When Enough Is Enough: Decision Criteria for Moving a Known Drug into Clinical Testing for a New Indication in the Absence of Preclinical Efficacy Data

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ABSTRACT

Many animal models of disease are suboptimal in their representation of human diseases and lack of predictive power in the success of pivotal human trials. In the context of repurposing drugs with known human safety, it is sometimes appropriate to conduct the "last experiment first," that is, progressing directly to human investigations. However, there are not accepted criteria for when to proceed straight to humans to test a new indication. We propose a specific set of criteria to guide the decision-making around when to initiate human proof of principle without preclinical efficacy studies in animal models. This

approach could accelerate the transition of novel therapeutic approaches to human applications.

Keywords: drug repurposing, translational research, animal models

INTRODUCTION

It is widely accepted that many animal models of disease are suboptimal in their representation of diseases humans experience and they are increasingly criticized for their lack of predictive power in the success of pivotal human trials needed for regulatory approval of new chemical entities.^{1,2} The literature is replete with examples of contradictions and discordance between animal and human effects, including many cases in which promising animal results have failed to translate to clinically significant efficacy in humans. This is particularly true in some therapeutic areas such as neurodegenerative, psychiatric, and central nervous system diseases,^{3–5} as well as sepsis⁶ and inflammatory diseases.⁷

These complexities inherent in translational research present an important opportunity for exploring novel approaches that successfully and efficiently yield outcomes as proximal as possible to eventual human benefit. Supported by several illustrative examples encountered in our drug repurposing program,⁸ we propose herein an approach for assessing when it is appropriate to conduct the "last experiment first," that is, progressing directly to human investigations when animal work would likely fail to provide data appropriate for translation into human applications of interest. This represents a significant—and we suggest, avoidable—barrier to drug introduction. This commentary focuses on rodents as the most commonly used animal models of disease, although general themes and principles would apply to all small animals.

STRENGTHS OF ANIMAL MODELS

Small animals such as mice are widely used as models for toxicology screening and preclinical efficacy testing of

investigational drugs. For example, rodent models have a long history as the gold standard for exploring the pro-convulsant potential of novel therapeutics,⁹ as well as for assessing potential retinal and other ocular adverse effects of investigational agents.¹⁰ It is undeniable that animal models can provide critically important information, particularly with respect to a new drug's mechanism of action and potential toxicity. Undoubtedly, the use of animal modeling in assessing safety has prevented the exposure of humans to drugs with the potential to cause countless serious adverse events. Importantly, animal models can potentially predict both on-target toxicity through the use of orthogonal approaches, such as genetic perturbation of a drug target's expression and/or activity, and off-target toxicity through the use of phenotypic screening in organisms, such as zebrafish and mice.^{11–14}

Animal models also have significant strengths for some diseases and types of pathway and therapeutic exploration. For example, various kinds of rodent experiments have been tremendously valuable for initial assessment of the antianxiety and antidepressant activities of novel antipsychotics¹⁵ and other therapeutics for psychiatric disorders,¹⁶ serving as important gatekeeping steps in preventing less effective agents from proceeding to human investigations. Canine models have led to key insights regarding pathophysiology and control pathways that have been translated to therapeutic gains in both human asthma and chronic obstructive pulmonary disease (COPD)¹⁷; similarly, experimental autoimmune encephalomyelitis is a widely accepted rodent model of multiple sclerosis that has led to valuable leaps forward in our understanding and treatment of this complex disease.¹⁸ Given such examples, it is clear that animal models remain an important part of the investigative pathway for a range of topics in biomedical research.

Model systems such as cells, mice, or zebrafish can also be particularly valuable for interrogating the biological effects of human variants that have not yet been characterized in the literature. This type of scientific inquiry can support drug repurposing efforts when a given variant under study (1) occurs in a druggable target for which safe human-tested drugs already exist and (2) has been found to have a strong association with a human phenotype/disease through a phenome- or genome-wide association study. Even if the organism carrying the variant does not exactly mimic the corresponding human disease, simply seeing a functional consequence of the presence of that variant in a model system can give additional, independent target validation. Experiments in these model systems should be carefully designed so that the resulting

information about variant effects works to advance treatments for human diseases.

ANIMAL MODEL LIMITATIONS

When animals do not naturally or spontaneously develop a given disease or condition, as is the case for most human diseases, these conditions are simulated or induced, resulting in experimental approximations. Techniques such as xenografting can model a human disease state in recipient animals, as in the case of patient-derived xenograft (PDX) mouse models, where malignant tissue resected from a human cancer patient is grafted into an immunocompromised mouse. Some PDX models have shown high accuracy in predicting drug toxicity and clinical efficacy.^{19–27} However, small differences in engraftment protocols and graft stability yield highly variable outcomes.^{28,29} Moreover, the use of immunodeficient animals limits translation of novel therapeutic pathways into human applications. One review of animal models in cancer summarizes several compelling examples of failure of translation from animals to humans, noting that the average rate of successful progression from animal models to clinical trials in this field is less than 8%.³⁰ Engraftment of intestinal microorganisms might enable investigations of microbiota in a variety of human diseases.³¹ Mice can also be engrafted with human immune system cells, for example, to study virulent infectious diseases.³² Yet these, too, require changes to the host environment to accept the graft, which do not mirror the human host environment.

Diseases can be chemically induced, behaviorally stimulated, or genetically driven. Genetic manipulation, such as in generating loss-of-function or gain-of-function mutations or complete gene knockouts in the animal ortholog of the human disease, can be extremely helpful in studies of monogenic disorders. However, even in purely genetic disorders, there are often challenges encountered using this approach; in Down Syndrome, orthologs of human chromosome 21 genes map to segments of three different mouse chromosomes, and no current mouse model successfully incorporates trisomy affecting all three segments.³³ Furthermore, in common complex diseases, such as cardiovascular and metabolic disorders, there is no way to reliably recapitulate the heterogeneity of human disease through gene editing.^{34–36} Autoimmune diseases are generally studied in genetically modified mice or following immunization with a specific antigen plus adjuvant. These techniques fail to recapitulate the full expression of human autoimmune inflammatory diseases; *Figure 1* illustrates several meaningful differences, for example, between a key animal model of granulomatosis with polyangiitis (GPA) and the human experience of this devastating disease. Other

Fig. 1. Depiction of the relative value of murine versus human testing in granulomatosis with polyangiitis, based on model developed by Little *et al.*⁸⁵

animal models of autoimmune diseases are also affected by translation-hampering disconnects with their human counterparts, including multiple sclerosis,³⁷ rheumatic diseases,³⁸ and autoimmune hepatitis.³⁹

Experimental exposure to biologic or environmental toxins and carcinogens can induce disease, such as replicating significant infectious and inflammatory insults such as sepsis,⁴⁰ simulating human occupational exposures to trigger interstitial and malignant respiratory disease,⁴¹ or inducing carcinogenesis through cigarette smoke exposure in murine models.^{42,43} In a recent development, researchers have even generated a new COPD mouse model by exposing mice to high levels of ozone.⁴⁴ All of these methods are increasing in accuracy and precision and are valuable in many contexts. However, it is also known that the methods to generate the disease model will, by their very nature, change the course of the disease, not just in onset, but in terms of features and phenotypes, progression, and lethality, with unknown effects on eventual generalizability, or lack thereof, to the corresponding human disease.^{45–47} It is also notable that these limitations may have negative implications related to effective experimental design in neglected and orphan diseases, which may have particularly complex or even completely unknown mechanisms, making an animal model that precisely recapitulates the disease observed in humans extremely difficult, if not impossible, to design.

In addition, even when an animal model is theoretically reliable and valid, it has been theorized that the acquisition, husbandry, and facilities commonly used reduce reproducibility.⁴⁸ Historically, animal colonies have tended to be genetically homogeneous and disproportionately male. Besides the biasing effect of inbreeding on immunological responses, fundamental differences in both innate and adaptive immune responses^{49–51} have led to numerous failed attempts to translate effects of therapeutics in humans. Furthermore, laboratory animals live in restricted and unvarying environments that tend to not be analogous to their natural environments.⁵² For example, housing mice below their thermoneutral zone might affect the animal in important ways, exhibiting phenotypes under thermal stress that are less consistent with human biology, thereby further limiting the ability to model human diseases.⁵³

Subtle differences in animal husbandry methods and housing environment can have implications for the reproducibility of experimental outcomes,^{54–56} many of which are poorly understood.

Other hurdles can arise in well-established animal models of human disease when administering drugs and attempting to establish preliminary data regarding metabolism, pharmacokinetics, and efficacy. A critical component of successful drug development is predicting human drug metabolism and pharmacokinetics (DMPK) and toxicology properties with absorption, distribution, metabolism, and elimination studies in animals. The pharmaceutical industry uses a complex array of multispecies *in vitro* and *in vivo* correlations to extrapolate expected parameters in humans.⁵⁷ These predictions may be challenging to generate in cases where a mismatch exists between the species correlate that more reliably predicts human DMPK and the species correlate that more reliably models the human disease. In addition to the failure of animal pharmacokinetics and metabolism to reliably predict these parameters in humans, differences in targets, pathways, metabolites, or other dynamics can all work to increase uncertainty regarding the strength of any extrapolations from animal model data to humans.^{58,59}

Finally, in even a perfect animal model of disease, the measures used to assess responses are often limited, and in some cases the animal model could not reasonably support the establishment of efficacy; complex diseases such as stroke,

traumatic brain injury, and a number of inflammatory conditions have been particularly challenging to successfully model in a laboratory animal in a manner that translates meaningfully to humans.⁵² This issue also notably affects research assessing treatments for diseases which, in humans, rely largely or even wholly on patient-reported symptom relief. Thus, for example, even when researchers can reliably mimic the pathophysiology of migraine in an animal model, there is no direct way of assessing whether the migraine is diminished by the treatment.⁶⁰ This issue is particularly prevalent in mood/psychiatric/neurological disorders contingent on patient-reported experiential outcomes.

APPLICATION OF ANIMAL TESTING TO DRUG REPURPOSING

None of these strengths and limitations is new. Animal models have historically been essential for the drug development process and will continue to be for the near future, simply because there are no commonly and widely accepted alternatives. However, in the field of drug repurposing, this might not be the case, and the decision criteria for including animals in an overall experimental plan should be revisited. Drug repurposing has resulted in some notable successes; however, few of these cases to date have moved straight to human testing without preclinical efficacy established in animals. The development story of sildenafil, repurposed for erectile dysfunction and pulmonary hypertension after significant clinical investigations as an antihypertensive therapy, is often offered as a classic case of successful repurposing,^{61,62} but it fits this paradigm almost by accident. The observation that penile erections were a common side effect of sildenafil, coupled with a growing understanding of the pathophysiology of erectile dysfunction, supported the decision to pursue its development for this new indication.⁶² A more recent example of an intentional repurposing that skipped animal testing is the growing off-label use of ketamine for treatment of treatment-refractory depression and suicidal ideation.^{63–65} Based on the decision criteria we outline below, this is a good example of when animals do not belong in an experimental plan due to several factors, including significant limitations in animal modeling of treatment-resistant depression and suicidality,^{66,67} substantial unmet medical need, and established dosing in a similar population.

Drug discovery methods that utilize big data, machine learning, and artificial intelligence are increasingly being deployed early in the development process to bolster confidence in therapeutic concepts. Vanderbilt's drug repurposing program⁸ uses a new approach that is both *in vivo* and *in silico*, using phenome-wide association studies (PheWAS^{68–71}) to take advantage of genetic variation of humans to recapitulate

the effect of a targeted drug. The big data resources leveraged by this approach are BioVU, a large repository of deidentified DNA samples assembled by Vanderbilt University Medical Center, which are linked to corresponding demographic and clinical data from the Synthetic Derivative, our deidentified database of electronic health records.^{72–75}

In applying PheWAS methods to the drug repurposing context, we identify variants in drug target genes that recapitulate drug effects; for example, a loss of function variant in the target gene would mimic the effect of a pharmacologic antagonist of that protein, while a gain of function variant in a gene would mimic the effect of a pharmacologic agonist of that protein. We then execute PheWAS to find all phenotypes across the human phenome (based on PheWAS codes derived from ICD-9/10 codes and manually grouped to define clinical phenotypes) that are associated with carrying at least one copy of a minor allele of interest within the target gene, leveraging the odds ratio to understand directionality of the variant's effects on risk of phenotypes of interest. Of note, in addition to using known variant effects combined with phenotype odds ratios to identify new therapeutic opportunities, we have also recently used these methods to predict potential *adverse effects* of a novel therapy.⁷⁶ Disease candidates for novel drug indications are then more precisely defined by thorough chart reviews, examining the clinical phenotypes and disease etiologies in carriers. Next, we formulate follow-on investigations to further explore the nature and strength of each identified repurposing prospect.

Every repurposed drug in our program, however, faces a preclinical development pathway with major limitations in the available animal models. Issues faced by our specific programs are briefly mentioned in this study to illustrate real-world examples of potential opportunities for rejecting inadequate animal options in favor of progressing directly to clinically meaningful human investigations.

In systemic lupus erythematosus (SLE), existing mouse models include both spontaneous and externally induced triggers, yet none of these is able to reflect the complex clinical spectrum of disease we observe in our human SLE patients and none mimic the specific phenotype we are pursuing, which comprises complex cognitive and neuropsychiatric characteristics not well captured by any existing model, but deeply problematic among affected patients with lupus.⁷⁷

Nearly all humans with neurofibromatosis type 1 develop pigmentary lesions and neurofibromas, but mouse models incompletely replicate this pathophysiology and develop only some of these features,⁷⁸ significantly limiting our ability to explore key neurofibromatosis treatment repurposing hypotheses in mice.

Animal testing of another repurposing candidate for trigeminal neuralgia (TN) relies on rodent models in which TN is induced

by administration of cobra venom to the infraorbital nerve, chronic constriction injury, or orofacial formalin injection, none of which recapitulate conditions recorded in humans.^{79–81}

Rodent models of chronic fatigue syndrome (CFS) include restraint stress and forced exercise (swimming in cold water⁸²), which poorly mirror the pathophysiology of our patients with severe CFS.

Another indication being sought, granulomatosis with polyangiitis, is a rare autoimmune disorder that involves inflammation of small- and medium-sized blood vessels, resulting in damage to the respiratory tract, kidneys, and various other organ systems of the body. GPA has a large number of human presentations, but ear–nose–throat disease and kidney abnormalities (sometimes progressing to kidney failure) are some of the most common. Given the diversity of symptom profiles, treatments vary by individual and target the specific symptoms they experience.⁸³ The underlying cause of GPA is unknown, although it is associated with the gene for its autoantigen, proteinase 3 (PR3), which confirms the importance of PR3 in the pathogenesis of the disease.⁸⁴ Since PR3 does not have a direct murine homolog, a team created a mouse model with a humanized immune system and was able to induce an acute vasculitis phenotype similar to GPA (*Fig. 1*).⁸⁵ The mice developed several symptoms that overlap with human GPA symptoms, rendering it a very useful tool in elucidating the mechanisms of microvascular injury. However, given that some key phenotypes are missing from or are different in the mouse model (*e.g.*, ear–nose–throat, cardiac, lung, and kidney manifestations),⁸⁵ extrapolation from this model to human GPA has some limitations. This is particularly true if one were to propose using this model to test the efficacy of certain therapeutic approaches. While this model can offer some clinical benefit by answering some questions about GPA pathogenesis, it offers less value than a “straight to humans” approach in the context of repurposing a safe generic drug to address this unmet medical need.

Finally, we identified a therapy that could be repurposed for the treatment of suicidality among polysubstance abusers. Again, in this instance, there is simply no animal model of suicidality. Thus, a key question arises across this portfolio of repurposing projects actively being pursued: *what is the value of animal testing in this context relative to the harm to the animals, costs of the research activity, ability to interpret experimental outcomes, and the potential for translation to future clinical work?*

CONSIDERATIONS TO EVALUATE HUMAN CLINICAL TRIALS WITHOUT PRECLINICAL DATA

We propose that in some therapeutic-indication pairings the limitations of the most analogous animal model often

render the model uninformative or misleading in predicting the efficacy of a repurposed therapeutic in humans. An established set of criteria to acceptably omit preclinical efficacy studies would be beneficial in decision-making. In this study, we propose a set of those criteria and argue that if a drug meets the criteria below, the ethical balance—which justifiably values human protection over animal sacrifice—is shifted. In the setting of drug repurposing, the acceptable deliberate avoidance of animal efficacy testing can be one mechanism for facilitating more rapid and efficient timelines from idea and hypothesis generation to regulatory approval in cases when it is desirable to leverage existing safety data to support the repurposed indication, such as in the 505(b)(2) New Drug Application (NDA) in the United States. In addition, this approach avoids unnecessary use of animals in research. It can also save costs, which is important in the repurposing of generic medications.

PROPOSED CRITERIA FOR INITIATING HUMAN PROOF-OF-PRINCIPLE WITHOUT PRECLINICAL EFFICACY STUDIES IN ANIMAL MODELS

1. The drug has been shown to be appropriately safe and well tolerated in multiple human safety studies. Thus, the drug should have a firm body of valid and comprehensive safety data, as is true for therapeutics already approved by the Food & Drug Administration (FDA) for clinical use. The purpose of preclinical work—animal pharmacology/toxicology testing—is to develop adequate data to undergird a decision that it is reasonably safe to proceed with human trials of the drug.⁸⁶ Drugs that are unapproved for a particular indication but still proven safe and well tolerated in human trials also fit into this category.
2. The animal model does not adequately recapitulate the human disease for which an indication is sought. In this case, the animal model could not reasonably provide the needed relevant information, so it effectively puts the animal in harm’s way with no corresponding potential for benefit to human health.
3. The patient candidates for the proposed novel therapeutic investigation have substantial unmet medical need with current standard of care therapies, and therefore, the research protocol either would not require discontinuation of any existing effective therapy or could be administered preceding, following, or as an adjuvant to existing therapy in hopes of improved benefit. In the case where no effective therapy exists, participants in the human study would not be at potentially increased risk due to changing an effective regimen in favor of an unproven therapeutic approach.

Of note, if the proposed novel therapeutic is being considered as an adjuvant to existing therapy, and an existing body of data is not available to assess potential drug–drug interactions, then this may necessitate additional preclinical work.

4. Data from *in vitro* or *in silico* experiments are supportive. These include tissues-on-a-chip and other human target validation resources. It has been theorized that with rigorous target validation in human tissues, it may be justifiable to skip the animal model assessment of efficacy altogether.⁸⁷ In our repurposing method, we use human genetic variation as an *in silico* proxy for the effects of pharmacologic therapies, which also provides a way of predicting both positive and negative effects without administering a drug. Thus, our approach leverages a strong connection between an observed phenotype and putative changes in the target protein of interest to increase confidence in each novel therapeutic concept.
5. Dosing and administration are consistent with prior human experience for the therapeutic agent of interest. In the broader context of repurposing, it is sometimes preferable to alter the dose, formulation, and/or route of administration for a new indication; in such situations, a straight to humans approach may not be reasonable. However, when seeking approval through a mechanism such as the FDA 505(b)(2) pathway, this criterion applies when the proposed protocol does not involve a modification to dose, route of administration, or formulation, as changes in any of those dimensions could compromise the safety profile of the FDA approved product. For example, a change in the route of administration would require a reformulation based on results of preclinical animal work in which it is determined how much of a drug is absorbed into the blood, how it is broken down chemically in the body, the toxicity of the drug and its metabolites, and how quickly the drug and its metabolites are excreted.⁸⁸ An increase or decrease in dose or dosing frequency compared to the approved dosing regimen of the product can also significantly increase risk, particularly related to potential alterations in the adverse effect profile with an increase, or reduced efficacy with a decrease, also indicating a decision point at which further preclinical work would be required.

PRESERVING SAFETY IN CLINICAL TRIALS WITHOUT ESTABLISHMENT OF PRECLINICAL EFFICACY

Implementing research models based on these decision criteria requires that additional caution be exercised in several areas.

Although this approach leverages agents with a strong, existing safety profile, applying a therapeutic in a novel clinical population will require thorough consideration of the implications of the drug's safety profile as it relates to the participants to be included in the investigation; inclusion and exclusion criteria, as well as safety monitoring plans, will need to be as precise and thorough as possible to prevent any untoward sequelae.

CONCLUSIONS

Historically, preclinical investigations have been pursued to increase confidence in hypothesized safety and/or efficacy in advance of human study. However, we argue that such data hold little prospect of import when the associated animal models are irrelevant to the target human phenotype of interest and, thus, can be forgone in some situations. The proposed criteria for bypassing animal testing and initiating a clinical trial might seem straightforward and even obvious; however, it remains true that research sponsors often expect to see the results of preclinical animal experiments in grant submissions and protocols. In the past, incorrect extrapolations⁸⁹ (including both false positives and false negatives) have thwarted progress by leading researchers down development paths based on faulty logic, contributing to further delays in the development of effective therapeutics; this might be especially problematic when pathogenesis is only partly understood because the limitations of the animal model are not clear. We propose that those research protocols that meet these criteria can proceed to human studies significantly more proximal to real clinical application. This change could have the aggregate effect of accelerating the transition of novel therapeutic approaches to human applications without untoward effects on risk benefit, reducing the overall burden/cost/waste of insufficient animal models and reducing costs over the longer term by improving the precision of research efforts.

ACKNOWLEDGMENTS

The authors are grateful for the participation of all individuals contributing to this research. The project described herein is supported by the CTSA award No. UL1TR000445 from the National Center for Advancing Translational Sciences. Its contents are solely the responsibility of the authors and do not necessarily represent official views of the National Center for Advancing Translational Sciences or the National Institutes of Health.

DISCLOSURE STATEMENT

No competing financial interests exist.

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Abbreviations Used

CFS = chronic fatigue syndrome
 COPD = chronic obstructive pulmonary disease
 DMPK = drug metabolism and pharmacokinetics
 FDA = Food & Drug Administration
 GPA = granulomatosis with polyangiitis
 NDA = new drug application
 PDX = patient-derived xenograft
 PheWAS = phenome-wide association study
 PR3 = proteinase 3
 SLE = systemic lupus erythematosus
 TN = trigeminal neuralgia