Alzheimer's & Dementia 12 (2016) 1177-1185

Perspective

Guidelines to improve animal study design and reproducibility for Alzheimer's disease and related dementias: For funders and researchers

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Abstract The reproducibility of laboratory experiments is fundamental to the scientific process. There have been increasing reports regarding challenges in reproducing and translating preclinical experiments in animal models. In Alzheimer's disease and related dementias, there have been similar reports and growing interest from funding organizations, researchers, and the broader scientific community to set parameters around experimental design, statistical power, and reporting requirements. A number of efforts in recent years have attempted to develop standard guidelines; however, these have not yet been widely implemented by researchers or by funding agencies. A workgroup of the International Alzheimer's disease Research Funder Consortium, a group of over 30 research funding agencies from around the world, worked to compile the best practices identified in these prior efforts for preclinical biomedical research. This article represents a consensus of this work group's review and includes recommendations for researchers and funding agencies on designing, performing, reviewing, and funding preclinical research studies.

Keywords: Alzheimer's disease; Reproducibility; Animal models; Preclinical; Drug development

1. Introduction

There is growing interest and concern across the biomedical research community about the reproducibility and translatability of published research findings into clinical results [1–3]. This topic is highly relevant to a number of stakeholders, including academic and industry researchers, medical journal editors, and funding organizations (government and non-governmental). The credibility of scientific researchers, institutions, journals, and funding agencies around the world depends on the production of robust, high-quality, and reproducible research. Across the spectrum, consensus guidelines for biomedical research have been developed to ensure the overall integrity of findings is secure.

This article builds on the large body of excellent prior work that targeted different aspects of the reproducibility issue across the biomedical research spectrum [4,5]. Although much of this is generalizable to the broader research community, there is a need to communicate the specific challenges and opportunities of reproducibility for

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http://dx.doi.org/10.1016/j.jalz.2016.07.001
1552-5260/

preclinical studies for Alzheimer's disease (AD) and related dementias from the unique perspective of funding organizations from around the world [6]. This effort specifically references models of AD-related pathologies; however, the recommendations described below may also be relevant to non-AD dementias as well as the broader research community.

One important concept for this document is the categorization of different types of animal studies and their corresponding requirements for design rigor. In the context of drug discovery, Shineman et al. distinguished between "exploratory" and "therapeutic" animal studies (described below) [6]. In this article, the scope is expanded to include "mechanistic" experiments, which usually precede the identification of a compound or therapeutic agent (i.e., small molecule, biologic, gene therapy) used in exploratory and therapeutic studies. "Exploratory" or "therapeutic" studies may also comprise "mechanistic" aspects. The practical relevance of these categories will be explored in the guidelines in Tables 1 and 2 and after a fuller exploration of their meaning.

Mechanistic animal studies focus on understanding the underlying biological processes contributing to a disease state. For example, these studies may include transgenic overexpression or knockout of a particular gene and/or gene product or pharmacological manipulation of a biological pathway to identify or validate a disease-related target. These studies do not include investigation of a specific experimental compound; however, they require appropriate statistical and power analyses and study design considerations.

Exploratory animal studies are similar to pilot or early proof-of-concept studies for a particular therapeutic intervention and must include rigor in the design (as described in Table 1). Exploratory studies confirm that a compound is accessing a specific target and/or disease mechanism. These studies are designed with defined outcome measures to assess target engagement and should include some initial absorption, distribution, metabolism, and excretion (ADME) profiling, as well as tolerability assessments. The main focus of limited ADME studies at this stage is to demonstrate brain penetrance (if required) and target engagement at a relevant concentration/dose. Exploratory studies can form the basis of full therapeutic studies through the exploration and selection of appropriate endpoints and corresponding power calculations. It is imperative that exploratory studies are labeled, interpreted, and reported as such.

Therapeutic animal studies are comprehensive animal studies focused on a specific compound or series of compounds and are generally larger, more costly, and statistically rigorous than exploratory studies. In addition to predefined efficacy measures, therapeutic studies require full pharmacokinetic (PK) and pharmacodynamics (PD) assessments to determine appropriate dosing and a toxicology profile in the model being studied. These animal studies should be designed and executed with the same rigor that is appropriate for human clinical trials.

2. Background

The profile of research into Alzheimer's disease has arguably never been higher than at present, at both national and international levels. Through the 2013 Group of Eight or the G8 Summit on Dementia, these government agencies committed to the ambitious goal of identifying a cure or disease-modifying treatment for dementia by 2025 [7], a goal commensurate with the US National Plan to address Alzheimer's disease [8]. In the meantime, a number of funding initiatives from around the world have or are being established to address this plan [9]. With increased funding and profile come increased expectations of research and a focused effort across multiple stakeholders is needed to actively address this issue of research reproducibility.

Over the past three decades, significant progress has been achieved in understanding the neurobiology of AD. Notwithstanding these advances, the development of effective AD therapies has been challenging, as is evidenced by the extremely high attrition rate for AD treatments, with 99.6% of compounds being tested not meeting their primary endpoints in or before phase III [10]. Many of the AD clinical failures have occurred with both small molecules and immunotherapies failing to show efficacy or having unacceptable toxicity.

There are many possible factors that could contribute to the failure of AD therapies in clinical trials from demonstrating efficacy. One could be the drugs themselves or nature of the trials [11]. Another possible factor is poor predictive power of preclinical efficacy seen in transgenic models of AD pathology. Such models are widely used by researchers but their limitations in relation to human Alzheimer's disease are also well recognized [12,13]. For example, of more than 300 potential therapeutic agents reported, in leading scientific journals, to ameliorate pathology and/or cognitive deficits in AD Tg mouse models, there has been limited translation into a clinical benefit in human trials, with the last drug being approved for symptomatic use over more than a decade ago [10]. When inferring from published mouse studies, it may be helpful to consider the extent to which a positive treatment outcome with one pathologic endpoint (such as plaque removal) relates to another—such as a neurodegenerative one. Another key consideration is the extent to which early, preventative-type intervention studies in models can be compared human studies with more advanced disease. A number of more general factors have been identified as contributors to the poor translation of efficacy from AD Tg models to humans; these include lack of standards in the design, conduct, and data analyses in preclinical efficacy studies and publication bias as the result of under-reporting of negative results in the scientific literature [14–16].

Table 1
Guidelines for preclinical study design

Recommendation	Mechanistic	Exploratory	Therapeutic
Before all studies: choice and use of animal models			
Use models of disease targets rather than the entirety of the disease; understand the context of the mechanism or disease target in the selection of the animal model	+	+	+
Choose model based on the specific mechanism that will be tested and at an age/stage of progression with the strongest rationale for that mechanism	+	+	+
Obtain animals from trusted sources; confirm genotype and phenotype through regular characterization based on model selection and experimental design	+	+	+
Characterization of experimental drug compound, antibody and so forth before drug efficacy studies is needed to confirm that it will provide quality and interpretable data			
Characterize experimental drug compound/agents before drug efficacy studies, including • Analytic characterization to confirm the structure and purity of the experimental drug (i.e., LC/MS, NMR, and so forth) when these are not provided by the supplier or if storage conditions/stability may be in question • Pharmacokinetic/absorption, distribution, metabolism, and excretion studies to establish free-drug concentrations at the site of action are essential for conducting in vivo mechanism of action or efficacy studies		+	+
Demonstrate target engagement of experimental compound at a dose which is well tolerated. When a target-related mechanism cannot be directly measured, a sufficiently proximal measure may be used		+	+
Compare control group data to published data using the same background strain. Be alert to unexpected variations in disease symptoms and/or deaths	+	+	+
Study design			
Define a clear hypothesis with disease-relevant primary and secondary outcome measures	+	+	+
Include study design outcome measures which cover disease predictive phenotypes and translatable biomarkers, as known and/or as applicable		+	+
Establish inclusion and exclusion for enrollment of animals into experimental groups. Match groups for sex, age, strain, split littermates then use randomization	+	+	+
Use double-blinded allocation of animals into treatment groups and assessment of efficacy/ outcome	+	+	+
Use appropriate power calculations. When applicable (i.e., exploratory/therapeutic), sample size estimates must use previously measured variability in the outcome measures	+	+	+
Address potentially confounding variables relating to the animals (gender, litter, copy number), testing parameters (time, lighting conditions, stress, human operator factors)	+	+	+
Use sensitive positive and negative controls (e.g., vehicle) where possible; report dose-response data		+	+
Data analysis			
Analyze the data with a statistical model capable of handling multiple variables	+	+	+
Engage with statistician from the outset to optimize study design	+	+	+
Engage with a statistician familiar with clinical trial design			+
Include procedures for dealing with dropouts and deaths of animals in statistical analyses	+	+	+
Use intent-to-treat analysis in statistical analyses			+
Control group data should be compared to published data using the same background strain. Be alert to unexpected variations in disease symptoms and/or deaths	+	+	+
Further work			
Reproduce studies in independent cohorts of the same model	+	+	+
Reproduce studies in independent cohorts of a related model	+	+	+

Certain outcomes in Alzheimer's disease have translated from animal studies into human clinical trials. Examples of efficacy have been reported for cholinesterase inhibitors, nicotinic agonists, memantine, and muscarinic agonists/ PAM in animal models and in the clinic, including phase II of drugs in development. Regarding antiamyloid immunotherapies, care must be exercised in comparing preventative studies in mice with therapeutic trials in individuals with AD [17], but some consistencies were seen between the models and patients in relation to semiquantitative measures of plaque load [11]. Although we may never have animal models that recapitulate all aspects of human AD, these models are tools to test pharmacodynamic readouts on specific disease targets and, when done in a rigorous way, should translate into human clinical results.

The development of the consensus guidelines outlined in this article for AD builds on past efforts, such as the Alzheimer's Drug Discovery Foundation Best Practices for Preclinical Animal Studies [6] and is being led by multiple funding organizations from around the world under the umbrella of the International Alzheimer's Disease Research Funder Consortium (IADRFC). The goal of this article

Table 2
Guidelines for reporting

Recommendation	Mechanistic	Exploratory	Therapeutic
Reporting criteria to improve replicability. Please describe			
Details of the animals used, including species, strain, sex, age, and weight	+	+	+
Relevant information such as the source of animals, international strain nomenclature, genetic modification status, genotype, health/immune status, drug or test naïve, previous procedures, and so forth	+	+	+
Details of housing (e.g., type of facility; type of cage or housing; bedding material; number of companions) and husbandry conditions (e.g., breeding program, light/dark cycle, temperature, access to food and water, environmental enrichment)	+	+	+
Details of welfare-related assessments and interventions that were carried out before, during, or after the experiment	+	+	+
Details of the ethical review permissions, relevant licenses, and national or institutional guidelines for the care and use of animals that cover the research	+	+	+
Details of how animals were allocated to experimental groups, including randomization or matching if done	+	+	+
Notate any modifications to the experimental protocols made to reduce adverse events	+	+	+
Details of primary and secondary experimental outcomes assessed (e.g., cell death, molecular markers, behavioral changes)	+	+	+
Details of the statistical methods used for each analysis	+	+	+
Reporting criteria to improve validity. Please include			
How and why the model being used can address the scientific objectives and, where appropriate, the study's relevance to human biology/pathology	+	+	+
Primary and any secondary objectives of the study or specific hypotheses being tested	+	+	+
Number of animals in each experimental group and explain how the number of animals was arrived at. Provide details of any sample size calculations	+	+	+
Details of all important adverse events in each experimental group	+	+	+
Raw data as supplemental	+	+	+
Number of independent replications of each experiment, if relevant	+	+	+
If any animals or data were not included in the analysis, explain why	+	+	+
The results for each analysis carried out, with a measure of precision (e.g., standard error or confidence interval)	+	+	+
Any methods used to assess whether the data met the assumptions of the statistical approach	+	+	+
Potential conflicts of interest and whether investigators are third-party or primary investigators invested in the hypothesis	+	+	+
List all funding sources (including grant number) and the role of the funder(s) in the study	+	+	+

was to provide guidelines to funding organizations, scientists applying for funding from these organizations, and those individuals providing peer-review expertise of applications related to preclinical research. The following sections outline the methodology used to develop these guidelines and specific recommendations for the various stakeholders.

3. Consensus guidelines: Methodology of development

The IADRFC convenes over 35 organizations from nearly 15 countries to discuss issues relevant to funding scientific pursuits for Alzheimer's and related dementias around the world. From the IADRFC, a working group for reproducibility was formed (IADRFC Reproducibility Working Group or IRWG). This IRWG comprised representatives from the Alzheimer's Association, Alzheimer's Australia, Alzheimer's Drug Discovery Foundation, Alzheimer's Research UK, Alzheimer's Society (UK), National Institute on Aging, and the journal Alzheimer's & Dementia. A nonsystematic literature review was conducted on the topic of reproducibility of preclinical studies. Individual recommendations and guidelines were compiled together with commentary and shared with a wider group of research funders and researchers expert in preclinical efficacy studies from academia and industry.

A survey was also sent to the IADRFC to gather additional information on the international funding community views on research reproducibility and actions underway within organizations to address this issue. There were 13 organizations that provided responses to the survey (Alzheimer Society of Canada, Alzheimer's Association, Alzheimer's Drug Discovery Foundation, Alzheimer's Research UK, American Federation for Aging Research, Brain Canada Foundation, Czech Alzheimer foundation, Department of Defense, FBRI, Internationale Stichting Alzheimer Onderzoek, Medical Research Council (UK), and multiple institutes from National Institutes of Health) representing five countries and more than $40 million in annual funding to preclinical animal studies.

The report underwent further refinement following external review before being finalized with the agreement of all the contributing authors on behalf of their respective organizations.

4. Issues surrounding reproducibility in preclinical studies

Although the need for new and better models that simulate human disease continues, this article focuses on how we can make the best use of our existing models and improve preclinical study design to enhance reproducibility and translatability. A recent survey of multiple funding organizations identified several interrelated challenges that impact research quality and efficiency, and each are described in further details below. These major challenges include but are not limited to

- Levels of funding for the project and/or study design considerations;
- Validation of studies for follow-up;
- Access and sharing of animal models;
- Access to informational and educational resources;
- Reporting of methodology and outcomes; and
- Rigor of review process, both journals and funding agencies.

Of note, close to 70% of organizations who responded to the survey expressed research reproducibility is an important issue for their organization and something that funding organizations need to address. We also include examples of initiatives that aim to directly address these challenges and discuss how the funding community can continue to incentivize the field to endorse overall best practices for preclinical drug discovery in animal models. These challenges represent opportunities for the Alzheimer's research community to work together to address issues of reproducibility in preclinical scientific investigations.

4.1. Level of funding for project/study design

Funders and journals alike have issued guidelines to reviewers on how preclinical studies should be evaluated. Including these study design considerations into an experimental plan can significantly increase the cost of the study. Most funders would prefer to pay more for a good study that will result in a clear positive or negative readout over a less expensive but poorly designed study that provides results that are either uninterpretable or misleading. In support of this statement, $<$10% of organizations responding to the survey indicated that they would not be willing to increase the size of their grants to improve study design. Yet, not every study is at the stage that necessitates a large, expensive study. Shineman et al. propose distinguishing between an exploratory and therapeutic animal study [6]. Once a compound or therapeutic candidate has been identified, a much larger, statistically rigorous, and expensive study is necessary and must be backed by strong pharmacological data and safety on the therapeutic candidate. These study design categories are further defined and detailed in Tables 1 and 2.

4.2. Validation of studies for follow-up

Key finding should be replicated by the originator group with a different animal cohort as the first as part of any first publication. The options and considerations for external validation/replication are more complex. Other groups have been established that seek to provide direct external validation of studies. Science Exchange, for example, will reproduce academic research findings in a clinical research organization (CRO) or independent academic setting, providing validation. One challenge is in securing funding for such studies. Although there can be logistical challenges to perform external validation studies on proprietary drug candidates, this is a model that has already been established within the cancer field [18] where experiments from "highly impactful" research articles are independently replicated and the findings made publically accessible; they have also faced criticism from some in the research community [19]. Replication is dependent on the quality of the replication laboratory, which may not have the expertise or the precise research tools needed to replicate the experiment. In addition, some experimental design factors will have a deterministic effect on the ability to be replicated, simply because they are at the base of what affects replication, for example, the power of a study. A replication study performed under the same experimental conditions as the original study will therefore face the same issues, and hence, it remains open which finding is correct if they differ. "Negative" studies should also include and report the estimated effect size needed for a positive outcome in power calculations. Nonetheless, funding organization can support these types of validation studies in situations where they make sense.

4.3. Access and sharing of animal models

Biotechnology companies and academic scientists new to the Alzheimer's field are significantly limited by the lack of available, well-characterized animal models. There are a number of academic centers that have large, well-characterized cohorts, but the availability of these animals, especially to for-profit entities, is significantly limited. Funding organizations could come together to support the characterization and production of designated cohorts in CRO settings that could be widely available to the research community. The National Institute of Aging's Interventions Testing Program is an interesting model by which a centrally run resource and evaluate interventions suggested by the research community. The challenge is that there is not one optimal model for Alzheimer's disease and likely a series of animal models that express different pathologies (i.e., amyloid, tau, inflammation, and neurodegeneration) would be most useful to the research community. The characterization of these models could include detailed analysis of pharmacodynamic endpoints for assessing drug target engagement, although these will be target specific. There is a need for more translatable biomarkers beyond cognitive testing. In

addition, there is a need to develop new models which display pathologies more relevant to the human disease and which potential issues such as intellectual property which could limit the distribution potential. There is an opportunity for funding organizations to work together to provide funding that address these needs.

4.4. Access to informational and educational resources

There has also been a significant amount of progress made in the development of resources for the research community. Alzforum holds a database [20] which lists over 100 different animal models with information on their neuropathologic and cognitive/behavioral phenotypes as well as data gaps. This highlights the variability between models and the partial characterization of many models. The National Institute on Aging is developing an Alzheimer's Disease Preclinical Efficacy Database analogous to clinicatrials.gov [21]. Incentives and opportunities to stimulate investigators to share negative data are also in the development. PLOS ONE, for example, has a short publication format [22]. Although these tools are a step in the right direction, there are obstacles in the peer review process for journal publications, for instance, that are expanded on further below.

There are also a number of training opportunities available for researchers such as the NINDS Neurotherapeutics Summit and the ADDF's annual Drug Discovery for Neurodegeneration Conference. The NIH has also provided resources on training for researchers, guidance to peer reviewers, primary data depository, and reporting on their website [23,24].

Finally, as an interest in big data and quantitative systems pharmacology has grown, tying animal research findings back to human disease data helps to build confidence in a particular disease pathway. The emergence of these fields has increased the need for biostatisticians and biostatistics training, which is critical to proper animal model study design and analysis of results. Prespecifying outcomes is important, including a power analysis for animal model studies to determine sample size and using proper statistics in analyzing results should become standard. Researchers should be encouraged the use of resources like NC3Rs' Experimental Design Assistant [25].

4.5. Reporting of methodology and outcomes

Beyond the model selection and study design, the reproducibility of studies depends in large part on studies being accurately reported in the scientific literature. There have been many efforts to standardize reporting including the ARRIVE guidelines for experiments involving animals, the STARD checklist for diagnostic studies, the CONSORT statement for randomized trials, and the PRISMA statement for systematic reviews and meta-analyses [26].

4.6. Rigor of review process for both journals and funding agencies

There have been several efforts, referenced throughout this article, shared with the research community to date on the issue of reproducibility. From these efforts, there has been tangible progress made in both modifying processes for evaluation and for funding preclinical research [27]. There have also been tangible changes in the peer review of scientific publications by many publishers. For instance, some scientific journals have altered their requirements for what should be included in the Methods sections. Nature journals have issued specific guidelines on what information is required to include in the Methods section, so that others can reproduce studies more reliably [28,29]. The field could benefit by increased rigor of publications to include better defined scientific methodology (i.e. experimental design) and sufficient space allowed for this [30]. Also, in response to the growing trend of journal editors and reviewers for additional details and the tension placed on authors to adhere to word count limits, online supplemental material and technical appendices provide additional avenues to report expanded methods and should be used when applicable.

Research funders are also incorporating reproducibility considerations into their grant application and review processes. In a recent survey of international AD funders, over 50% of organizations who responded noted having made changes to the grant application process with the goal of improving study design and reproducibility. The Alzheimer's Drug Discovery Foundation currently requires a table for all preclinical studies that highlights key considerations from the work of Shineman et al., highlighting the need to address these issues for applicants and making it easier for reviewers to evaluate [6,31]. Other funding agencies have added a separate section on power calculation in the application forms or have included a larger emphasis on statistical support for experimental design, and the importance of interdisciplinary teams. Many organizations directly link to published guidelines. In addition, funding agencies have increased efforts to confirm the identity of cell lines used for cell culture experiments.

Funding agencies do not consistently provide the same reviewer guidelines for review of preclinical guidelines, some organizations are directly asking reviewers for feedback on study design quality and to pay special attention to the choice of experimental animals in relation to the target of interest. More specific reviewer guidelines for the research funding community may help to standardize the review process and improve the quality of funded studies. Another opportunity for funders and publishers to address these issues of optimal preclinical study design and reproducibility is through the contracting process. Grant recipients can be required to share their data into a shared resource portal. A number of such portals are in development as described above.

In recent years, there has been a growing consideration of the need to refine, reduce, and replace the use of animals in

research and an adoption of this principle by many funders. There are many reasons why this is desirable. However, in doing so, it should also be understood that small, underpowered studies that do not replicate and do not in the long run reduce the use of animals in research are also not desirable. Funders are in a position to highlight this point to researchers and other interested parties.

Although there has been some progress made in the AD research community toward improving preclinical study design and research reproducibility and translatability, there is much more that can be done. In acknowledging these challenges, this article provides a distillation focused on the reproducibility of preclinical efficacy studies within AD and related dementias, summarizes efforts underway, and outlines potential opportunities for funders to work together through targeted initiatives to reach these stated goals. Given the current interest and investment in research to identify and understand disease mechanisms, discover and validate potential drug targets, and build preclinical pipelines leading to clinical trials, it is clear that proper preclinical study design is a critical step and should be emphasized.

5. Guidelines to improve preclinical study design and reporting

The tables below represent a core set of recommendations for researchers in the Alzheimer's disease and related dementia community. Recommendations are included for each stage of the study planning process. However, it is important to note that for Alzheimer's disease, there is still no model that recapitulates all aspects of the disease. Disease models show aspects of the human disease pathology and should be used to model disease targets. These tables could be adapted into application and reviewer instructions [32] for both funding agencies and journal publications to improve reproducibility.

6. Discussion and future directions

The goal of this manuscript is to provide common guidance and recommendations covering general procedures and practice of research funding of preclinical efficacy studies. These recommendations were developed in collaboration with several funding agencies and input from the scientific community. As new knowledge and its application develop, these guidelines will be evaluated and updated periodically.

Another core goal of this initiative was to identify resources or funding programs which are or that could be developed to improve the reproducibility of future research or which directly address replication of prior research. Funding agencies are increasingly collaborating through private-public partnerships, various consortia, and information sharing venues like the IADRFC. With continued focus and effort on improving collaboration, there are tangible actions funding agencies, as well as researchers and journals, can implement to improve preclinical research reproducibility in AD. Although joint activities may be preferable, funders will then consider individually whether to participate in, develop, or co-develop any such initiatives. These recommendations and potential future programs are applicable beyond AD and related dementias for potential adoption to the broader research spectrum.

In addition, it is critically important to tackle the issue of publication bias. Funders can actively encourage researchers to share and publish both positive and negative data in publications and in open source databases in development as discussed below. In order for researchers to interpret accurately the data in a publication, the study should be clearly labeled as either mechanistic or in the case of more drug/compound-focused, exploratory, or therapeutic as discussed above.

Although there are certainly challenges for implementing these recommendations, there must be a paradigm shift in how we conduct preclinical research such that reproducible science carries forward to further investigation in downstream clinical paths. Funding agencies can work together to create an environment that improves research application and review processes. For example, this could include reviews that evaluate study design and statistics more fully, funding mechanisms that provide new and innovative resources for education and data sharing to the research community to improve study design and translatability and opportunities to work together to address issues of animal model quality and availability.

Although these recommendations are reflective of the collective discussion from the IRWG, there remain challenges in international implementation across both the nonprofit and governmental sectors of funding agencies. Some considerations for funding agencies include the potential additive burden on applicants and reviewers to include and evaluate, respectively, the additional level of information summarized above. Current incentive and reward structures for academic research around the globe together with the types of measures of success have contributed to challenges surrounding reproducibility [4]. Together with universities and journal editors and publishers, funding agencies have the ability to consider the incentives and rewards that they use and how these may influence reproducibility in either direction.

Organizations may need to consider mechanisms to provide dedicated or increased funding to investigators to abide by guidelines of study design and statistical analysis. Given that budgets are usually limited, it would be important that they are not all consumed by a small number of therapeutic studies at the expense of the larger numbers of well-conducted mechanistic or exploratory studies, although funders with wide-ranging grant portfolios should be used to such balancing. In addition, the funding community should consider implementing incentives to researchers to produce highly reproducible research; in the chemistry research community, the professional society facilitates reproducibility of various chemical reactions and produces an annual compendium for the broader field. Analogous processes could be

developed to incentivize reproduction of preclinical animal studies, such as the "Preclinical Reproducibility and Robustness channel" by the open access publisher F1000Research [31,33].

For funding organizations, these recommendations are the first step toward increased success of reproducibility and translation from preclinical to clinical outcomes. Furthermore, for the AD research enterprise to achieve the ultimate goal of developing therapy(s) to stop or slow the progression of this devastating disease, open communication and collaboration between funding agencies, scientific journals, academic, and industry scientists are necessary.

Acknowledgments

The authors thank the International Alzheimer's Disease Research Funder Consortium for both incubating this effort and for input throughout the development of this article. In addition, multiple stakeholders were engaged in reviewing and providing commentary on the guidelines including David Morgan (University of South Florida), Barry Greenberg (Toronto Dementia Research Alliance and University Health Network), Bruce Lamb (University of Indiana Medical School), Cynthia Lemere (Harvard University), Michael Coleman (University of Cambridge), Bettina Platt (University of Aberdeen), Fiona Marshall (Heptares), Tara Spires-Jones (University of Edinburgh), Stuart Allen (University of Manchester), Thomas Steckler (Janssen Research & Development), Guy Eakin (formerly at BrightFocus Foundation), Neil Cashman (University of British Columbia), team at the Center for Open Science (University of Virginia), and Eric Karran (formerly at Alzheimer's Research UK and currently at AbbVie).

RESEARCH IN CONTEXT

1. Systematic Review: The reproducibility of laboratory experiments is fundamental to the scientific process. There have been increasing reports regarding challenges in reproducing and translating preclinical experiments in animal models.
2. Interpretation: A workgroup of the International Alzheimer's Disease Research Funder Consortium, a group of over 30 research funding agencies from around the world, worked to compile the best practices identified in these prior efforts for preclinical biomedical research in Alzheimer's disease.
3. Future Directors: These published guidelines include recommendations for researchers and funding agencies on designing, performing, reviewing and funding preclinical research studies.

References

[1] Begley CG, Ioannidis JP. Reproducibility in science: improving the standard for basic and preclinical research. Circ Res 2015; 116:116–26.
[2] Collins FS, Tabak LA. NIH plans to enhance reproducibility. Nature 2014;505:612–3.
[3] Freedman LP, Cockburn IM, Simcoe TS. The Economics of Reproducibility in Preclinical Research. PLoS Biol 2015;13:e1002165.
[4] Ioannidis JPA. How to Make More Published Research True. PLoS Med 2014;11:e1001747.
[5] Perrin S. Make mouse studies work. Nature 2014;507:423–5.
[6] Shineman DW, Basi GS, Bizon JL. Accelerating drug discovery for Alzheimer's disease: best practices for preclinical animal studies. Alzheimers Res Ther 2011;3:28.
[7] G8 Health Ministers' Dementia Summit Declaration (2013), UK Department of Health and Prime Minister's Office [cited 2016 Feb 4]. Available from: https://www.gov.uk/government/publications/g8-dementia-summit-agreements/g8-dementia-summit-declaration.
[8] National Plan to Address Alzheimer's Disease: 2015 Update, Office of The Assistant Secretary for Planning and Evaluation [cited 2016 Feb 4]. Available from: https://aspe.hhs.gov/national-plan-address-alzheimer%E2%80%99s-disease-2015-update.
[9] The World Dementia Council's Year-On Report 2014/15 [cited 2016 Feb 4]. Available from: https://s3-eu-west-1.amazonaws.com/media.dh.gov.uk/network/353/files/2015/03/WDC-Annual-Report.pdf.
[10] Cummings JL, Morstorf T, Zhong K. Alzheimer's disease drug-development pipeline: few candidates, frequent failures. Alzheimers Res Ther 2014;6:37.
[11] Karran E, Hardy J. A critique of the drug discovery and Phase 3 clinical programs targeting the amyloid hypothesis for Alzheimer's disease. Ann Neurol 2014;76:185–205.
[12] Bales KR. The value and limitations of transgenic mouse models used in drug discovery for Alzheimer's disease: an update. Expert Opin Drug Discov 2012;7:281–97.
[13] Zahs KR, Ashe KH. Too much good news – are Alzheimer mouse models trying to tell us how to prevent, not cure, Alzheimer's disease? Trends Neurosci 2010;33:381–9.
[14] Sena ES. Publication Bias in Reports of Animal Stroke Studies Leads to Major Overstatement of Efficacy. PLoS Biol 2010;8:e1000344.
[15] Institute of Medicine (US) Forum on Neuroscience and Nervous System Disorders. Improving the Utility and Translation of Animal Models for Nervous System Disorders: Workshop Summary. Washington, DC: National Academies Press; 2013.
[16] Institute of Medicine (US) Forum n Neuroscience and Nervous System Disorders. Improving and Accelerating Therapeutic Development for Nervous System Disorders: Workshop Summary. Washington, DC: National Academies Press; 2014.
[17] Agadjanyan MG, Petrovsky N, Ghochikyan A. A fresh perspective from immunologists and vaccine researchers: Active vaccination strategies to prevent and reverse Alzheimer's disease. Alzheimers Dement 2015;11:1246–59.
[18] Reproducibility Project: Cancer Biology (2015). Available at: https://osf.io/e81xl/. Accessed Feburary 4, 2016.
[19] Kaiser J. The Cancer Test. Science 2015;348:1411–3.
[20] Alzforum. Research Models. Available at: http://www.alzforum.org/research-models. Accessed June 10, 2016.
[21] Alzheimer's Disease Preclinical Efficacy Database. Available at: https://alzped.nia.nih.gov/. Accessed March 1, 2016.
[22] Validation by the Science Exchange Network. Validating key experimental results via independent replication. Available at: http://validation.scienceexchange.com/#/. Accessed February 4, 2016.
[23] Moher D, Avey M, Antes G, Altman DG. The National Institutes of Health and guidance for reporting preclinical research. BMC Med 2014;13:34.

[24] National Institutes of Health. Rigor and Reproducibility. Available at: https://www.nih.gov/research-training/rigor-reproducibility. Accessed Feburary 4, 2016.
[25] National Centre for the Refinement & Reduction of Animals in Research. The Experimental Design Assistant - EDA. Available at https://www.nc3rs.org.uk/experimental-design-assistant-eda. Accessed January 3, 2016.
[26] Weuve J, Proust-Lima C, Power MC, Gross AL, Hofer SM, Thiébaut R, et al. Guidelines for reporting methodological challenges and evaluating potential bias in dementia research. Alzheimers Dement 2015;11:1098–109.
[27] Medical Research Council. Updated RCUK guidance for funding applications involving animal research. Available at: http://www.mrc.ac.uk/news/browse/updated-rcuk-guidance-for-funding-applications-involving-animal-research/. Accessed March 29, 2016.
[28] Journals unite for reproducibility. Nature 2014;515:7. Available at: http://www.nature.com/news/journals-unite-for-reproducibility-1.16259. Accessed February 4, 2016.
[29] Landis SC, Amara SG, Asadullah K, Austin CP, Blumenstein R, Bradley EW, et al. A call for transparent reporting to optimize the predictive value of preclinical research. Nature 2012;490:187–91.
[30] Baker D, Lidster K, Sottomayor A, Amor S, et al. Two years later: journals are not yet enforcing the ARRIVE guidelines on reporting standards for pre-clinical animal studies. PLoS Biol 2014;12:e1001756.
[31] Alzheimer's Drug Discovery Foundation. Apply for Funding. Available at: http://www.alzdiscovery.org/research-and-grants/applyforfunding. Accessed February 4, 2016.
[32] Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving Bioscience Research Reporting: The ARRIVE Guidelines for Reporting Animal Research. PLoS Biol 2010;8:e1000412.
[33] Articles from channel Preclinical Reproducibility and Robustness. Available at: https://f1000research.com/channels/PRR. Accessed February 12, 2016.