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Alzheimer's & Dementia
Translational Research & Clinical Interventions

PERSPECTIVE

Translational animal models for Alzheimer's disease: An Alzheimer's Association Business Consortium Think Tank

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Abstract

Over 5 million Americans and 50 million individuals worldwide are living with Alzheimer's disease (AD). The progressive dementia associated with AD currently has no cure. Although clinical trials in patients are ultimately required to find safe and effective drugs, animal models of AD permit the integration of brain pathologies with learning and memory deficits that are the first step in developing these new drugs. The purpose of the Alzheimer's Association Business Consortium Think Tank meeting was to address the unmet need to improve the discovery and successful development of Alzheimer's therapies. We hypothesize that positive responses to new therapies observed in validated models of AD will provide predictive evidence for positive responses to these same therapies in AD patients. To achieve this goal, we convened a meeting of experts to explore the current state of AD animal models, identify knowledge gaps, and recommend actions for development of next-generation models with better predictability. Among our findings, we all recognize that models reflecting only single aspects of AD pathogenesis do not mimic AD. Models or combinations of new models are needed that incorporate genetics with environmental interactions, timing of disease development, heterogeneous mechanisms and pathways, comorbidities, and other pathologies that lead to AD and related dementias. Selection of the best models requires us to address the following: (1) which animal species, strains, and genetic backgrounds are most appropriate; (2) which models permit efficient use throughout the drug development pipeline; (3) the translatability of behavioral-cognitive assays from animals to patients; and (4) how to match potential AD therapeutics with particular models. Best practice guidelines to improve reproducibility also need to be developed for consistent use of these models in different research settings. To enhance translational predictability, we discuss a multi-model evaluation strategy to de-risk the successful transition of pre-clinical drug assets to the clinic.

KEYWORDS
Alzheimer's disease, animal models, *APOE*, drug development, EOAD, LOAD, transgenic, translational, TREM2

1 | INTRODUCTION

More than 5 million Americans and ≈50 million individuals worldwide are living with Alzheimer's disease (AD)–related dementia in 2020, and these numbers are projected to skyrocket due to the aging population.[1,2] Prevalence statistics highlight the urgent need for effective therapeutics. Moreover, risk factors associated with age, sex, race, ethnicity, genetics, comorbidities, and many other factors, not only highlight the complex nature of the disease, but also raise concerns of how best to engage in therapeutic discovery and evaluate outcomes in human patients. There is a great need for models that better recapitulate the human condition, as well as represent the diversity and sex of individuals living with AD. Ultimately, successful drug discovery is strongly supported by animal models that are able to predict clinical outcomes in human patients.

Existing AD animal models have provided important insights into the disease. For example, the concept that amyloid beta (Aβ) toxicity depends on the presence of tau illuminated an added dimension to anti-tau antibodies as a therapeutic approach to AD.[3,4] However, the inability of these models to reflect the entire biology of the disease, much less predict efficacy in clinical trials, has contributed to the high failure rate (99.6%) of AD drugs in clinical development.[5] The National Institute on Aging (NIA) is building the infrastructure to enable precision medicine approaches to AD. This involves (1) defining disease traits using human data with the goal of integrating data from genomic, proteomic, transcriptomic, metabolomic, immunological, epigenetic, and other such unbiased studies to build more granular molecular maps of disease mechanisms; (2) inventing new animal models that more accurately reflect the complexity and greater genetic disease diversity to enable dynamic modeling of disease risk and resilience factors by disease stage; and (3) improving pre-clinical translation of therapeutic efficacy testing by building a standardized and rigorous drug testing platform, as well as matching the model to the treatment to be tested. These models endeavor to provide translational tools for predictive and personalized drug development.

In November 2018, the Alzheimer's Association Business Consortium convened a think tank event by bringing together experts in the field to explore the current state of the science in animal models, identify knowledge gaps, and recommend specific actions for the development of next-generation AD animal models that display translational relevance by more accurately predicting outcomes in human AD patients.

2 | CURRENT STATUS OF ALZHEIMER'S DISEASE ANIMAL MODELS

According to AlzForum, there are at least 168 AD animal models in addition to invertebrate and non-mammalian models. Most of these are transgenic mice that overexpress human genes involved in the production of amyloid plaques and neurofibrillary tangles.[6] Fly, worm, and fish models have employed the same approach of overexpressing human genes to generate aspects of plaque and tangle pathologies.[7–10] Although these models have fueled much of the progress in understanding aspects of AD pathogenesis, their value is limited by a number of factors including the realities that most of the AD mice do not develop neurodegeneration, the models are focused largely on familial AD (FAD) with early onset AD (EOAD) mechanisms, the genetic backgrounds of the mouse strains have not been standardized, and the models incompletely recapitulate the human neuropathology phenotype of typical late-onset Alzheimer's dementia (LOAD). These factors limit the translatability of findings not only from one transgenic mouse strain to another, but to the human disease condition as well. For example, the massive and non-physiologic overexpression of genes needed to produce pathology and symptomatology including behavioral deficits and hyperactivity observed in mouse models relates poorly to symptoms seen in the majority of humans with AD. However, some newer models do feature more physiological levels of human amyloid proteins with measurable levels of amyloid plaque and cerebrovascular amyloid pathologies, but they still lack the neurodegeneration that is key to human AD.[11–13] Other limitations of mouse models are expected as a result of their smaller and less-developed prefrontal cortex and a shorter lifespan that may not be useful in studying age-related neurodegenerative diseases such as AD. Critically, there are also substantial differences between mouse and human immune systems. Reliance on a singular model, an under-appreciation of background strain influences, and a tendency to "anthropomorphize" outcomes in behavioral-cognitive evaluations may model responses to disease factors that insufficiently translate beyond the specific model utilized.[14] This must be addressed to adequately model human disease.

Other rodents including transgenic rats have also been used as animal models of AD.[15] Although AD pathology does not develop naturally in most rodent brains, one exception may be the aged *Octodon degus*, a ground-dwelling, diurnal rodent from the upper mountain regions of Chile.[16] This rodent shares features of neurodegeneration including production of extracellular Aβ deposits and intracellular tau, and replicates other characteristics of the AD brain.[17] Its utility as a translational model for drug development, however, is at a very early stage and new data suggest that degus raised in captivity do not reproducibly demonstrate the hallmarks of AD reported previously.[18] Similarly, guinea pigs are a developing animal model of AD featuring a human-like Aβ sequence with age-dependent diffuse accumulation of amyloid pathology.[19]

Canine and non-human primate models are also used to study AD. Phylogenetically, they are more closely related to humans, both functionally and neuroanatomically, and their larger body and brain sizes allow for larger sampling volumes in biomarker studies. Genetic

Research in Context

1. **Systematic review**: The Alzheimer's Association Business Consortium convened a Translational Animal Models for Alzheimer's Disease Think Tank meeting to assess the state of the field and the need to provide recommendations for next-generation animal model development to enhance therapeutic development pipelines.
2. **Interpretation**: Experts across academia, government, and industry demonstrated that continued investment and increased funding for basic and translational science has advanced our understanding of both the strengths and weaknesses of current animal models used for AD drug development.
3. **Future directions**: This review proposes that the advancement of new AD therapeutics requires both new translational animal models that capture genetic heterogeneity across populations, comorbidities. and other pathologies that lead to AD and the use of more than one animal model in drug-testing studies to predict the efficacy of selected therapeutic approaches more accurately.

Highlights

- More than 60 researchers from academia, industry, and government were convened.
- The current state of models across rodent, canine, and non-human primate species.
- Translational therapeutic development requires new rational models to test efficacy.
- New models must capture the genetic heterogeneity across human populations.
- Environmental interactions, comorbidities, and other pathologies must be modeled.

heterogeneity may also better model human populations than inbred rodent models where several species develop early AD-like features including cognitive decline and Aβ pathology. In addition, because age is the greatest risk factor for AD, the aging trajectory in canines and non-human primates affords greater consideration of lifespan-related changes than that of a rodent. However, husbandry costs are higher for these animals, which often has resulted in pre-clinical studies utilizing small group sizes, which limits the statistical power of the results. Moreover, neurofibrillary tangles are absent in most canine and non-human primate species, as well as most mammals. However, hyperphosphorylated tau has been reported in aged dogs, and cerebrospinal fluid (CSF) levels of tau species have been used to evaluate novel therapeutics targeting tauopathies.[20]

Aged dogs offer an advantage over non-transgenic aged rodent species because they naturally produce Aβ, which has the identical amino acid sequence as the human protein and results from similar biochemical processing of the amyloid precursor protein. Moreover, the pattern of cerebral plaque distribution parallels that of humans, both temporally and anatomically. Aged beagles have been shown to develop cognitive dysfunction associated with neuropathology that resembles certain aspects of human AD-related cognitive deficits.[21] In addition, diet notwithstanding, domesticated dogs are also are exposed to environmental risk factors that are more similar to humans than in rodent models.[22] The pattern of Aβ deposition in aged dogs correlates positively with cognitive impairment and negatively with CSF levels of Aβ42. Although aged dogs accumulate Aβ plaques, and show significant cortical atrophy and hippocampal cell loss with increasing age, they do not develop neurofibrillary tangles, although cerebral levels of hyperphosphorylated tau are associated with behavioral changes linked to brain aging and cognitive dysfunction.[23–25] Of interest, canines develop significant cerebrovascular pathology and cerebral amyloid angiopathy, which is associated with soluble Aβ40,[26] and may represent a partial model of vascular changes associated with AD patients. Pre-clinical studies in aged beagles have been used successfully to predict the efficacy of some AD treatments in humans.[27,28] Dogs are also easier to work with, and have a far more sophisticated behavioral repertoire than rodents, affording more nuanced cognitive studies. However, when planning intervention studies in the canine model it is important to consider that they are also expensive to house, cognitive outcome measures require weeks to months to complete, cognitive evaluations in an experimental setting are time and labor intensive, and comparative brain structures related to sensory perception and environmental orientation differ from those in humans. On the other hand, colonies of aged dogs that are highly trained on cognitive tasks can be employed rapidly in studies evaluating putative therapeutics permitting study designs that are similar to AD clinical trials (eg, InterVivo Solutions). Moreover, parameters such as cognitive neuroprotection can be assessed longitudinally in conjunction with CSF and/or brain imaging end points currently used in AD clinical trials.

Non-human primates have multiple advantages as animal models including genetic similarity to humans, a well-developed prefrontal cortex, and development of age-related cognitive deficits in executive function, working memory, and attention.[29] Many aged non-human primate species may develop amyloid plaques and cerebral amyloid angiopathy (CAA) without readily observable tauopathy or neuronal loss[30]; however, the extent of pathological changes varies greatly among and within non-human primate species. Although apes are most closely related to humans and may show diffuse amyloid plaques,[31] they have been largely excluded from research for ethical reasons. The exploration of Old World monkeys such as the rhesus macaque and the cynomogolous macaque as models of human aging and AD has been limited by the availability of aged subjects and their inconsistent presentation of AD pathology.[32,33] The vervet, or African green monkey develops amyloid pathology and tau paired-helical filaments

in the absence of neurofibrillary tangles, as well as transcriptional, pathological, cognitive, and behavioral signs of AD.[34–36] New World monkeys (squirrel, marmoset, tamarins) and primitive monkeys such as mouse lemurs have also proved useful for studying aspects of human aging.[37] Marmosets are perhaps the most well-established model, since a robust research platform and toolbox exists for gene editing, imaging, neuropathology studies, and neurophysiological and cognitive testing.[38] Lemurs develop early amyloid deposits, cerebral amyloid angiopathy, and cerebral atrophy.[39] However, as appropriate, these animal species are rigorously protected, and access to aged subjects is limited as are longitudinal data enabling stratification of subjects. In this respect, the literature suggests that non-human primates may better model non-pathological human aging rather than AD per se, possibly due to differences in amyloid biochemistry and absence of a full spectrum of AD-like brain pathology. By contrast, recent data more relevant to current AD paradigms suggest that aged rhesus macaques and other monkeys demonstrate AD-like pathology and biomarkers that may be relevant for evaluating novel therapeutics,[33] although more thorough investigation of AD neuropathological biomarkers would be required if they are to be useful as models of AD.[40–43]

At a more granular level, one of the most overlooked factors in translating from an animal model to the human condition is the substantial inter-species differences in immune response pathways.[44] Different terminology has been used to describe pro-inflammatory or classical immune activation versus anti-inflammatory or alternative activation of the immune system, all of which are dependent on the immune stimulus causing the response. Of particular note is the immune system control of reductive-oxidative stress and its associated redox balance, which has been demonstrated to actively play a role in AD. One of the main components of the redox status/oxidative stress is nitric oxide, which is produced by the three different nitric oxide synthases across all mammalian species. Although qualitatively similar across species, the nitric oxide synthase 2 (*NOS2*) gene encoding inducible nitric oxide synthase is not highly expressed or strongly induced in the immune system of humans when compared to its mouse counterpart.[45] In contrast, rodents typically produce on the order of 10-fold or higher levels of nitric oxide than do humans.[46–48] Because the quality and quantity of oxidative stress is clearly different between mice and humans, one of the limiting principles for translation from animals to humans is recreation of a physiological redox balance. Colton and colleagues at Duke have addressed this issue by first moving amyloid precursor protein (APP) mouse models onto a NOS2 knockout background and then adding the entire human *NOS2* gene to recreate the human redox balance in a mouse model. Unlike singular APP-overexpressing mice, *APPSwDI+/+/mNos2−/−* (CVN-AD) mice expressing physiological levels of APP on a NOS2 knockout background and CVN-AD mice expressing physiological levels of APP on a humanized NOS2 background *(APPSwDI(+)/(-)/HuNOS2(tg+)/(+)/mNos2(-/-))* both display significant learning and memory deficits, neuronal loss, amyloid plaques, and neurofibrillary tangle pathologies.[49] These changes include regional brain volume changes and altered connectivity as shown by magnetic resonance imaging (MRI).[50] The dramatic shift in pathological and behavioral phenotypes from transgenic mice on a wild-type background to one with a humanized NOS2 background, which now includes the full spectrum of AD pathologies, provides important clues for development of accurate models that will enable full translation from models to the human condition.

With the dominating strength of the genetic association between apolipoprotein E (*APOE*) genotypes and risk of AD, inclusion of human *APOE* isoforms in all animal AD models is a critical requirement to improve translatability between these models and humans. Unlike animals, humans are the only species to express multiple isoforms of apoE protein, which derives from a single polymorphic *APOE* gene. Despite the *APOE ε*4 allele being carried by only 14% of the human population, roughly half of all AD patients are *APOE ε*4 carriers, whereas *APOE ε*2 carriers (8% prevalence) are relatively protected from the disease and tend to live to 100 years or more.[51] Non-human primates, dogs, and rodents all express single versions of apolipoprotein E that are similar, but non-identical to the human episilon-4 APOE protein.[52] The contribution of apoE proteins to the development of amyloid plaque and neurofibrillary tangle pathologies, at least in mice, is well proven by the lack of such pathologies in *APOE* knockout mice. Holtzman and LaDu have clearly shown that transgenic mice with human *APOE ε*4 backgrounds demonstrate significantly increased behavioral and pathological deficits that mimic the human condition.[53–55] With this strong genetic foundation in diseased humans and clear parallel influences of *APOE* genotypes in AD patients and *APOE*-transgenic mice, new models including humanized *APOE* backgrounds may provide improvements that may better define the predictability of these AD models. This addition can be realistically exploited for AD drug development.

The promising number of animal species and transgenic models that we have discussed support a place for the use of animal models to translate therapeutic efficacy outcomes into the clinic, and ultimately into AD patient populations. The strongest translatable drug development association to date has been with drugs designed to lower amyloid/Aβ levels. Many reports and clinical trials accurately show that anti-Aβ antibody therapies and/or secretase inhibitor therapies will lower amyloid/Aβ levels in animals and in humans. The lack of effectiveness of anti-amyloid therapies to slow, halt, and/or reverse cognitive decline in AD patients, however, was unanticipated and now requires more thoughtful evaluations of the complexities of the disease process. On the other hand, several groups reported safety concerns with secretase inhibitors,[56] and lack of efficacy of anti-amyloid immunization was also reported in aged dogs.[57,58] Such findings further underscore the importance of animal models and the use of more than one animal model not only for studies of potential efficacy, but also for studies undertaken to define safe and effective drugs for testing in human clinical trials.

A major of the complexity in human disease results from the presence of multiple or mixed pathologies.[59] Most LOAD is associated not only with plaques and tangles, but with multiple other neurodegenerative pathologies including Lewy bodies[60] and TDP-43 pathology.[61] Moreover, various vascular pathologies such as small vessel disease and infarcts are extraordinarily common in the aging brain and are well known to contribute to cognitive impairment, decline, and the threshold to dementia.[62] Although common mechanisms such as *APOE*

ε4 pathways may underlie some of the pathologic disease processes, animal models of AD do not classically show these additional "mixed" neurodegenerative changes. Some animal models have incorporated the common co-occurrence of vascular disease by using hypoperfusion, diabetes, hypertension, or hyperhomocysteinemia in combination with an AD animal model. As one example, induction of diet-induced obesity and diabetes in human mutant APP mice resulted in impaired nitric oxide production, vascular relaxation, and raised blood pressure, which were all corrected by treatment with a β-site amyloid precursor protein–cleaving (APP-cleaving) enzyme 1 (BACE1) inhibitor.[63] In another example, chronic hypertension induced by feeding of a nitric oxide synthase inhibitor to human APP mice resulted in hypertension, impaired cognitive performance, enhanced cerebrovascular amyloid deposition, and enhanced blood-brain barrier leakage, conditions frequently observed in AD patients.[64] One exception to this trend is the use of naturally aged models such as canines and macaques, which typically demonstrate multiple aspects of AD.[65] While studies of comorbidities using animal models are important, they must take into account the physiological differences between species to better address the relevance of perturbations seen in the animals to those seen in AD patients in order to adequately design informative models.

3 | NEXT-GENERATION AD ANIMAL MODELS

The creation of animal models that accurately predict clinical efficacy in humans will depend on the integration of specific humanized biological systems into those animal models that result in measurable changes to permit reliable testing of novel therapeutics. As discussed above, suppression and/or activation of immune system responses underlie the development of all diseases with human immune responses being different from most animals.[44] Similarly, distinctly different transcriptional changes have been identified in several cell types in human AD brains compared with those of 5XFAD mice that begin to identify resistive versus pathogenic responses in different species.[66] *APOE* backgrounds have also been demonstrated to affect AD-related outcomes in a variety of transgenic mouse models and could likely further influence disease outcomes in higher order animals.[53–55] Creating polygenic models on "backgrounds" in model animals that better mimic human immunity may further enhance our ability to recreate a human like pathologic, disease-based change. Confirmation of model reliability will depend on comprehensive statistical analyses and the application of single nucleus transcriptomic studies and new statistical platforms to compare human and mouse data sets.[67] These systems may represent the tip of the iceberg, especially as more genes are identified in subpopulations of AD patients, and cell-type specific changes in gene expression related to disease progression and resistance are better defined to permit comparisons between model systems and human subjects.

In addition to ongoing efforts in individual laboratories throughout the world, the NIA established the Model Organism Development & Evaluation for Late-Onset Alzheimer's Disease (MODEL-AD) consortium in 2016 with Indiana University, The Jackson Laboratory (JAX), The University of Pittsburgh (PITT), Sage Bionetworks, and the University of California, Irvine, to develop better *in vivo* models that may accelerate the discovery and development of new therapies. These MODEL-AD partners plan to establish a pre-clinical testing pipeline by generating more than 50 new rodent models based on human data sets, screening at least 30 of these models, and deeply phenotyping at least 15 of them. MODEL-AD partners also plan to develop a platform for modeling the disease that incorporates prioritized genetic variants of LOAD, albeit in the rodent milieu with corresponding inherent limitations discussed above. To further its goals, the MODEL-AD consortium will engage other National Institutes of Health (NIH) genetics and systems biology resources including the Accelerating Medicines Partnership – Alzheimer's Disease (AMP-AD), the AD Sequencing Project (ADSP), and the AD Neuroimaging Initiative (ADNI). AMP-AD has funded two projects: a project focusing on tau imaging and fluid biomarkers to track disease progression and response to treatment; and a target discovery and pre-clinical validation project, which includes studies of genomic, transcriptomic, proteomic, and metabolomic networks that contribute to AD pathogenesis. Data derived from AMP-AD are already informing the development of novel animal models of AD as described later here. All data from this consortium will be publicly available and all models will be available from the JAX mouse repository without restrictions.

As a generalized approach, next-generation models of AD should incorporate genes that are genetically linked to AD by incorporating them into platform mice that are humanized for the human Aβ peptide sequence and human tau sequences, with all transgenes being expressed at human-like physiological levels. By doing so, these next-generation polygenic models may show better concordance with human genetics and pathology. Each of these approaches may generate a more accurate disease phenotype or provide a more accurate assessment of all experimental therapeutic intervention strategies. As discussed below, the combination of detailed assessments of metabolomics and proteomics in conjunction with corresponding gene changes is essential to fully phenotype any model. As described at the meeting, Dr. Lutz detailed novel unpublished data and rigorous statistical methods that can be used to provide verification of pathologic pathway communality, which is defined as the extent that pathways between human disease and the animal models correlate.[67] Generating these modified animal models is now feasible because of recent advances in genome editing, particularly CRISPR/Cas technology, which has dramatically reduced the time and cost of gene targeting and modification[68] and enabled the creation of novel *in vivo* models of human disease,[69] potentially making them more predictive of aspects of AD in humans.

4 | GENOMICS AND PROTEOMICS

4.1 | Genomics

Genetic linkage approaches have been invaluable for identifying genes associated with EOAD versus LOAD. In rough terms, genetic forms

of inherited or familial AD (FAD) that are typically early onset AD (or EOAD) represent about 1% of all AD patients, whereas all EOAD represent as much as 5% of all AD patients. On the other hand, late-onset AD (or LOAD) represents the vast majority (>95%) of AD patients.[70] Initially, genetics pointed to the central role of Aβ metabolism in the development of FAD/EOAD.[71,72] Genetic linkage studies led to the identification of mutations in the *APP*, *PSEN1*, and *PSEN2* genes as main autosomal dominant causal factors for FAD/EOAD. In contrast, genome-wide association studies (GWAS) have identified numerous gene loci as risk factors falling within several potential physiological pathways, many of which suggest inflammatory mechanisms that increase susceptibility to LOAD. The contribution of *APOE* to LOAD has estimated heritability of at least 50%.

A major objective of the field is to create new genetic models of LOAD that exhibit the hallmark features of the disease including progressive cognitive impairments that inform corresponding changes in humans, amyloid and tau accumulation, immune-mediated responses, and neuronal dysfunction and degeneration. Multiple genetics resources and approaches in human populations exist with the objective of identifying new genes that mediate AD susceptibility, including the International Genomics of Alzheimer's Project (IGAP) consortium, the ADSP, and initiatives associated with the NIA Genetics of Alzheimer's Disease Data Storage Site (NIAGADS), among others. As new genes are identified, new models will be engineered to strategically express combinations of gene variants associated with AD, including those identified in humans as polygenic risk factors. Many susceptibility genes that have already been identified are involved in immune system regulation, lipid homeostasis, mitochondrial function, cellular metabolism, maintenance of the extracellular matrix of the plasma membrane, and synaptic signaling. Deep phenotyping of these new models may facilitate clarification of the linkage between these presumptive AD genes and the pathophysiology of AD,[73] and identify potential targets for drug development.

APOE is the strongest and most well-studied LOAD risk factor.[74] Other novel biologic pathways identified through genetic studies that are important in AD pathogenesis cluster around cholesterol transport and metabolism, endocytosis, and immune mechanisms. By comparing functional gene regulatory networks to neuropathology present in large numbers of human brain samples, Zhang and colleagues showed that the immune/microglia network is most strongly associated with LOAD pathology.[75] However, mouse immune pathways differ in potentially significant processes from human immune pathways.[44] For example, CR1, a GWAS identified risk factor for human AD, differs genetically, functionally, and in expression patterns from animals to humans.[76–78] Another significant difference is in the nitric oxide synthase 2 (*NOS2*) gene where NOS2 knockout mice more closely mimic human immune responses. Moreover, APP mutant mice crossed with NOS2 knockouts have been shown to develop typical AD-like pathology, perform poorly on cognitive tests, and show brain volume changes and altered connectivity similar to that seen in humans with AD.[79] These novel models strongly support that fidelity of modeling human disease in animals may require mimicking the immune background of the human brain to the degree that is possible in this inter-species context.

4.2 | Integrative proteomics for novel target and biomarker discovery

The Accelerating Medicine Partnership AD (AMP-AD) consortium is taking an agnostic approach to look at the AD brain proteome in up to 2000 individuals using both a high-throughput label-free and isobaric tandem mass tag mass spectrometry (MS)–based approaches across the disease continuum.[80] Tissues are being obtained from several large brain banks and longitudinal cohort studies including the Baltimore Longitudinal Study of Aging (BLSA), Adult Changes of Thought (ACT), Mount Sinai Brain Bank (MSBB), Banner Sun Health Research Institute (Banner), Emory Brain Bank (Emory), University of Pennsylvania (UPenn) and Mayo Brain Bank (Mayo), and the Rush University ROS/MAP cohort. Using systems biology approaches and predictive modeling, clusters of inter-related proteins were found to be associated with key clinical and pathological phenotypes. Weighted gene coexpression network analysis (WGCNA) represents one such approach and is used to understand disease processes at a systems level by resolving groups of proteins (ie, modules) with highly correlated expression profiles.[81] These modules can reflect specific cell types and functional protein complexes not always seen at the RNA level.[82] WGCNA can be used to define biological functions of modules and correlate those to specific AD phenotypes (eg, neuropathology, cognitive status).[82–84] LOAD genetic risk factors, including *APOE*, have also been shown to correlate with these modules.[80,82,83,85] For example, using samples from the BLSA study and a validation set from the Emory Brain Bank, Seyfried et al.[82] identified protein modules enriched for LOAD GWAS targets within glial cell pathways in patients with AD. In contrast, genetic risk factors linked with schizophrenia and autism mapped to modules associated with neuronal cell biology. These data support an emerging hypothesis that glial dysfunction plays a causal role in LOAD.

Moving forward, these investigators are building deeper networks in human samples[83,86–88] and mapping them to various mouse models to assess how well mouse models reflect the underlying pathophysiology in AD. For example, efforts are ongoing to investigate whether familial AD (FAD) mouse models recapitulate some of the cellular phenotypes and modules (neuronal and glial) in human AD brain. These studies are also being performed on an APOE4/TREM2*R47H LOAD mouse model. Going further, magnetic resonance imaging (MRI) analyses of *APOE* ε4 mice on a humanized *NOS2* background (E4HN) have also shown extensive brain shrinkage consistent with neuronal loss and loss of neuronal tracts associated with normal brain function compared to *APOE* ε3 mice on a humanized *NOS2* background *(E3HN)*. Behavioral testing confirmed that E4HN mice were significantly impaired on learning and memory tests compared to E3HN mice.[50,89,90] These types of deep phenotyping efforts not only validate the characteristics of each animal model, but provide data for comparative analysis with human

AD patients. In this regard, Lutz et al.[67] presented a novel bioinformatics strategy to compare genomics, transcriptomics, and proteomics data from human AD patients with an AD animal model to help decipher how strongly different AD models align with the human disease. These types of approaches will clearly guide model development, biomarker development, and drug development in the Alzheimer's arena. Ultimately, the consortium plans to couple the results from cell-type specific enrichment and confirm them with mass spectrometry from neurons, astrocytes, oligodendrocyte, microglia, and endothelial cell populations in AD models.[91–94] With this complementary approach to assess the impact of the AMP-AD nominated targets in pre-clinical mouse models in a cell-type specific manner, it remains necessary to also consider the above-mentioned distinct differences in cellular and immune pathways that exist between humans and rodents. "Advanced" disease in the respective species potentially represents very different disease severity scenarios, with rodents demonstrating greater protective responses in "late rodent disease" than are seen in advanced human AD (ie, Refs. 95,96). Altogether, the recapitulation of human disease traits in each animal model can iteratively be combined to represent the human disease more accurately, and ultimately the value of that model to predict responses to novel therapeutics.

5 | MODERN BEHAVIORAL APPROACHES

Translating behavioral changes observed in animal models to the cognitive deficits observed in humans with AD has proved to be challenging. Historical assays for assessing cognition in rodents are not readily translated to human outcomes. Although the newer methodologies discussed below have improved the correlation between behavior and pathophysiology, the question remains as to which, and to what degree, important cognitive and behavioral domains impaired in humans with AD (eg, episodic memory, working memory, short-term memory, executive function, activities of daily living, and mood/neuropsychiatric outcomes) have reasonable endophenotypic analogs in animal models.[97] The dominant behavioral assays used in mice—fear conditioning, water maze, and novel object recognition—all have limited face and predictive validity. More subtle behaviors like burrowing, nest construction, and hoarding may be useful alternative measures.[98] On the other hand, confounding factors like the impact of stress, hyperactivity, and/or visual impairments may be improved by careful selection of background strains for genetically engineered mice. In fact, Howell and colleagues presented strategies of back-breeding transgenic mice to multiple background strains to exploit the resulting differences in phenotypes that can then be traced back genetically to illuminate novel genes and/or gene networks that control desirable traits and/or outcomes.[99,100] Of course, standardization of testing protocols across sex, age, and genotype will continue to improve best practices with application of principles like those in Animal Research: Reporting of *In* Vivo Experiments (ARRIVE) guidelines.[101] Although it may never be possible to create a fully translatable menu of cognitive and behavioral outcomes in animals that reflect corresponding phenotypes in humans, it will be critical to determine the degree to which any outcomes can map between models and humans in order for the models to be useful in predicting outcomes from pre-clinical drug discovery efforts.

Several speakers detailed plans to emphasize pharmacokinetic and translational pharmacodynamics in addition to behavior as a primary screen for pre-clinical efficacy. However, improved behavioral and cognitive assessment tools for characterizing animal models of AD are also being created. Such assays must be robust and reliable to overcome subtleties resulting from background strain effects and different operating environments. The clear reporting of environmental conditions for both housing and during experimental procedures is one straightforward way to reduce variability of results from one laboratory to another. These assays must also be capable of consistently demonstrating normal aging-related effects in each sex with the awareness that the nature and/or timing of such changes may be different depending upon the sex of the individual.[102] This becomes particularly important in light of wide-spread recognition that the female sex is a risk factor in AD.[103–105] Because mice, like humans, manifest a spectrum of aging-related characteristics including increased vulnerability to physiological decline, Sukoff et al.[106] have proposed a composite score with components of the Neuropsychiatric Inventory questionnaire (NPI-Q) that captures decline in function across multiple physiological systems through assessments of frailty, cognition, hearing, vision, olfaction, motor, and fine motor function. Notably, recent findings have demonstrated that composite frailty indexes that have value in tracking normal healthy aging may also predict cognitive decline and AD progression to dementia.[107–109] Therefore, frailty may be used as a translational biomarker to capture global sensory and motor decline longitudinally prior to the onset of cognitive decline. These frailty measurements allow capture of the divergence between normal gradual progression of change through age, versus an acceleration and increase in severity as a harbinger of disease onset.

Leahy and colleagues at PsychoGenics have developed proprietary platforms that incorporate high-throughput, unbiased, modern behavioral and physiological assays with innovative computational, machine learning, and signal-processing tools to identify novel compounds that demonstrate efficacy in rodents.[110] They have demonstrated the ability of these behavioral assessment platforms to identify early and robust phenotypes in a variety of disease models including Alzheimer's and Huntington disease mouse models.[111] These types of data-intensive behavioral assessments clearly open the doorway to similar intensive data collection in humans, which through unobtrusive technologies can be incorporated into the home environment or reasonably inexpensive wearables to collect data both day and night. Alternatively, operant behavioral paradigms aimed at evaluating specific cognitive processes in rodents such as motoric versus cognitive impulsivity deficits, which potentially contribute to executive deficits and attentional processes, can be measured using tasks such as the five-choice serial reaction time test or age-related changes in motivation.[112] Such testing that is reflective of anhedonia, provides validated methods for evaluating subtle effects of genetic modification or drug interventions on cognitive processes impacted across various diseases.[113,114] Of course, the rigor and reproducibility of such studies in animal models will need to be demonstrated across multiple

laboratory groups and sufficiently validated to be informative of translatable comparisons with humans.

6 | TRANSLATIONAL CHALLENGES

A significant translational advantage of animal models is that biomarker changes can be compared with brain pathology, thus avoiding the diagnostic uncertainty in human cohorts. Although even inbred models have been shown to yield phenotypic variations, the careful use of the overall reduced variability in genetically homogeneous mouse models enables cross-sectional studies to provide pseudo-longitudinal data.[115] Studies in genetically engineered mouse models can also provide mechanistic insight regarding biomarker changes.

However, the small size of mice makes the analysis of CSF biomarkers challenging. Current commercial assays lack the sensitivity needed for small murine samples, and available assays may not recognize animal counterparts of human proteins that are important for translational work. Despite these challenges, some mouse CSF biomarker studies have demonstrated their usefulness in predicting biomarker changes in humans. For example, in two APP transgenic mouse models, CSF levels of Aβ40, Aβ42, and tau mirror the temporal sequence and magnitude of changes seen in patients with AD.[116] Increases in mouse neurofilament light chain (NfL) in CSF and blood were found to correlate with the progression of neurodegenerative pathology, similar to reported observations in humans with AD.[117]

The small size of the mouse brain also makes translational positron emission tomography (PET) and MRI studies challenging, but does not prevent the performance of these types of critical translational studies. Device manufacturers have been encouraged to develop technologies that provide adequate informative resolution for mouse and rat. For example, head coils designed specifically for mice, longer scanning times, and higher field strengths can produce extremely detailed images for structural analysis.[27,60] In addition, novel analysis routines that provide translational relevance when evaluating animal MRI data via similar and/or parallel clinical routines used to evaluate human MRI data are also required. Standardization and quality control of collection, storage, and analytic protocols as well as instrument qualification and calibration are needed for both fluid and imaging mouse biomarker assays. Moreover, optimization and validation of these assays will be critical for implementation. Badea and colleagues have established tool sets for MRI analyses and shown their usefulness in mouse models of AD.[50,89] Again, part of the challenge is to develop guidelines that show how a specific biomarker and/or image correctly predicts a desired outcome in the human AD patient and thus validates this approach.

By contrast, aged animal models, such as the dog or monkey, may also demonstrate biomarker changes that model those seen in AD. The larger size of these models enables evaluation of multiple fluid biomarkers relevant to disease progression and for evaluating therapeutic efficacy. Moreover, imaging sequences employed in human studies are easily transitioned to studies employing larger species.

Designing *in vivo* studies to create confidence in clinical translation will require asking the right questions and selecting the model or groups of models that can best answer those questions.[14] A methodical and complete characterization of models will be needed to achieve this. When appropriate models have been selected, proof-of-concept studies should be conducted first to ensure that the study design is appropriate and account for differences in age, sex, and litter lines as per the ARRIVE guidelines.

7 | EXPERIMENTAL/OPERATIONAL ISSUES

Some laboratories are using a systems biology approach to develop improved models for LOAD.[99,100] Because genetic context matters, initial development of all models on at least one common C57BL/6J background will permit transgene to transgene comparisons. Deep phenotyping of each mouse background strain including metabolic, behavioral, genomics, metabolomics, transcriptomics, proteomics, imaging, electrophysiology, fluid biomarkers, and neuropathology will be required. Furthermore, sex differences have emerged as important considerations with respect to timing and extent of changes (ie,[95,118], and will help to define the host's contribution to the disease in much the same way that genetic diversity exists in different human sub-populations). To validate the combination of transgene with background mouse strains, data from both males and females should be analyzed separately and together, and the same assays must be performed at multiple sites to assess the reproducibility of inter-operator and intra-operator variability in much the same process used to perform clinical trials at multiple sites. In addition, availability of all mouse models through commercial vendors without the need for licensing fees and other restrictive covenants will alleviate obstacles that prevent researchers from academia and industry alike from using the proper translational model for drug development.

Platform strains already completed include a humanized Aβ-knock-in model, as well as strains with humanized *APOE* ε2, *APOE* ε3, and *APOE* ε4, humanized tau, two different *TREM2* variants, and strains that express APOE4/TREM2^{R47H}, APOE4/Aβ-KI, and APOE4/Aβ-KI /TREM2^{R47H}. Other strains in progress will include a humanized tau knock-in model. Addition of genetic variants to these platform strains with CRISPR technology, will be followed by a primary screen to select strains relevant to LOAD for deep phenotyping and use in the preclinical testing core. Even strains that are not selected based on the primary screen may provide useful information about different aspects of the disease. Future models will explore not just genetic risk but also genetic context and environmental changes. Although difficult to compare across conditions associated with human aging and disease, efforts to develop animal models are still necessary and warranted. The Foundational Neuroscience Center is generating models that include age, comorbidities, and other types of dementia to enable testing of targets in the context of multiple pathologies.

8 | CONCLUSIONS AND NEXT STEPS

The lack of success of clinical trials of disease-modifying therapies for AD has been attributed to many possible reasons. Although one can conclude that pre-clinical models may accurately reflect individual pathogenic mechanisms but may not fully model human aging, disease, and co-morbidities, new models are currently being developed to improve the translation from animal to human. The ability of anti-amyloid therapies to reduce brain amyloid in transgenic models and in human patients does not address the more complex issue of whether reducing amyloid corrects cognitive decline in Alzheimer's patients. This sort of issue must be addressed in human subjects, but informed if possible using predictive pre-clinical models. Meanwhile, the aging of the world's population has increased the urgency of finding new and effective treatments for AD. In the United States alone, by 2050, the number of people with Alzheimer's dementia may grow from over 5 million to nearly 14 million barring the development of medical breakthroughs to prevent, slow, or cure AD {Alzheimer's Association, 2019 #1}. Taken together, these factors have propelled efforts to develop next-generation animal models that will more accurately predict efficacy of experimental treatments thereby enabling the translation of drugs from bench to bedside.

The Translational Animal Models for AD Think Tank participants explored the promise and shortcomings of existing animal models and key attributes needed for the next generation of animal models to be successful. Although efforts are currently underway to develop those models, gaps remain to be addressed in developing tools that will facilitate outcomes in all models to improve translation and predictive validity.

This effort starts with recognizing that animal models that reflect single aspects of AD pathogenesis do not mimic AD, and that multiple models or combinations of new models are needed that encompass the heterogeneous mechanisms and pathways, comorbidities, and other pathologies that lead to AD and other dementias. Elucidating genetic and environmental interactions along these pathophysiological pathways could lead to novel therapeutic interventional approaches. To maximize the usefulness of new models, phenotypes should be interpreted in the context of the recently proposed Research Framework.[119]

Continued investment and increased funding for basic and translational science will eventually lead to new therapeutic targets, requiring better and more rational models to test efficacy as accurately and early as possible early in the research and development pipeline. No single model will likely be able to fully recapitulate human AD, and the choice of the best models will depend on the question being asked.[14] To select optimal models, many issues will need to be addressed including: (1) which animal species, strains, and genetic backgrounds are most appropriate to answer specific questions; (2) how specific models can be most efficiently used throughout the drug development pipeline; (3) the meaningfulness of these models and behavioral-cognitive assays to human aging and disease; and (4) and how to match potential AD therapeutic approaches with particular animal models. Best practice guidelines will also need to be developed for the consistent use of these models in different research settings.

Finally, it is important to note that the novel murine models assessing impact of genetic risk factors, both alone and in combination, will potentially yield opportunities to elucidate and evaluate the impact of specific risk factors related to AD subpopulations and to potential targeted therapies. However, current transgenic models incorporating high-impact mutations directly linked to heritable familial AD have not been successful, which raises concerns that novel models evaluating genetic risk factors that are less penetrant may not improve the predictive validity essential to drug development. Given the immediate need to develop novel disease-modifying therapeutics and the knowledge that no model accurately recapitulates all aspects of AD, a strategically-driven multi-model evaluation strategy incorporating both genetic models and currently available "agnostic" aged animal models of AD-like progression should be considered for drug development to de-risk the transition of pre-clinical drug assets to the clinic.

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DECLARATION OF INTEREST

M. P. Vitek is a principal and stockholder in Cognosci, Inc. J. A. Araujo is a shareholder of Vivocore Inc., the parent company of InterVivo Solutions Inc. and CanCog Inc.; a director in all three companies; the CEO and president of InterVivo Solutions Inc.; and an advisor to Telocyte. M. Fossel is president of Telocyte. M. Windisch is CEO and president of NeuroScios GmbH. A. Ross was a full-time employee of the Alzheimer's Association during the time of manuscript review. B. T. Lamb is a consultant for AvroBio and Eli-Lilly. M. C. Carrillo and R. M. Edelmayer are full-time employees of the Alzheimer's Association. B. D. Greenberg, G. R. Howell, S. J. Sukoff Rizzo, N. T. Seyfried, A. J. Tenner, P. R. Territo, and L. J. Bain have no declarations of conflicts of interest.

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