

Preclinical Models of Alzheimer's Disease: Relevance and Translational Validity

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The only drugs currently approved for the treatment of Alzheimer's Disease (AD) are four acetylcholinesterase inhibitors and the NMDA antagonist memantine. Apart from these drugs, which have minimal to no clinical benefit, the 40-year search for effective therapeutics to treat AD has resulted in a clinical failure rate of 100% not only for compounds that prevent brain amyloid deposition or remove existing amyloid plaques but also those acting by a variety of other putative disease-associated mechanisms. This indicates that the preclinical data generated from current AD targets to support the selection, optimization, and translation of new chemical entities (NCEs) and biologics to clinical trials is seriously compromised. While many of these failures reflect flawed hypotheses or a lack of adequate characterization of the preclinical pharmacodynamic and pharmacokinetic (PD/PK) properties of lead NCEs—including their bioavailability and toxicity—the conceptualization, validation, and interrogation of the current animal models of AD represent key limitations. The overwhelming majority of these AD models are transgenic, based on aspects of the amyloid hypothesis and the genetics of the familial form of the disease. As a result, these generally lack construct and predictive validity for the sporadic form of the human disease. The 170 or so transgenic models, perhaps the largest number ever focused on a single disease, use rodents, mainly mice, and in addition to amyloid also address aspects of tau causality with more complex multigene models including other presumed causative factors together with amyloid. This overview discusses the current animal models of AD in the context of both the controversies surrounding the causative role of amyloid in the disease and the need to develop validated models of cognitive function/dysfunction that more appropriately reflect the phenotype(s) of human aged-related dementias. © 2019 by John Wiley & Sons, Inc.

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INTRODUCTION

Alzheimer's Disease (AD) is a chronic neurodegenerative disease that represents 60% to 80% of diagnosed cases of age-related dementia (Alzheimer's Association, 2018). It has been termed “the 4th or 5th most common cause of death in the United States” (Katzman, 1976). Accordingly, its incidence is considered

as “the greatest challenge for health and social care in the 21st century” (Livingston et al., 2017).

As a result, considerable resources have been expended over the past forty years in searching for therapeutics to treat AD, all of which have failed—sometimes repeatedly—in late-stage clinical trials (Khachaturian, 2018;

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Martin, 2018; Morris, Clark, & Vissel, 2018; Mullane & Williams, 2018a; Panza et al., 2019). Among the reasons for these failures are:

(i) The politicization of AD, which has tended to confuse the guidelines for the diagnosis and treatment of a disease that is increasingly viewed as multifactorial and non-linear in both causality and progression (De Strooper & Karran, 2016; Medina, Khachaturian, Rossor, Avila, & Cedazo-Minguez, 2017). AD was first described in 1910 as a “peculiar disease process” on the basis of four patients with early-onset/presenile dementia that was severe in its pathology and associated with the post-mortem identification of hallmark amyloid plaques and neurofibrillary tangles (NFTs) in the brain (Maurer, 2006). The delineation of AD from senile dementia (SD) appears to have been politically motivated in order to enhance the prestige of Kraepelin’s laboratory where Alzheimer did his research. However, both Alzheimer and his contemporaries, Fischer and Fuller, considered AD as an “atypical” form of SD (Mullane & Williams, 2019). Some sixty years later, the conclusion was made that “Alzheimer disease and senile dementia are . . . a single disease” (Katzman, 1976), which led to SD being “reframed” under the umbrella of AD, the latter of which progressed overnight from “an obscure, rarely applied medical diagnosis” to a crisis that aided in focusing public opinion and federal funding on age-related research (Fox, 1989; Mullane & Williams, 2019). The situation has been further complicated by the recent, controversial (McCleery, Flicker, Richard, & Quinn, 2018; Mullane & Williams, 2018b) biomarker-based ATN (amyloid/tau/neurodegeneration) NIA-AA Research Framework (Jack et al., 2018) that has been proposed as a replacement for the appellation AD, and may reflect an initial step in the “De-Alzheimerization” of AD (Mullane & Williams, 2019), which should in time lead to a more logical description of age-related dementias that can be qualified in terms of their presumed causality, e.g., age-related vascular dementia.

(ii) A subjective and incomplete understanding of the complexity and mechanism(s) of AD causality that resulted in an overarching focus on the amyloid hypothesis of AD (Hardy & Higgins, 1992), despite clinical studies with a 100% failure rate that question its relevance, to the almost total exclusion of other potential mechanisms, even though 30% to 40% of individuals with measurable brain amyloid de-

posits have repeatedly shown no measurable cognitive impairment during their life span (Mullane & Williams, 2019).

(iii) A conflation of the familial/gene-based early-onset form of AD, *EOAD*, which represents approximately 1% of AD cases and involves mutations in presenilin genes, with the sporadic, late-onset form, *LOAD*, which comprises up to 99% of diagnosed AD cases. While *EOAD* “exhibits all of the behavioral and pathological hallmarks of sporadic AD” (Barrett & McGonigle, 2017), preclinical models based on the genetics of *EOAD* do not fully recapitulate the full spectrum of human *LOAD* in terms of genetics (Newman et al., 2017) or in terms of the onset, progression and duration, of the *LOAD* disease phenotype which is often non-physiological (Drummond & Wisniewski, 2017; Puzzo, Lee, Palmeri, Calabrese, & Arancio, 2014; Sabbagh, Kinney, & Cummings, 2013; Webster et al., 2014). Some transgenic models can present a very aggressive disease phenotype compared to the human form of the disease (Mazi et al., 2018; Sasaguri et al., 2017), the possible result of post-translational modifications of A β in the transgenic models being different from those in the human (Morris et al., 2018), while others fail to demonstrate aspects of neuronal loss and dysfunction, including synaptic and axonal function (Drummond & Wisniewski, 2017; Onos, Sukoff Rizzo, Howell, & Sasner, 2016). As in the case of many other transgenic models, this can lead to both false positives and false negatives, in the present instance putative amyloid clearing therapeutics that are either more effective in the models than they are in the clinic (Drummond & Wisniewski, 2017) or inactive, in which instance clinical data is unlikely to be generated. In either instance, the translational challenges are formidable. Few of the currently available transgenic rodent models are representative of *LOAD* (King, 2018), with the present *EOAD/LOAD* conflation being comparable to modeling Type 1 and Type 2 diabetes as closely related mechanistically based on the hyperglycemia that is associated with the former, the cause of which is a loss of insulin production due to an autoimmune reaction that destroys the insulin-producing islet cells in the pancreas, whereas that of Type 2 diabetes involves insulin resistance and lifestyle including exercise and diet.

(iv) The flawed and incomplete preclinical assessment of AD drug candidates where their target selectivity, efficacy, pharmacodynamic properties, and pharmacokinetic

properties—including bioavailability, blood-brain barrier access, toxicity, and target engagement/residence time (de Witte, Danhof, van der Graaf, & de Lange, 2019; Kleiman & Ehlers, 2016; Morgan et al., 2012)—have not been properly assessed or reproduced (Shine-man et al., 2011; Snyder et al., 2016), overlooked (Karran & Hardy, 2014), or both. This has led to compounds lacking drug-like properties or an adequate proof of concept being advanced into clinical trials based on commercial priorities rather than scientific considerations (Gold, 2017; Gray, Fleet, & Winblad, 2015).

(v) Animal models that, while having a certain degree of face validity, lacked content, construct validity, and predictive validity (Laurijssens, Aujard, & Rahman, 2013). In addition to natural, e.g., wild-type, models that have been developed in a variety of species, approximately 170 AD transgenic models have been described to date (<https://www.alzforum.org/research-models>), 164 in mice and 4 in rat. These range in complexity from the PDAPP mouse that expresses a single gene mutation, the human familial Indiana APP V717F (Games et al., 1995), to the recently developed triple transgenic mouse, App KO/APOE4/Trem2**R47*, which carries a humanized APOE4 gene, the p.R47H mutation knocked into mouse Trem2, and a CRISPR/Cas-generated 94-bp deletion in exon 14 (APP₆₉₅ numbering) of the mouse APP gene that is considered a potential model for LOAD (<https://www.jax.org/strain/031722>). While invoking a contribution from the innate immune system, the R47H variant of TREM-2 confers a loss-of-function that, while rare in AD, has been associated with a tripling of the risk for the disease (Ulland & Colonna, 2018; Ulrich, Ulland, Colonna, & Holtzman, 2017). There are currently no published data on this new model.

Of additional concern is the fact that mouse models often fail to show a substantive neuronal loss even in the presence of amyloid deposits and generate amyloid peptides different from those found in human brain. In addition to expressing the human *APP* gene, these mice also express endogenous, non-human APP (Morris et al., 2018).

Animal models are also limited by the fact that the majority of AD patients also suffer from co-morbid disease states—often more than one—that include hypertension, atherosclerosis, diabetes, chronic inflammatory and/or immunological disorders, as well as other CNS disorders including depression,

etc., that could impact the severity and progression of the AD phenotype and represent additional dementia domains, the absence of which in the transgenic models further highlights the reductionist nature of current animal models and their limitations in modeling the full clinical picture.

ENHANCING COGNITION

The concept that xenobiotics can be used to improve cognition in humans has its practical origins in folk medicine with the widespread use of caffeine and nicotine in their various forms and, more recently, stimulants and performance-enhancing drugs like amphetamine, methylphenidate, and modafinil (Sahakian & Morein-Zamir, 2015) as well as the nootropics (Gouliarov & Senning, 1994) being used as cognitive enhancers (Mehlman, 2004). These psychoactive substances are widely used in healthy individuals to improve cognitive performance, leading to their evaluation in treating cognitive impairment in patients with neurodegenerative diseases that include age-related dementias and AD.

Obvious issues in the translation of findings from healthy individuals to patients are whether the behavioral substrates for cognition in health, aging, and neurodegeneration are part of a similar spectrum—which appears unlikely given the changes in brain architecture that occur with aging and disease—and whether animal models exist or can be developed that can model and integrate the relevant substrates in a context that is valid for identifying potential drugs to treat AD based on the central feature of the disease, cognitive failure (Laurijssens et al., 2013; Puzzo et al., 2014).

The Cholinergic Hypothesis of AD

Initial efforts in the latter half of the 20th century to find therapeutics for AD focused on the cholinergic hypothesis (Bartus, Dean, Beer, & Lippa, 1982; Terry & Buccafusco, 2003), which was based on the association between cholinergic dysfunction, including degeneration of cortically projecting cholinergic neurons in the basal forebrain, and cognitive abnormalities in aging and AD. This suggested that improving central cholinergic tone via the use of inhibitors of the catabolic enzyme acetylcholinesterase (AChase) or agonists of muscarinic or nicotinic receptors would lead to improvements in cognitive function.

While the AChase inhibitors—tacrine, donepezil, galantamine, and rivastigmine—were developed and approved for the treatment of AD, issues with their side effects and modest efficacy (Blanco-Silvente et al., 2017) have limited their use. Considerable efforts were also expended in the 1990s in developing selective muscarinic M₁ (xanomeline; Bodick et al., 1997) and nicotinic α 4 β 2 (epiboxidine; Arneric, Holladay, & Williams, 2007) cholinergic agonists for the treatment of AD. While promising results were observed for these compounds preclinically and also in Phase II clinical trials, side effects curtailed the further development of both compound classes. Newer M₁ receptor cholinergic agonists are now under evaluation for AD (Douchamps & Mathis, 2017; Lebois et al., 2017; Lowe, 2016) but have yet to move into the clinic.

While recombinant DNA technologies had been used to determine the structure and clone subunits of the pentameric nicotinic ligand ion channel (Changeux, 2012) and the muscarinic receptor family was the first GPCR family to be cloned (Bonner, 1989), the genetic revolution in biology played a minimal role in the phenotypic characterization of the first generation of muscarinic and nicotinic receptor agonists, such that the efficacy of candidate drugs was usually tested in wild-type or “natural” rodent models, not transgenic models.

ANIMAL MODELS OF COGNITION, DEMENTIA, AND AD

Rodents [rats and mice (wild-type and transgenic)] and various non-human primates (Crawley, 1999; Sarter, Hagan, & Dudchenko, 1992a, 1992b) are the species typically used to model cognition, dementia, and AD in drug discovery. Rodents have a long history of being used in behavioral research, are readily available and validated as reflected in a copious database of research findings, and are cost effective. A variety of other species including *C. elegans*, *Drosophila*, chicken, frog, zebrafish, dog, cat, goat, wolverine, lemur, rabbit, guinea pig, degu, Syrian hamster, sheep, chimpanzee, Arctic ground squirrel, and black bear (Laurijssens et al., 2013; Drummond & Wisniewski, 2017; Newman et al., 2017) have also been considered as experimental models of human dementia/AD. Some of these have no behaviors that are obviously relevant to the human disease, some have yet to be validated behaviorally, while others are limited in supply due to cost, ethical considerations, or patent issues.

Wild-Type Animal Models of Dementia and AD

While aged rodents, both rats and mice, do not spontaneously develop AD-like pathologies, wild-type or ‘natural’ animal models can be used to evaluate cognitive dysfunction as a surrogate for human dementia. These models can be divided into *spontaneous* models that include normal, age-related, and senescence accelerated mouse (SAM) models and *chemical/lesion-* induced models (Van Dam & De Deyn, 2011; Lecanu & Papadopoulos, 2013; Neha, Jaggi, & Singh, 2014; Table 1). Of the former, the SAMP-8 (SAM-Prone 8) mouse is a naturally occurring line that was selected due to an accelerated aging phenotype that was accompanied by age-associated increases in hippocampal A β and behavioral impairments that include learning and memory difficulties but no plaque-like structures (Miyamoto et al., 1986).

The remainder of the models listed in Table 1 are focused on the chemical lesioning of cholinergic pathways using various excitotoxins, or are models based on hypoxia and/or stroke induction (Cada, de la Torre, & Gonzalez-Lima, 2000; Nguyen et al., 2018; Yang et al., 2013; Yasuda et al., 2014).

By the late 1990s, wild-type/natural animal models of AD were increasingly being replaced by transgenic models in mice (Table 2) and rats (Table 3) such that in the encyclopedic consideration of animal models of AD by Newman et al. (2017), these were not included.

Transgenic AD Models

Nearly 170 transgenic/knock-in/knock-out models of AD have been developed to date (ALZ FORUM Research Models Database; <https://www.alzforum.org/research-models>) that are principally focused on human gene mutations in *APP* (Amyloid precursor protein), *PSEN1* (presenilin 1), *MAPT* (microtubule-associated protein tau), and *Trem2* (Triggering receptor expressed on myeloid cells2), and *APOE* (apolipoprotein E), as well as transfection of the amyloid processing enzyme, BACE1 (Beta-Secretase 1), alone or in combination with one another. With the exception of Trem2, a cell-surface receptor which is present on microglia and regulates the innate immune system response to A β pathology (Ulrich et al., 2017; Yeh et al., 2017), the gene mutations in these models are almost exclusively focused on amyloid, and to a far lesser extent tau, the two hallmarks of AD originally identified by Alzheimer (Maurer, 2006).

Table 1 Selected Wild-Type/Natural Animal Models of Dementia and Cognitive Impairment

	Animal/treatment paradigm	Phenotype	References
Spontaneous	Normal and aged rodents	Normal aging	Gallagher et al. (1993); Erickson & Barnes (2003); Mitchell et al. (2015)
	Senescence-accelerated mouse (SAM).	Accelerated senescence with age-associated increases in hippocampal A β and behavioral impairment	Miyamoto et al. (1986); Takeda et al. (1997)
Chemically induced	Scopolamine Mecamylamine	Muscarinic learning impairment Nicotinic learning impairment	Ebert & Kirch, (1998); Gilles & Ertlé, (2000); Estapé & Steckler (2002)
	STZ (streptozotocin) icv	Progressive memory loss similar to AD	Lannert & Hoyer (1998)
	A β infusion	Acute or chronic ICV or intrahippocampal injection of A β 40, A β 42 or FAB (ferrous amyloid buthionine) induce learning and cognitive deficits	Lawlor & Young, (2010); Lecanu & Papadopoulos (2013)
	Okadaic acid icv	14-day infusion induces cognitive deficits thought to result from increases in p-tau and A β	Song et al. (2013)
	Ibotenic acid and other excitotoxins (NMDA, quinolinic, kainic, and quisqualic acids)	Excitotoxins alone or in combination with A β leads to loss of hippocampal cholinergic neurons and cognitive dysfunction (Hasselmo & Sarter, 2010).	Wenk (1993); Wallace et al. (1991, 1993); Morimoto et al. (1998); Toledano and Alvarez, (2004); Liu et al. (2015)
	192-IgG-saporin icv	Produces cholinergic hypofunction and a persistent impairment in the acquisition and performance of standard Morris water maze task as well as a cued version of the task	Walsh et al. (1995)
	AF64A	Selective cholinotoxin reduces levels of ACh, AChE, ChAT, HAcHT, and K ⁺ and ouabain stimulated release of ACh	Hanin (1996)
	Hypoxia/stroke	Memory deficits, vascular dementia	Cada et al. (2000); Yang et al. (2013); Yasuda et al. (2014); Nguyen et al. (2018)

Table 2 Selected Mouse Transgenic Models of AD^a

Model	Transgene/ mutation(s)	Pathology	Behavior	References
PDAPP	APP V717F (Indiana)	A β plaques (6-8 months)	Impaired spatial memory (3–4 months) Cued fear conditioning (11 months)	Games et al. (1995)
APP _{Lon}	APP V717I (London)	A β plaques (10 months)	Spatial and non-spatial orientation and memory deficits (6 months)	Moechars et al. (1999)
TG2576	APP KM670/671NL (Swedish)	A β plaques (6-9 months) activated microglia, decreased adrenergic and cholinergic innervation	Deficits in working and spatial memory, cued and contextual fear conditioning (10 months)	Hsiao et al. (1996); Holcomb et al. (1998)
APP23	APP KM670/671NL (Swedish)	A β plaques (6 months) with cerebral amyloid angiopathy	Deficits in working and spatial memory (3 months)	Sturchler-Pierrat et al. (1997)
J20	APP KM670/671NL (Swedish), APP V717F (Indiana)	A β plaques (2-9 months, elevated complement proteins C1q and C3 in hippocampus and frontal cortex (1 month)	Deficits in working and spatial memory (6 months)	Mucke et al. (2000)
PS1	PSEN1 A246E	None		Schneider et al., (2001)
APP/ PS1	APP V717I (London), PSEN1 A246E	A β plaques (6-9 months), activated microglia, decreased adrenergic innervation	Deficits in working and spatial memory (5 months)	Dewachter et al. (2000)
APP _{swe} / PSEN1 Δ E9	APP KM670/671NL (Swedish), PSEN1: Δ E9	A β plaques (6 months), activated microglia, modest neuronal loss (8-10 months)	Deficit in contextual memory (6 months of age), spatial learning impaired (12 months)	Jankowsky et al. (2004)
3 \times Tg	APP KM670/671NL (Swedish), MAPT P301L, PSEN1 M146V	Soluble A β , plaques (6 months,) activated microglia, hyper- phosphorylated tau, NFTs	Deficits in working and spatial memory, cued and contextual fear conditioning (4-5 months)	Oddo et al. (2003)

continued

Table 2 Selected Mouse Transgenic Models of AD^a, *continued*

Model	Transgene/ mutation(s)	Pathology	Behavior	References
5×FAD	APP KM670/671NL (Swedish), APP I716V (Florida), APP V717I (London), PSEN1 M146L (A>C), PSEN1 L286V	Aβ plaques (2 months), activated microglia, decreased adrenergic and cholinergic innervation	Deficits in working and spatial memory, cued and contextual fear conditioning (6 months)	Oakley et al. (2006)
APPswe 2576/ TauJNPL3	APPswe (Tg2576), Tau4RP301L (JNPL3)	Tangles with cerebral amyloid angiopathy and NFTs (9-11 months)	Motor disturbances 4-6 months	Lewis et al. (2001)
rTg4510	MAPT P301L inducible	Tangles and neuronal loss (4-6 months)	Deficits in working, spatial memory, novel object recognition (1.5-4 months) and cued fear conditioning	Santacruz et al. (2005); Ramsden et al. (2005)
hTau	Human tau	Tau hyper- phosphorylation (6 months), tangles and cell loss (15 months)	Deficits in working and spatial memory at 12 months	Andorfer et al. (2003)
APOE2 Knock-In	APOE2 inserted with expression regulated by endogenous regulatory elements and the mouse APOE gene inactivated			Mann et al. (2004)

^aTable collated from ALZ FORUM Research Models Database (<https://www.alzforum.org/research-models>) February, 2019. Additional information added from Barrett and McGonigle (2017), Drummond and Wisniewski (2017), Newman et al. (2017), Onos et al. (2016), Sukoff Rizzo and Crawley (2017), and Zeiss (2015).

Thus, the majority of transgenic animals that overexpress various forms of human amyloid in the brain are more models of a pathology that is *assumed* to be causal to AD than AD *per se*.

In a systematic review Foley, Ammar, Lee, and Mitchell (2015) examined the relationship between Aβ levels (Aβ₄₀, Aβ₄₂ and the ratio of soluble Aβ₄₂ to Aβ₄₀) and cognitive function in the Morris water maze (MWM) or novel object recognition test in five mouse AD models, Tg2576, APP, PS1, 3×Tg, and APP(OSK)-Tg, and found that there was no

correlation between quantified Aβ levels and cognitive function, leading to the conclusion “that mice bred to show elevated levels of Aβ do not perform significantly worse in cognitive tests than mice that do not have elevated Aβ levels.”

If the amyloid hypothesis is incorrect—as the Foley et al. (2015) analysis and the numerous clinical failures of therapeutics targeted to remove amyloid or prevent its formation would appear to indicate (Martin, 2018; Morris et al., 2018; Mullane & Williams, 2018a; Panza et al., 2019)—then these models

Table 3 Rat Transgenic Models of AD^a

Model	Transgene/ mutation(s)	Pathology	Behavior	References
APP21	APP KM670/671NL (Swedish), APP V717F (Indiana)	Diffuse plaques and perivascular amyloid deposits 9 months after intra-hippocampal injection of extracts from AD brain. Cerebral amyloid angiopathy.	Deficits in LTP and reversal learning 19 months	Agca et al. (2008)
APP+PS1	APP KM670/671NL (Swedish), APP V717F (Indiana), PSEN1 L166P	A β plaques and cerebral amyloid angiopathy (19-months) in female rats (no data on males)	Memory deficits (12-14 months)	Agca et al. (2016)
McGill-R- Thy1-APP	APP KM670/671NL (Swedish), APP V717F (Indiana)	Intraneuronal A β in hippocampus and cortex at 1 week. Extracellular A β plaques and increased microglial density (6 months,) neuronal loss (18 months), decreased cholinergic innervation (20 months)	Deficits in fear response acquisition (3 months), spatial and working memory Severe deficits in operant visual discrimination (4-6 months)	Leon et al. (2010); Galeano et al. (2014); Iulita et al. (2014); Wilson et al. (2017); Petrasek et al. (2018)
TgF344- AD	APP KM670/671NL (Swedish), PSEN1: deltaE9	A β plaques in hippocampus and cortex (6-26 months) Hyperphosphorylated tau in <i>locus coeruleus</i> at 6 months	Deficits in spatial and working memory (6-24 months)	Cohen et al. (2013)

^aTable collated from ALZ FORUM Research Models Database (<https://www.alzforum.org/research-models>) February, 2019. Additional information added from Barrett and McGonigle (2017), Drummond and Wisniewski (2017), Newman et al. (2017), Onos et al. (2016), Sabbagh et al. (2013), Sukoff Rizzo and Crawley (2017).

are potentially irrelevant artifacts. Nonetheless, while they may be of questionable relevance in identifying and characterizing putative AD therapeutics, amyloid transgenics may still have utility in demonstrating that putative therapeutics are still effective in the setting of AD brain pathology, to show that any amyloid-associated disruptions in the

blood brain barrier, synaptic connectivity, neuronal function, etc. do not impede the ability of a compound/biologic that is acting in an amyloid-independent manner, to improve cognitive or neuronal outcomes (Voorhees et al., 2018).

Fourteen mouse-based models in common use are listed in Table 2, while Table 3 shows

the four rat models currently available, in terms of their transgenes/mutations, pathology and behavioral responses including their time to onset. Transgenic rats are thought to have advantages over transgenic mice in that they are more similar to humans in their genetic, morphological, and physiological characteristics and have a richer behavioral repertoire facilitating complex behavioral testing while their larger brains make it easier to collect larger CSF samples and to conduct electrophysiological, optogenetic and imaging studies (Do Carmo & Cuello, 2013).

THE RELATIVE MERITS OF AD TRANSGENIC RODENT MODELS

Given the choice of 170 or so AD models, the researcher faces a considerable challenge in determining which model or models are the most appropriate (Drummond & Wisniewski, 2017), and in which order (Sabbagh et al., 2013; Onos et al., 2016). This requires consideration of whether these animals are being used to understand and validate disease hypotheses or as screening tools to identify, optimize, and prioritize lead compounds to translate these to clinical trials.

Behavior

In assessing compounds in the various AD models, behavioral outcomes have routinely been the primary outcome measure, since changes in these are related to the cognitive failure associated with AD (Laurijsens et al., 2013; Puzzo et al., 2014), with the aim of identifying drug-like compounds that can robustly improve cognitive function. How cognitive failure in aged humans can be validly measured in non-humans, e.g., rodent and non-human primates, has been the topic of extensive and often metaphysical debate (LeDoux, 2003; Decker, 2006; Van der Staay, Arndt, & Nordquist, 2009) that lies well outside the scope of this overview and has plagued the use of animal behavioral models in drug discovery for many decades.

In transgenic animals, behavior has been less consistently and rigorously measured than the biochemical changes associated with the transgenic AD phenotype(s) (Sabbagh et al., 2013; Onos et al., 2016; Barrett & McGonigle, 2017). This markedly limits comparisons between the various models even when these are standardized. Indeed, is often unclear whether new therapeutic candidates are directly evaluated and benchmarked in behavioral studies in transgenic models against reference standards

or whether it is assumed, based on the initial validation of the transgenic disease model, that evidence of a reduction in the mechanistic phenotype of the transgene construct, often at a single dose, can be automatically equated with an improved cognitive outcome.

In a landmark paper, Webster, Bachstetter, Nelson, Schmitt, and Van Eldik (2014) used ten different mouse AD models (PDAPP, TG2576, APP23, TgCRND8, J20, APP/PS1, TG2576 + PS1 (M146L), APP/PS1 KI, 5×FAD, and 3×Tg-AD) as models of human AD in behavioral tests of *spatial memory* [MWM, the radial arm water maze (RAWM), and Barnes maze], *associative learning* (passive avoidance, fear conditioning), *alternation* (Y- and T-maze), recognition memory (NOR), attention (3- and 5-choice serial reaction time), set-shifting tasks, and reversal learning tasks, the background to some of which is provided in Table 4.

From their comprehensive evaluation, Webster et al. (2014) concluded that:

- No animal model fully recapitulates all of the cognitive deficits observed in human AD pathogenesis but model different aspects of the disease.
- Each mouse model provides insight into different aspects of cognition related to AD due to the anatomical makeup of the mouse and the cognitive ability related to the genotype.
- The temporal time course and progression of cognitive deficits in a specific cognitive domain/behavioral task can be quite different among the different mouse models.
- Most models display deficits in spatial working memory earlier than deficits in other cognitive domains.
- Most studies using mouse models of AD have focused behaviorally on understanding/reversing the cognitive deficits associated with the disease. However, given that AD is a complex disease that is also associated with a variety of non-cognitive symptoms also present in the different mouse models of AD, these should be considered along with the cognitive deficits of the disease in assessing the behavioral phenotype.

The reader is referred to Webster et al. (2014) for additional detail and insights.

Issues with Transgenic AD Models

Transgenic models of AD generally involve a static overexpression of APP and a similarly static expression of the other transgenes used to create the model. At face value, while this provides a logical genetic manipulation

Table 4 Behavioral Testing in AD Models. Commonly Used Models

Behavior	Description	Cognitive domains	References
Morris water maze (MWM)	Rats and mice assessed for their ability to find a stable platform in a circular water tank containing opaque fluid based on learned visual clues external to the tank. The time taken to find these platforms is a measure of cognitive function.	Reference and working memory	Morris et al. (1982); Decker (1998); Vorhees & Williams (2006); Rose and Rowe (2012)
Radial arm maze (RAM)	A maze with 6-8 arms radiating from a central space. One arm contains a food reward that the animal has to find. Can be modified to include a water component (Diamond et al., 1999).	Reference and working memory	Olton and Samuelson (1976)
Barnes maze	Circular table with up to 20 circular holes at its edge each of which has visual cues and under one of which is an “escape box” that the rodent can access via the corresponding hole on the table top. The model is based on rodent aversion to open spaces, which motivates the animal to find the escape box.	Reference and working memory	Barnes (1979); Klakotskaia et al. (2018)
Novel object recognition (NOR)	Model based on the spontaneous tendency of rodents to spend more time exploring a novel object than a familiar one. Animals are placed in an open field with two different types of object that are consistent in height and volume, but different in shape and appearance. During habituation period, rodents explore an empty arena. 24 hr later, they are placed in the arena with two equidistant <i>identical</i> objects. The next day, the rodents are placed in the open field in the presence of the familiar object and a novel object to test long-term recognition memory.	Recognition memory	Ennaceur and Delacour (1988)
Contextual and cued fear conditioning	Rodents, usually mice, are placed in a conditioning chamber and given pairings of a conditioned stimulus (auditory cue) and an aversive unconditioned stimulus (electric foot shock). After a delay, mice are exposed to the same conditioning chamber and a differently shaped chamber with presentation of the auditory cue. Freezing behavior, a common response to fearful situations, can be measured as an index of fear memory.	Reference memory	Curzon et al. (2009)

to model the amyloid hypothesis, overt APP overexpression has the potential to obscure and distort the nuanced dynamics and context of the normal disease state that may compromise animal survivability (Sasaguri et al., 2017). Similarly, the key pleiotropic effects of the causal gene product(s) and those related to increased A β and other APP fragments may also be obscured. APP-related peptide modulation of α 7 nAChR (Wang et al., 2009) and GABA_B receptor activity (Rice et al., 2019) may also disrupt synaptic connectivity and neuronal function. As a result, the addition of other genetic manipulations to the APP knock-in (Tables 3 and 4) to create more sophisticated AD transgene models, while heuristically compelling, may be several orders removed from the original conceptualization of the AD model based on the human pathology with overabundant gene products potentially altering the stoichiometry of APP overexpression, leading to an overt and generalized brain dyshomeostasis.

A number of generic limitations, some real, some conceptual, exist related to the various genetic manipulations that are required to create a transgenic disease model, which may result in the production of similar but non-identical models that complicate their use as the models, as they may not be routinely validated or even be capable of being validated. These limitations (Zeiss, 2015; Sasaguri et al., 2017; Jacobson et al., 2017) include:

- Destruction or alteration of host gene loci by transgene insertion
- Variable insertion site (random transgene insertion), integration, transgene copy number, and isoform generation that can result in the expression of normally silent or minimally expressed genes unrelated to the target gene.
- An absence of non-coding regions that precludes APP mRNA analysis and the transcriptional regulation conferred by these gene regions
- Promiscuous, non-physiological, non-stoichiometric interactions of overexpressed APP with other cell proteins
- Overexpression of APP in organs other than brain that may affect the phenotype of the animal
- Nonspecific ER stress in APP/PS overexpressing mice
- A β species generated in these models that are different from those in human AD brain
- Atypical regional specificity of A β pathology due to use of different promoters to drive APP transgene expression in different transgenic mice that may affect A β propaga-

tion *in vivo* and lead to differences in expression level and A β stability

- Strain background as a genetic confounder, e.g., the autosomal recessive allele, *rd-1*, that leads to photoreceptor degeneration making animals blind by 6 to 8 weeks of age. The lack of awareness of this allele and the inadvertent use of blind animals in behavioral tests involving visual cues, e.g., the MWM (Table 5), can unknowingly contribute to inconsistent results and high within-group variability.

Backcrossing of such models with another species strain can remove genetic confounders like *rd-1*, as in the case of the congenic 129S6/Tg2576 mouse that was derived from the B6;SJL/Tg2576 mouse (Wolf, Bauer, Abner, Ashkenazy-Frolinger, & Hartz, 2016). Newer transgenic models may also be anticipated to address these limitations (King, 2018), but this will require that previous behavioral findings be revalidated in the newer models (Sasaguri et al. (2017). Additional issues with transgenics include: developmental adaptation and physiological compensation secondary to insertion of the transgene due to unknown systems redundancy and host homeostasis, passenger gene mutations that function in the host background (Vanden Berghe et al., 2015), and breeding errors (Perrin, 2014), all of which can markedly influence animal phenotypes, experimental reproducibility, and translational outcomes.

GENDER DIFFERENCES IN ANIMAL MODELS OF AD

Studies have reported that the incidence of AD is higher in females, with a more aggressive phenotype (Knopman, 2014; Mielke, Vemuri, & Rocca, 2014). These studies have however proven difficult to repeat, often due to an absence of the critical postmortem neuropathological confirmation, e.g., amyloid deposition, of the disease diagnosis in the original studies. This has made the role of gender a controversial issue in AD. A study of 1,028 deceased subjects reported that while the age of onset to dementia did not differ on the basis of gender, females, once they were diagnosed, were more likely to proceed to a severe pathological phenotype with a greater severity of neurofibrillary changes and changes in brain weight as compared to males (Filon et al., 2016). Similar controversies exist preclinically with reported sex differences in rat and mouse models of learning and memory (Jonasson, 2005), and in transgenic mice (Rae & Brown, 2015), where reports of females

outliving males among 3×Tg-AD and Tg2576 mice have not been confirmed in other transgenic AD models (Brown et al., 2018). These conflicting data will no doubt be resolved as the result of the NIH initiative to “monitor compliance of sex and gender inclusion in preclinical research funded by the agency” (Clayton & Collins, 2014) such that the contributions of sex can be considered alongside genotype in considering the results generated from animal models of AD.

REPRODUCIBILITY ISSUES WITH TRANSGENIC RODENT AD MODELS

A key concern related to the translational value of the mouse and rat transgenic AD models, in fact for any transgenic model, is the rigor and objectivity with which the behavioral/functional phenotypes have been interrogated, reproduced, and reported. Behavioral studies in animal models have traditionally involved small effect sizes that as a result require experiments that are appropriately and rigorously designed to reduce bias i.e., blinded, randomized and adequately powered with relevant controls and predefined endpoints (Shineman et al., 2011; Gore & Stanley, 2015; Egan et al., 2016; Snyder et al., 2016; Mullane, Curtis, & Williams, 2018; Marino, 2018). Despite this, efficacy outcomes for new AD therapeutics are often reported as significant based on small effect sizes, minimal animal numbers that have not been repeated such that their value is overstated and questionable, reflecting reporting and significance bias (Tsilidis et al., 2013). Such results should not be used in isolation to prioritize compounds for translational activities (Egan et al., 2016).

This point has been emphasized in the systematic analysis/meta-analysis of interventions conducted by Egan et al. (2016). This study, based on 427 papers describing 357 interventions in 55 transgenic models of AD that reported both behavioral (e.g., MWM, NOR, fear conditioning) and pathological (extracellular plaque burden, abundance of Aβ40, Aβ42 and tau) outcomes, involved over 11,000 animals in 838 experiments. The conclusion from this analysis was that evidence for compound efficacy from preclinical studies should not be taken at face value but requires a detailed critique to ensure that the results are not false positives. These outcomes were related to poor study quality (a lack of blinding and randomization) with overt publication

bias (non-reporting of neutral or negative studies) and a “remarkable variation in the experimental approaches and the outcomes measures used.” As examples, the authors noted that in the 83 papers that used the MWM, the water bath temperature varied between 16°C and 28°C, the tank size between 85 and 200 cm, the number of trials per day between 2 and 12, and the number of training days between 1 and 15 days, and that in some studies such detail was not provided. Similarly, in the 129S6/TG2576 mouse model (Wolf et al., 2016), the subject mouse was behaviorally tested at 11 to 13 months of age, while the 129S6/SvEvTac mouse strain from which it was derived was tested at 3 to 5 months of age for unstated reasons.

REPRODUCIBILITY GUIDELINES

Guidelines for preclinical study design and reporting in AD research are shown in Table 5, where the types of experiment are divided into *mechanistic*, *exploratory*, and *therapeutic* based on original guidelines developed by Shineman et al. (2011) and elaborated by Snyder et al. (2016).

Mechanistic studies precede exploratory and therapeutic studies and are focused on the biological processes thought to be involved in the disease etiology. Snyder et al. (2016) cite animal models of transgenic overexpression or knockout of a particular gene or its product, or pharmacological manipulation of a biological pathway, as being useful to identify or validate a disease-related target.

Exploratory studies are pilot or early proof-of-concept studies that are target-focused and typically involve interrogation of the animal model using tool compounds that can define the involvement of a particular molecular target in the disease process. Exploratory studies do not involve *extensive* lead optimization, PK/PD, and toxicity assessment but require predefined outcome measures based on compound bioavailability via a particular route, access to the brain, and target engagement to obtain preliminary assessments of absorption (A), distribution (D), metabolism (M), and excretion (E) studies, along with tolerability (T) assessments, to give an AD-MET profile. These studies, using both single and repeat dosing, provide the basis for subsequent therapeutic studies, as they can provide the flexibility to explore and validate appropriate endpoints and power calculations. Repeated administration of compound doses that singly result in plasma and tissue

Table 5 Guidelines for Preclinical Study Design, Execution, and Reporting^a

Recommendation	Detail
Criteria for choice and use of animal models	<ul style="list-style-type: none">• Use models of the disease target(s) rather than entire disease; understand the context of the mechanism or disease target in the selection of the animal model• Provide animal details—species, strain, sex, age, weight, genetic modification status, source and ethical review/institutional guidelines for studies (see Jones-Bolin, 2012)• Select model based on the specific mechanism to be tested at a stage of progression with the strongest rationale for the mechanism• Obtain animals from trusted sources; regularly validate genotype and phenotype
Fully characterize experimental therapeutic (compound, antibody, etc.) prior to conducting efficacy studies to ensure data can be objectively interpreted	<ul style="list-style-type: none">• Confirm structure, purity and solubility and stability if this information is not provided by a reputable supplier• Determine pharmacokinetic profile to establish free compound concentrations in plasma and, if possible, at the site of action <i>in vivo</i>• For mechanism of action or efficacy studies. It is imperative that the half-life ($t_{1/2}$), time of maximal plasma concentration (t_{max}) and maximal plasma concentration (c_{max}) of the compound are known so that its effects can be measured at an appropriate time point• Demonstrate target engagement over a range of doses that are well tolerated• Compare control data to published data using the same species/background strain
Study design (with statistician input)	<p>Define</p> <ul style="list-style-type: none">○ A clear experimental hypothesis with disease-relevant primary and secondary outcome measures.○ The minimal effect response anticipated that can address disease predictive phenotypes and translatable biomarkers-if any○ Measures for internal and external validation that address study quality items, e.g., blinding and randomization and variation, respectively○ Power calculations to determine group sample size and balance group sizes. When planning exploratory or therapeutic studies, sample size estimates should be based on previously assessed variability in the effect size.○ The number of different groups required to support the study outcomes <p>State</p> <ul style="list-style-type: none">○ Outcome measures together with their required precision as defined by standard error or confidence limits/intervals in the experimental protocol to prevent data dredging and misinterpretation of results○ Level of uncertainty required for acceptable decision-making○ Sources of variability that need to be understood and controlled to achieve the required precision as reflected in the standard errors and confidence limits/intervals for the key endpoints <ul style="list-style-type: none">• When dose-response studies are required, the data should allow facile estimation of the key parameters (slope, maximum and $IC_{50}/EC_{50}/K_i$ value) required to determine the specificity and the nature of the agonism (full/partial) or antagonism (competitive, noncompetitive/uncompetitive)

continued

Table 5 Guidelines for Preclinical Study Design, Execution, and Reporting^a, *continued*

Recommendation	Detail
	<ul style="list-style-type: none">• The doses used should ideally be logarithmic (0.1, 0.3, 1.0, 3, 10 mg/kg, etc.) to bracket the IC₅₀/EC₅₀ value.• When multiple interventions of a compound or compounds at single doses are required along with single or multiple control groups (vehicle controls, positive and negative compound/treatment controls), the experimental design can become very complex with an equally complex data analysis:<ul style="list-style-type: none">◦ When a study becomes large and tactically unwieldy, it should be divided into smaller parts, each of which involves a maximum of five groups and as few as possible analysis sets on multiple ancillary readouts where the variables may be dependent on or independent of the primary readout. The more ways that a data set is analyzed, the less reliable the significance (<i>p</i>-value) that is generated, with a greater occurrence of false positives.• Express experimental outcomes in terms of the plasma exposure of the compound as well as the dose• Establish inclusion and exclusion criteria for animals in the experimental groups that are matched for gender, age, strain• Randomize the inclusion of animals in controls and treatment groups to avoid bias. This process can be greatly facilitated by using software like Research Randomizer (https://www.randomizer.org).• Double-blind the allocation of animals into treatment groups and assessment of efficacy/outcome to avoid bias.• Control for confounding variables:<ul style="list-style-type: none">◦ Animals—gender, litter, copy number/expression level/genetic drift◦ Testing—environment (housing type, density, light cycle, temperature, humidity, health, noise, stress, investigator\gender) and time (before or after compound administration/experimental manipulation and how often)
Data analysis	<ul style="list-style-type: none">• Use a statistical model able to handle multiple variables (see Marino, 2018)• Avoid selective data reporting or P-hacking/data dredging and define raw data processing prior to statistical analysis, e.g., raw response data, change from baseline or log-transformed data that are to be analyzed; or are raw data summarized into an area under the curve or average? See Gore and Stanley (2015); Marino (2018).
Mandatory reproduction of original study	<ul style="list-style-type: none">• Additional studies (<i>n</i> = 3-5) in independent cohorts of the same animal model, e.g., Morris Water Maze• Additional studies (<i>n</i> = 3-5) in independent cohorts of same animal model using a different but related model, e.g., fear conditioning
Test compound in different species	<ul style="list-style-type: none">• After initial evaluation of compound in one animal model, repeat testing in a second animal model and/or species, e.g., a different transgenic, a non-transgenic, and a non-human primate.
Assess efficacy in a behavioral battery	<ul style="list-style-type: none">• Rather than evaluate the lead clinical candidate in a single model of the disease with one or two behavioral tests, use a battery of tests (see Wolf et al., 2016 and text); provide a systematic paradigm to test a variety of animal behaviors related to AD to ensure that compounds being advanced to clinical trials are more completely tested (Sabbagh et al., 2013; King, 2018).

^aDeveloped from Shineman et al. (2011); Sabbagh et al. (2013); Egan et al. (2016); Gore and Stanley (2015); Snyder et al. (2016); Wolf et al. (2016); Mullane et al. (2018).

concentrations below the toxicity/tolerability range can approach the limits of tolerability, resulting in phenotypic outcomes that are unrelated to the therapeutic target.

A key point of interrogation in exploratory studies in rodent models of AD is whether the putative therapeutic target outcomes of a study can be practically measured and have intrinsic value. As examples, using compounds in the exploratory setting where the PK/PD properties are unknown, as is often done, can result in situations where: the route of compound administration (e.g., p.o.) results in no measurable compound in the plasma at any time such that efficacy can never be measured; the time to maximal plasma concentration (t_{\max}) and the maximal plasma concentration (c_{\max}) of a compound are temporally disconnected from the measurement of activity; compounds are extensively bound (> 99%) to plasma proteins; blood-brain barrier penetration is unknown; or multiple dosing regimens either rapidly attenuate the efficacy response (Bespalov, Müller, Relo, & Hudzik, 2016) or result in compound accumulation that leads to toxicities. In this context, an investigator reporting lack of compound toxicity requires more than subjective observations that the animals “looked okay.”

Therapeutic studies are more comprehensive and compound-focused than exploratory studies and require full PK/PD and ADMET profiles to ensure appropriate dosing (route and plasma compound concentration) and timing of outcomes with respect to exposure to the compound (Morgan et al., 2012). Toxicity considerations are a high priority in order to minimize potential off-target phenotypic impacts on outcome measures and should be assessed by defined outcomes rather than mere observation. The design, conduct, analysis, and reporting of a therapeutic animal study should be analogous in rigor to that required for human clinical trials (Kimmelman, Mogil, & Dirnagl, 2014) and are distinct from an exploratory study and should be analyzed, interpreted, and reported as such.

Kimmelman et al. (2014) have delineated animal studies as exploratory and confirmatory in a manner that is akin to the exploratory and therapeutic designations defined here. In intent, confirmatory studies are not designed to validate a mechanistic hypothesis in animals but rather are intended to improve the predictive translatability of animal models. These include “large sample sizes” that have been extensively reproduced, using multiple

compound doses, and involve more than one animal model of the targeted disease, in this instance AD (Table 5), and “aim less at elaborating theories or mechanisms of a drug’s action than rigorously testing a drug’s clinical potential and restricting the advance of ineffective interventions advanced into clinical testing” (Kimmelman et al., 2014). The comparison of a therapeutic or confirmatory animal study to a human clinical trial has been taken one step further by Mogul and Macleod (2017), who segue the Kimmelman therapeutic/confirmatory study into a *preclinical trial*, a final “impeccable” confirmatory study without which a putative definitive data set should not be published. In the worldview of Mogul and Macleod (2017), a preclinical trial would “incorporate an independent, statistically rigorous confirmation of a researcher’s central hypothesis” that would: (i) adhere to the highest standards of rigor in design, analysis, and reporting; (ii) be held to the $p < 0.01$ or $p < 0.005$ rather than the current $p < 0.05$ level of statistical significance (Benjamin et al., 2018); (iii) be performed by an independent laboratory or research consortium; and (iv) increase the number of animals used six-fold. The authors further argue that the preclinical trial approach would provide the core behavioral competencies via “researchers with strong expertise in the relevant animal model” that molecular biology labs/research groups often lack and that there should be “no publication without confirmation.” While the preclinical trial concept is a logical recommendation to improve the rigor and relevance of the animal model data used as the basis for the selection of a compound to be tested in humans, its adoption has been mixed due to practical issues with implementation.

The first is the cost, which the authors address by assuming that “government funders and industry partners, which have spent billions of dollars on disappointing clinical trials, would be prepared to shift resources to support such an improved system.” Indeed, it would be logical to ratchet up the quality, depth, and relevance of such studies, but this would inevitably require a shift in resources from existing preclinical research *per se*, which is already suffering in a major way from funding constraints (Alberts, Kirschner, Tilghman, & Varmus, 2014; Bourne & Vermillion, 2017). The second issue relates to the ethical guidelines regarding the use of animals, especially non-human primates, in biomedical research, a topic that has engendered

considerable controversy, addressed over the past half century by initiatives like the ARRIVE (Animal Research: Reporting of In Vivo Experiments) Guidelines (Kilkenny, Browne, Cuthill, Emerson, & Altman, 2010; Percie du Sert et al., 2018) that reflect the 3Rs initiative—replacement, refinement, and reduction—to reduce animal usage in research (Burden, Chapman, Sewell, & Robinson, 2015).

While this is topic is outside the scope of this overview, there is a cognitive dissonance in efforts to minimize the use of animals in biomedical research while trying to characterize disease pathophysiology and develop therapeutics. Frequently instances arise where federal ethical guidelines can result in an institutional IACUC (Institutional Animal Care and Use Committee) *mandating* approval of underpowered studies that are incapable of providing useful data such that should not even be done. Shifting resources to fund well-intended initiatives like the preclinical trial approach does not, and will not, address these ethical issues.

An additional confound reflects the transition of a unique expertise in a laboratory that is not readily replicated by an independent third party without months of development. For example, stem cell therapy to “replace” lost neurons requires expertise in stem cell production, transformation, maturation, integration, and surgical implantation—all in a highly reproducible manner over prolonged time periods.

TRANSLATIONAL VALUE OF AD MODELS

Animal data is of inestimable value in the translational research paradigm to provide context and relevance, especially when such data can be used as a demonstration of compound efficacy with intrinsic predictive value (McGonigle & Ruggeri, 2014; Drucker, 2016; Tsukamoto, 2016). However, as numerous failures in the clinic attest, especially in the case of AD, these models frequently lack predictive value and are more reductive mechanistic representations than facsimiles of the targeted human disease.

In this context, Hargis & Blalock (2017) reported a meta-analysis of 29 studies on brain transcriptional profiles from human, mouse, and rat animal models. Comparisons of young versus aged or control versus AD were made, the latter as represented by idiopathic human AD and five AD transgenic mouse models—

J20, Tg2576, 3×Tg, 5×FAD, and CK-p25. Idiopathic AD brain transcriptional signatures were highly concordant with one another as assessed by the number of genes that were commonly significant versus those expected to be significant by chance, the proportion of significant genes showing directional agreement among studies, and the correlation in the magnitude of change among commonly significant genes, but were markedly different from the aging signature, being primarily composed of downregulated mitochondrial and neuronal signatures. The transcriptional profiles of brain aging were also concordant across human and rodents in multiple brain regions, with the hippocampal aging profiles in human and rat showing a marked upregulation of immune/inflammatory signaling. For the five transgenic mouse models, upregulated genes in J20, Tg2576, and 3×Tg showed a poor concordance with one other and with human AD, while the 5×FAD and CK-p25 mice had moderate agreement with one another and with human AD. The authors concluded that normal brain aging was similar in humans and rodents, but that different transgenic AD model mice, while recapitulating some anatomic and behavioral aspects of human AD, did not appear to consistently model the strong transcriptional influence observed in the human disease.

In some instances, the failures encountered with animal transgene models reflect the fact that they are based on intrinsically flawed hypotheses and the constructs used to interrogate these; in other instances, they reflect a lack of diligence on the part of investigators to ensure best practices in the husbandry and use of these models. Despite their limitations, these flawed models become widely utilized, with their relevance being overstated because of the lack of any viable alternatives, while only lip-service is paid to their validity as they become *de rigor* and self-perpetuating—driving the field down a blind alley

Issues with the Rigor of Animal Validation and Quality Control: Meta-Analysis of the Translational Failures in ALS

An example of the latter involves mouse models of the neurodegenerative disorder amyotrophic lateral sclerosis (ALS). In 2008, a series of 50 papers had been published on compounds that had shown a survival benefit in a standard model of ALS, the SOD1G93A transgenic mouse. However, when these compounds were advanced into the clinic, they

failed to provide benefit to ALS patients. A subsequent analysis of these studies concluded that many of those reported failed to use blinding or randomization as part of the experimental design, limitations that coupled with “the high noise floor of the model . . . support the conclusion that the bulk of published studies using the SOD1G93A mouse model may unfortunately be measurements of biological variability due to inappropriate study design” (Scott et al., 2008).

An additional 100 compounds from the literature that were reported to attenuate ALS symptoms in animal models were evaluated by the ALS Therapy Development Institute (ALS TDI) who found that none of the preclinical findings could be reproduced (Perrin, 2014). Eight compounds that had been advanced to the clinic and riluzole, a compound approved for use in ALS, failed to show efficacy in the ALS TDI studies, leading to the conclusion that the original preclinical data reported were false positives resulting from limitations in either the ALS mouse model or in the design, conduct, and analysis of the studies in which it was generated. Additionally, the lack of activity of riluzole in the ALS TDI studies would indicate a false negative, again questioning the predictive value of the model.

In ALS patients and some mouse models of the disease, the paralysis caused by deterioration of the neurons innervating skeletal muscle progresses over time. However, an ALS mouse model involving a mutant form of the RNA-binding protein TDP43 and a defective version of the SOD1 gene (which is mutated in 10% of the familial ALS population) was associated with motor neuron loss, protein aggregation, and progressive muscle atrophy, but the progressive deterioration seen in the human disease did not occur (Perrin, 2014). Assessment of the reasons for this by the ALS TDI identified issues with gender balance and the lack of *a priori* exclusion and inclusion criteria in the experimental planning and with the loss of the transgenic disease phenotype. When initially developed, the TDP-43 mutant mice had a life span of approximately 200 days. A subsequent generation of this model lived for 400 days and showed no signs of ALS-like symptoms. This was due to the presence of multiple copies of the disease-causing gene that were not stably inherited, making the phenotype unstable, and that led to mandatory genotyping of the transgenic model to ensure that subsequent generations of TDP-43 mice did not have fewer

copies of the transgene, and consequently less severe disease (Table 5).

An analysis similar to that reported for ALS (Perrin, 2014) has yet to be conducted for the multiple clinical failures for current AD therapeutics and may not be possible given the variety of transgenic models used and the oversights in the preclinical characterization of lead candidates (Karran & Hardy, 2014). However, it can be safely assumed that most of the 200 to 300 compounds/biologics that reportedly have entered into, and subsequently failed, clinical trials for the treatment of AD, did so after demonstrating some “positive” effects in an *in vivo* rodent model, be this in terms of changes in a biochemical or a behavioral substrate. Whether this is attributable solely to the model or flawed experimental design, or some combination of the two, is difficult to glean, but conducting routine genotyping of transgenic models of neurodegenerative diseases is a logical standard operating procedure to aid in ensuring experimental reproducibility.

Studies focused on improving the predictive value of AD models have been conducted by Possin et al. (2016) and Zeiss (2015). The first involved a cross-species translation of the MWM from the J20 hAPP transgenic mouse (Table 2) to patients with mild cognitive impairment (MCI), the stage prior to AD, using a virtual version of the MWM that recapitulated the visible-target training, hidden-target training, and probe aspects of the mouse protocol in human subjects (Possin et al., 2016). The authors concluded that: the MWM was able to detect similar deficits in spatial learning and memory across the two species; a rank summary score avoids the limitations inherent in a traditional repeated measures ANOVA while retaining the sensitivity of the latter; the rank-summary score for distance in the MWM represented a sensitive cross-species measurement of learning that may be superior to latency; and adequate power could be obtained using these methods to detect clinically relevant treatment effects in human trials.

The Zeiss (2015) study involved an *in silico* networks-system analysis of 448 various AD interventions across 752 animal and human clinical trials. These interventions included exercise and a variety of known/repurposed and experimental therapeutics that included BACE and γ -secretase inhibitors, active and passive β -amyloid immunotherapy, statins, retinoids, and three approved therapeutics, rivastigmine,

memantine, and donepezil. Of the interventions, 75% of the animal studies reported positive outcomes based on biomarker, pathological scores, and functional scores of which only 25 (~ 5%) were also tested in human studies. Of the latter, three interventions—donepezil, memantine, and exercise—reported positive outcomes.

The networks-system analysis concluded that the predictive value of the animal AD models was limited due to issues with genetic confounders like *rd-1* and DBA/2-associated blinding alleles that caused blindness, the magnitude of the positive outcomes (that may represent methodological false positives; Tsilidis et al., 2013), publication bias as reflected in a reluctance to report negative outcomes, a lack of detail on the animal models used (the synonym APP/PS1 denoting four different models), and/or the background host strain together with limitations in the clinical trials, the results of which were often not reported. Several of these same issues were also identified in the behavioral meta-analysis conducted by Egan et al. (2016). Given the four years that have elapsed since the Zeiss study was done and the multitude of clinical trials conducted since then, it would be interesting to see if a (re)analysis based on broader data sets yields the same conclusions.

Additional efforts to improve the translational utility of AD animal model testing have focused on behavioral phenotyping beyond the primary outcome measure of cognition (Sabbagh et al., 2013; Onos et al., 2016). This includes this evaluation of human comorbidity phenotypes, e.g., anxiety, depression, motivation, and sleep, as well as locomotor and exploratory behaviors and age-related impairment in vision, hearing, olfaction, fine motor skills, and metabolism, the latter including the impact of the models on the PK/PD properties of the compounds administered.

BEYOND AMYLOID AND TAU

As already discussed, transgenic AD models are largely based on the amyloid and tau hypotheses of the disease, and in those where amyloid is deemed to be causal, genetically reflect the familial EOAD form rather than LOAD. While newer models like the triple transgenic, App KO/APOE4/Trem2**R47* (King, 2018), may conceptually represent more relevant models of evolving aspects of human LOAD causality, these are still in the early stages of their validation, and, given the clinical failures of

AD therapeutics, may also turn out to be reductionist artifacts.

Given the large body of data that amyloid is not causal to AD but rather the result of a protective response (Krstic & Knuesel, 2013) where amyloid may function as an endogenous antimicrobial peptide (AMP; Moir, Lathe, & Tanzi, 2018) in response to microbial infection (Itzhaki, 2018; Dominy et al., 2019), the current focus on amyloid-based transgenics may actually be antithetical to AD causality, instead leading to the creation of models of the overexpression of an AMP. Amyloid-based transgenics also have conceptual limitations when they are used as animal models for non-amyloid approaches to AD causality like those focused on neuronal network dysfunction, innate immune dysfunction, and chronic inflammation, neurotoxic protein accumulation, cerebrovascular disease, and metabolically-related diseases that involve mitochondrial dysfunction (Mullane & Williams, 2018b).

If the causal hypothesis for a new approach to AD does not involve amyloid, why use decreases in brain amyloid as an outcome measure? In this context, Voorhees et al. (2018) used the TgF344AD rat model (Table 3) to evaluate the effects of the proneurogenic nicotinamide phosphoribosyltransferase (NAMPT) activator, (–)-P7C3-S243, and showed that it prevented hippocampal and cortical neuronal loss without altering the AD markers, amyloid, phosphorylated tau, and reactive gliosis, suggesting that in this amyloid transgenic model, the observed neuronal loss is independent of amyloid pathology and markedly differentiating (–)-P7C3-S243 from other putative therapeutics for AD.

Newer models of AD/age-related dementia will inevitably continue to incorporate aspects of these newer mechanisms in the form of specific transgenes, but stand to recapitulate many of the issues already identified with current AD models and those for ALS (Perrin, 2014) and HD (Jacobsen et al., 2017). The incorporation of models from other therapeutic areas that involve aspects of the human immune response (Tao and Reese, 2017; Friedman et al., 2018) or diabetes (Drucker, 2016), may lead to additional challenges in testing cognitive deficits.

FUTURE DIRECTIONS: NEW MODELS AND BROADER TESTING

The predictive value of animal models in the translational process in drug discovery has been the subject of active controversy for at least the past 20 years, irrespective of the therapeutic area (McGonigle & Ruggeri, 2014:

Justice & Dhillon, 2016). Thus, compounds with robust activity in disease-related animal models, often more than one, repetitively fail to recapitulate this efficacy in the clinic. The need to understand in better detail the PK/PD properties of lead candidates may be a key aspect of this disconnect (Morgan et al., 2012; McGonigle & Ruggeri, 2014; Kleiman & Ehlers, 2016). Other issues reflect the potential for compound tolerance (Bespalov et al., 2016) and the actual relevance of the animal model to the human disease which, in AD, currently relates to the amyloid hypothesis (Morris et al., 2018; Mullane & Williams, 2018a, 2018b).

Knowledge of the PK/PD properties of a drug candidate is reflected in the “three pillars of survival” concept discussed by Morgan et al. (2012), where knowledge of compound exposure at the target site, target engagement, and the expression of pharmacological activity (Table 5) was found to enhance the translational success of compounds through Phase II clinical studies.

In reimagining AD based on approaches distinct from the amyloid hypothesis, the key element in their validation is that they can robustly demonstrate the central feature of AD, cognitive failure (Laurijssens et al., 2013). To this end, initiatives like the NIA MODEL-AD initiative (King, 2018), a collaboration between Indiana University, the Jackson Laboratory, and Sage Bionetworks that led to the App KO/APOE4/Trem2*^{R47} mouse, is funded to produce 10 new mouse models per year for the next 5 years to add yet another 50 AD rodent transgenic models to the current 170, surely the largest number available for any single disease state, and a perfect example of technology driving reductionism in biomedical research. Additionally, the EU’s IMPRiND (Inhibiting Misfolded protein Propagation In Neurodegenerative Diseases; https://www.imprind.org/IMPRiND_PSI_0.7.pdf) consortium is assessing the value of *Drosophila* or zebrafish models [the latter of which have shown promise as first-in-class high-throughput phenotypic compound screens for appetite modulators (Jordi et al., 2018)] in neurodegenerative diseases research and the enhanced development of whole animal models of AD by transplanting human iPSC neurons into the macaque monkey, which leads to plaque and tangle development, as well as cognitive impairment. A marmoset model of AD is also under development at RIKEN using CRISPR to insert mutations of the gene *PSENI* into fertilized eggs.

The development and validation of a humanized macaque AD model, although expensive, has been posited as the missing link in compound translation based on the suggestion (King, 2018) that the use of a validated non-human primate model *may* have provided data that plaque removal did not improve the cognitive status of primates before clinical trials were initiated, thus avoiding the massive costs of the numerous failed clinical trials. At this time, definitive data to support this contention does not appear to be available, no doubt due to the limited studies characterizing AD pathology in non-human primates (Drummond & Wisniewski, 2017). While ethical and cost considerations may limit the practical use of such models, they may prove to be extremely valuable and far more convincing in guiding decision making than the rodent pre-translational animal models, given their similarities to humans (King, 2018).

The questionable translatability of animal models led Tsukamoto (2016) to suggest “skipping them altogether” given the bias towards animal models that exhibit “profound preclinical efficacy” (Tsilidis et al., 2013) except where they have a proven ability to predict clinical safety. Rather, Tsukamoto advocated a focus on Morgan’s “three pillars,” with increased efforts on developing biomarkers for the expression of pharmacological activity, itself a far from easy task. In AD, there is a high level of comfort with current biochemical and imaging readouts focused on measuring increased amyloid clearance and/or blockade of its formation as the key pharmacological endpoints, which is unwarranted, especially as these readouts have not been approved for use to assess compound efficacy in clinical trials (Mullane & Williams, 2018b; 2019). Similarly, Ransohoff (2018) in an article entitled *All (animal) models (of neurodegeneration) are wrong. Are they also useful?* discussed the various experimental and genetic limitations of current AD models in the context of the “incontestable complexity of neurodegeneration” and concluded that these models remain “extraordinarily valuable for investigating biological processes in vivo [and] . . . as a preferred testing platform for prospective pharmacodynamic biomarkers.” In a similar vein, Bartus and Dean (2009) noted that “the dramatic shift in focus away from behavioral outcomes in animal neurodegenerative research . . . has compromised further progress and continues to impede our ability to understand how these diseases impair human cognition

and what pathways might lead to effective therapies.”

Systematic Compound Testing to Improve Translational Predictivity

One way to improve the predictivity of the translational decision-making process is via the use of a more systematic tiered approach (Sabbagh et al., 2013; Onos et al., 2016) that involves greater consistency in the design, execution, analysis, reporting, interpretation, and reproduction of the penultimate animal studies used to inform the decision to initiate pre-IND studies for a novel therapeutic. Given the large number of animal models of AD available that are iterations on the theme of amyloid causality, the diversity of the behavioral test procedures, the inevitable concerns with the real-time validation of both the animals and procedures, and the costs and time involved in generating these data, there is often a tendency to evaluate the lead clinical candidate in a single model of the disease with one or two behavioral tests and to make a decision based on this output. Questions then arise, especially when a selected compound fails in Phase IIa as to whether appropriate rigor was used in generating the preclinical data to make this critical decision. Should more than one animal model of AD have been used? Was a single dose adequate or should a repeat-dosing paradigm have been used? Were the ADME data used sufficient to support the dosing regimen or should a full dose response curve have been generated? Were two behavioral tests sufficient? and so on.

In a behavioral assessment of the 129S6/Tg2546 transgenic mouse, Wolf et al. (2016) used a battery of tests (Fig. 1) in two mouse models having the same 129S6 background, the scopolamine-treated 3- to 5-month-old 129S6/SvEvTac mouse and the 11 to 13-month-old 129S6/Tg2576 transgenic mouse to assess learning and memory to create a cognitive profile. Five behavioral tests, the Y-maze forced alternation task, a NOR test, the MWM, the RAWM, and a Y-maze spontaneous alternation task were used to evaluate different aspects of cognitive impairment—learning memory (MWM, RAWM), working memory (Y-maze), object discrimination/recognition memory (NOR), spatial memory (MWM, RAWM), long-term memory (RAWM), and episodic-like memory (RAWM). The order of these tests (Fig. 1) was the same for each mouse, with each mouse being tested once in every test over an 8-week period in group sizes of 10 mice

per group. The two mouse models exhibited different patterns of cognitive impairment in varying patterns. Scopolamine-treated 129S6/SvEvTac mice showed impairments in spatial learning and memory, episodic-like learning, retention, and long-term memory, but had no effects on cognitive performance in the NOR or in alternation tasks. The 129S6/Tg2576 mice showed impaired spatial and episodic-like learning, impaired working memory (forced alternation test), and impaired long-term memory (MWM, RAWM) with no cognitive deficits in the NOR or the spontaneous alternation tests; retention and long-term memory in the RAWM test were also unimpaired. Once established, a behavioral battery of this type can be used with various AD models in combination, e.g., the SAM-8 mouse with the 129S6/Tg2576 (to avoid the *rd1* confounder retinal degeneration effect), or the PS1 mouse with the APP/PS1 mouse, or even validated models that lack an AD phenotype. While the effects of cognitive enhancers were not evaluated in this behavioral test battery, it, together with relevant ADME evaluation, can provide a systematic paradigm to test a variety of animal behaviors related to AD to ensure that compounds being advanced to clinical trials are more completely tested (Sabbagh et al., 2013; King, 2018). Thus, rather than selecting a compound that has shown robust efficacy at a single dose in the MWM (learning and spatial memory) and/or NOR (object discrimination) test procedures, this battery can provide information on working, long-term, and episodic-like memory via additional testing in the Y-maze and RAWM, as well as adding dose-response curves, repeat dosing, repeat testing procedures, and even compound combinations (Von Radovitz, 2016; Strickland, 2018) to provide a broader, more nuanced data set for an informed go/no-go decision that can identify compounds that can then be iteratively benchmarked in clinical trials to determine the optimal translational profile for an AD therapeutic.

While more expensive and time consuming at the preclinical stage, ultimately the systematic battery-based approach will be far more cost- and time-effective than jumping straight into clinical trials with minimal data. It may also be adopted in the yet-to-be developed humanized non-human primate models prior to approving a compound for clinical testing (King, 2018).

In conclusion, future success in developing more useful animal models for a better understanding of disease complexity that are

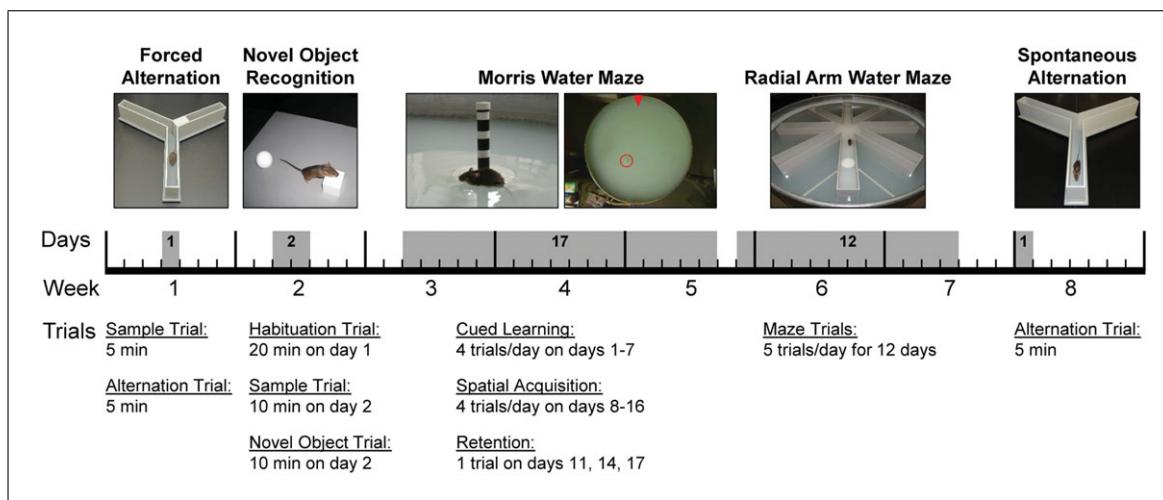


Figure 1 Behavioral Test Battery. Mice can be tested in a behavioral test battery consisting of forced alternation (Y-maze), novel object recognition, Morris water maze, radial arm water maze, and spontaneous alternation (Y-maze) over an 8-week period. Top panel: Behavioral tests sequentially conducted in the battery. Middle panel: Testing timeline. Bottom panel: Trials conducted per behavioral test. Tests were conducted in the order of increasing invasiveness. The spontaneous alternation test was the last to be performed, to maximize the interval to the forced alternation test, which utilized the same maze. Reproduced from Wolf et al. (2016) under the Creative Commons [CC BY 4] license.

more predictive and that can inform decision making in AD/age-related dementia research may lie less in extending the already extensive repertoire of transgenic models, especially those based on the amyloid hypothesis (King, 2018), and more in improving unbiased high throughput analysis in a battery of wild-type/natural phenotypic models (Alexandrov, Brunner, Hanania, & Leahy, 2015) while remaining focused on the challenge of improving biomarkers relevant to, and predictive of, an appropriate non-anthropomorphic behavioral phenotype that can improve both the process of translation and clinical trial design. Example of this are models of olfactory dysfunction, a key feature of AD (Franks, Chuah, King, & Vickers, 2015; Roberts et al., 2016) that may represent more relevant AD animal phenotypes to assess compounds than navigating a maze (Zhou, Wang, Pan, Lu, & Xia, 2015).

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