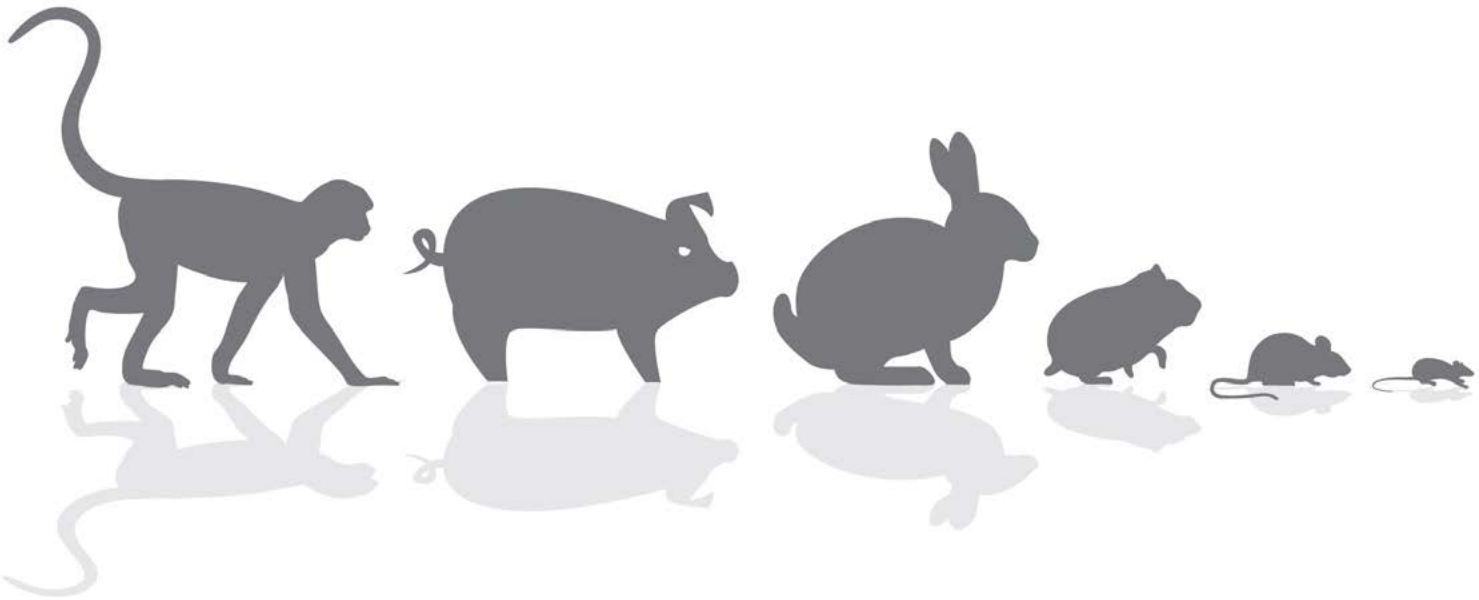
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THE IMPORTANCE OF SELECTING THE RIGHT ANIMAL MODELS TO EVALUATE GENE THERAPIES

Gene therapies aim to correct genetic defects by editing or replacing different types of mutations and the therapeutic aim is to directly treat or correct the disease driver and not the symptoms. The development of gene therapies is a high interest area, which is driven by clinical success.

As of 2026, about 40 gene therapy products have been approved[1] and the clinical trial landscape is robust with ~2,000 open clinical trials across multiple disease indications including various cancers, metabolic diseases, rare diseases, cardiovascular conditions and neurological diseases.[1,2]

The clinical pipeline is populated with gene therapy assets that have been successfully validated preclinically using a combination of *in vitro* and *in vivo* models. While initial studies can be performed in cell-based models, *in vivo* models are essential to understand disease biology and evaluate therapeutic responses for viral and non-viral gene therapies. *In vitro* models are rapid and cost-effective to test infectivity and replication of engineered viral vectors, but these models provide limited information on tissue specificity and efficacy. In contrast, *in vivo* models provide comprehensive information on efficacy and safety of gene therapy drug candidates. Some of the critical endpoints include the assessment of infectivity (viral vectors), biodistribution, delivery efficiency, tissue targeting and safety including off target effects.

Additional readouts include the host response to the payload expression and the duration of expression to gain an understanding of the clinical dosing frequency.

Animal models have a complete interactive physiology that allows therapeutic evaluation in a translationally relevant context and can also be used to identify definitive biomarkers that translate to the clinic and can be used for prognostic monitoring. However, for gene therapies, unmodified or wild-type models may not be optimal as the target gene may not be present, especially if the disease is caused by a mutation. Genetically modified animal models such as knockouts, transgenic or humanized models that express the disease-causing gene and recapitulate some, if not all, of the disease pathophysiology are more suitable for gene therapy testing. The characterization of genetically modified animal models during gene therapy studies can be done using biochemical readouts, imaging, histological analyses, behavioral and cognitive endpoints. Animal models are also being used to identify clinically relevant biomarkers. Biomarkers are critical tools for clinical success and are used to measure disease development and the impact of therapeutic interventions. Biomarkers can be of various types – genetic, biochemical, physiological, behavioral, developmental and pathological and, often, a battery of biomarker tests is used for diagnostic and prognostic testing. Given the wide range of biomarkers that are available, it is essential to identify reliable, robust endpoints that translate from preclinical animal models to humans.

The selection of small or large animal models to evaluate gene therapy requires careful consideration as both options have benefits and challenges.

Small animal models are easy to handle and house and are relatively cost-effective. More importantly, genetically modified rodent models can be generated efficiently and in a short timespan. However, small animal models only partially recapitulate human disease biology and are less ideal to estimate clinical doses and routes of administration. Additionally, longitudinal studies in small animals are typically shorter and provide limited information on long-term exposure. While genetic engineering is well established for rodent models, it is important to note that mouse and rat strains are genetically uniform due to inbreeding limiting the clinical relevance of the data. In contrast, large animal models better recapitulate human anatomy and physiology and are well suited to establish dosage and routes of administration. Depending on the species, large animal models have diverse genetic backgrounds that mimic the human population. However, studies in large animal models can be expensive and require specialized housing and handling that is available through select CROs and institutes. Modeling disease states via genetic engineering is still a challenge due to scientific and ethical concerns and disease models can take a long time to develop and validate[3].

Selecting the optimal model for gene therapy studies should include scientific and practical considerations. Transgenic or knockout mouse models that recapitulate the disease phenotype via gene editing (CRISPR, TALENS or zinc finger nucleases) are usually the first option. It is also important to select preclinical models that closely recapitulate human physiology. For example, an engineered AAV9 (adeno-associated vector serotype 9) crossed the blood brain barrier in mice but not in marmosets[4], suggesting that the specific AAV may not work well in humans. Rabbits are widely used models for eye research due to the comparatively large eye size, similarities to the human eye and the availability of detailed information about anatomy and physiology.

Similarly, the brains of non-human primates (NHPs) are very similar to humans in terms of anatomy, network organization and abilities so NHPs are well suited to study gene delivery to the CNS5. Some large animals are also natural disease models where they develop similar diseases as humans – an example of a natural model is the development of arthritis in dogs. An IL-10 boosting gene therapy was found to be effective against canine arthritis paving the way for human clinical trials[6].

Some of the practical considerations for selecting an animal model for gene therapy studies are availability of the animal model, associated costs and maintenance of the animal colony. Large animal colonies are typically more expensive and require special vivarium spaces with experienced staff. These colonies have to be maintained for longer periods of time as large animal studies can take a long time (months to years) to be completed. Murine vivarium spaces are more cost effective, and more animals can be housed per square foot. Instrumentation requirements for small animal studies are typically more cost effective and easier to handle compared to large animal studies[7].

Small Animal Models in Gene Therapy Studies

Rodent models are used to evaluate gene therapies for various diseases including cancer, metabolic diseases and neurological diseases. Since gene therapies target gene defects, genetically engineered mouse models (GEMMs) are optimal to model the disease state and test novel therapies. GEMMs include transgenic mice where a human gene is expressed under the control of a tissue-specific or ubiquitous promoter, knock-in mice where the human gene is substituted for the mouse gene and knockout mice that have an inactive gene. Gene editing technologies such as CRISPR and base editors have accelerated and improved GEMMs to model various disease states.
An example of how GEMMs have helped advance drug development is Huntington's disease. Various transgenic and knock in mouse models[8] as well as humanized models have been developed to test various therapies. Gene therapies were found to be the most promising as the therapy directly targets the mutant *Htt* gene. Indeed, a microRNA-based therapy aimed at reducing mutant Huntingtin protein levels is currently in clinical trials[9] where the AAV encoded miRNA was tested in the Hu128/21 mouse model of Huntington's disease[10].

The use of transgenic rats is not as widespread as mice due to technical complexities, but specific diseases can be modeled better in rats than mice. One example is Alzheimer's disease where transgenic rat models have distinct benefits compared to GEMMs[11]. Rats have larger brains for easier drug administration and tissue/biofluid sampling and more importantly, rats express all 6 forms of the tau protein which mice do not. The complex behavioral habits of rats support more sophisticated behavioral modeling as a readout for cognitive outcomes.

Large Animal Models in Gene Therapy Studies

Large animal models have traditionally been technically complex and expensive but increasingly, there is a shift towards evaluating both viral and LNP therapies in large animal models. Rabbit models are used when human disease cannot be adequately modeled by rodents or non-mammalian species. Transgenic rabbit models are being used to study cardiovascular diseases, ocular diseases, osteo-arthritis etc. Cardiovascular diseases including atherosclerosis, long QT syndrome and cardiomyopathy have been successfully modeled in transgenic rabbits[12]. Ocular diseases are an area where rabbit models are being used to evaluate gene therapies.
Rabbits are used extensively to test the safety

and pharmacokinetics of drugs injected directly into the eye (e.g. intravitreally) and the large eyes simplifies gene delivery. Both viral[13] and nonviral[14] gene therapies have been reported. Some large animal models lend themselves to studying specific diseases as they naturally develop the disease or are susceptible to developing the disease. One example is the development of cystic fibrosis in pigs[15] and these models have been used to evaluate the efficacy of an aerosolized lentivirus gene therapy[16] as well as an AAV mediated delivery of the *Cftr* gene[17]. A knock-in pig model expressing full-length mutant Huntingtin protein was shown to accurately recapitulate key pathological features of Huntington's disease[18] and was used to test a microRNA-based HD therapy that is currently in clinical trials[19].

There are documented cases where data from canine models have been used to design clinical trials in retinal diseases[20] and hemophilia[21]. Canine models can often be natural models for specific diseases and one such example is the development of dilated cardiomyopathies in Doberman pinscher dogs due to a missense mutation in the *Ttn* gene that is also seen in humans[22]. Another well documented natural model is the Golden retriever muscular dystrophy model that has similar disease development as Duchenne muscular dystrophy seen in humans[23] and is ideally suited to test late-stage gene therapies.

Non-human primates or NHPs are closely related to humans and are very useful preclinical animal models. NHPs are widely used to evaluate ADME characteristics including biodistribution and clearance of gene therapies and are ideally suited to identify clinical doses and routes of administration. NHP studies are particularly well-suited to specific disease indications. For example, NHP brains have similar anatomy and physiology as humans and are a good model to study gene transport across the blood brain barrier (BBB) since the NHP BBB has a similar distribution of uptake and efflux transporters as the human brain[24]. The first genetically modified NHP model was developed in 2001[25] and since then gene-edited NHP models have been developed for several disease states[25]. One example of the application of a transgenic NHP model is the evaluation of cell therapies for Parkinson's disease (PD)[26] where a PD like phenotype is induced using neurotoxins and motor end-points can be more accurately measured. Another area where data from NHP models translate well to humans is eye diseases due to similarities in the eye anatomy and physiology and the availability of ocular instrumentation such as OCT to image the NHP eye that is close to the size of the human eye[27].

Given the range of available preclinical models, it is important to identify the most relevant species for each stage of therapeutic evaluation and design studies that answer critical scientific questions and are practically feasible.

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