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# THE IMPORTANCE OF SELECTING RELEVANT ANIMAL MODELS FOR GENE THERAPY STUDIES

## INTRODUCTION

Gene therapies aim to correct genetic defects by editing or replacing mutated genetic material, so the therapeutic aim is to directly treat the disease cause and not focus on symptoms. Development of gene therapies is a high interest area, which is driven by clinical success. To date, the FDA has approved 4 gene therapy products[1] and the clinical trial landscape is robust with over 3,400 active clinical trials across multiple disease areas including various cancers, metabolic diseases, rare diseases, cardiovascular conditions and neurological diseases.[2]

The growing clinical pipeline is populated with gene therapy assets that have been successfully validated preclinically using relevant *in vitro* and *in vivo* models. Preclinical animal models are an essential component to understanding disease biology and evaluating the therapeutic response to gene therapy. Some of the critical endpoints include the assessment of infectivity, biodistribution, delivery efficiency and safety. Additional readouts include the host response to the payload expression and the duration of expression to gain an understanding of the dosing frequency.

*In vitro* models are rapid and cost-effective to test infectivity and replication of engineered viral vectors but these models provide limited information on specificity and efficacy. In contrast, *in vivo* models provide more comprehensive information on gene therapy assets. Animal models have a complete interactive

physiology that allows therapeutic evaluation in a more meaningful and relevant context and can also be used to identify definitive bio-markers that translate to the clinic and can be used for prognostic monitoring. However, standard animal models may not be optimal to test gene therapies for various reasons including the fact that the target gene may not be present. Genetically modified animal models such as knockouts, transgenic or humanized models that express the gene target and recapitulate some if not all, disease pathophysiology are more suitable for gene therapy testing.

Animal models are also being used to identify clinically relevant biomarkers. Biomarkers are critical to measure changes in disease states and the impact of therapeutic interventions and are essential tools to measure clinical success. Biomarkers can be of various types – genetic, biochemical, physiological, behavioral, developmental and pathological. Given the wide range of biomarkers that are available, it is essential to identify reliable, robust endpoints that translate across small and large animal models as well as humans. The characterization of genetically modified animal models during gene therapy studies can be done using biochemical readouts, imaging, histological analyses, behavioral and cognitive endpoints.

Both small and large animal models have benefits and challenges. Small animal models are easy to handle and relatively cost-effective, and genetically modified models can be generated quickly due to short gestation. However, small animal models only partially recapitulate human disease pathophysiology and cannot be used to estimate clinical dosages. Additionally, longitudinal studies in small animals are of limited duration. An additional drawback of mouse and rat models is the genetic uniformity due to inbreeding. In contrast, large animal models recapitulate human anatomy and physiology so disease states can be more accurately modeled and clinical dosages can be estimated. Additionally, large animal models have diverse genetic backgrounds that mimics the human population but are expensive and require specialized handling. Furthermore, due to the long gestation times, studies in genetically modified models take time[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 to develop a disease state model. It is also important to evaluate therapies in models that more 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 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 the 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 brain[5].

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 and human trials are underway[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, traditional mouse or rat models may not be relevant and genetically engineered mouse models (GEMMs) may be more optimal. 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. In the past few years, gene editing technologies such as CRISPR have accelerated the generation of GEMMs for 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. gene therapies. Indeed, a microRNA-based therapy aimed at reducing mutant Huntingtin protein levels is currently in clinical trials[9] and 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.

Rabbit models are used when human disease cannot be adequately modeled by rodents or nonmammalian species. Transgenic rabbit models are being used to study cardiovascular diseases, ocular diseases, osteoarthritis 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 intravitreally and the large eyes simplifies gene delivery. Additionally, both viral[13] and nonviral[14] gene therapies have been tested in rabbit models.

## LARGE ANIMAL MODELS IN GENE THERAPY STUDIES

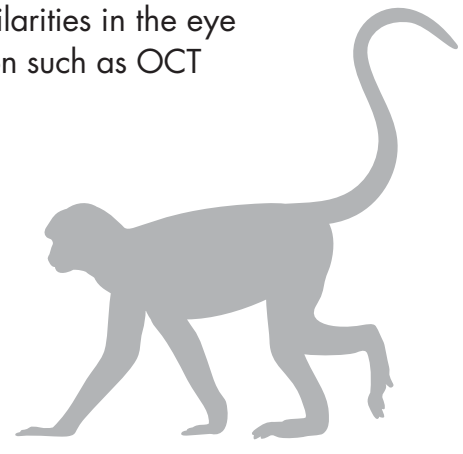
Large animal models have traditionally been considered to be technical complex and expensive but increasingly, there is a shift towards evaluating gene therapies in relevant large animal models.

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. 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]. Additionally, NHP models are being widely used to study cell therapies for Parkinson's disease (PD)[25] where a PD like phenotype is induced using neurotoxins and motor endpoints 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[26].

Taken together, it is critical to develop an animal model that recapitulates disease pathophysiology and has the most relevant anatomy and physiology but is also feasible from a practical standpoint.



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