FROM MOUSE TO MEDICINE: OPTIMIZING THE PREDICTIVE VALUE OF PRECLINICAL RESEARCH

INTRODUCTION

One of the major challenges to the successful development of therapies for Alzheimer’s disease (AD) is the poor translation of preclinical efficacy from animal models to the clinic. Assessments of preclinical animal studies have highlighted the need for an emphasis on rigor in study design, methodology and data analysis, transparent reporting methods, mitigation of publication bias due to under-reporting of negative results, and the development of a set of best practices to optimize the predictive value of preclinical research testing candidate AD therapies.

To address this challenge and ameliorate some of the factors contributing to the preclinical to clinical gap in the development of AD therapies the National Institute on Aging (NIA) and the National Institutes of Health (NIH) Library have created a publicly available data repository – the Alzheimer’s Preclinical Efficacy Database, or AlzPED. AlzPED is designed as a web-based portal for housing, sharing and mining of preclinical efficacy data. The data are submitted to AlzPED through a curator and gleaned from at least two sources; 1) the scientific literature; 2) directly from researchers. These data include information on AD animal models, therapeutic agents, therapeutic targets, outcome measures, related clinical trials, patents and study design. Most importantly, AlzPED is designed to help identify critical experimental design elements and methodology missing from studies that make them susceptible to misinterpretation and reduce their reproducibility and translational value. Through this capability, AlzPED is intended to guide the development and implementation of strategies and recommendations for standardized best practices for the rigorous preclinical testing of AD candidate therapeutics.

This growing knowledge platform currently houses 1030 preclinical efficacy studies published between 1996 and the present (Table 1), collected from databases like PubMed and EMBASE using key word search strings specific to AD. Each study is carefully curated by 2 experts in AD research prior to publication in the database. Efforts are underway to expand the database further and balance the number of studies curated based on the year of publication.

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Table 1: The table shows the number of preclinical efficacy studies collected, curated and available in the database and the year of publication.
EXPERIMENTAL DESIGN ELEMENTS

AlzPED identifies 24 experimental design elements that should be included in any preclinical efficacy study to improve its rigor, reproducibility and translational value (Figure 1). Comprehensive analysis of the 1030 published preclinical efficacy studies compiled in AlzPED demonstrate considerable variation in the frequency of including and reporting these elements of experimental design. For example, experimental design elements like dose and formulation of the therapeutic agent being examined and treatment paradigms are included and reported with consistency (at 95% or greater) whereas other critical design elements like power calculation, blinding for treatment allocation and outcome measures, randomization, and balancing a study for sex as a biological variable are less frequently reported (less than 35%).

AlzPED further defines 9 core experimental design elements that are critical for ensuring scientific rigor and reproducibility of a preclinical efficacy study, derived from Shineman et al., 2011, Landis et al., 2012, Snyder et al., 2016 and ARRIVE guidelines (Figure 2). These include power/sample size calculation, randomization, blinding for treatment allocation and outcome measures, sex as a biological variable and balancing a study for sex as a biological variable, animal genetic background, financial conflict of interest statement and inclusion and exclusion criteria. Of these 9 core design elements, sex as a biological variable and genetic background of the animals used in the study as well as a financial conflict of interest statement from authors are well reported, though this is reflective of changes in data reporting and publication policies and requirements recommended by federal funding agencies, private foundations and peer-reviewed scientific journals. The remaining 6 core design elements including power/sample
size calculation, randomization, blinding for treatment and outcomes, balancing a study for sex as a biological variable, and inclusion and exclusion criteria are very poorly reported.

Further evaluation of the reporting trends in the 9 core experimental design elements demonstrates that few studies report more than 5 core design elements, most studies reporting only 2-4 core design elements. From the 1030 curated preclinical studies in the database, 5% report none of the core design elements, 14% report at least 1 core design element, 25% report at least 2, 26% report at least 3, 18% report 4, 8% report 5, 3% report 6, 2% report 7, and 0.2% report all 9 core design elements (Figure 3).

Figure 2: Percentage of studies that reported 9 core experimental design elements derived from Shineman et al., 2011, Landis et al., 2012, Snyder et al., 2016 and ARRIVE guidelines, calculated from 1030 published preclinical studies curated to AlzPED.

Figure 3: Percentage of studies reporting 0-9 core experimental design elements, calculated from 1030 published preclinical studies curated to AlzPED.
NIH-issued policies to enhance the rigor, reproducibility and translatability of its supported research place significant emphasis on rigorous scientific method/study design and consideration of biological variables including sex. Analysis of study design and methodology of NIH-funded published research curated in AlzPED demonstrates a positive impact of these policies. NIH-funded studies show significantly higher frequency of reporting of sex as a biological variable, study balanced for sex as a biological variable, blinding for outcome measures, randomization, and conflict of interest when compared with studies supported by non-NIH funding agencies (Figure 4). Non-NIH funded studies include those funded by nonprofit organizations, pharmaceutical companies, the European Union, and the Chinese, Japanese and Korean governments.

Figure 4: Comparison of reporting trends in 9 core experimental design elements between NIH-funded and non-NIH funded studies. Data are presented as percentages calculated from 75 NIH-funded studies, 128 studies funded by the Chinese Government, 65 Nonprofit-funded studies, 53 Pharmaceutical Industry-funded studies, 103 European Union-funded studies, and 69 studies funded by the Japanese or Korean Governments. Data are analyzed using the Fisher’s Exact Test, *p<0.05, **p<0.01 and ***p<0.001.

The lack of rigor and reproducibility of research findings in the scientific publishing arena is well documented. A systematic review of the rigor and translatability of highly cited animal studies published in leading scientific journals (including Science, Nature, Cell, and others) demonstrates a lack of scientific rigor in study design. A comprehensive review of reporting trends for critical experimental design elements in highly cited studies published in leading journals that are curated in AlzPED revealed a similar pattern of poor study design and reporting practices. These analyses are described in greater detail in the next segment.

Reporting trends for the 9 core and all 24 experimental design elements were evaluated based on the impact factor of the journal in which the curated preclinical study was published (Figure 5). These studies were categorized into 4 groups based on 2019 journal impact factor values. Curated studies published in journals with impact factors below 3 were sorted in Group 1, and those published in
journals with impact factors between 3 and 4.99, or between 5 and 9.99 were sorted in Groups 2 and 3 respectively. Studies published in high impact journals with impact factors greater than 10 were sorted in Group 4. While t-tests show that there are statistically significant differences in reporting the 9 core elements as well as all 24 elements of experimental design between these four groups, overall, the data demonstrate poor reporting practices irrespective of journal impact factor.

![Figure 5: Reporting trends for the 9 core and all 24 experimental design elements based on journal impact factor.](image)

Figure 5: Reporting trends for the 9 core and all 24 experimental design elements based on journal impact factor. Data are presented as Mean ± SEM and analyzed using two-tailed t-tests, *p<0.05, **p<0.01 and ***p<0.001, from 220, 424, 301 and 85 curated studies published in journals with impact factors below 3, between 3 and 4.99, between 5 and 9.99, and above 10 respectively.

Reporting trends for the 9 core and all 24 experimental design elements were evaluated based on the relative number of citations per year of each curated study published between 1996 and 2019 (Figure 6). Relative number of citations for each curated study was calculated by dividing the total number of citations for that study by the number of years since publication. For example, for a study published in 2018, the total number of citations for that study was divided by 2, or for a study published in 2017, the total number of citations for that study was divided by 3, and so on. These studies were categorized into 3 groups based on the relative number of citations per year. Curated studies with less than 3 relative number of citations per year were sorted into Group 1, those with relative number of citations per year between 3 and 7 or those with relative number of citations per year greater than 7 were sorted into Groups 2 and 3 respectively. While t-tests show that there are statistically significant differences in reporting the 9 core elements as well as all 24 elements of experimental design between these three groups, overall, the data demonstrate poor reporting practices irrespective of relative number of citations per year.
Therefore, it is clear that there are serious deficiencies in reporting critical elements of methodology such as power/sample size calculation, blinding for treatment/outcomes, randomization, and others, even in high impact factor journals as well as in highly cited published preclinical research. Consequently, the scientific rigor, reproducibility and translational value of preclinical studies is diminished.

In light of these results, it is evident that a standardized set of best practices is required for successful translation of therapeutic efficacy in AD research. To this end, several meetings and workshops have been held between the NIH and journal publishers to discuss the issue of reproducibility and rigor of research findings and identify common opportunities to enhance rigor and support research that reproducible and transparent. It is imperative that the NIH, other federal funding agencies, private foundations and scientific journal publishers continue to collaborate on this issue and enforce a standardized set of best practices.

Figure 6: Reporting trends for the 9 core and all 24 experimental design elements based on relative citation number per year. Data are presented as Mean ± SEM and analyzed using two-tailed t-tests, ***p<0.001, from 291, 388 and 349 curated studies with relative citation numbers per year below 3, between 3 and 7, and above 7, respectively.
THERAPEUTICS

A diverse array of therapeutic agents and targets are reported in the 1030 studies curated in AlzPED. The database catalogues 890 novel therapeutic agents into 14 distinct categories (Figure 7) based on agent source (natural product or synthetic), molecular structure (biologic or small molecule), chemical nature (peptide, nucleic acid, or hormone) and mechanism of action (immunotherapy – active or passive).

Figure 7: The graph shows the diverse types of therapeutic agents tested, presented as percentages from 1030 published preclinical studies curated to AlzPED.

Therapeutic agents tested in curated studies were sorted into four groups based on source and molecular structure as small molecules, natural products, dietary supplements, and biologics. Trends for the testing of therapeutic agents in these groups were evaluated over 5-year spans from 1996 to 2019 (Figure 8). The analysis revealed decreased use of biologic-based therapeutic agents over the past ten years (2010-2019) with concomitant increase in the use of small molecule therapeutics and natural products.
This decrease in the use of biologic-based therapeutics over the past ten years is accounted for by decreased active and passive immunotherapy. During this period, there is an increase in the variety of biology-based therapies for AD (Figure 9).

Currently, AlzPED stores information on 173 therapeutic targets that aim to reduce beta amyloid and tau-related pathology and address disease-associated inflammation, oxidative stress, metabolic, synaptic and behavioral dysfunction. These assorted targets are categorized into amyloidogenic proteins, tau protein, non-amyloid proteins, enzymes, receptors and transporters, metal ions, free radicals and multi target (Figure 10). Notably, numerous therapeutic agents demonstrate varying extents of anti-inflammatory, antioxidant, beta amyloid-reducing, neuroprotective and cognition enhancing properties and are categorized as multi target therapeutics. Agents that have 2 or more documented targets are also categorized as multi target therapeutics.
Trends for these therapeutic target categories were evaluated over 5-year spans from 1996 to 2019 (Figure 11). The analysis revealed decreased targeting of amyloidogenic proteins including beta amyloid over the past ten years (2010-2019) with concomitant increase in the use of multi target therapeutics.

Figure 10: The graph shows the diverse categories of therapeutic targets, presented as percentages from 1030 published preclinical studies curated to AlzPED.

Figure 11: The graph shows the diverse therapeutic targets tested, over 5-year spans from 1996 – 2019. Data are presented as percentages from 121, 246, 287 and 376 curated studies published between 1996-2004, 2005-2009, 2010-2014 and 2015-2019 respectively.
Within this diverse group of targets catalogued in AlzPED, the most frequently targeted are beta amyloid peptides, beta and gamma secretases, tau protein, cholesterol metabolism regulator HMG CoA reductase, inflammatory response regulating enzyme cyclooxygenase (1 and 2), glucose metabolism regulator peroxisome proliferator-activated receptor gamma (PPAR gamma), and critical neurotransmission and synaptic signaling molecules like NMDA receptors and acetylcholinesterase (Figure 12).

Trends for these ten therapeutic targets were evaluated over 5-year spans from 1996 to 2019 (Figure 13). The analysis revealed decreased targeting of beta amyloid over the past ten years (2010-2019) with concomitant increase in other non-amyloid therapeutic targets.

Figure 12: The graph shows the diverse cellular and signaling mechanisms targeted, presented as percentages from 1030 published preclinical studies curated to AlzPED.

Figure 13: The graph shows the top ten therapeutic targets catalogued in AlzPED, over 5-year spans from 1996 – 2019. Data are presented as percentages from 121, 246, 287 and 376 curated studies published between 1996-2004, 2005-2009, 2010-2014 and 2015-2019 respectively.
These results are representative of the varied agents and targets currently being studied in AD therapeutics research and show a shift from beta amyloid as the primary target to more wide-ranging set of targets. This analysis also supports similar analyses from related databases like the International Alzheimer’s and Related Dementias Research Portfolio (IADRP).

Within the 890 therapeutic agents catalogued in AlzPED, 140 agents (or 16% of the total number of agents) are currently in AD clinical trials, and 172 agents (or 19%) are in clinical trials for non-AD indications like cancer, diabetes, cardiovascular ailments, neuropsychiatric disorders, movement disorders and various other conditions (Figure 14). Of the 140 agents that are in AD clinical trials, 110 are also in clinical trials for various non-AD indications. 580 (65%) therapeutic agents are in the preclinical testing phase.

According to ClinicalTrials.gov, 140 AlzPED-catalogued therapeutic agents are currently in 1029 AD clinical trials (Figure 15). These therapeutic agents include small molecules, dietary supplements, natural products, and various biologic-based compounds. Within the AlzPED-catalogued therapeutics, NMDA receptor antagonist memantine, and acetylcholinesterase inhibitors like donepezil, galantamine and rivastigmine account for 37% of the agents that are in AD clinical trials. Additionally, these compounds account for 57% of the AlzPED-catalogued small molecule therapeutics currently in AD clinical trials.
Data from ClinicalTrials.gov also show clinical trial status and clinical trial phase of the 140 AlzPED-catalogued therapeutic agents that are in AD clinical trials (Figure 16). A large percentage of phase 2 clinical trials are completed with these agents.

Figure 16: The graph shows the clinical trial status and phase of the 140 AlzPED-catalogued therapeutic agents in AD clinical trials. Data are presented as percentages of total number of AD clinical trials for these therapeutic agents.

AlzPED also includes 20 therapeutic targets from a list of more than 500 nascent drug targets that have been nominated by researchers from the NIA’s Accelerating Medicines Partnership in Alzheimer’s Disease (AMP-AD) Consortium.
ANIMAL MODELS

Within the 1030 curated studies compiled in AlzPED, 6 different animal species have been utilized, a majority of which are mouse models of AD (Figure 17). Other animal species include rat, guinea pig, rabbit, dog, and non-human primate models of AD.

Preclinical efficacy data from 41 model types and 188 different AD animal models are currently available in AlzPED (Figure 18). Preclinical efficacy data from new AD animal models generated in the NIA-established Model Organism Development and Evaluation for Late-Onset Alzheimer’s Disease (MODEL-AD) Consortium will be included as they become available.

Figure 17: Six different animal species utilized in preclinical efficacy studies, presented as percentages calculated from 1030 published preclinical studies curated to AlzPED.

Figure 18: 41 different animal model types and 188 AD animal models are utilized in preclinical efficacy studies, presented as numbers calculated from 1030 published preclinical studies curated to AlzPED.
OUTCOME MEASURES

Each curated study provides an individual snapshot of the measures tested and outcomes achieved in response to the therapeutic agent tested. AlzPED defines 21 different outcome measures that are categorized as either functional or descriptive. Functional measures include behavioral, motor, electrophysiological and imaging outcomes (Figure 19).

Figure 19: The graph shows the percentage of studies reporting functional measures, calculated from 1030 published preclinical studies curated in AlzPED.

Of these functional measures, behavioral outcomes are most commonly tested. There are 77 unique behavioral outcomes measured, from which the Morris water maze, novel object recognition, open field tests and Y maze are the most frequently studied. Within the 19 different motor function outcomes measured, locomotor activity, swimming speed, path length and the rotarod test are the most frequently studied. 68 diverse electrophysiological outcomes are measured, the most frequently measured being long term potentiation (LTP), field excitatory postsynaptic potentials (fEPSP), paired pulse facilitation (PPF) and input/output (I/O) curve. Within the 40 unique imaging outcomes measured, cerebral blood flow, structural MRI and in vivo two-photon amyloid and calcium imaging are the most frequently studied (Figure 20).
Descriptive measures include ADME, biochemical, biomarker, cell biology, chemistry, electron microscopy, histopathological, immunochemical, immunological, microscopy, omics (proteomics, lipidomics, metabolomics, transcriptomics, and others), pharmacodynamic, pharmacokinetic, pharmacological, physiological, spectroscopy and toxicology outcomes (Figure 21).
Within the descriptive measures tested, beta amyloid pathology-related biochemical, histopathological, immunochrometical and biomarker outcomes are a major focus in the studies curated to AlzPED. These measures analyze several species of beta amyloid including soluble, insoluble, monomers, oligomers, fibrils and plaques. Other measures in these categories include evaluation of several species of tau (soluble, insoluble, aggregated, hyperphosphorylated and others), and astrocytic, microglial and synaptic markers.

Notably, even though beta amyloid and tau species, and glial markers are a major focus, an extraordinary range of factors and molecules are investigated within these 3 descriptive measures. In total, information from 1193 biochemical, 36 histopathological and 380 immunochrometical measures are currently available in AlzPED. As many as 28 different biomarkers have been analyzed, and beta amyloid markers in plasma, serum or CSF constitute a large proportion (Figure 22).
Other frequently studied descriptive measures used to characterize the therapeutic agent being tested include ADME, pharmacokinetic, pharmacodynamic and toxicology outcomes (Figure 23). Of the 25 ADME measures studied, the most commonly tested are biodistribution, metabolic stability and cytochrome p450 inhibition capability of therapeutic agent. Similarly, 93 different pharmacodynamic measures are examined with key focus on reducing beta amyloid species. As many as 54 pharmacokinetic measures have been analyzed, and drug concentration in brain and plasma are most frequently evaluated. A comprehensive listing of at least 94 toxicology measures such as Ames tests, enzyme profiles, organ histology and others are available in the database as well. Of these, the most frequently evaluated are body weight, general behavior and food and water intake.
AlzPED reports on 14 different physiological measures from which blood pressure and cerebral blood flow are most frequently evaluated and 7 pharmacological measures from the most commonly tested are binding affinity and target selectivity of the therapeutic agent. As many as 93 cell biology outcomes are measured, and cell viability and cytotoxicity are the most common measures. Of the 34 immunological measures reported, antibody titers and target specificity are most frequently evaluated (Figure 24).
AlzPED also informs on 15 OMICS-related measures such as metabolomics and gene expression profiles. Finally, AlzPED also reports on 34 electron microscopy outcomes, 58 microscopy outcomes and 15 spectroscopy outcomes (Figure 25).
SUMMARY

In summary, AlzPED provides the AD research community free access to a treasure trove of information pertaining to rigorous experimental design and methodology, AD animal models, therapeutic agents and targets, outcome measures, and principal findings from preclinical studies, along with related PubMed and PubChem literature, clinical trials, patents, funding sources and financial conflict of interest. AlzPED is also designed to serve as a platform for reporting unpublished negative findings to mitigate publication bias favoring reporting of positive findings. Researchers can use this resource to survey existing preclinical therapy developments, understand the requirements for rigorous study design and transparent reporting and plan preclinical intervention studies.

**Figure 24:** The graph shows the most frequently measured microscopy, electron microscopy, spectroscopy, and omics outcomes, presented as percentage reported, calculated from 193, 58, 68 and 47 curated studies that reported microscopy, electron microscopy, spectroscopy, and omics measures, respectively.