Future NEUROLOGY

REVIEW

For reprint orders, please contact: reprints@futuremedicine.com

Optimizing mouse models of neurodegenerative disorders: are therapeutics in sight?

Cathleen M Lutz*[1] & Melissa A Osborne[1]

ABSTRACT: The genomic and biologic conservation between mice and humans, along with our increasing ability to manipulate the mouse genome, places the mouse as a premier model for deciphering disease mechanisms and testing potential new therapies. Despite these advantages, mouse models of neurodegenerative disease are sometimes difficult to generate and can present challenges that must be carefully addressed when used for preclinical studies. For those models that do exist, the standardization and optimization of the models is a critical step in ensuring success in both basic research and preclinical use. This review looks back on the history of model development for neurodegenerative diseases and highlights the key strategies that have been learned in order to improve the design, development and use of mouse models in the study of neurodegenerative disease.

KEYWORDS
• ALS • Friedreich's ataxia
• genetic background
• mouse models
• neurodegeneration
• preclinical trials • SMA

The mouse has long been recognized to be a powerful tool in elucidating the genetics and pathophysiology of human disease [1]. However, neurodegenerative mouse models are particularly scarce and can be challenging to use for preclinical studies. Many neurodegenerative diseases seen in humans simply do not occur naturally in mice, implying that that underlying disease mechanisms and biology may drastically differ between the two species. In addition, many neurodegenerative diseases seen in humans involve an aging component. Diseases such as Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis (ALS) can take decades to manifest in humans. The lifespan of a mouse may not be long enough for such deficits to be revealed. However, the ease by which we can manipulate the mouse genome has resulted in a number of transgenic and knockout mouse models over the last decade that have allowed us to push the onset of neurodegenerative disease manifestation in the mouse through overexpression and tissue-specific expression. Even with these systems, it remains unclear how well these models recapitulate the deficits seen in the CNS of patients, or how directly the data obtained from these models will translate into clinical trials. Failed clinical trials, especially in the field of ALS, have caused many researchers to question whether the mouse is a good model for translating therapeutic efficacy (reviewed in [2], and see [3,4]). The answer to this question may vary depending on the disease or the model used, but invariably, we can improve the way in which we approach the design of clinical trials, as well as the rigor with which preclinical studies are performed. Recent attention has focused on calling for greater transparency and rigor in preclinical efficacy testing studies, citing a number of reasons for reporting biases [5]. Adherence to better preclinical trial design will no doubt improve the reproducibility of published findings. In the spirit of improving preclinical studies, this review takes one step further in terms of the mouse models themselves. While no mouse model is likely to recapitulate all aspects of a disease, we can greatly improve their usefulness by applying higher standards and scrutiny to model design, standardization, use and the interpretation of research results. We will use our experience

[1]The Jackson Laboratory, Genetic Resource Sciences, 600 Main Street, Bar Harbor, ME 04609, USA
*Author for correspondence: Tel.: +1 207 288 6341; Fax: +1 207 288 6195; cat.lutz@jax.org

Future Medicine part of fsg

in ALS, Friedreich's ataxia (FRDA) and spinal muscular atrophy (SMA) in order to examine what we have learned that is changing the way we move forward in mouse model development.

Human genetics & the mouse models

When considering the prospect of modeling a human neurodegenerative disease in mice, it is important to evaluate not only the genetics of the disease, but also the clinical heterogeneity and the disease penetrance within a patient population. Diseases that are inherited in a Mendelian manner, where the gene(s) underlying the disease is known and present clinically with high disease penetrance, are good candidates to model in mice. For example, proximal 5q SMA is a disease in which the genetic etiology and clinical manifestations of the disease are fairly well defined. SMA is a neuromuscular disease characterized by degeneration of motor neurons, resulting in progressive muscle wasting and weakness. SMA is caused by mutations in the *SMN1* gene [6,7], and a wide range of clinical severity can be observed due to the differential expression of a compensatory gene of *SMN1*, called *SMN2*. As such, disease severity inversely correlates with patient copy number of *SMN2* and thus SMA is a disease of insufficient levels of SMN, rather than a complete lack of the functional protein [7]. With SMN2 as the target, SMA becomes a very 'drugable' disease, as SMA therapies need only upregulate an existing gene. To model the human condition in mice, a genetic engineering strategy can be employed by simply knocking out the murine *Smn1* gene and then genetically adding back, in varying amounts, human *SMN2* via SMN2-expressing transgenes. This approach was successfully utilized by three different laboratories in order to generate many of the severe and mild SMA models with clear neuromuscular junction abnormalities [8–10]. These mice have been heavily utilized for both the purposes of understanding the disease mechanism and therapeutic efficacy testing. The construct validity in these mouse models closely mirrors the human genetic condition, and the use of transgenic expression of human *SMN2* allows for similarity in the drug target between the mouse model and the human patient.

Nevertheless, other diseases of seemingly straightforward Mendelian inheritance have not been as easy to model in mice. FRDA is an autosomal-recessive ataxia caused by a mutation in the *FXN* gene [11]. This mutation is characterized by an expanded trinucleotide (GAA) repeat within the first intron of the gene. This expansion leads to reduced expression of frataxin, a ubiquitously expressed protein that acts in iron–sulfur cluster and heme biosynthesis. The greater the repeat in patients, the less frataxin is produced and the more severe the disease. Patients with FRDA exhibit symptoms of incoordination, muscle weakness and sensory loss in addition to non-neuronal pathologies, including cardiomyopathy, which is the leading cause of death in FRDA patients. FRDA is similar to SMA in that it is a disease of low protein, making this disease an excellent candidate for developing therapeutics. The approach to model FRDA in the laboratory mouse entails knocking out endogenous *Fxn* expression and replacing it with mutant *FXN* containing large GAA repeats either through transgenesis or a targeted approach [12,13]. Repeat expansions can be especially difficult to clone into targeting constructs, as the repeat often contracts when introduced into bacteria in the cloning phase. Mice do not have a naturally occurring repeat in their *Fxn* gene. Normal individuals in the human population harbor between five and 30 GAA repeats, while those affected by FRDA most commonly carry 600–900 repeats [14]. While many groups have successfully generated what would be considered the genetic equivalent of FRDA in mice, the phenotypes of the mice are exceptionally mild, manifesting as a subtle late-onset rotarod or gait phenotype with no cardiac deficits [13,15,16]. It has been speculated that larger GAA repeats might be needed to lower frataxin levels enough in order to elicit a more robust phenotype; however, recent reports indicate a mouse model containing less than 10% frataxin levels still demonstrated no robust neurological phenotype [Sarsero J, Pers. Comm.]. With these particular mouse models of FRDA, we have learned that although good construct validity is achieved, the phenotypic validity has some shortcomings. These models can be very useful for testing the therapeutic ability to upregulate frataxin expression, but translating therapeutic efficacy into amelioration of disease symptoms using these models is challenging due to their mild phenotype.

ALS is set apart from SMA and FRDA on a number of fronts. First, the majority of cases of ALS are considered sporadic, in that there is no known family history of the disease. Only 10% of all cases of ALS are considered inherited or familial in nature. Mutations in the *SOD1* gene

were known to be the most prevalent causes of familial ALS, with an incidence of only 10–15% in the familial ALS population [17], and for many years, transgenic models carrying *SOD1* patient mutations were the primary source for mouse models. The B6SJL-TG(SOD1G93A) mouse model developed by Gurney *et al.* in 1994 is by far the most widely used model of ALS [18]. In this model, high expression of the SOD1G93A mutant protein accelerated the onset of the disease and provided a rapid onset of disease and mortality caused by motor neuron loss that was exceptionally attractive for therapeutic trials. Recent advances in genomics have enabled the identification of many more disease-causing genes and new mouse models, such as the TDP43 transgenics [19], and the yet-to-be published C9ORF72 models. These new models for ALS offer the potential for additional resources to study neurodegeneration and to test potential new therapies for ALS and frontal temporal lobe dementia. When we examine the available mouse models for ALS, we can certainly realize that the SOD1 mouse models provide invaluable tools for studying disease mechanisms and testing therapies for ALS. However, among their limitations is that their construct validity only represents a very small proportion of the patient population. This caveat certainly brings into question the predictive validity of the model, a question that has arisen in the wake of numerous failed clinical drug trials for ALS. However, the recent discovery of several other genes implicated in ALS, and the subsequent generation of mouse models harboring these genes, may lead us to a collective dataset that can better steer therapeutic development to reach a broader patient population.

In each of these disease areas (SMA, FRDA and ALS), we have encountered challenges in our ability to completely achieve construct validity, phenotypic validity and predictive ability in our mouse models. Despite this, mouse models in these diseases have furthered our understanding of the disease pathology that leads to therapeutic discovery. We can recognize that the mouse only represents part of the disease process and is a research tool that enables us to uncover clues about diseases through research that simply cannot be performed in humans. Our experience with these mouse models has also guided us to improve not only the way we interpret our findings, but also how we can generate better models of disease.

Importance of genetic standardization

With gene identification and basic modeling in hand, how do we most efficiently and effectively use mouse models? A lack of reproducibility involving phenotypic and efficacy data in studies of neurological mouse models has been the subject of numerous publications [5,20]. Multiple reasons have been cited for the lack of reproducibility in experiments, including small sample sizes and the failure to properly blind researchers from advanced knowledge of drug and placebo arms, as well as other sources of unintentional experimental bias [5]. However, one of the biggest and most overlooked reasons why experimental results cannot be replicated in mouse studies is that phenotypes on segregating backgrounds drift and change over time. This is particularly problematic when strains of mixed segregating genetic backgrounds are dispersed throughout the scientific community, creating founder-like effects in different breeding colonies across different laboratories. All too often, phenotypes that existed in the initial characterization are less prominent over time, and new phenotypes emerge. In this case, data produced in laboratory X can no longer be reproduced by laboratory Y.

In 2005, multiple mouse models of SMA were imported into The Jackson Laboratory (ME, USA) for distribution to the scientific community. The severe SMA mouse FVB.Cg-Tg(SMN2)89Ahmb*Smn1*^{tm1Msd}/J was described as having a median life span of 5 days. However, survival studies at The Jackson Laboratory indicated a drift to a more severe SMA phenotype, resulting in earlier death and a median lifespan of ≤1 day. While environment and nutritional state have been shown to play a role in the survival of severe SMA pups [21,22], genome scans revealed a significant shift in the genetic background from the original strain. A similar phenomenon was observed with the SMA mouse model known as 'Δ7', but with more pronounced effects. The severe Δ7 mouse was found to be an incipient congenic by high-density genome scanning, with 15% of the genome typing as non-FVB. After its arrival, this stock was subsequently backcrossed to generate complete congenics on both the FVB/NJ and C57BL/6J genetic background. Phenotypic survival analysis of these crosses confirmed that genetic background plays a key role in the survival of mutant animals. The Δ7 mouse on a C57BL/6J background was largely embryonic lethal, with only 8% of the expected

25% of Δ7 animals surviving to birth compared with the FVB-incipient congenic that survives to 15 days. These findings illustrate the importance of considering genetic background when attempting to create a mouse model for a particular disease. The effects of genetic background on phenotype in mice have been well documented over many disease areas, and the SMA strains were no exception. In the early days of genetic engineering, most embryonic stem cells used for gene targeting were derived from F1 hybrids, and many researchers took advantage of coat color genetics in the assessment of germline transmission animals by mating their chimeras to a different strain. Pronuclear injections frequently employed F1 hybrids or CD-1-outbred animals in order to take advantage of their large pronuclei and big litter sizes. Understandably, many genetically engineered models were created on mixed genetic backgrounds. While some models were backcrossed to congenicity by either traditional backcrossing of ten generations or marker-assisted backcrossing, the process was perceived by many researchers as both time consuming and expensive. However, a major advantage of using inbred mouse strains is that every mouse from that strain is genetically identical and can be housed in environmentally similar conditions, providing tremendous consistency in phenotype.

Today, we have access to more resources, such as embryonic stem cells from inbred backgrounds, C57BL/6J albino mice for assessing the coat color of chimeras and even newer nuclease technologies, such as transcription activator-like effector nucleases, zinc fingers and clustered regularly interspaced short palindromic repeats, which allow us to engineer mutations directly into inbred backgrounds [23,24]. In constructing future models of neurodegenerative disease, or any disease, the use of inbred backgrounds and a stable phenotype should always outweigh the convenience of using traditional transgenic core resources. Starting from an inbred strain also more readily enables the assessment of modifier genes through deliberate crosses with other inbred lines.

While differences in strain genetic backgrounds have contributed to variation in experimental results across different disease areas, these observations have also given us insight into the ways that we can modify disease phenotypes in order to improve the face validity of our mouse models. As discussed in the next section, we can now incorporate the use of new technologies to generate mutant alleles with our current working knowledge of genetic background effects in order to improve our mouse models of disease.

Leveraging technologies to explore genetic modifiers

Many neurological diseases present with tremendous clinical heterogeneity. For example, some ALS patients can progress very rapidly in their disease, while others experience only weakness for more extended periods of time [4,25]. In addition to variation in the age of onset, ALS patients may present with either bulbar or limb onset, and may even exhibit tremendous variation in the sequence of limbs affected and how the disease progresses. Given its high sporadic incidence, ALS is considered to be a complex disease that is influenced by many environmental triggers and likely many genetic susceptibility and resistance loci.

The SOD1G93A mouse model has been the premier model for preclinical testing to predict efficacy in ALS clinical trials. However, much of the preclinical work carried out in the SOD1G93A model has failed to translate to the clinic. This failure to translate is often attributed to poor preclinical trial design, as revealed in sobering and impactful publications by Benatar and Scott *et al.* [4,26]. However, even with proper preclinical trial designs, are we expecting too much from a single model? *SOD1* mutations represent such a small proportion of the ALS patient population and are also likely under-represented in clinical trials, if they are represented at all. Is it, therefore, unrealistic to expect a single gene mutation on a single genetic mouse background to represent a diverse patient population of such a complex disease? Indeed, the field of ALS research has been hindered by a lack of animal models; validation across numerous models would increase the confidence that preclinical work will translate to human trials.

Today, advances in next-generation sequencing technologies and massive reductions in sequencing costs have generated a wealth of data that are revealing the genetic basis for many human neurodegenerative diseases. This is an especially exciting time for gene discoveries in ALS. With new gene discoveries comes a new opportunity to engineer new mouse models. However, getting the most out of these mouse models will also depend on how we leverage both sequencing and new engineering technologies in order to explore modifier genes in mice. It is known that genetic modifiers exist and can significantly influence disease onset in the

SOD1G93A model. Male mice in this model on a B6SJL genetic background survive to 129 ± 9 days (mean ± standard deviation) [27]. On a C57BL/6J genetic background, male survival is significantly extended to 157 ± 9 days (mean ± standard deviation). Other genetic backgrounds have also been shown to influence survival in these mice, and gene identification is underway [28]. Recent studies also demonstrated that survival of the transgenic mouse (Prnp-TARDBP*A315T)95Balo/J was also influenced by genetic background [29]. In a dominant modifier screen, male survival was significantly different across a number of F1 genetic crosses and, in addition, males were always significantly more affected than females, regardless of genetic background, giving this mouse model both gender-specific and genetic background-specific influences [29]. Advances in high-throughput exome sequencing will significantly enhance our ability to identify these genetic modifiers, even in complex genetic interactions.

New technologies in genome editing with site-specific nucleases will also play a powerful role in our ability to create and validate new disease genes and allelic variants associated with human ALS. Although homologous recombination proved to be a powerful tool in genetic engineering, the creation of the models through construct design and traditional targeting is often hindered by the low efficiency of the technology. Recent advances in site-specific nuclease technology allows for a more directed approach to introducing mutations into the mouse genome, with the significant advantage of introducing multiple mutations that are not restricted to the genetic background of the embryonic stem cell. This is potentially very interesting from the standpoint of genetic modifiers, as a single transcription activator-like effector nuclease, zinc finger or clustered regularly interspaced short palindromic repeat could be used to introduce mutations in multiple genetic backgrounds.

Optimization & fine tuning

While it may be starting to sound clichéd, the answer to the question of whether the mouse is a good model for human disease does indeed depend on the question being asked. Even more important than the question being asked is the selection of the appropriate model to answer the question. The mouse is not a human and never will be; fine tuning mouse models may not be so much about finding the perfect mouse model, but rather finding one that is sufficiently able to answer the questions at hand. This concept is well illustrated in the development of different mouse models for SMA.

The first generation of transgenic-based SMA mouse models provided (and still provide) excellent research tools for SMA investigators; however, the models did not demonstrate the phenotypic spectrum of disease seen in patients. The mice were either embryonic lethal or very mild in their presentation. A mouse model of intermediate survival that was more representative of a type II/type III patient was difficult to achieve. The importance of achieving this intermediate model became more evident as a number of groups begin to define a critical therapeutic window in the severe Δ7 mouse model. In 2010–2011, multiple groups demonstrated that postsymptomatic rescue of Δ7 mice was achievable, but that intervention in this model, either by AAV9-mediated gene therapy or through the use of cre-mediated conditional inversion alleles, needed to happen by postnatal day (PND) 4 in order to achieve significant survival [30–32]. Restoration of SMN levels at PND 6 in the conditional inversion allele provided some efficacy, while induction of SMN at PND 8–10 had little to no effect on survival in the Δ7 mouse. However, the rapid kinetics of disease progression in severe mouse models of SMA present several obstacles to the preclinical testing of therapeutics. Among these is the narrow window for which intervention can be demonstrated, given the short overall survival time of 17 days. The severe model also does not fully represent the spectrum of disease observed in the patient population; thus, a lack of efficacy of a potential therapeutic in the severe mouse model of SMA may not necessarily preclude its benefit in a milder form of the disease.

A number of very promising therapies are being pursued in SMA [33]. For each of these therapies, efficacy studies were performed using the severe Δ7 mouse model of SMA at early neonatal time points. As SMA therapies start to move out of the laboratory and into the clinic, critical questions in the timing of therapeutic delivery remain. Will some therapies only be efficacious in either mild or severe patients, but not both? Will efficacy be limited to presymptomatic stages, or will it extend to a later postsymptomatic time point? If efficacy does extend to later time points, how late in the disease course can we expect to see a benefit from treatment? Insights into these questions are critical in the design, patient selection and assessment of a clinical trial, especially in the absence of a clear biomarker and changing natural history data from patients.

An intermediate-to-mild model of SMA is an essential tool that is needed to thoroughly interrogate the potential treatment options for the SMA patient population. With this in mind, an allelic series was designed to essentially titrate the level of SMN in the mouse by using a recombineering approach in order to target varying copy numbers of *SMN2* directly into the murine *Smn1* locus [34]. Once again, the survival analysis on mutants generated from the allelic series revealed a sharp delineation between embryonic lethality and long-lived animals, with no intermediate lifespan observed in the series. While this was initially disappointing from the aspect of achieving an intermediate survival phenotype, it was an exciting prospect for patients, as it demonstrated that, at least in mice, only a small increase in SMN levels was needed to completely correct the disease. The allelic series in SMA validated what many past transgenic experiments were suggesting – in the mouse, a very tight threshold of SMN exists. This phenomenon in SMA, frequently referred to as the 'Goldilocks effect' has challenged many SMA researchers to titrate SMN levels through the combination of existing alleles in order to produce a model with SMN levels that are 'just right'. To this end, the allelic series was later combined with existing alleles in order to ultimately achieve a mouse model with an average lifespan of 80 days [Lutz CM, Osborne MA, Unpublished Data]. Several groups are pursuing intermediate models that will also provide additional data on the therapeutic window of drug delivery [Didonato C, Pers. Comm.] [35].

This experience in SMA raises interesting questions for FRDA. Does such a threshold also exist in FRDA models, where no or too low frataxin results in embryonic lethality, but levels of 10% or more result in mice that are phenotypcially normal? Can mice simply tolerate low levels of frataxin? Alternatively, perhaps FRDA is not just a disease of low frataxin protein, but insread is one in which the GAA repeat itself plays a greater role in the disease course, beyond just inhibiting transcription. Would mouse models of higher repeat length or uninterupted repeats produce a more robust phenotype? Additional FRDA models are desperately needed in order to help address these questions.

What matters in a phenotype?

When modeling a human disease in mice, the question of recapitulating clinically relevant phenotypes is understandably used as a means of assessing the model and its face and predictive validity. While the SOD1G93A mouse model may take some criticism for the high levels of overexpression needed to recapitulate the disease phenotype, few would argue whether the robustness of motor neuron death and obvious denervation is responsible for the mortality in the mice. Similarly, while some may use FRDA models for pharmacokinetic/pharmacodynic studies and to test whether the upregulation of frataxin induced by a drug, they may be less likely to assess gait or rotarod as the first-pass assessment of efficacy, given the subtlety and late onset of these phenotypes. Transgenic mouse models of ALS, representing mutations in the *TARDP43* gene, have demonstrated mild motor neuron loss, but recent publications have suggested that the mortality of some of these transgenic models is the result of a gut ileus [36,37]. While on the surface this does not seem like a clinically relevant phenotype for ALS, data from our laboratory indicate that the ileus is the result of rapid neurodegeneration in the myenteric plexus [29]. Referencing SMA again, characterization of the most severe mouse model of SMA that survives only 5–8 days would indicate that the model recapitulates many of the main features observed in the most severe SMA type I patients. Within 48 h of birth, pups reportedly exhibit decreased suckling and movement, labored breathing and tremoring limbs. Histological analysis indicates that affected mice that survive to day 5 exhibit a 35% loss of motor neurons from the spinal cord and a 40% loss from the facial nucleus. The Δ7 mouse is the model that has been most widely used in SMA research. While this model is still considered severe, in that the mean lifespan of the mice is 17 days, the mice have more subtle deficits at the level of the motor neuron and neuromuscular junction. The neuromuscular junctions are generally described as immature, with disorganization of acetylcholine receptor subunits and more plaque-like structures, as opposed to the well-innervated muscle that presents with more pretzel-like morphology at the motor endplate [38]. The Δ7 model appears to recapitulate key aspects of severe SMA, including severe weakness and early death [9,39]; however, the mice show surprisingly limited muscle denervation and motor neuron loss. Years after the original publication describing the Δ7 mouse by Burghes and colleagues [9], multiple laboratories have reported significant structural and functional cardiac deficits in the hearts of SMA mice

that likely contribute to their premature death [40–42]. Milder models of SMA generated by multiple groups also reported cardiac deficits, as well as vascular defects in the form of mild-to-severe necrosis. While these phenotypes were originally viewed as comorbidity phenotypes that were specific to mice, a recent paper by Shababi *et al.* summarizes a number of case studies that provide evidence for the presence of these autonomic defects in severe SMA type I patients [43]. Interestingly, there have also been isolated reports of peripheral necrosis in type I human patients [44]. Indeed, several groups have found that therapeutics that upregulate SMN expression in SMA model mice will ameliorate this necrosis phenotype, and also correct the cardiac defects [45]. Therefore, while alleviation of necrosis may not be a clinically meaningful outcome measure, it is certainly a direct reflection of the SMN load in the mouse. This raises important questions regarding which outcome measures give us the most confidence when attempting to translate preclinical data to patient trials. Given that deficits in the autonomic nervous system likely contribute to the mortality in Δ7 mice, and that deficits in the autonomic nervous system are, to date, only recognized in a small number of case studies involving type I patients, one might question whether survival is the most clinically relevant outcome to use in preclinical mouse studies. To this end, many laboratories have started to incorporate compound muscle action potential and motor unit number estimation as outcome measures in their preclinical trials. These electrophysiological measurements are very indicative of the changes at the neuromuscular junction and are also used to assess disease progression in many patients [46].

Conclusion

Mouse models have proven essential in both basic research and preclinical discovery for bringing promising therapeutics to the clinic. While some mouse models of neurodegenerative diseases may be more or less robust in their research applications, it is important that we approach not only the preclinical data, but also the mouse models themselves, with rigor. The optimization and standardization of mouse models is an important step in ensuring their proper use. In many cases, tenacity in model design and development is a key requirement for success. New technologies in genetic engineering will be instrumental in efficiently and effectively creating new genetic mutations in mice that are discovered in the human population for both rare and oligogenic disorders.

Mice and humans may have greater than 95% of their genes conserved, but there exists 65 million years of evolutionary diversity between the species. As such, we must manage our own expectations when attempting to use preclinical data in order to predict human clinical trial results. It is important to remember that the mouse is being utilized as an assay, and should not be expected to be reflective of an entire disease population. For an accurate interpretation of results, it is essential to understand the differences that exist in the spectrum of disease as it is modeled by a mouse – not only on a phenotypic level, but also on the molecular defect level – and to recognize that not all phenotypes displayed by mouse models may be relevant in the clinic.

Exciting times lie ahead for clinical trials in SMA. Multiple therapies have demonstrated exceptional promise in both the areas of gene therapy and SMN2 upregulation. How well the preclinical studies in mice have informed clinical trials is a question that researchers, clinicians and patients are eager to understand.

Future perspective

Mouse models still represent our best mammalian models for studying human disease. As ingenuity and genomic technologies increase, so will our ability to understand how allelic variations influence biology from a systems-based approach. Advances in genetic engineering in mice will allow for quick and efficient modeling of allelic variations, enabling us to better model oligogenic diseases. Immunodeficient mice for human tissue engraftment studies will be widely used in order not only to study the disease course, but also to assess therapies. Induced pluripotent stem cells from patients will play a powerful role in understanding human disease and will compliment work performed in the mouse models, as well as other biological systems. Finally, as clinical trials for neurodegenerative diseases continue to evolve, much more data will be gleaned from the patients themselves, making them, and not the mouse, the better predictor of future successful trials.

Acknowledgements

The authors would like to thank the researchers who deposited their models into The Jackson Laboratory, and especially A Burghes for all of the outstanding models he generously made available to the spinal muscular atrophy

community. Finally, a special thanks is given to D Kobayashi for her helpful advice and support over the years.

Financial & competing interests disclosure

The authors would like to acknowledge funding from the SMA Foundation, the Friedreich's Ataxia Research Alliance, the ALS Association, the Tow foundation and the ALS Therapy Foundation, and for their support in funding disease-specific mouse repositories. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

EXECUTIVE SUMMARY

- Across different mouse models for neurodegenerative disease, achieving construct, face and predictive validity is challenging.
- Recognizing that the mouse model represents only a part of the disease pathology is essential.
- A lack of reproducibility in experiments can be largely attributed to differences in strain genetic backgrounds.
- New technologies allow for engineering into inbred backgrounds, saving time and resources.
- Differences in genetic backgrounds can be exploited in order to modify phenotype.
- New gene discoveries are leading to new models of disease.
- Amyotrophic lateral sclerosis therapeutic development is hindered, in part, by work carried out in one mouse model representing only a small proportion of the patient population.
- Experimental data sets may prove to be more powerful and translatable to the clinic if therapeutics are tested across multiple models, representing a broader patient population.
- Multiple mouse models are often needed in order to answer questions regarding clinically heterogeneous diseases.
- Some phenotypes that are present in mouse models may not be clinically relevant, but still provide information regarding disease processes and therapeutic effectiveness.
- It is important to find measurable features in mouse models that can be translated into the patient population.
- It is important to recognize that the mouse is an assay to be used in order to help understand disease mechanisms – humans develop human diseases, and mice do not develop human diseases.
- Both the development of mouse models and the analysis of preclinical data must be approached with rigor.

References

Papers of special note have been highlighted as:

- • of interest
- •• of considerable interest

1 Justice MJ, Siracusa LD, Stewart AF. Technical approaches for mouse models of human disease. *Dis. Model Mech.* 4(3), 305–310 (2011).

•• **Examines the reasons why experimental results in the laboratory fail to be replicated in the clinic – observations include experimental design, blinding and the interpretation of results.**

2 Wilkins HM, Bouchard RJ, Lorenzon NM, Linseman DA. Poor correlation between drug efficacies in the mutant *SOD1* mouse model versus clinical trials of ALS necessitates the development of novel animal models for sporadic motor neuron disease. In: *Horizons in Neuroscience Research.* Costa A, Villalba E (Eds). Nova Science Publishers, Inc., NY, USA (2011).

3 Gordon P, Corcia P, Meininger V. New therapy options for amyotrophic lateral sclerosis. *Expert Opin. Pharmacother.* 14(14), 1907–1917 (2013).

4 Benatar M. Lost in translation: treatment trials in the *SOD1* mouse and in human ALS. *Neurobiol. Dis.* 26(1), 1–13 (2007).

5 Landis SC, Amara SG, Asadullah K *et al.* A call for transparent reporting to optimize the predictive value of preclinical research. *Nature* 490(7419), 187–191 (2012).

6 Lefebvre S, Burglen L, Reboullet S *et al.* Identification and characterization of a spinal muscular atrophy-determining gene. *Cell* 80(1), 155–165 (1995).

7 Lefebvre S, Burlet P, Liu Q *et al.* Correlation between severity and SMN protein level in spinal muscular atrophy. *Nat. Genet.* 16(3), 265–269 (1997).

8 Michaud M, Arnoux T, Bielli S *et al.* Neuromuscular defects and breathing disorders in a new mouse model of spinal muscular atrophy. *Neurobiol. Dis.* 38(1), 125–135 (2010).

9 Le TT, Pham LT, Butchbach ME *et al.* SMNDelta7, the major product of the centromeric survival motor neuron (*SMN2*) gene, extends survival in mice with spinal muscular atrophy and associates with full-length SMN. *Hum. Mol. Genet.* 14(6), 845–857 (2005).

10 Hsieh-Li HM, Chang JG, Jong YJ *et al.* A mouse model for spinal muscular atrophy. *Nat. Genet.* 24(1), 66–70 (2000).

11 Campuzano V, Montermini L, Molto MD *et al.* Friedreich's ataxia: autosomal recessive disease caused by an intronic GAA triplet repeat expansion. *Science* 271(5254), 1423–1427 (1996).

12 Al-Mahdawi S, Pinto RM, Ruddle P, Carroll C, Webster Z, Pook M. GAA repeat instability in Friedreich ataxia YAC transgenic mice. *Genomics* 84(2), 301–310 (2004).

13 Miranda CJ, Santos MM, Ohshima K *et al.* Frataxin knockin mouse. *FEBS Lett.* 512(1–3), 291–297 (2002).

14 Pandolfo M. The molecular basis of Friedreich ataxia. *Adv. Exp. Med. Biol.* 516, 99–118 (2002).

15 Pook MA, Al-Mahdawi S, Carroll CJ *et al.* Rescue of the Friedreich's ataxia knockout mouse by human YAC transgenesis. *Neurogenetics* 3(4), 185–193 (2001).

16 Perdomini M, Hick A, Puccio H, Pook MA. Animal and cellular models of Friedreich ataxia. *J. Neurochem.* 126(Suppl. 1), S65–S79 (2013).

17 Rosen DR, Siddique T, Patterson D *et al.* Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature* 362(6415), 59–62 (1993).

18 Gurney ME, Pu H, Chiu AY *et al.* Motor neuron degeneration in mice that express a human Cu,Zn superoxide dismutase mutation. *Science* 264(5166), 1772–1775 (1994).

19 Tsao W, Jeong YH, Lin S *et al.* Rodent models of TDP-43: recent advances. *Brain Res.* 1462, 26–39 (2012).

• **Comprehensive review of the lack of reproducible results in amyotrophic lateral sclerosis therapy development.**

20 Mancuso R, Olivan S, Mancera P *et al.* Effect of genetic background on onset and disease progression in the *SOD1*-G93A model of amyotrophic lateral sclerosis. *Amyotroph. Lateral Scler.* 13(3), 302–310 (2012).

• **Uses computer modeling and statistical analysis of a cohort of greater than 5000 mutant animals in order to identify confounding biological variables that contribute to the measurement of noise in amyotrophic lateral sclerosis experimental settings.**

21 Butchbach ME, Singh J, Thorsteinsdottir M *et al.* Effects of 2,4-diaminoquinazoline derivatives on SMN expression and phenotype in a mouse model for spinal muscular atrophy. *Hum. Mol. Genet.* 19(3), 454–467 (2010).

22 Narver HL, Kong L, Burnett BG *et al.* Sustained improvement of spinal muscular atrophy mice treated with trichostatin A plus nutrition. *Ann. Neurol.* 64(4), 465–470 (2008).

23 Carlson DF, Fahrenkrug SC, Hackett PB. Targeting DNA with fingers and TALENs. *Mol. Ther. Nucleic Acids* 1, e3 (2012).

24 Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science* 337(6096), 816–821 (2012).

25 Chio A, Calvo A, Moglia C, Mazzini L, Mora G. Phenotypic heterogeneity of amyotrophic lateral sclerosis: a population based study. *J. Neurol. Neurosurg. Psychiatry* 82(7), 740–746 (2011).

26 Scott S, Kranz JE, Cole J *et al.* Design, power, and interpretation of studies in the standard murine model of ALS. *Amyotroph. Lateral Scler.* 9(1), 4–15 (2008).

27 Heiman-Patterson TD, Deitch JS, Blankenhorn EP *et al.* Background and gender effects on survival in the TgN(SOD1-G93A)1Gur mouse model of ALS. *J. Neurol. Sci.* 236(1–2), 1–7 (2005).

28 Wooley CM, Xing S, Burgess RW, Cox GA, Seburn KL. Age, experience and genetic background influence treadmill walking in mice. *Physiol. Behav.* 96(2), 350–361 (2009).

29 Hatzipetros T, Bogdanik LP, Tassinari VR *et al.* Prp-TDP43A315T mice develop progressive neurodegeneration in the myenteric plexus of the colon without exhibiting key features of ALS. *Brain Res.* doi:10.1016/j.brainres.2013.10.013 (2013) (Epub ahead of print).

30 Lutz CM, Kariya S, Patruni S *et al.* Postsymptomatic restoration of SMN rescues the disease phenotype in a mouse model of severe spinal muscular atrophy. *J. Clin. Invest.* 121(8), 3029–3041 (2011).

31 Foust KD, Wang X, Mcgovern VL *et al.* Rescue of the spinal muscular atrophy phenotype in a mouse model by early postnatal delivery of SMN. *Nat. Biotechnol.* 28(3), 271–274 (2010).

32 Passini MA, Bu J, Roskelley EM *et al.* CNS-targeted gene therapy improves survival and motor function in a mouse model of spinal muscular atrophy. *J. Clin. Invest.* 120(4), 1253–1264 (2010).

33 Nurputra DK, Lai PS, Harahap NI *et al.* Spinal muscular atrophy: from gene discovery to clinical trials. *Ann. Hum. Genet.* doi:10.1111/ahg.12031 (2013) (Epub ahead of print).

34 Osborne M, Gomez D, Feng Z *et al.* Characterization of behavioral and neuromuscular junction phenotypes in a novel allelic series of SMA mouse models. *Hum. Mol. Genet.* 21(20), 4431–4447 (2012).

35 Cobb MS, Rose FF, Rindt H *et al.* Development and characterization of an SMN2-based intermediate mouse model of spinal muscular atrophy. *Hum. Mol. Genet.* 22(9), 1843–1855 (2013).

36 Esmaeili MA, Panahi M, Yadav S, Hennings L, Kiaei M. Premature death of *TDP-43* (A315T) transgenic mice due to gastrointestinal complications prior to development of full neurological symptoms of amyotrophic lateral sclerosis. *Int. J. Exp. Pathol.* 94(1), 56–64 (2013).

37 Guo Y, Wang Q, Zhang K *et al.* HO-1 induction in motor cortex and intestinal dysfunction in *TDP-43* A315T transgenic mice. *Brain Res.* 1460, 88–95 (2012).

38 Kong L, Wang X, Choe DW *et al.* Impaired synaptic vesicle release and immaturity of neuromuscular junctions in spinal muscular atrophy mice. *J. Neurosci.* 29(3), 842–851 (2009).

39 Park GH, Maeno-Hikichi Y, Awano T, Landmesser LT, Monani UR. Reduced survival of motor neuron (SMN) protein in motor neuronal progenitors functions cell autonomously to cause spinal muscular atrophy in model mice expressing the human centromeric (*SMN2*) gene. *J. Neurosci.* 30(36), 12005–12019 (2010).

40 Bevan AK, Hutchinson KR, Foust KD *et al.* Early heart failure in the SMNDelta7 model of spinal muscular atrophy and correction by postnatal scAAV9-SMN delivery. *Hum. Mol. Genet.* 19(20), 3895–3905 (2010).

41 Heier CR, Satta R, Lutz C, Didonato CJ. Arrhythmia and cardiac defects are a feature of spinal muscular atrophy model mice. *Hum. Mol. Genet.* 19(20), 3906–3918 (2010).

42 Shababi M, Habibi J, Yang HT, Vale SM, Sewell WA, Lorson CL. Cardiac defects contribute to the pathology of spinal muscular atrophy models. *Hum. Mol. Genet.* 19(20), 4059–4071 (2010).

43 Shababi M, Lorson CL, Rudnik-Schoneborn SS. Spinal muscular atrophy: a motor neuron disorder or a multi-organ disease? *J. Anat.* doi:10.1111/joa.12083 (2013) (Epub ahead of print).

44 Araujo A, Araujo M, Swoboda KJ. Vascular perfusion abnormalities in infants with spinal muscular atrophy. *J. Pediatr.* 155(2), 292–294 (2009).

45 Shababi M, Habibi J, Ma L, Glascock JJ, Sowers JR, Lorson CL. Partial restoration of cardio-vascular defects in a rescued severe model of spinal muscular atrophy. *J. Mol. Cell. Cardiol.* 52(5), 1074–1082 (2012).

46 Arnold WD, Burghes AH. Spinal muscular atrophy: the development and implementation of potential treatments. *Ann. Neurol.* 74(3), 348–362 (2013).