Accelerating Drug Discovery for Alzheimer's Disease: Best Practices for Preclinical Animal Studies

Alzheimer's Drug Discovery Foundation

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Animal models have contributed significantly to our understanding of the underlying mechanisms of Alzheimer's disease (AD). As a result, over 300 interventions have been investigated and reported to ameliorate pathological phenotypes and/or improve behavior in AD animal models [1]. To date, however, these findings have not resulted in target validation in humans and successful translation to disease-modifying therapies. Challenges in translating preclinical studies to clinical trials include the inability of mouse models to adequately recapitulate the human disease, variations in breeding and colony maintenance practices, lack of standards in design, conduct and analysis of animal trials, and publication bias due to under reporting of negative results in the scientific literature [2-4].

The quality of animal model research on novel therapeutics can be improved by bringing the rigor of human clinical trials to animal studies. To this end, the Alzheimer's Drug Discovery Foundation (ADDF) partnered with Charles River Discovery Research Services and convened an expert advisory panel of academic, industry and government scientists to make recommendations on best practices for animal studies testing investigational AD therapies. By promoting best practices, these recommendations can improve the methodological quality and predictive value of AD animal studies and make the translation to human clinical trials more efficient and reliable.

Animal Models of AD: Modeling Targets, Not Disease

• Despite failing to accurately model all aspects of human AD, animal models offer a means for testing pharmacodynamic properties of candidate molecules on drug targets that may be involved in AD pathogenesis.

• It is critical to choose a chronic model of disease that exhibits significant, well characterized pathology relevant to the target of interest (i.e. amyloid plaques, tau pathology, neuronal loss, oxidative stress/inflammatory changes, etc) based on the hypothesized mechanism of action of the therapeutic being investigated. There is no "one model" for AD. Testing in multiple models is necessary to provide preclinical validation.

• Animal models that better integrate the effects of multiple AD pathologies and models that demonstrate pathology on understudied targets (such as mitochondria function and energy metabolism, neural plasticity, vascular changes, lipid metabolism, oxidative stress and inflammation pathway targets among others) are needed.

• Models that do not solely rely on mutated human genes as the basis of the induced pathology should also be employed, including non-transgenic mice, pharmacologically and surgically-induced models and aged rodent models.

Know Your Model

Many genetically engineered animals show high variability in extent and time course of expression of disease phenotypes. **Table 1** illustrates common factors effecting phenotype variability, including environment, age, sex, genetic background, litter, transgene copy number, and health status. Not all of these variables can be controlled, but measures can be taken so that changes in phenotype can be properly noted and potentially corrected.

Most importantly:

• Maintain good communication among laboratory members to track deviations from expected phenotypes. Keep careful records to track if a change in phenotype occurs

- Identify issue with breeding, such as longer litter intervals, smaller litter sizes, and fewer pregnancies. Identifying such problems early will help keep production on track.

• Screen gene copy number and transgene expression level regularly. Document and report.

• Freeze embryos early during characterization of the transgene in case phenotypic drift necessitates re-derivation of colony.

• Consider your genetic background: Mice may be healthier and more viable on a hybrid background, but genetic drift must be controlled to avoid confounding variables. Certain inbred strains are more prone to characteristics like blindness, hearing loss and aggression.

• If working with a contract research organization (CRO), ask to see historical data on the colony. These data should include rearing conditions (light cycle, housing type, diet, and health status) and breeding scheme to assess genetic management of the strain background(s) in the colony.

The pharmacokinetics of drugs, along with their pharmacodynamic effects, can also differ among genetically engineered lines and genetic backgrounds. When possible, it is informative to validate target effects of a therapeutic in a second model or in a non-transgenic model.

Improving Rigor in Study Design

By paying careful attention to study design before starting experiments, investigators can reduce the likelihood of false positive or false negative results. **Table 2** outlines key study design considerations.

Develop and Employ Translatable Biomarkers for Animal Preclinical Studies

Although more validation is needed, biomarker methods under development in rodents include imaging (MRI, MRS, fMRI, ASL-MRI, FDG-PET, PET amyloid imaging, PET-tau imaging, SPECT/CT and others) and biochemical assays on biological fluids such as plasma and cerebrospinal fluid. Whenever possible, biomarker measurements should be incorporated into study design.

Biomarkers can be used in animal studies to:

- Assess target engagement of investigational treatments
- Monitor biological responses to treatment in real-time
- Characterize the translatability of AD models
- Determine the translatability of a novel therapeutic if the same biomarker can be used in a human clinical trial

Timing of Treatment

Treatment timing should depend on whether the therapeutic goal is ***disease prevention***, ***therapeutic intervention*** (i.e. slowing/reversal of established pathology), or ***symptomatic relief***.

- Tissue should be collected from a proper cohort of animals at the time when treatment is initiated to determine whether the treatment reduced pre-existing pathology in the brain, or simply slowed the age-associated accumulation. Where a longitudinal assessment is possible (i.e. peripheral biomarkers, imaging, certain behavioral responses), taking repeated measures of the same animal can be especially informative and add statistical power.
- Treatment should be timed based on optimal stage of pathology development in the animal, which will allow for acceptable signal to background ratio and dynamic range for experimental treatments. Optimally, demonstration of assay validation should be a pre-requisite to embarking on therapeutic studies.

• Pathology can vary widely with animal age. Control and treatment groups should be age-matched to the greatest extent possible (i.e. within days of one another).

• Pathology and biochemical readouts can also vary widely among animals within a genetically engineered line. The variability in pathology with age and in outcome measures must be assessed in order to power the animal studies properly.

Pharmacokinetics(PK)/Pharmacodynamics (PD), ADME-Toxicology

Studies should include pharmacokinetic (PK) and pharamacodynamic (PD) assessments to determine whether the compound exposure is sufficient and whether it is interacting with the target of interest. Depending on whether a study is *exploratory* or *therapeutic* (page 6), the degree to which Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) are profiled should be considered as part of the prospective study design. More information about these types of studies can be found in the ADDF/Alzheimer's Research Forum Drug Development Tutorial [5]. It is important to note that genetically engineered models may not always be the most cost-effective and translatable model when measuring PK/PD. Wild-type mice are often preferable for use in these studies, but correspondence with genetic background strains in the transgenic studies should be considered.

Statistical Methods

Statistical methods should be chosen before a study is begun, with the anticipated direction of change (1-sided or 2-sided) in mind. Statistical considerations should be clearly stated in the methods. Assessment of endpoint variability in a large sample size is necessary, as the type of variability (normal distribution vs. skewed) dictates a parametric vs. non-parametric statistical analysis of the data. Guidance or consultation of a statistician should be enlisted in the design of the study once the endpoint variability has been characterized.

Proper Quantification

For tissue sections, the area of the pathology as well as the magnitude of pathology counts should be quantified and reported. Adequate tissue sampling is critical. Typically, at least 6-7 fields/section, 6-7 sections/mouse, across multiple affected brain regions should be measured. Staining and field sampling methods should be stated in methods, and sampling should be guided by statistical considerations of the variability in the end point being

interrogated. Analysis and quantification of pathology should be conducted by someone blind to the treatment condition.

Sample Size

Studies are frequently underpowered. This has been reported to be the single most important factor in influencing spurious research results with animal models [3]. Sample size depends on the expected magnitude of the biological effect, the inherent variability of the target being measured (e.g., CSF Aβ is much more variable than hippocampal Aβ), variability in behavioral measures, and other factors such as variations in survival within the particular cohort of animals. The sample size needed to achieve substantial significant differences given the variability of disease outcomes in most AD mouse models has been estimated to be on the order of 20-30 per group.

Exclusion Criteria

Animals whose physiological condition appears compromised by factors unrelated to the normal progression of the disease should be excluded from the study. Exclusion criteria should be established prior to the study and not on a post hoc basis and records kept of which animals were excluded and why.

Balancing and Randomization

Sex and age-matching is critical in study design as both of these factors significantly affect pathological expression. For example, Aβ plaque loads can increase exponentially during the first stages of plaque deposition, and spurious drug effects may be seen in animals analyzed at this stage unless control and treatments groups are age-matched to within days of one another. Mice should be separated into groups by sex, age and litter and then randomly assigned to either control or treatment groups. In addition, wild-type and/or young controls should be included into study design as a reference point.

Blinding

Individuals conducting the experiments and those analyzing the results should be blinded to treatment. In the event that a test compound may have a phenotypic impact on the treated animals, these potentially unblinding observations should be noted by the animal handler but kept segregated to the degree possible from the analyst until the experiment is unblinded. If this is not possible, a full re-design of the experiment may be required. For example, a compound that results in reduced feeding activity (phenotypic observation – reduced rate of weight gain) may have an impact on Abeta levels for reasons unrelated to its therapeutic target.

Reporting

Investigators should report full details of target assay methods and detailed information on the animal model used, including genetic background, copy number, exclusion criteria and statistical analyses. For behavioral assays, training as well as testing phases should be reported. When possible, scatter-plots should be shown rather than, or in addition to, bar graphs.

Publication bias fueled by a decreased ability or desire to publish negative results represents a huge problem for the field [6]. To increase efficiency, decrease redundant efforts, and learn from others' experiences, it is crucial that negative results be reported. Forums for discussing the quality of negative results, and results that differ from laboratory to laboratory, would aid in the interpretation of negative studies.

Definition: Exploratory vs. Therapeutic Studies

Many investigators, particularly in academic settings, lack the infrastructure and budget to perform extensive preclinical studies incorporating all the design, methodolgical and statistical considerations recommended here. In addition, comprehensive analyses are not always warranted when the compound or target is being assessed in early stages. As a result, we propose to distinguish between ***Exploratory*** and ***Therapeutic*** studies (**Table 3**).

• Exploratory studies should demonstrate that a particular molecular target is involved in a disease process. While exploratory studies do not require the extensive lead optimization, PK/PD, and toxicity analyses undertaken in therapeutic studies, they nonetheless should provide sufficient data to inform the decision of whether to proceed to a therapeutic animal study. Exploratory studies should contain a tolerability/toxicity assay to verify that selected doses are not causing an adverse effect. Furthermore, terminal blood and brain tissue samples should be collected for possible later PK verification, as the half-life of the test compound may or may not have been consistent with the timing of the putative therapeutic readout.

• ***Therapeutic*** studies should be compound focused and include a full PK/PD and ADMET profile to ensure appropriate dosing and timing of outcomes with respect to exposure of the compound. Toxicity considerations are important for minimizing potential off-target phenotypic impacts on outcome measures. The design, conduct, analysis and reporting of a therapeutic animal study should be analogous in rigor to those required for human clinical trials.

Summary

In conclusion, our advisory panel identified opportunities for improvement and made resultant recommendations regarding the ***measurement, design, analysis and reporting of pre-clinical animal studies in AD***. We hope these recommendations will significantly advance the field by making animal studies more consistent and predictive of subsequent clinical trial outcomes.

Future Direction

Models

- Improve access, characterization and standardization of existing AD mouse models
- Develop more animal models to non-traditional targets, and make more use of available non-transgenic models, such as aging rodents

Methods

- Standardize commonly used protocols for target assessment
- Develop new and more high throughput methods for measuring outcomes
- Focus on novel targets and outcome measures

Study Design

- Develop biomarkers in animal models that can be translated to humans in clinical trials
- Standardize review of animal studies in grant applications and scientific publications

Resource building

- Establish a public data repository for both positive and negative animal studies
- Create a historical data compendium on AD animal models that investigators can continually update

Table 1

Major Factors Effecting Phenotypic Variability in Mice

Environmental	Biological
Housing system (cages/enrichment)	Age
Housing (#mice/cage)	Sex
Handler/investigator	Body condition
Light cycle	Genetic drift
Temperature and moisture	Genetic background (mixed vs. inbred)
Noise and vibration	Type of background strain
Diet	Transgene copy number
Health status	Transgene expression level

Table 2

Key Considerations in Preclinical Animal Studies

Clearly delineate an a priori hypothesis for the study that includes primary and secondary outcomes

- Pre-specify specific measure to assess the primary and secondary outcomes
- Attempt to employ translatable biomarkers
- Consider issues of sex, timing of treatment, age of animals
- Determine inclusion and exclusion criteria

Carefully design a statistical analysis plan prior to initiation of the study

- Perform power analysis and sample size estimates prior to initiation of the study taking into account previously measured variability in the outcome measures
- Include randomization methods for treatment groups and blinding procedures for those doing assessments
- Include procedures for dealing with dropouts and deaths of animals in statistical analyses

Table 2 continued

Key Considerations in Preclinical Animal Studies continued

Reduce publication bias

- Report both positive AND negative results in peer-reviewed journals or other open-access format
- Report details of strain, housing, diet, drop-out events/in-trial exlusions, etc. so variables can be assessed
- Analogous to clinical trials, report the flow of animals through the treatment plan of the study
- Report potential conflicts of interest and whether investigators are third party or primary investigators invested in the hypothesis

Table 3

Exploratory vs. Therapeutic Preclinical Studies

Goal	Exploratory Studies: Mechanism/Target Focused	Therapeutic Studies: Compound Focused
Study Design	Efficacy data assessed through multiple outcome measures	Efficacy results in more than one model
	Randomized, placebo controlled, blinded, with dose response	
	Consideration of in vivo model – pathogenic stage, age, length of treatment required, exclusion criteria defined	
ADME	Initial physicochemical property considerations and terminal blood and brain tissue sampling for possible PK verification	ADME profiling and full PKPD analysis – Distribution/exposure of parent compound and metabolites
Toxicity	Defined toxicity assessment not needed at this stage, but efficacy study should have a simple drug tolerability assay included.	Assess toxicology in model being studied, with treatment conducted at levels reliably below adverse event doses
Statistics Plan	While statistical considerations are not as stringent, prospective power analysis should take into account variability in the model itself and in outcome measure readouts	Prospective study design issues should include sample size power analyses, statistical evaluation plan, primary and secondary outcome measures, blinding and randomization

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