

Large Animal Models of Genetic Disease: Pertinent IACUC Issues

N. Matthew Ellinwood and Colin M. Clay

The editors of this issue have assembled an exemplary group of expert reviews that provide an effective overview of the current use of large animal models in genetic disease research and describe the importance of these models in bringing new, safe, and efficacious treatments to clinical practice. In this essay we focus on both practical and technical considerations for the conduct of this type of research from the standpoint of those who prepare and review institutional animal care and use committee (IACUC) protocols. We discuss the history of these types of research subjects and practical considerations for the use of these models. As IACUC membership often varies in expertise, we spend some time on issues that may seem obvious to the expert but that may not be readily apparent to nonscientist IACUC members. Many of our observations are based on our own and our colleagues' experiences in the preparation and review of protocols, reflecting common questions or themes raised during the review process. We do not address issues relevant to individual species but rather the general considerations, including the advantages and constraints, of maintaining and using these large animal models for the study of genetic disease.

History of Animal Models of Genetic Diseases

The history of the use and development of large animal models goes back decades—researchers first identified and characterized a canine model of a genetic disease now known as a lysosomal storage disease (Krabbe disease) more than 45 years ago (Fankhauser et al. 1963; Haskins 2009). The current use of these models has led to the development of therapies with literally biblical qualities, allowing lame dogs to walk (as with mucopolysaccharidosis [MPS] type VII; Haskins 2009) and blind dogs to see (as with Leber congenital amaurosis; Stieger et al. 2009). These models have particular assets that may not be well known to a biomedical

research community that often focuses on rodent and other small animal models. Specifically, the size and reproductive physiology of the larger species may (or may not) favor their use in most genetic disease research—which is to say not all large animal model species are created equal. Thus, although some of the articles in this issue discuss bovine, nonhuman primate, and other large animal models of some disorders (e.g., Bauer et al. 2009; Stieger et al. 2009), these species may make poor models to study interventional therapy because of constraints of time, space, and cost. In cases where the species (e.g., nonhuman primates) mirrors human development (as with brain physiology) or human infectious diseases (such as HIV), these large animal species have become indispensable to the study of conditions such as Parkinsonism and neuroAIDS (Gagliardi and Bunnell 2009). The models may also stand in a hierarchy of appropriateness as therapeutic development progresses; thus the murine model of Krabbe disease supersedes as a first line of study the canine model, which itself precedes the macaque model (Haskins 2009; Gagliardi and Bunnell 2009).

In most large animal models of genetic disorders the disease is recessive (since dominant mutations are seldom propagated and their sporadic occurrences often escape the attention of the veterinary and research communities) and represents an accurate homologue of the corresponding human condition. In some cases the models differ significantly from the human condition (e.g., gray collie syndrome and human cyclic hematopoiesis; Bauer et al. 2009) and the differences influence how the models can be used. Less commonly, large animal models may represent as yet unelucidated genetic conditions that could provide important insight to human disease, as is the case with the juvenile form of dilated cardiomyopathy that occurs in the Portuguese water dog (Sleeper et al. 2009).

General Considerations for the Use of Large Animal Models

Any significant research using large animal models of genetic disease requires a viable breeding colony. The reproductive physiology of the larger species (e.g., a bitch's reproductive cycle is 6 months) and the autosomal recessive nature of many of the conditions under investigation may require large numbers of breeding animals and production of a large number of offspring. In studies of significant diseases where models exist but no viable therapy is on the horizon, IACUCs

N. Matthew Ellinwood, DVM, PhD, is an assistant professor of Companion Animal Genomics in the Animal Breeding and Genetics Section of the Department of Animal Science at Iowa State University in Ames. Colin M. Clay, PhD, is a professor in the Department of Biomedical Sciences at Colorado State University in Fort Collins.

Address correspondence and reprint requests to Dr. Matthew Ellinwood, Department of Animal Science, 2356D Kildee Hall, Iowa State University, Ames, Iowa 50011-3150 or email mellinwo@iastate.edu.

may review protocols that focus on little more than maintenance of viable breeding stock. Such efforts are important because, although cryopreservation of these models is critical and can serve as a backup to existing stocks, the time required for reconstitution of a canine model from cryopreserved semen is up to 2 years, which represents a serious constraint given the timeline of most sponsored research.

Models may use pure stock of the breed of origin of a given disease or cross-bred stock. Unless there is compelling evidence to the contrary, investigators and IACUCs can appreciate the benefits of crossing purebred stock:

- Outcrossing increases colony production through hybrid vigor and decreases the number of animals needed for production.
- It increases genetic heterozygosity, which may better mimic human genetics.
- It may improve the well-being of the animals by resulting in a line of dogs that may be more adaptable to a research environment.

From our experience monitoring and maintaining such colonies, something as simple as maintaining a short-haired dog of medium size, versus long-haired animals or very large or small animals, can significantly affect the husbandry and by extension the potential welfare of the animal.

It is critical when using these models, especially for a gene or protein-based therapy, to know the underlying molecular defect or at least be able to predict how the mutation may affect therapy. The nature of the mutation, and supporting protein-based evaluations, may clearly indicate whether the model in question is positive or negative for so-called cross-reacting immunological material (CRIM⁺ or CRIM⁻). The difference has an important impact on the research and protocols considered for review. For models that are well characterized, as with those for hemophilia A or B, a literature exists on the nature of the canine immune response and the need for immune tolerance or immunosuppression (Nichols et al. 2009). In other cases, as in the MPS I feline and canine models, research has identified critical windows or methods for tolerizing animals to gene therapy (Haskins 2009). Regardless of specifics, principal investigators and IACUC members alike must be mindful of difficulties that may affect the health and welfare of the research subjects, based on the approach to therapy, the source of the cDNA to be used (e.g., human vs. species-specific), the nature of the mutation involved, and the need for immunosuppression or immune tolerizing regimens (Wang et al. 2009). In some cases of gene therapy, even with species-specific cDNAs in CRIM⁺ animals, severe and life-threatening sequelae may involve neutralizing auto-antibodies (e.g., in macaques receiving erythropoietin gene therapy; Chenuaud et al. 2004).

Advantages and Constraints

A distinct advantage of the large animal models is that they manifest clinical signs, which originally brought them to the attention of the veterinary and research communities. In

contrast, murine targeted genetic disease models may manifest biochemical characteristics but not the clinical characteristics of the human disorder, as in the mouse model of Tay-Sachs disease (Yamanaka et al. 1994). In this and other respects, large animal models have some distinct advantages over murine and other small animal models, and it is worthwhile to discuss these advantages and under what circumstances they apply, given the goal of IACUCs to determine the suitability of a particular species to a given research application.

The most important uses of large animal models are to advance knowledge of pathogenesis and to support the development and testing of human targeted therapies, especially as such efforts pass from proof of principle to application. Such models are critical to this process for several reasons: They are orders of magnitude closer to humans in size, so the scale-up to human patients is much less than from mice to humans. And the larger animals live longer and allow long-term evaluation well beyond that offered by mouse models. For example, MPS VII dogs that have received gene therapy have been under evaluation for more than 8 years, during which time they have shown stable transgene expression, with no adverse gene therapy-associated sequelae (Haskins 2009). Monitoring on this time scale is a critical asset for evaluating safety and efficacy, especially in light of some gene therapy trials in humans that resulted in vector-induced oncogenesis (Hacein-Bey-Abina et al. 2003; see also Bauer et al. 2009).

In addition, large animal model species are, relative to murine models, outbred, better reflecting human patients, and they mount robust immune responses, as mentioned above. However, these qualities do not always ensure an evaluation predictive of the potential response in humans. For example, preliminary work in both canines and nonhuman primates on the use of adeno-associated viral vectors to treat hemophilia B in humans (Manno et al. 2006; Nichols et al. 2009) failed to reveal immune-mediated responses that limited the effectiveness of the gene therapy in humans.

In terms of constraints, large animal models are more expensive to maintain and experiments that use them require a longer time scale. These factors, coupled with the fact that these species are more phylogenetically advanced, place the proper use of these large animal models in IACUC protocols that, among other things, involve the following areas: colony maintenance; basic disease research in cases where no other or suboptimal models exist; and applied or preclinical research directed at development of human targeted therapeutics for serious or significant diseases that need improved therapies.

IACUC Considerations

Because of the expense of large animal models, and because of their use in preclinical therapy development, their use most often follows preliminary or proof-of-principle experiments in more efficient, less phylogenetically advanced

models. There is thus a high expectation of efficacy when evaluating therapies in these models, which are, conversely, a poor method of evaluating incremental or small effects of potential therapies. This fact may lead IACUC reviewers accustomed to evaluating murine protocols to think that the number of experimental animals is too small. But the effect anticipated should be large enough to warrant the use of as few animals as statistically possible, as production and economic constraints will limit the animal resources available to investigators.

In fact, the published research record demonstrates that large animal models were critical and indispensable in the early development of human therapies (Bauer et al. 2009) and hematopoietic stem cell transplantation. The species and animals used are second only to humans in terms of the degree of medical attention, knowledge, and technology devoted to them, and the combined resources and knowledge of the clinical and research veterinary communities converge to make these models very well suited as preclinical models to test and evaluate potential human therapies.

With the exception of swine, the current state of the art of reproductive and stem cell technologies is not adequate for the targeted creation of genetic disease models in canines and other large animal species. This limitation aside, advances such as the sequencing of the canine genome (Lindblad-Toh et al. 2005) and developments in feline genomics (Murphy 2006) have ensured the development of important research resources associated with these species, a process that will only continue to expand. Because existing genetic disease models occur spontaneously, they do not require review by an Institutional Biosafety Committee (IBC), whereas the use of gene therapies and other DNA-based approaches does require IBC as well as IACUC review.

Concluding Observations

The genetic diseases discussed in this issue represent the results of a long history of work on identifying, characterizing, and using these models, a history well documented in the articles and their references. The physiological systems and disease classes covered by the reviews reflect many facets of model development. Scientists identified many of these diseases because of striking and obvious clinical signs, often in the veterinary pediatric period (e.g., Haskins 2009; Bauer et al. 2009; Sleeper et al. 2009; Koeberl et al. 2009; Wang et al. 2009). Some of the diseases are serious multisystem disorders—the lysosomal and glycogen storage diseases, metabolic diseases, and neurologic disorders (Gagliardi and Bunnell 2009; Haskins 2009; Koeberl et al. 2009). Others are evident at birth, such as gray collie syndrome, or emerge shortly thereafter in conditions such as pneumonia or omphalophlebitis (with canine leukocyte adhesion deficiency or canine X-linked severe combined immune deficiency; Bauer et al. 2008). The development of some classes of disease models has been aided by fairly simple screening and phenotyping methods, as with ophthalmological models

(Stieger et al. 2009). Most of these disorders have been identified in the genetic isolates of purebred dogs (Ostrander et al. 2000) and in cats, but exceptions exist (for example, the feline model of the lysosomal storage disease MPS I was identified in a domestic short-haired cat that was likely the offspring of a mother-son mating in an urban neighborhood; Haskins et al. 1979).

Regardless of the origins of these models, they have some elements in common. They are usually models of diseases that in humans are associated with significant morbidity or mortality. In some cases, such as muscular dystrophy (Wang et al. 2009), the disease in humans may be relatively common, while other diseases may be ultrarare genetic disorders, such as some of the lysosomal storage diseases and metabolic disorders (Haskins 2009; Koeberl et al. 2009). Treatments may exist, but they are fraught with serious problems and constraints; examples include hematopoietic stem cell transplantation (for hematopoietic disease and lysosomal storage disease), recombinant protein-based therapies (for clotting disorders and lysosomal storage diseases), and drug therapies (for HIV infection and AIDS). On the other hand, for diseases for which successful therapy exists, researchers have discovered that it may not be necessary to achieve normal levels. For example, hemophilia A and B, most nonneuropathic lysosomal storage diseases, and many immunological diseases may require only a small percentage (5-25%) of normal levels of cells, proteins, or function to achieve substantial clinical benefit or cure. Some approaches focus on types of tissues or discrete targets such as the retina (Stieger et al. 2009), the central nervous system (Gagliardi and Bunnell 2009), skeletal muscle (Wang et al. 2009), or cardiomyocytes (Sleeper et al. 2009), and in many cases achieve therapeutic levels with current techniques. As interventional genetic methods improve, scientists may develop and use new models suitable for the next generation of more challenging diseases, which may include difficult or widespread targets for therapy, such as skin or connective tissue disorders.

In conclusion, large animal models of genetic disorders are often crucial to the development of effective treatments of human disease. When evaluating the appropriateness of these large animal models and the specifics of protocols involving them, it is important to bear in mind that such models fill an important niche between the use of murine and other small animal models and translation to human patients. When large animal models are available, they should be the model of choice for preclinical studies preliminary to human clinical trials.

References

- Bauer TR Jr, Adler RL, Hickstein DD. 2009. Potential large animal models for gene therapy of human genetic diseases of immune and blood cell systems. *ILAR J* 50:168-186.
- Chenuaud P, Larcher T, Rabinowitz JE, Provost N, Cherel Y, Casadevall N, Samulski RJ, Moullier P. 2004. Autoimmune anemia in macaques following erythropoietin gene therapy. *Blood* 103:3303-3304.

- Fankhauser R, Luginbuhl H, Hartley WJ. 1963. Leukodystrophie vom typus Krabbe beim hund. *Schweiz Arch Tierheilk* 105:198-207.
- Gagliardi C, Bunnell BA. 2009. Large animal models of neurological disorders for gene therapy. *ILAR J* 50:128-143.
- Hacein-Bey-Abina S, von Kalle C, Schmidt M, Le Deist F, Wulffraat N, McIntyre E, Radford I, Villeval JL, Fraser CC, Cavazzana-Calvo M, Fischer A. 2003. A serious adverse event after successful gene therapy for X-linked severe combined immunodeficiency. *N Engl J Med* 348:255-256.
- Haskins M. 2009. Gene therapy for lysosomal storage diseases (LSDs) in large animal models. *ILAR J* 50:112-121.
- Haskins ME, Jezyk PF, Desnick RJ, McDonough SK, Patterson DF. 1979. Alpha-L-iduronidase deficiency in a cat: A model of mucopolysaccharidosis I. *Pediatr Res* 13:1294-1297.
- Koeberl DD, Pinto C, Brown T, Chen YT. 2009. Gene therapy for inherited metabolic disorders in companion animals. *ILAR J* 50:122-127.
- Lindblad-Toh K, Wade CM, Mikkelsen TS, Karlsson EK, Jaffe DB, Kamal M, Clamp M, Chang JL, Kulbokas EJ 3rd, Zody MC, Mauceli E, Xie X, Breen M, Wayne RK, Ostrander EA, Ponting CP, Galibert F, Smith DR, DeJong PJ, Kirkness E, Alvarez P, Biagi T, Brockman W, Butler J, Chin CW, Cook A, Cuff J, Daly MJ, DeCaprio D, Gnerre S, Grabherr M, Kellis M, Kleber M, Bardeleben C, Goodstadt L, Heger A, Hitte C, Kim L, Koepfli KP, Parker HG, Pollinger JP, Searle SM, Sutter NB, Thomas R, Webber C, Baldwin J, Abebe A, Abouelleil A, Aftuck L, Ait-Zahra M, Aldredge T, Allen N, An P, Anderson S, Antoine C, Arachchi H, Aslam A, Ayotte L, Bachantsang P, Barry A, Bayul T, Benamara M, Berlin A, Bessette D, Blitshteyn B, Bloom T, Blye J, Boguslavskiy L, Bonnet C, Boukhgalter B, Brown A, Cahill P, Calixte N, Camarata J, Cheshatsang Y, Chu J, Citroen M, Collymore A, Cooke P, Dawoe T, Daza R, Decktor K, DeGray S, Dhargay N, Dooley K, Dooley K, Dorje P, Dorjee K, Dorris L, Duffey N, Dupes A, Egbiremolun O, Elong R, Falk J, Farina A, Faro S, Ferguson D, Ferreira P, Fisher S, FitzGerald M, Foley K, Foley C, Franke A, Friedrich D, Gage D, Garber M, Gearin G, Giannoukos G, Goode T, Goyette A, Graham J, Grandbois E, Gyaltsen K, Hafez N, Hagopian D, Hagos B, Hall J, Healy C, Hegarty R, Honan T, Horn A, Houde N, Hughes L, Hunnicutt L, Husby M, Jester B, Jones C, Kamat A, Kanga B, Kells C, Khazanovich D, Kieu AC, Kisner P, Kumar M, Lance K, Landers T, Lara M, Lee W, Leger JP, Lennon N, Leuper L, LeVine S, Liu J, Liu X, Lokyitsang Y, Lokyitsang T, Lui A, Macdonald J, Major J, Marabella R, Maru K, Matthews C, McDonough S, Mehta T, Meldrim J, Melnikov A, Meneus L, Mihalev A, Mihova T, Miller K, Mittelman R, Mlenga V, Mulrain L, Munson G, Navidi A, Naylor J, Nguyen T, Nguyen N, Nguyen C, Nguyen T, Nicol R, Norbu N, Norbu C, Novod N, Nyima T, Olandt P, O'Neill B, O'Neill K, Osman S, Oyono L, Patti C, Perrin D, Phunkhang P, Pierre F, Priest M, Rachupka A, Raghuraman S, Rameau R, Ray V, Raymond C, Rege F, Rise C, Rogers J, Rogov P, Sahalie J, Settipalli S, Sharpe T, Shea T, Sheehan M, Sherpa N, Shi J, Shih D, Sloan J, Smith C, Sparrow T, Stalker J, Stange-Thomann N, Stavropoulos S, Stone C, Stone S, Sykes S, Tchuinga P, Tenzing P, Tesfaye S, Thoulutsang D, Thoulutsang Y, Topham K, Topping I, Tsamla T, Vassiliev H, Venkataraman V, Vo A, Wangchuk T, Wangdi T, Weiland M, Wilkinson J, Wilson A, Yadav S, Yang S, Yang X, Young G, Yu Q, Zainoun J, Zembek L, Zimmer A, Lander ES. 2005. Genome sequence, comparative analysis and haplotype structure of the domestic dog. *Nature* 438:803-819.
- Manno CS, Pierce GF, Arruda VR, Glader B, Ragni M, Rasko JJ, Ozelo MC, Hoots K, Blatt P, Konkle B, Dake M, Kaye R, Razavi M, Zajko A, Zehnder J, Rustagi PK, Nakai H, Chew A, Leonard D, Wright JF, Lessard RR, Sommer JM, Tigges M, Sabatino D, Luk A, Jiang H, Mingozzi F, Couto L, Ertl HC, High KA, Kay MA. 2006. Successful transduction of liver in hemophilia by AAV-Factor IX and limitations imposed by the host immune response. *Nat Med* 12:342-347.
- Murphy WJ. 2006. The feline genome. *Genome Dyn* 2:60-68.
- Nichols TC, Dillow AM, Franck HWG, Merricks EP, Raymer RA, Bellinger DA, Arruda VR, High KA. 2009. Protein replacement therapy and gene transfer in canine models of hemophilia A, hemophilia B, von Willebrand disease, and factor VII deficiency. *ILAR J* 50:144-167.
- Ostrander EA, Galibert F, Patterson DF. 2000. Canine genetics comes of age. *Trends Genet* 16:117-124.
- Sleeper MM, Bish LT, Sweeney HL. 2009. Gene therapy in large animal models of human cardiovascular genetic disease. *ILAR J* 50:199-205.
- Stieger K, Lhériteau E, Moullier P, Rolling F. 2009. AAV-mediated gene therapy for retinal disorders in large animal models. *ILAR J* 50:206-224.
- Wang Z, Chamberlain JS, Tapscott SJ, Storb R. 2009. Gene therapy in large animal models of muscular dystrophy. *ILAR J* 50:187-198.
- Yamanaka S, Johnson MD, Grinberg A, Westphal H, Crawley JN, Taniike M, Suzuki K, Proia RL. 1994. Targeted disruption of the Hexa gene results in mice with biochemical and pathologic features of Tay-Sachs disease. *Proc Natl Acad Sci U S A* 91:9975-9979.