-treated animals. Yuen et al. [72] employed a more common transgenic model of *C. elegans* which expresses human $A\beta_{1-42}$ in body-wall muscle cells causing paralysis. Danshen, a traditional Chinese

Table 2
Summary of common *in vivo* models of AD; including the pathological relevance of each model to AD, the studies that can be performed, and the advantages and disadvantages of the models.

Model		Pathological Relevance to AD	Phenotype & Assessments	Advantages	Disadvantages	Ref
Transgenic	*C. elegans*	Aβ- or tau-expressing models e.g. CL4176: $A\beta_{1-42}$ in muscle cells CL2355: $A\beta_{1-42}$ in neurons	Study AD mechanisms, including paralysis and uncoordinated motility	• Simple genetic manipulation • Short lifespan • Several orthologues of human AD-related genes and pathways • Low cost	• Expression in muscle • Simple nervous system; lack of defined brain • Basic measures for cognitive decline	[62]
	Zebrafish	Express APP or tau e.g. APPsw: Aβ deposition hTAU-P301L: tau hyper-phosphorylation and aggregation	Study APP processing and other AD pathways	• Share the same major organs/ tissues with humans • Similar genetic structure to humans • Cheap to maintain • Large quantity of eggs with short generation time	• Genetic manipulation is more challenging • Require strictly controlled environmental variables • Basic measures for cognitive decline	[63]
	Drosophila	Transgenic expression of APP or tau e.g. UAS-Aβ42: Aβ in retinal neurons UAS-tau: tau aggregation	Study Aβ and tau toxicity	• Short lifespan • Low cost • Orthologues of AD-related genes and some functional conservation of proteins	• Brain anatomy and major organs differ substantially from humans • Basic measures for cognitive decline • Unable to conserve permanently as frozen stocks	[64]
	Rat	APP, tau, PSEN1, and combination transgenic models e.g. TgF344-AD: Aβ aggregation APP + PS1: Aβ aggregation	Study AD mechanisms including Aβ, tau and inflammatory pathways	• Brain surgery easier as brains are larger than mice • Easier to handle compared to mice	• Model of FAD rather than more common SAD • Difficult to reproduce complete AD pathology	[65]
	Mouse	APP, tau, PSEN1, and combination transgenic models e.g. 5xFAD: Aβ aggregation	Study AD mechanisms including Aβ, tau and inflammatory pathways	• Technically easier to inject DNA into embryos than rats • Ease of breeding and relatively low maintenance costs	• Difficult to reproduce complete AD pathology • NFTs do not develop without tau mutations which do not occur in human AD	[66]
Chemically/ mechanically induced	Rodents (mouse, rat)	Induce cholinergic hypofunction, memory dysfunction, brain inflammation e.g. $AlCl_3$: Aβ and tau aggregation	Study AD changes not directly related to APP/tau	• Rapid and easy to attain • Specific neurotransmitter pathway explored	• Can lack hallmarks of AD (Aβ plaques and NFTs)	[67]
Spontaneous	Dog	Progressive Aβ pathology e.g. aged canine: Aβ aggregation	Study age-related Aβ aggregation and oxidative stress	• Share several key molecular pathways of human AD • Model of more common, sporadic form of AD	• Late-onset of disease compared to transgenic models • High costs • Strict ethical considerations	[68]
	Rodents (mouse, rat)	Accelerated aging and APP overproduction e.g. SAMP8: Aβ in brain	Study AD hallmarks in old age	• Assessable behaviours • Age-related cognitive decline	• Longer period of pathology development than transgenic models	[69]
	Non-human primates	Develop Aβ and tau aggregates, and brain atrophy e.g. aged vervet: Aβ plaques and tau	Study AD pathology in model most relevant to human	• Similar brain anatomy to humans • Close genetic proximity	• Strict ethical constraints • High costs • Extended period of pathology development • Inconsistent disease pathology	[70]

medicine obtained from Salvia miltiorrhiza, was able to reduce the toxicity of $A\beta_{1-42}$ in the nematodes as demonstrated by the delay of the onset of paralysis in treated worms. However, no significant reduction in $A\beta_{1-42}$ levels was detected although the extract was able to prevent $A\beta_{1-42}$ aggregation *in vitro*. Treatment with Danshen extract was shown to significantly reduce ROS levels, therefore the authors postulated that the delay in paralysis in treated worms may be due to the protection against $A\beta_{1-42}$-induced toxicity *via* ROS inhibition.

In general, rodents (i.e. mouse and rat) are the most popular *in vivo* models for AD. These models are employed most frequently due to their relatively low maintenance costs, and ease of genetic manipulation and breeding. Furthermore, the nervous systems of rodents are similar to that of humans and their behaviours are complex which allows for the study of AD-relevant cognitive impairment in these models. An AD-like disease state can be induced in these models using a variety of methods; including transgenic, chemically/mechanically induced, and spontaneous. However, no one model can completely emulate the complex pathology of human AD. Table 3 provides a summary of the common rodent models for AD, with the corresponding phenotype of each model relevant to AD.

Numerous experiments are performed in rodents to assess the disease state of the animal, the phenotypic relevance to human AD, and for therapeutic screening. Behavioural tests which study the cognitive function of the rodents are commonly employed for AD experiments, as these are relevant to the major AD symptom of memory impairment. Table 4 below lists the common behavioural tests employed in AD studies with rodent models.

Two widely used mouse models are the 3xTg and the 5xFAD models, which develop Aβ plaques at 6 months and 2 months respectively [37]. The 3xTg model exhibits behavioural symptoms at 4 months, while the corresponding age for 5xFAD mice is 2–4 months. The 3xTg mice overexpress transgenic APP and tau and display a progressive onset of symptoms, but the 5xFAD model overexpresses transgenic APP and develops a significantly more severe and rapid-onset disease with severe amyloid pathology [94]. Therefore, the 3xTg model is considered a more appropriate model of the age-related sporadic AD (SAD) while the 5xFAD mice are used to model familial AD (FAD). Esquerda-Canals et al. [95] used several of the common behavioural tests listed in Table 4 for their study of 3xTg mice treated with an *anti*-Aβ antibody, including the Morris water maze and the object recognition test. In the Morris water maze, an

Table 3
Common rodent models for AD.

Model			Phenotype	Ref
Transgenic	3xTg	PSEN1 M146V, APP KM670/671NL (Swedish), MAPT P301L (mouse Thy1.2 promoter)	Aβ plaques, tau tangles, synaptic plasticity deficit, cognitive impairment, learning and memory deficits	[73]
	5xFAD	APP KM670/671NL (Swedish), APP I716V (Florida), APP V717I (London), PSEN1 M146L (A > C), PSEN1 L286V (mouse Thy1 promoter)	Aβ plaques, neuronal loss, synaptic loss and plasticity deficit, cognitive impairment, impaired spatial memory, learning and memory deficits, impaired social recognition, motor impairments	[74]
	APOE-KO	ApoE knockout	High serum cholesterol, Aβ plaques, tau tangles, potential cognitive impairment	[75]
	APP/PS1	APP V717I (London), PSEN1 A246E (mouse Thy1 promoter)	Aβ plaques, neuron and synaptic loss, cognitive impairment, spatial learning and memory deficits	[76]
	J2O	APP KM670/671NL (Swedish), APP V717F (Indiana) (human PDGF-β promoter)	Aβ plaques, neuron loss, synaptic loss and plasticity deficit, cognitive impairment, spatial learning and memory deficits	[66]
	Tg2576	APP KM670/671NL (Swedish) (hamster PrP promoter)	Aβ plaques, synaptic loss and plasticity deficit, cognitive impairment, spatial learning and working memory deficits	[77]
Induced	$AlCl_3$	Aluminium chloride	Aβ plaques and tau tangles, cholinergic deficit, cognitive impairment, spatial learning and memory deficits	[78]
	HFCD	High fat-cholesterol diet	Aβ plaques, high serum cholesterol, inflammation, cognitive impairment, memory and behavioural deficits	[79]
	OKA	Okadaic acid	Tau tangles, inflammation, neuron loss, cognitive impairment, memory deficits	[80]
	SCO	Scopolamine	Aβ plaques, tau tangles, cholinergic deficit, cognitive impairment, learning and memory and behaviour deficits	[81]
	STZ	Streptozotocin	Aβ plaques, tau tangles, neuron loss, reduced glucose uptake, cholinergic deficit, cognitive impairment, spatial learning and working memory deficits	[82]
	TBI	Traumatic brain injury	Aβ plaques, inflammation, neuron loss, cognitive impairment, learning and memory deficits	[83]
Spontaneous	Age	Aging	Inflammation, synaptic plasticity deficit, cognitive impairment, memory deficit	[84]
	KKAy	Diabetic type 2	Aβ plaques, tau tangles, inflammation, cognitive impairment, spatial learning and memory deficits	[85]
	SAMP8	Senescence accelerated mouse-prone 8	Aβ plaques, tau tangles, inflammation, cognitive impairment, learning and memory deficits	[86]

Table 4
Common rodent behavioural tests for AD.

Task		Cognitive test	Description	Ref
Contextual memory	Fear conditioning	Reference memory, hippocampal-dependent associative learning	Animal is exposed to aversive stimulus (mild shock) associated with a conditioned stimulus (tone). Freezing response associated with tone alone is measured	[87]
	Passive-avoidance learning	Reference memory, associative learning	Animal learns to avoid mild aversive stimulus associated with entering desired compartment (darkness)	[88]
Spatial memory	Morris water maze	Reference memory, working memory, hippocampal spatial memory	Animal must find stable platform in circular pool based on prior learned visual clues	[89]
	Radial arm (water) maze	Reference memory, working memory, spatial memory	Animal placed in maze with several arms radiating from central platform and must guide themselves towards food reward (in water, maze is submerged and escape platform used in place of food reward)	[90]
	Barnes maze	Reference memory, working memory	Animal placed on circular platform with several holes around circumference and must find escape box accessed through one of the holes	[91]
Working memory	Y-maze/T-maze	Reference memory, working memory	Animal placed in 3-arm maze and alternations (explorations of each arm) are recorded	[92]
	Object recognition	Learning and recognition memory	Animal given different objects to explore then positions of objects are changed and some novel objects introduced to test recognition	[93]

improvement in spatial memory was observed in treated mice and an improvement in recognition memory was evident in the object recognition test, however no significant improvements were detected in exploratory behaviour or anxiety. The authors attributed this to a reduced clearance of Aβ in the amygdala compared to other brain regions. Despite a reduction in Aβ compared to the untreated mice, the amygdala remained the most affected region with Aβ following treatment which could explain the amelioration of hippocampal-dependent tasks but not those associated with the amygdala.

Based on the multi-target approach for drug development, Kupershmidt et al. [96] generated M30 with the active group from rasagiline (a monoamine oxidase B (MAO-B) inhibitor) and an antioxidant-iron chelator moiety. The authors previously reported improved cognition following M30 treatment in the APP/PS1 model. In this study, Kupershmidt et al. [96] employed an aging mouse model. An improvement in recognition memory was observed in M30-treated mice using the object recognition test with an increase in recognition index of around 2.5-fold. Furthermore, M30 could reportedly reduce cortical iron levels and Aβ deposition as well as inhibit MAO-B activity in aged mice by around 37%.

Following the above transgenic and spontaneous examples, an alternative method for inducing an AD phenotype in rodent models is through chemical or mechanical administration. Chemically-induced AD models are particularly common, and a variety of chemicals are available for this purpose. Aluminium chloride ($AlCl_3$) is commonly employed as it induces an AD-like phenotype with cognitive impairments and increased acetylcholinesterase activity. Khalaf et al. [97] and Ahmed et al. [98] applied the $AlCl_3$-induced rat model for testing clopidogrel (an antiplatelet medication) and an extract of Lepidium sativum respectively as potential AD therapeutics. While Khalaf et al. [97] administered $AlCl_3$ and treatment orally, Ahmed et al. [98] administered $AlCl_3$ *via* intra-peritoneal injection and treatment was given by oral gavage. Khalaf et al. [97] employed the popular Morris water maze and object recognition test, whereas Ahmed et al. [98] used only one, less common behavioural test – the dipping hole test, where the animal is placed in a chamber with several holes in the base and scored based on the number of times they dipped their head through a hole. In both studies, exploratory behaviour was negatively affected following exposure to $AlCl_3$ as demonstrated in the object recognition test by Khalaf et al. [97] (around 2-fold reduction in recognition index) and the dipping hole test by Ahmed et al. [98] (around 1.7-fold reduction in head poking). However, the treatments in both studies were able to improve this phenotype. An alternative to metals as chemical inducers of AD is streptozotocin. Pilipenko et al. [99] and Zhang et al. [100] administered streptozotocin to rats *via* intracerebroventricular injection at a sub-diabetogenic dose. Pilipenko et al. [99] tested the therapeutic effects of metformin in this model using the Morris water maze. Zhang et al. [100] studied the therapeutic potential of silver nanoparticles using the object recognition test and the Barnes maze test. Spatial memory was impaired in the streptozotocin-induced rats as shown in the Morris water maze by Pilipenko et al. [99] and the Barnes maze by Zhang et al. [100], with an increase in escape latency of around 3-fold and 1.4-fold respectively. Zhang et al. [100] also observed a negative effect on recognition memory in the object recognition test. Metformin reportedly improved spatial memory but had no effect on motor function, while silver nanoparticles prevented deficits in spatial and recognition memory.

10. *Ex vivo* models of AD

Ex vivo models can combine the advantages of both *in vitro* and *in vivo* systems, through the direct investigation of intact affected tissues with the ability to control the extracellular environment [101]. Most commonly, primary cell and tissue cultures and brain slices are employed as *ex vivo* models taken from genetically modified AD rodents [102]. Primary cells are better representations of *in vivo* conditions compared to cell lines and avoid the high costs of animal experiments. On the other hand, primary cells often lack consistency between donors and depending on the sub-culturing conditions applied. Beggiato et al. [103] employed a co-culture of astrocytes and neurons derived from a triple-transgenic murine model of AD. By using primary cell culture rather than the animal model, detailed studies into cell physiology and effects of drug treatments can be carried out at a cellular level. As a result, Beggiato et al. [103] were able to establish that palmitoylethanolamide (PEA) exerts its protective effects against neurodegeneration by counteracting reactive astrogliosis. Salau et al. [104] also tested the neuroprotective effects of a natural product, but used primary tissue culture. Rat brain tissue was harvested, treated with vanillin, and subjected to Fe^{2+}-induced neurotoxicity. By using primary tissue, the therapeutic effects of vanillin could be studied in a model which represents *in vivo* conditions whilst also allowing investigation of the mechanisms of the neuroprotective activity – in this case, vanillin could ameliorate oxidative imbalance and dysregulated metabolic pathways, elevate ATPase activity, and inhibit cholinergic enzymatic activities. Brain slices, for example from mice as reported by Kniewallner et al. [102], can be studied *ex vivo* to observe effects of stress and/or drug treatments on each cell and tissue type. Kniewallner et al. [102] explored the effects of platelets isolated from AD mice on healthy mouse brain slices. They reported previous attempts to generate a similar *in vivo* model, however infused platelets did not enter the brains of the mice therefore this model was not successful. However, the authors also noted the drawbacks of the *ex vivo* model – specifically, that the model lacks blood flow and therefore the platelet localisation and adhesion to vessels may not reflect an *in vivo* condition. Human samples have also been used as *ex vivo* models; for example, post-mortem brain or tissue samples from AD patients. These samples provide direct insight into the disease pathology, but have limited accessibility [105]. Furthermore, the acquisition of appropriately matched controls can be challenging, and differing handling practices between various sources can affect comparability. As Scholefield et al. [105] reported when studying post-mortem brain tissue with *ex vivo* rat brain tissue, the human samples can be highly variable depending on the methodology used in addition to which brain region is being tested. Platelets and lymphocytes, or induced pluripotent stem cells, have the benefit of ease of accessibility from AD patients [1]. While post-mortem human brain tissue provides the most direct insight into pathological changes, platelets and lymphocytes can allow the investigation of cellular pathological mechanisms and are not susceptible to rapid degradation as with post-mortem tissues.

11. Clinical trials

As mentioned above in 'Drug discovery process' (section 5), clinical trials for AD therapeutics are expensive, time-consuming, and have a high failure rate. The current design for clinical trials involves three main phases (Fig. 6). First in human (FIH) Phase 1 studies employ a small

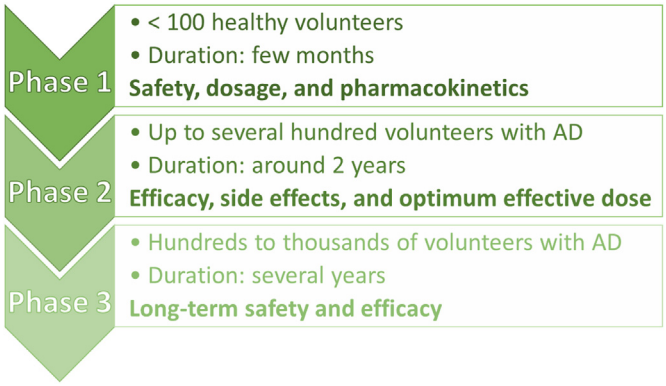

Fig. 6. Clinical trial process with 3 main phases, in addition to the number of participants typically used, the duration, and the key aims at each stage.

group of volunteers (20–100 people) to test the safety and dosage of the drug. This study typically lasts a few months, and employs healthy participants. In some cases, including the testing of genotoxic drugs for terminal cancer, volunteers with the condition may be used. The objectives of Phase 1 clinical trials also include pharmacokinetics, i.e. ensuring that the drug can pass to the targeted area and remain in the body for a duration sufficient to exert its effect. Preliminary studies into its therapeutic capacity in humans may also be performed at this stage. Next, Phase 2 studies are carried out with the drugs that have succeeded in Phase 1. This stage is performed on a larger scale (up to several hundreds of volunteers with the condition) and can last around 2 years. The purpose of these studies is to investigate the efficacy and any side effects of the drug, as well as determining an optimum effective dose. Around 80% of drugs do not pass Phase 1 or 2, often due to toxicity or lack of efficacy. For those that do enter Phase 3, long-term studies (around several years) on the safety and efficacy in hundreds to thousands of participants are carried out. Any adverse side effects are monitored, and the effect of the drug is compared to existing treatments. Where a drug is successful in Phase 3, an application is submitted to the regulatory body after which the drug can be marketed. A final Phase 4 may occur at this stage where the long-term safety and efficacy is evaluated in patients who have been prescribed this medication.

So, why are so many drugs failing AD clinical trials? This issue has been discussed in numerous reviews [27,106–108], which have posed similar potential explanations for these failures. Issues have been identified with drug design as well as clinical trial design which could contribute to the widespread lack of efficacy observed in drugs aimed at AD (Table 5). With regards to drug design, the difficulty in identifying suitable therapeutic targets is attributed to the poor understanding of the mechanisms of the disease. Furthermore, poor drug delivery and penetration as a result of an inability to cross the blood brain barrier (BBB) is a prevalent reason for the failure of drug candidates in clinical trials that have otherwise demonstrated promise in early drug development. In terms of the clinical trial design, concerns have been raised regarding the length of the studies and the variability in clinical endpoints between trials [108]. Due to the progressive nature of the disease, extended durations may be required to detect any disease-modifying effects. Anderson et al. [108] employed a clinical trial simulator to show that, even with a study which lasted 5 years, measurement variability between individuals results in difficulty identifying a treatment with 80% efficacy. However, increasing the trial duration would consequently incur higher costs and potentially result in a higher drop-out rate. The use of patients with mild to moderate stage AD has also been questioned, as the progression of the disease may be too advanced by this point for the drugs to be effective [27]. As a result, participants with earlier stages of the disease are being employed in trials and several prevention studies are also being performed. Biomarkers for amyloid and tau are currently used in clinical trials to identify participants at risk of developing AD. However, novel biomarker analyses are being investigated as a technique for monitoring target engagement and drug efficiency. In addition to amyloid and tau, biomarkers for several other AD targets such as oxidative stress and inflammation are increasingly employed as investigational agents during clinical trials [106]. A key case demonstrating an issue with clinical trial design is that of aducanumab. An interim futility analysis of aducanumab (an anti-amyloid monoclonal antibody developed by Biogen) deemed that the drug would not achieve statistically significant clinical effects by the end of the trial based on the data obtained so far [109]. In the 3-month period between the completion of the futility analysis and the announcement of drug futility, trial participants had the opportunity to complete the trial. Upon reanalysis of the data to include participants that continued during this 3-month period, significant clinical effects were found. Subsequently, Biogen applied for FDA approval for aducanumab [107] and it was granted in June 2021 [110]. However, this decision has been met with great controversy – in particular, due to the fact that the FDA approved aducanumab against the recommendations of its expert advisory committee which had agreed that there was insufficient evidence of any clinical benefit to approve the drug [111].

Table 5
AD drug Phase 3 failures between 2016 and 2019. Drugs which had been discontinued were identified by comparing the lists of Phase 3 drugs between years using Cummings et al. [112–116]. Drug targets and reasons for failure were identified using alzforum.org.

Year	Name	Target	Reason for Failure
2019	Crenezumab	Anti-amyloid	Lack of efficacy
	Umibecestat (CNP520)	BACE-1 inhibitor	Cognitive worsening
	Elenbecestat (E2609)	BACE-1 inhibitor	Lack of efficacy
2018	ITI-007	Serotonin receptor (5-HT2A) antagonist	Lack of efficacy
	Verubecestat (MK-8931)	BACE-1 inhibitor	Cognitive worsening
	Lanabecestat (AZD3293)	BACE-1 inhibitor	Lack of efficacy
	Insulin	Unknown	Lack of efficacy
	Atabecestat (JNJ54861911)	BACE-1 inhibitor	Cognitive worsening
	GV-971	Unknown	Lack of efficacy
2017	Intepirdine (RVT-101)	Serotonin receptor (5-HT6) antagonist	Lack of efficacy
	Idalopirdine (Lu AE58054)	Serotonin receptor (5-HT6) antagonist	Lack of efficacy
	Tricaprilin (AC-1204)	Cellular metabolism	Lack of efficacy
	Pioglitazone	Inflammation	Lack of efficacy
	Nilvadipine	Anti-amyloid	Lack of efficacy
2016	Azeliragon (TTP488)	Anti-amyloid	Lack of efficacy

12. Future of experimental models for AD

Efforts continue to develop an experimental model which can mimic the pathology of AD. In recent years, *in silico* methods for modelling AD as well as drug development have gained attention due to the lack of ethical considerations and relatively low costs. Computer simulations can easily be updated and the parameters adapted as new information about AD is learned. These methods can be used for designing and screening new drugs against protein targets, but have also been used to help elucidate disease mechanisms [117]. *In silico* methods are typically used alongside traditional *in vitro* experiments to validate the results. By using *in silico* modelling for drug design, predictions can be made about pharmacokinetics as well as target affinity [30]. Therefore, large libraries of potential ligands can be screened to identify leads with the greatest predicted target affinity which can then be synthesised and tested. This saves considerable costs and time as only a selected number of ligands need to be synthesised following the virtual ligand screening. Possible improvements on the ligand structure to optimise affinity for the target can also be recommended using *in silico* modelling [118]. Based on the size of the active site on the target, side chains can be added or removed to enhance ligand binding interactions. Furthermore, based on the properties of residues within the active site, substituents can be altered on the ligand structure to form interactions with these areas (e.g. whether hydrophobic or hydrophilic substituents would be more appropriate). One example of disease modelling from Anastasio [119] demonstrated that cerebrovascular disease can contribute to amyloid dysregulation and, in turn, the progression of AD. By modelling the various elements which are associated with the amyloid regulatory pathway, it was possible to identify alternative therapeutic targets, and therefore recommend potential treatments. By developing this model further, the authors could make predictions on the response to pharmacological interventions, and were able to demonstrate the potential for oestrogen to significantly reduce amyloid levels. A more recent model from Madrasi et al. [120] based on quantitative systems pharmacology (QSR) was developed to rationalise the lack of clinical efficacy of amyloid-modulating therapeutics. With the growing availability of artificial intelligence (AI) and machine learning,

these techniques have recently been applied to AD research in several capacities – for example, determining individual risk of AD, drug development, and in efforts to decipher the cause of AD. AI is capable of processing large datasets and analysing it with a high degree of accuracy. However, this relies significantly on the quality of the data input. A machine learning diagnostic platform to detect AD by analysing retinal images was reported by Wisely et al. [121]. Rodriguez et al. [122] applied AI to identify potential candidates for repurposing as AD therapeutics by studying differentially expressed genes in relation to disease progression then recommending potential treatments which have an affinity for the identified targets. Despite the clear benefits of AI including rapid processing and low error compared to human methods, these techniques remain extremely costly to implement which currently limits their application and regular use.

The generation of brain organoids from iPSCs is another example of an AD experimental model which is likely to be increasingly employed in the future. Brain organoids allow the study of brain development and the mechanisms of neurological and neurodegenerative disorders, in addition to the screening of therapeutic compounds [123,124]. Furthermore, by employing patient-derived iPSCs for the generation of brain organoids, personalised therapeutic strategies could be developed and novel insights into molecular and genetic disease mechanisms may be revealed [123]. While significant advances have been made in the last decade, a number of challenges exist with the use of brain organoids including the technical difficulty in culturing these models and the lack of reproducibility [124]. Due to the lack of immune and vascular systems, these models can be improved to enhance their physiological relevance [125]. As with current AD experimental models, brain organoids are currently not able to completely simulate the pathological features of the disease. However, with continued research, the brain organoid is a promising preclinical model that has the potential to bridge the translational gap between animal models and clinical trials.

13. Conclusion

Experimental models of AD are important for both the advancement of the knowledge on disease pathogenesis as well as the development of novel therapeutics. At present, no experimental model can fully replicate the pathophysiology of human AD. The high failure rate of clinical trials for AD drugs indicates that there is an issue with the current systems for modelling the disease, as the positive results observed in these models often do not translate into clinical benefits. However, by acknowledging the limitations of each model, it is possible to continue gaining useful information on AD. Employing multiple experimental models which mimic various aspects of the disease in preclinical studies can provide a more representative depiction of the human condition. Furthermore, as the current models are adapted and new experimental models are generated, these systems continue to gain translational power and produce more reliable results. With the approval of the first novel therapeutic for AD in two decades, the future of drug development to combat this debilitating disease is increasingly hopeful.

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