

Animal models for metabolic, neuromuscular and ophthalmological rare diseases

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Abstract | Animal models are important tools in the discovery and development of treatments for rare diseases, particularly given the small populations of patients in which to evaluate therapeutic candidates. Here, we provide a compilation of mammalian animal models for metabolic, neuromuscular and ophthalmological orphan-designated conditions based on information gathered by the European Medicines Agency's Committee for Orphan Medicinal Products (COMP) since its establishment in 2000, as well as from a review of the literature. We discuss the predictive value of the models and their advantages and limitations with the aim of highlighting those that are appropriate for the preclinical evaluation of novel therapies, thereby facilitating further drug development for rare diseases.

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In Europe and the United States alone, more than 55 million people suffer from a rare disease. It is estimated that approximately 5,000 to 7,000 rare diseases exist and in recent years approximately 250 new diseases have been described annually, which is partly due to the continual improvement of knowledge on disease biology and genomics¹. However, there are still only a small number of treatments available for rare diseases, although efforts to stimulate the development of new drugs through regulatory and economic incentives have catalysed progress; these incentives include those provided by the 1983 US Orphan Drug Act and the regulation on orphan medicinal products that was adopted in the European Union in 2000 (REF. 1). At present, there are 80 marketed orphan drugs in the European Union, which represent potential treatments for two to six million patients diagnosed with the rare disease for which the drugs are indicated.

One of the major issues hindering drug development for rare diseases is the use of animal models in preclinical studies that are not closely based on the knowledge of the molecular pathology of the human disease. For instance, photoreceptors are rare in rodents, which limits their utility as a model for ophthalmological rare diseases, whereas the Briard dog with a deficiency in retinal pigment epithelium-specific protein 65 kDa (RPE65) is considered to be an appropriate model for translation to the clinical setting. Choosing an appropriate and reliable animal model for evaluating potential

candidate therapies is particularly important for orphan drug development, as there is a limited number of patients available for enrolment into clinical trials for rare diseases.

Bringing together available knowledge on animal models of rare diseases could help in identifying which models are most appropriate for evaluating candidate therapies, while also clarifying areas in which further research is needed to improve the models. With this in mind, using the experience gained over the past decade by the European Medicines Agency (EMA)'s Committee for Orphan Medicinal Products (COMP), which reviews applications from companies seeking orphan medicinal product (OMP) designation in the European Union (BOX 1), we provide a comprehensive overview of the mammalian models used in research for rare diseases. The scope of this work was restricted to three therapeutic areas — metabolic diseases, neuromuscular diseases and ophthalmological diseases — and 57 different models that have been presented to the COMP are described (FIG. 1).

Data were collected from previously designated OMPs and from protocol assistance (BOX 1) given by the EMA's Scientific Advice Working Party on the recommendation of the Committee for Medicinal Products for Human Use (CHMP). In addition, documents such as the "Summary of product characteristics" within the [European public assessment reports](#) for the OMPs were reviewed. For each mammalian animal model identified,

Box 1 | Orphan medicinal product designation and incentives in the European Union

Regulation (EC) No. 141/2000 lays down a community procedure for the designation of medicinal products as orphan medicinal products and the criteria for the designation of orphan status. The Regulation establishes the Committee for Orphan Medicinal Products (COMP), within the European Medicines Agency (EMA), which is responsible for examining all applications for orphan medicinal product designation submitted to it in accordance with the Regulation.

Criteria for designation

A medicinal product shall be designated as an orphan medicinal product if its sponsor can establish:

- That it is intended for the diagnosis, prevention or treatment of a life-threatening or chronically debilitating condition affecting not more than 5 in 10,000 individuals in the European Community (EC; now the European Union (EU)) when the application is made (“prevalence criterion”)
- That it is intended for the diagnosis, prevention or treatment of a life-threatening, seriously debilitating or serious and chronic condition in the EU and that without incentives it is unlikely that the marketing of the medicinal product in the EU would generate sufficient return to justify the necessary investment (“insufficient return on investment criterion”)

And, in addition:

- That there exists no satisfactory method of diagnosis, prevention or treatment of the condition in question that has been authorized in the EU (“no satisfactory method criterion”)
- Or:
- If such a method exists, that the medicinal product will be of significant benefit to those affected by that condition (“significant benefit criterion”)

Incentives

Protocol assistance. Access to protocol assistance at the EMA is a strong incentive in drug development, in particular for small and medium-sized enterprises. A company can ask questions on the regulatory requirements regarding the quality of the product, the non-clinical aspects of development as well as on the design of the clinical trials that are necessary to fulfil the regulatory requirements for the demonstration of the efficacy and safety of the drug.

The centralized procedure. Regulation (EC) No. 726/2004 states that it is compulsory (as of 20 November 2005) that all orphan medicinal products be authorized via a centralized procedure that gives access to 29 countries in Europe (27 EU member states plus Norway and Iceland), with a reduction in the regular fee.

Market exclusivity. As stated in Article 8 of the Orphan Regulation, the 10-year market exclusivity protects against a “similar” drug being authorized in the EU for the same therapeutic indication. Three derogations from this rule exist: first, the sponsor’s consent; second, a lack of supply; and third, whether a new product — although similar — could be demonstrated to be “clinically superior”: that is, “safer, more effective or otherwise clinically superior” to the product already on the market.

National incentives. Article 9 of the Orphan Regulation calls for national incentives made by the member state. The member states shall communicate to the Commission detailed information concerning measures taken that should be updated regularly and published by the Commission (EU inventory).

Community research programmes. The EU Commission has continually supported research on rare diseases through its framework programmes, from the fifth framework programme (FP5) to the current FP7. The call for proposals in the rare disease area usually includes Europe-wide studies of the natural history of the rare disease, its pathophysiology and the development of preventive, diagnostic and therapeutic interventions.

information was compiled regarding its pathological characteristics, underlying genetic defect (or defects), origin of the specific phenotype displayed and the year when it was first described. Additionally, a search of the NCBI PubMed database was performed to obtain complete information on the different models described. Models described in the literature but not presented to the COMP are also briefly discussed.

Overall, the objective of this article is to highlight the broad range of animal models that are available for studying these rare diseases, from small transgenic rodents to large animals with naturally occurring disease. In addition, the relative merits and limitations of some of the animal models are discussed with the aim of encouraging more efficient and successful research and development of OMPs.

Metabolic diseases

Many lysosomal storage diseases (LSDs) are designated as orphan conditions, and the following LSDs are discussed below: Fabry’s disease, Gaucher’s disease, mucopolysaccharidosis (MPS) disorders, Niemann–Pick disease, Pompe’s disease and α -mannosidosis. These are characterized by deficiencies in normal lysosomal function, which are caused by mutations in genes encoding lysosomal enzymes and the subsequent accumulation of incompletely degraded substrates. Over 40 different genetic LSDs have been described and are generally classified by the nature of the primary stored material.

The underlying causes of LSDs vary from a primary deficiency in lysosomal enzyme function (as in Fabry’s disease and glycogen storage disease) to defective cellular transport of substrates (as in Niemann–Pick disease

types C and D), and are also genetically heterogeneous. Inheritance of such defects is autosomal recessive, with the exception of two X-linked diseases: type II MPS and Fabry's disease². Underlying genetic defects and inheritance are shared in humans and animals. Homologues for some human LSDs (except for type II MPS and Fabry's disease) are observed in common domestic animals, which can be used as models³.

In both animal models and patients with LSDs, high levels of enzyme substrates accumulate in cells. Accumulation can be systemic or tissue-specific (for example, in the spleen and liver in type 1 Gaucher's disease). The appearance of morphologically impaired cells in both humans and animals (that is, foam cells in the liver and lungs) is especially observed in sphingolipid storage disorders. In humans, disease severity varies substantially within each category of a particular disease as well as among the different diseases. Moreover, some diseases become apparent early in life and are fatal (for example, infantile Pompe's disease), whereas other diseases manifest later in life (in adolescence or adulthood) and are less severe (for example, type 1 Gaucher's disease).

Studies in preclinical animal models of LSDs have enabled the development of several therapeutic strategies, including enzyme replacement therapy (ERT) using recombinant forms of the relevant deficient enzyme, substrate deprivation, bone marrow transplantation and gene therapy. In addition to LSDs, acute intermittent porphyria (AIP) and hypophosphatasia are rare inherited metabolic diseases caused by enzyme deficiencies, and some animal models of these two diseases have been developed and used in proof-of-concept studies^{4,5}. Models of rare metabolic diseases that have been presented to the COMP are summarized in TABLE 1 and discussed further below.

Fabry's disease. In Fabry's disease, defects in the lysosomal enzyme α -galactosidase A (encoded by the *GLA* gene) lead to abnormal accumulation of globotriaosylceramide (Gb3), which is normally broken down by GLA. Accumulation of Gb3 results in numerous complications, including neurological (pain), cutaneous (angiokeratoma), renal (proteinuria and kidney failure), cardiovascular (cardiomyopathy and arrhythmia), cochleovestibular and cerebrovascular (transient ischaemic attacks and strokes) complications⁶.

Human and mouse genomic sequences of GLA share high homology. The GLA-knockout mouse model appears normal, with normal blood and urine analyses, a normal adult lifespan and fewer symptoms than seen in humans⁷, but shows progressive accumulation of substrate residues in the kidneys until 5 months of age. In addition, lipid analysis assays demonstrate a marked accumulation of Gb3 in the liver and kidneys. However, there are no obvious histological lesions visible with light microscopy in stained sections of the kidneys, liver, heart, spleen, lungs and brain. Typical lamellar inclusions have frequently been observed by electron microscopy in the lysosomes of Kupffer cells and, to a lesser extent, in hepatocytes from affected mice. In the brain, lamellar inclusions in lysosomes were identified in vascular

smooth muscle cells but not in neuronal or glial cells. Nevertheless, the model mimics major neurological features of the disease, including diminished locomotor activity, balance coordination and hypoalgesia⁸. Compared with wild-type mice, GLA-knockout mice exhibited increased numbers of lamellar bodies within proximal and distal tubular cells and, to a lesser extent, within glomerular epithelial cells and peritubular capillary endothelial cells in the kidney.

Overall, the GLA-knockout model is suitable for the development of ERTs; for example, the effectiveness of a therapy can be monitored by evaluating the clearance of accumulated Gb3 in the organs as well as the neurological features that affect the animals. At present, two ERTs are available to treat Fabry's disease: agalsidase alpha (Replagal; Shire) and agalsidase beta (Fabrazyme; Genzyme). However, one limitation of the model is that it is not suitable for assessing the potential of antibodies developing against recombinant enzymes in humans after treatment by ERT or gene therapies⁹, as there are substantial differences between the mouse and human immune systems. In addition, GLA-deficient mouse models cannot be used to assess the efficacy of active-site-specific chaperones, which have been proposed as a novel strategy for treating Fabry's disease by restoring the normal folding of the mutant GLA¹⁰. Such potential therapies can be evaluated in transgenic mouse models of Fabry's disease, in which the mutant form of the human *GLA* enzyme (R301Q substitution)¹¹ (TABLE 1) or α 1,4-galactosyltransferase (A4GALT; also known as Gb3 synthase) is expressed¹².

Gaucher's disease. Gaucher's disease (of which there are three types) is the most common LSD and is caused by inherited defects in the enzyme β -glucosidase (GBA; also known as β -glucocerebrosidase). It is characterized by the accumulation of glucocerebroside (also known as glucosylceramide) primarily in the lysosomal compartment of macrophages, which leads to organ enlargement (the spleen and liver), bone anomalies (pain and osteonecrosis) and cytopaenia in type 1 Gaucher's disease — the most frequently occurring type of the disease. Type 2 and type 3 Gaucher's disease also have the characteristics of type 1 Gaucher's disease but with additional neurological complications. The severity of Gaucher's disease is extremely variable, ranging from asymptomatic to early-diagnosed severe forms that are fatal. Several options are available for the treatment of type 1 and type 3 Gaucher's disease, including ERTs (imiglucerase (Cerezyme; Genzyme), velaglucerase alfa (Vpriv; Shire) and taliglucerase alfa (Elelyso; Pfizer)), bone marrow transplantation, and miglustat (Zavesca; Actelion) (discussed further below). There is currently no treatment available for type 2 Gaucher's disease.

Initial attempts to create knockout mouse models of Gaucher's disease with a GBA deficiency failed to establish a viable model¹³, as these mice show extensive lysosomal glucocerebroside storage but die within 24 hours of birth. In the early 2000s, there were no suitable direct animal models of Gaucher's disease for developers to test investigational therapeutics and so animal models

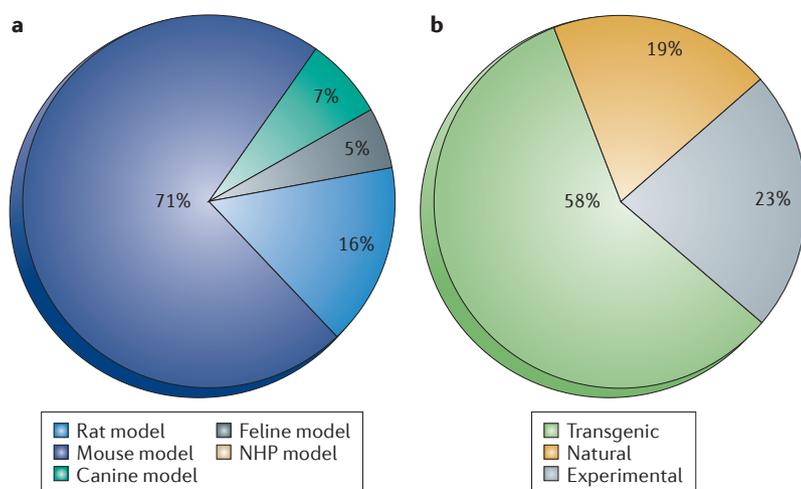


Figure 1 | Animal models presented to the European Medicines Agency's Committee for Orphan Medicinal Products. **a** | Proportion of animal model species for metabolic diseases, neuromuscular diseases and ophthalmological diseases designated as orphan conditions (overall 57 different models). **b** | Proportion of animal models following their origin for the three selected therapeutic areas: natural models (existing disease), transgenic models (inbred disease) or experimental models (induced disease). NHP, non-human primate.

of other LSDs were used for proof-of-concept studies for Gaucher's disease. For instance, miglustat, a small-molecule drug that inhibits the synthesis of multiple glycosphingolipids (including glucocerebroside) by the enzyme glucosylceramide synthase, was evaluated in mouse models of Tay–Sachs disease and Sandhoff disease¹⁴. Miglustat is approved in the European Union and the United States for treating type 1 Gaucher's disease and is also approved in the European Union and Japan for the treatment of type C Niemann–Pick disease.

The mouse models of Tay–Sachs disease¹⁵ and Sandhoff disease¹⁶ (TABLE 1) are models of the inherited LSDs GM2 gangliosidoses with different degrees of neurological impairment¹⁶. These two mouse models share biochemical and pathological features with type 2 and type 3 Gaucher's disease, and could have applications in the development of therapies targeting LSDs in general. Mice with defects in the lysosomal enzyme acid β -galactosidase also share similar features to Gaucher's disease, in which the accumulation of the substrates GM1-ganglioside and GA1 in gangliosides within the central nervous system (CNS) mimics the pathobiological abnormalities of human GM1 gangliosidosis¹⁷ (TABLE 1).

During the past decade, the COMP has observed a growing number of Gaucher's disease-specific transgenic mice being used in applications for OMPs. These mouse models have enhanced the understanding of the pathophysiology of the condition as well as the development of novel therapies¹⁸. Initial attempts used single-insertion mutagenesis to introduce human disease mutations into the mouse *Gba* gene, resulting in models of type 2 and type 3 Gaucher's disease¹⁹ (TABLE 1); however, the mice did not survive beyond 48 hours after birth. An important advancement was the generation of the *Gba*^{L444P/L444P}

transgenic mouse model, which enabled the mice to survive for up to 1 year²⁰. Although *Gba*^{L444P/L444P} mice did not manifest excess storage of glucocerebroside, they did exhibit several characteristics in common with the human form of the disease, such as a multisystem inflammatory reaction, decreased levels of GBA in several organs, moderate increases in the mass of the liver and spleen, as well as elevated plasma levels of chitin III (the mouse homologue of human chitotriosidase) and immunoglobulin G (IgG). The accumulation of glucocerebroside in the brain of this mouse model could make it suitable for studying therapies for neuropathic forms of Gaucher's disease, and this approach has been interpreted as a positive proof of concept model at the time of designation by the COMP (in the absence of further data). Nevertheless, the results provided by this animal model have to be evaluated with the knowledge that the abnormal accumulation of glucosylceramide in the liver, spleen or brain of mutant mice might not be detectable by thin-layer chromatography analysis, and this aspect of the animal model therefore needs to be further clarified.

In one study, four mouse models of Gaucher's disease were generated in which the following point mutations were introduced into the *Gba* locus: N370S, V394L, D409H and D409V²¹ (TABLE 1). In these models, the appearance and clearance of glucocerebroside storage in cells could be used as efficacy parameters for the development of inhibitors of glucosylceramide synthase. In another study, the level of residual activity of GBA needed to correct the neurological aspects of Gaucher's disease in D409H mice (in which point mutations were introduced in *Gba*) was evaluated²², thereby providing insights into how this could be achieved in humans. In another effort to model type 1 Gaucher's disease, a chimeric mouse model was generated in which aberrant glycolipid storage in the reticuloendothelial system was restored²³. This chimeric mouse model has not been used in COMP applications, but illustrates the continued efforts to develop more relevant and viable models of this disease.

MPS disorders. MPS disorders are caused by genetic mutations that result in the loss of function of enzymes involved in the degradation of glycosaminoglycans. There are several distinct medical types of MPS disorders, which are categorized according to the clinical features of the disease²⁴. There are currently three therapies — all of which are ERTs — available for MPS: laronidase (Aldurazyme; Biomarin/Genzyme) for type I MPS (also known as Hurler syndrome); idursulphase (Elaprase; Shire) for type II MPS (also known as Hunter syndrome); and galsulphase (Naglazyme; BioMarin) for type VI MPS.

There are several animal models available for each type of MPS, and some of these have been used to support OMP applications (for type I, II, IIIA and VI MPS; see TABLE 1). Naturally occurring feline and canine homologues have been described for all types of MPS except for type IVA MPS (also known as Morquio A disease); these homologues have many phenotypic similarities to the human disease and are thus useful for evaluating the efficacy of ERTs. More recently, transgenic models have

been developed for some MPS disorders and have proved to be useful for preclinical studies of more novel therapeutics as well as for disease characterization.

For type I MPS, which is caused by a deficiency in α -L-iduronidase, there are naturally occurring feline²⁵ and canine homologues²⁶, and more recently an immunodeficient mouse model of type I MPS was specifically generated to evaluate human stem cell and gene therapies²⁷ (TABLE 1). For other types of MPS, such as iduronate sulphatase deficiency (type II MPS), *N*-sulphoglucosamine sulphohydrolase (SGSH; also known as heparan-*N*-sulphatase) deficiency (type IIIA MPS) and arylsulphatase B deficiency (type VI MPS), animal models that have been chosen for the preclinical evaluation of therapeutics have included mouse models (transgenic or naturally occurring)^{28,29} or larger animal models (naturally occurring)^{30,31}, depending on the strategy being tested.

As there are no naturally occurring animal homologues of type IVA MPS, mouse models have been engineered to mimic the deficiency in *N*-acetylgalactosamine-6-sulphatase (GALNS)^{32,33} (TABLE 1). In the first attempt²³, homozygous *Galns*^{-/-} mice were generated that had excess lysosomal storage in organs, similar to the human form of the disorder, but these mice lacked the skeletal aspects of the disease and they generated immune responses to infusions of human GALNS. So, the same group generated a transgenic mouse model in which tolerance to human GALNS was induced through the ubiquitous expression of an inactive form of human GALNS²⁴. This immunotolerant model had a phenotype that was more similar to the human form of the disease than the original knockout model, and immunological reactions to purified human GALNS were not observed for the duration of the study (3 months).

Niemann–Pick disease. There are four types of Niemann–Pick disease, which are caused by the lack — or very low activity — of the lysosomal enzyme sphingomyelin phosphodiesterase 1 (SMPD1; also known as acid sphingomyelinase) or deficiencies in intracellular lipid trafficking. Types A and B Niemann–Pick disease are caused by a mutation in *SMPD1*, whereas type C Niemann–Pick disease is caused by mutations in Niemann–Pick C1 protein (*NPC1*) or *NPC2*. Type D Niemann–Pick disease was originally classified as a separate form of Niemann–Pick disease, as it is found only in a particular French Canadian population; however, it has the same genetic cause as type C and therefore falls under this category. There are natural (feline)³⁴ and transgenic (murine)³⁵ models of type C Niemann–Pick disease, both of which show neurological signs of the disease. In particular, the symptoms (tremors and hindlimb dysfunction) in the mouse model are similar to the clinical manifestations observed in patients with the disease, and this model was used for the development of miglustat, which is currently the only approved treatment for any of the Niemann–Pick disease types.

So far, proof-of-concept studies in mice deficient in SMPD1 (REF. 36) indicate that ERT should be an effective therapeutic approach for type B Niemann–Pick disease but it is unlikely to prevent the severe neurodegeneration associated with type A Niemann–Pick disease³⁷.

Pompe's disease. Mutations in the enzyme encoding the lysosomal enzyme α -glycosidase (GAA) cause Pompe's disease (also known as glycogen storage disease type II), which has symptoms that include an enlarged heart, respiratory difficulties and muscle weakness. There is currently one therapeutic available for Pompe's disease: the ERT α -glucosidase alfa (Myozyme; Genzyme). A gene replacement therapy — a recombinant adeno-associated viral (AAV) vector containing human GAA — had received orphan designation in Europe, but this was withdrawn by the sponsor. Both of these therapeutics were evaluated in the same *Gaa*-knockout mouse model (first described in 1998)³⁸, in which positive results were observed for the clearance of glycogen stored in the diaphragm as well as in cardiac and skeletal muscle³⁹ (see the “Summary of product characteristics” within the European public assessment report for α -glucosidase alfa for more details). This model is relevant for late-onset Pompe's disease and also reflects the neuronal pathology observed in the juvenile form of the disease⁴⁰.

With regard to other animal models of Pompe's disease, there are several naturally occurring homologues of the disease in large animals, including Brahman and shorthorn cattle, Lapland dogs, cats and sheep. The disease in the Lapland dog appears to be similar to the human infantile-onset disorder owing to its early onset and major cardiac involvement⁴¹. However, as noted in a study that examined the CNS effects in the *6^{neo}/6^{neo}* mouse model of Pompe's disease⁴² (in which *Gaa* expression is disrupted by the insertion of a neomycin resistance gene (*neo*) in exon 6), larger animals are not practical for evaluating therapeutics owing to the limited availability of such animals for studies. This study also highlights the utility of the *6^{neo}/6^{neo}* model in the assessment of ERT efficacy in the CNS, which remains a limitation of the marketed treatment. Notably, the study also demonstrated that the variability in the symptoms observed in genetic mouse models of Pompe's disease, generated by targeted disruption of the *Gaa* gene, is probably due to the genetic background of the mouse strains⁴².

α -mannosidosis. α -mannosidosis is an autosomal recessive LSD in which oligosaccharides accumulate as a result of a deficiency in the enzyme α -mannosidase. It is characterized by immune deficiency, skeletal abnormalities and progressive motor dysfunction. At present, there are no approved specific treatments for the disease.

There are at least three unrelated animal models for human α -mannosidosis, with the best known being the bovine model of mannosidosis: an autosomal recessive inherited disorder found in Aberdeen Angus cattle⁴³. Additionally, α -mannosidosis has been found in domestic short-haired cats and has similar clinical findings as human α -mannosidosis: that is, multiple skeletal deformities, retarded growth, ataxia, intention tremors (also known as cerebellar tremors; tremors that worsen with voluntary movement) and a deficiency in α -mannosidase activity⁴⁴. Finally, a mouse model of α -mannosidosis has been generated by the targeted disruption of the gene encoding lysosomal α -mannosidase⁴⁵ (TABLE 1) and it has been used as a proof-of-concept model for ERT⁴⁶.

Table 1 | Animal models used for proof-of-concept studies for rare metabolic diseases presented to the COMP

Model (year of description)	Method of generation	Phenotype and progression	Key factors for translational applicability
Fabry's disease			
Transgenic knockout mouse model ⁷ (1997)	Disruption of <i>GLA</i> by homologous recombination	Marked accumulation of ceramide trihexoside in the liver and the kidneys after 10 weeks of age; diminished locomotor activity and alteration of sensorimotor functions	Relevant neurological phenotype
Transgenic mouse model expressing human mutant enzyme ¹¹ (2004)	Introduction of human mutant enzyme (R301Q mutation) into <i>GLA</i> -knockout mice	Diminished enzyme activity in the heart, kidney, spleen and liver; accumulation of different α -galactosidase A substrate	Expression of the human enzyme
Gaucher's disease			
Transgenic mouse models with varied phenotypes ²¹ (2003)	Point mutations in N370S, V394L, D409H or D409V introduced into the mouse <i>Gba</i> locus	Enzyme produced is catalytically defective and unstable; only small amounts of glucosylceramide accumulation (none in brain); storage cells appear, especially in the lung	Clear signs of substrate accumulation
Transgenic <i>Gba</i> ^{L444P/L444P} mouse model ¹⁹ (1998)	Point mutation L444P introduced into the mouse <i>Gba</i> locus	Decreased levels of GBA similar to human disease; lack of neuronal and visceral glucosylceramide storage; absence of Gaucher cells	Clinical signs of inflammation
Transgenic mouse model of GM1 gangliosidosis ¹⁷ (1997)	Homologous recombination and embryonic stem cells deficient in β -galactosidase	Defects in GM1 ganglioside-hydrolysing capacity; storage materials already conspicuous in the brain at 3 weeks, but show no overt clinical phenotype for up to 4–5 months	Glucocerebroside accumulation measurable
Tay–Sachs disease transgenic mouse model of GM2 gangliosidosis ¹⁵ (1994)	Homologous recombination and embryonic stem cells deficient in β -hexosaminidase subunit- α (<i>Hexa</i> ^{-/-})	Biochemical and pathological features of the disease, but no neurological abnormalities as observed in human disease	Same symptomatology as in Gaucher's disease
Sandhoff disease transgenic mouse model of GM2 gangliosidosis ¹⁶ (1995)	Homologous recombination and embryonic stem cells deficient in β -hexosaminidase subunit- β (<i>Hexb</i> ^{-/-})	Neurological signs clearly severe, but differences in the ganglioside degradation pathway between mice and humans	Same symptomatology as in Gaucher's disease
Type I MPS disorder			
Immunodeficient mouse model of type I MPS ²⁷ (2007)	Homozygous <i>Idua</i> ^{-/-} mice bred from NOD.129(B6)- <i>Prkdc</i> ^{scid} <i>Idua</i> ^{tm1Clk} mice heterozygous for the IDUA mutation	Progressive development of morphological features (4 months) and biological signs of lysosomal storage	Clear morphological impairment; mice less likely to develop immune reactions to transplanted human or gene-corrected cells or secreted IDUA
Feline model ²⁵ (1983)	A naturally occurring three-base-pair deletion in the <i>IDUA</i> gene	Skeletal disease is not as significant, although feline models have facial deformity, lameness, corneal opacity and cardiac murmurs	Relevant disease phenotype
Canine model ²⁶ (1984)	A naturally occurring null mutation causing mRNA retention of intron 1 in the <i>IDUA</i> gene	Similar to the human disease, but the dogs show thickening and prolapse of the third eyelid (<i>membrana nictitans</i>), joint laxity instead of joint stiffness and no obvious cognitive impairment	Naturally occurring mutation, relevant disease phenotype
Type II MPS disorder			
Transgenic knockout mouse model ²⁸ (2002)	Homozygous <i>Ids</i> ^{-/-} mice	Progressive accumulation of glycosaminoglycans in many organs and excretion in urine; neuropathological defects and deformities	Relevant disease phenotype
Type IIIA MPS disorder			
Neonatal mouse model ²⁹ (1999)	Naturally occurring heterozygous mutation in <i>Sgsh</i>	1–3% of normal SGSH activity; neuropathological changes resembling human phenotype; mice usually die at 7–10 months of age	Naturally occurring mutation, relevant disease phenotype
Canine model ³⁰ (2007)	Naturally occurring mutation: single base insertion in the <i>SGSH</i> gene	Extensive CNS pathology by 12–18 months of age associated with cognitive dysfunction; reflects relatively long course of the disease	Naturally occurring mutation, relevant disease phenotype
Type IVA MPS disorder			
Transgenic knockout mouse model ³² (2003)	Exon 2 of <i>Galns</i> gene disrupted	Lysosomal storage is present (at 2 months of age) but no change is observed in skeletal bones of mice (up to 12 months old)	Substrate accumulation

Table 1 (cont.) | Animal models used for proof-of-concept studies for rare metabolic diseases presented to the COMP

Model (year of description)	Method of generation	Phenotype and progression	Key factors for translational applicability
Transgenic tolerogenic mouse model ³³ (2005)	Homologous recombination and embryonic stem cells containing point mutations in <i>GALNS</i> cDNA (C79S) and in <i>Galns</i> (C76S): <i>Galns</i> ^{tm(hC79S-mC76S)slu} mouse	Many similarities to human type IVA MPS, with obvious bone storage; reduction in enzyme activities of other sulphatases	Relevant disease phenotype
C76S point mutation mouse model ³³ (2005)	C76S active site mutation in <i>Galns</i> (model in development at time of publication and presented to the COMP during development)	No detectable GALNS activity; mice display fewer pathological findings compared to tolerant mouse	Attenuated form of the disease
Type VI MPS disorder			
Feline model ³¹ (1996)	Heterozygous mutation in arylsulphatase B	Presence of storage vacuoles; decrease in bone mineral volume; problems in mobility and some neurological symptoms are observed	Spontaneous disease in the model
Types A and B Niemann–Pick disease			
Transgenic SMPD1-deficient knockout mouse model ³⁶ (1995)	Gene targeting and embryonic stem cell transfer	5-month-old mice die prematurely; clinical, biochemical and pathological attributes mimic both human type B and type A Niemann–Pick disease	Relevant disease phenotype
Type C Niemann–Pick disease			
Mouse model ¹⁴⁰ (1980)	Spontaneous mutation in <i>Npc1</i> in BALB/c mice	Symptoms (tremors, hindlimb dysfunction) by 4–5 weeks of age; death by inanition at 70–80 days of age; resembles clinical manifestations in the human disease	Relevant disease phenotype
Feline model ³⁴ (1994)	Naturally occurring NPC1 missense mutation	Less severely affected than the NPC mouse; similar age of onset (8–12 weeks of age); progression of clinical neurological signs; autosomal recessive inheritance as in human form of the disease	Relevant neurological phenotype
Pompe's disease			
Transgenic knockout mouse model ³⁸ (1998)	Targeted disruption of the acid α -glycosidase gene (<i>Gaa</i>)	Recapitulates critical features of both infantile and adult forms of the disease; by 8–9 months of age, animals develop obvious muscle wasting and a weak, waddling gait	Relevant disease phenotype
α-mannosidosis			
Transgenic mouse model ⁴⁵ (1999)	Disruption of the gene for lysosomal α -mannosidase	Increase in oligosaccharides is observed in the kidney, liver and spleen	Substrate accumulation
Acute intermittent porphyria			
Transgenic mouse (T1) model ⁴⁸ (1996)	In homozygous animals: <i>Pbgd</i> ^{-/-} mouse generated by inserting the <i>neo</i> gene in antisense direction into SacII site of first exon	55.3% loss of PBGD activity in the liver, with subsequent biochemical perturbations	Intermediate model
Transgenic mouse (T2) model ⁴⁸ (1996)	Heterozygous animals: <i>Pbgd</i> ^{-/-} mouse generated by inserting a splice acceptor site in front of the coding sequence of the <i>neo</i> gene, which is then inserted into the first intron of <i>Pbgd</i>	56.6% loss of PBGD activity in the liver, with subsequent biochemical perturbations	Intermediate model
Transgenic mouse model of acute intermittent porphyria: crossbreeding of T1 mouse with T2 mouse ⁴⁸ (1996)	Compound heterozygote of T1 and T2 mouse models	30.7% loss of PBGD activity in the liver; biochemical abnormalities in the haem pathway, motor impairments and long-term neurohistological sequelae are observed with age	Relevant disease phenotype
Hypophosphatasia			
Transgenic mouse model ⁵¹ (1997)	Homologous recombination and embryonic stem cells containing TNSALP knockout	Mimics a severe form of hypophosphatasia; abnormal growth, defects in bone mineralization and abnormal tooth dentin; epileptic seizures and apnoea; die before weaning at approximately day 21; elevated plasma concentrations of intermediate products (such as PLP and PPI)	Severe phenotype and premature death of the model

CNS, central nervous system; COMP, Committee for Orphan Medicinal Products; GALNS, *N*-acetylgalactosamine-6-sulphatase; *Gba*, β -glucocerebrosidase gene; *GLA*, α -galactosidase A gene; *IDS*, iduronate 2-sulphatase; *IDUA*, α -L-iduronidase; *neo*, neomycin resistance gene; MPS, mucopolysaccharidosis; NPC1, Niemann–Pick C1 protein; PBGD, porphobilinogen deaminase; PLP, pyridoxal 5'-phosphate; PPI, inorganic pyrophosphate; PRKDC, protein kinase DNA-activated catalytic polypeptide; SGSH, *N*-sulphoglucosamine sulphohydrolase (also known as heparan-*N*-sulphatase); SMPD1, sphingomyelin phosphodiesterase 1; TNSALP, tissue-nonspecific alkaline phosphatase.

The experience gained by sponsors in developing a recombinant enzyme for ERT using mouse models of LSDs supports the choice of rodents in proof-of-concept studies. In addition to mouse models, feline models are of particular interest for CNS disorders such as α -mannosidosis, as feline brains have been well characterized both functionally and physiologically and, indeed, have been used to evaluate gene therapy for α -mannosidosis⁴⁷.

Acute intermittent porphyria. AIP is a rare autosomal dominant metabolic disorder that affects the production of haem, the oxygen-binding prosthetic group of haemoglobin. It is characterized by a deficiency of the enzyme porphobilinogen deaminase (PBGD), which leads to an accumulation of haem precursors. Patients suffer from neurovisceral attacks (intense abdominal pain with neurological and/or psychological symptoms)⁵. Treatment options consist of the administration of carbohydrates, haemin (an iron-containing porphyrin) or haem arginate, depending on the severity of the attack.

Three separate transgenic mouse models for AIP with mutations in the *PBGD* gene, which are deficient to various degrees in PBGD activity, have been reported⁴⁸ (TABLE 1). The transgenic mice are either homozygous or heterozygous, or a cross of the two former strains. These mice have enabled the characterization of the disease in terms of the accumulation and excretion patterns of the haem precursors, as well as the effects of ERT⁴⁹. Mice develop motor dysfunction and peripheral neuropathological features that closely mimic those observed in the human form of the disease. Another available animal model is the naturally occurring feline model of AIP⁵⁰.

Hypophosphatasia. Hypophosphatasia is a rare inherited metabolic bone disease caused by a deficiency in tissue-nonspecific alkaline phosphatase (TNSALP; also known as AKP2) in osteoblasts and chondrocytes, resulting in impairment of bone mineralization. Symptoms vary widely — from very mild to fatal — and affect all ages. There are currently no specific treatments approved for hypophosphatasia.

The *Tnsalp*^{-/-} mouse model⁵¹ mimics a severe form of hypophosphatasia, and the abnormal biochemical parameters of the phenotype (elevated concentrations of plasma pyridoxal-5'-phosphate, urinary inorganic pyrophosphate and urinary phosphoethanolamine) were used as clinical readouts for evaluating the efficacy of an ERT⁵².

Neuromuscular diseases

Neuromuscular diseases comprise a heterogeneous group of conditions with different causes and pathological mechanisms. Conditions that have been designated as orphan diseases include: amyotrophic lateral sclerosis (ALS); Huntington's disease; the neuromuscular junction disorders myasthenia gravis, Lambert–Eaton myasthenic syndrome (LEMS) and Guillain–Barré syndrome (GBS); sarcoglycanopathies; and calpainopathy. Riluzole (Rilutek; Sanofi), which is used for treating ALS, is the only drug that has been specifically approved for treating

a rare neuromuscular disease, although its mechanism of action is unclear. All of the animal models that have been presented to the COMP for neuromuscular diseases are rodent models, with the exception of a canine model for myasthenia gravis (TABLE 2).

Amyotrophic lateral sclerosis. ALS is a progressive disease of the nervous system, and its molecular basis and mechanism of pathology are complex⁵³. Two broad forms of ALS have been described: familial ALS and sporadic ALS; the latter comprises 90% of cases. Common phenotypic features include muscle weakness leading to paralysis, and the symptoms of ALS vary widely among patients.

Out of all the applications submitted to the COMP for ALS, the most common have been those involving mice that express a mutated form of the human gene that encodes superoxide dismutase 1 (SOD1), which is used as a model for familial ALS. Different strains of such mice are available and their characteristics are well described in terms of enzyme activity and gene structure⁵⁴. However, only 20% of cases of human familial ALS are caused by missense mutations in *SOD1* (REF. 55). It has been demonstrated that SOD1-mediated toxicity in ALS may not be caused by the loss of its catalytic activity but instead by a gain of function that confers one or more toxic properties independently of the levels of SOD1 activity⁵⁶.

The SOD1 animal model of ALS recapitulates elements of both the phenotype and the histopathology observed in patients. As in other neuromuscular degenerative disorders (for example, Parkinson's disease and Huntington's disease), misfolded protein aggregation is observed in SOD1-mutant mice, leading to changes in neurofilament composition. Mutant protein is expressed in tissue, supporting the acquired toxic function of mutant SOD1. The other clinical signs of the disease in these models include muscular fibrillations and atrophy, synaptic retraction, mitochondrial alterations, loss of motor neurons and reduced lifespan⁵⁷.

However, the need for high-level expression of mutant SOD1 in the CNS to cause the degeneration of motor neurons in mice is contrary to what is observed in patients with ALS, in whom a single copy of the mutant gene is sufficient to induce familial ALS⁵⁸. In addition, translation of mouse studies into clinical trials has not taken into account factors such as the dose, mode of administration of the drug or product being tested and penetration of the blood–brain barrier⁵⁸. Finally, because SOD1-mutant mice represent only a small subgroup of ALS, in our opinion this model has a limited predictive value. However, when considering other models proposed by sponsors to the COMP (TABLE 2), at present none appears to be better than the SOD1 model.

Animal models deficient in other genes that confer familial ALS also have limitations, as discussed in the literature. For example, mice deficient in *alsin* have defects in upper neurons rather than developing the human spastic paraplegia phenotype; however, they still have utility for studying the alterations in neuronal

physiology that occur before cellular death⁵⁹. In another example, a mouse model with dynactin mutations exhibits a motor neuron disease phenotype (muscle weakness accompanied by muscle wasting in hindlimbs); however, the histopathological hallmarks of ALS are missing and disease progression in these mice is variable⁶⁰.

There are fewer animal models for sporadic ALS than for familial ALS, and these are non-genetic spinal degeneration models. The excitatory neurotransmitter glutamate is widely distributed in the mammalian CNS; an excess of glutamate leads to excessive excitotoxic glutamate transmission, which is a relevant mechanism of spinal motor neuron degeneration in ALS. The main limitation of these animal models is the rapid onset of symptoms observed in rats after the injection of the excitotoxic agent (AMPA; α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid), whereas in patients these symptoms generally appear at the terminal stage of the disease⁶¹.

Because symptoms in ALS are similar to those observed in other motor neuron diseases, transgenic animal models of other diseases may be used to evaluate therapeutics for ALS. For example, the COMP has reviewed the Wobbler mouse (autosomal recessive Wobbler mutation)⁶² and progressive motor neuropathy mouse (*pnm* mouse; autosomal recessive mutation) for evaluating OMPs that are being developed for ALS⁶³. However, these mouse models display limited phenotypes and disease progression does not reflect that observed in ALS.

Nerve-damaged rats (axotomy in neonatal rats and sciatic nerve axotomy in Sprague Dawley rats, normal rats and diabetic rats) have also been presented to the COMP and are a well-characterized reversible model of motor neuron diseases; they are useful for monitoring the improvement of neuronal functions, motor coordination and muscle strength^{64,65}.

In summary, to increase the likelihood that the activity observed in animal models (in proof-of-concept studies for ALS) is translated to patients in clinical trials, it is important to consider using different models to cover the different types of ALS as well as the different symptoms, and also to consider using complementary *in vitro* experiments.

Huntington's disease. Huntington's disease is caused by an expanded CAG repeat in the huntingtin (*HTT*) gene, and is characterized by the formation of inclusions of the mutant HTT protein and cytoplasmic aggregates in neurons. These aggregates ultimately lead to symptoms such as involuntary movements, behavioural disturbances and mental deterioration appearing during the fourth or fifth decade of life.

Various transgenic approaches based on introducing mutant versions of *HTT* have been used to model Huntington's disease in mice. The R6/2 fragment mouse model⁶⁶ (TABLE 2), in which the mutant gene is inserted randomly into the mouse genome, leading to simultaneous expression of mutant HTT and native HTT, has been used in the preclinical evaluation of various potential OMPs, and is readily available commercially. As with the

human form of the disease, neurological impairments are observed in this animal model; however, neuronal degeneration is not seen before the premature death of the mice at week 15. In addition, the R6/2 mice do not show a clear cell death process as observed in humans or in the YAC128 (yeast artificial chromosome 128) mouse model (discussed below). This could explain the difficulties associated with translating the results obtained in this model to human Huntington's disease⁶⁶. Another mouse model engineered with the same approach is the N-171 mouse model, but this has not yet been used in the preclinical development of candidates designated as OMPs⁶⁷.

The YAC128 murine model (TABLE 2), which expresses the entire human mutant *HTT* gene, shows a more robust and uniform disease phenotype than the R6/2 model, with age-dependent striatal loss and subsequent cortical degeneration⁶⁸. Nuclear localization of human mutant HTT occurs at an early stage and extensively in the striatum, simultaneously with the appearance of behavioural abnormalities, and is thus consistent with the regional selectivity of human Huntington's disease. The model is appropriate for preclinical studies of potential therapies owing to its fidelity to the pathological mechanisms observed in patients, thereby enabling the definition of clear experimental end points (progression of the disease and survival). However, the variability and slow developmental phenotype of YAC128 mice must be taken into account when using this animal model⁶⁹.

A knock-in mouse model has also been developed in which an expanded CAG repeat is inserted into the endogenous mouse *Htt* gene⁷⁰. This is possible because the genomic sequence of human HTT is well conserved in mice. Consequently, the expression of HTT is controlled under the endogenous promoter. However, these mice display very late disease onset (after 10–18 months) and cell death has not been reported, which is consistent with their less severe phenotype⁷¹.

As with other neurodegenerative diseases (such as ALS and Parkinson's disease), an excitotoxic lesion model of Huntington's disease is also available for study⁷² (TABLE 2). Direct injection of excitatory amino acids into the striatum leads to neuronal death associated with neuropathological features that closely resemble Huntington's disease, such as hyperkinesias, impaired motor skills and deficits in spatial maze learning. In addition, this approach is feasible in transgenic rats and in mouse models of Huntington's disease (except for the R6/2 mouse, which is resistant to excitotoxicity)⁷². An indirect excitotoxic lesion model, using various mitochondrial toxins such as 3-nitropropionic acid (3-NP), is often cited in the literature despite the high inter-animal variability and the high incidence of gross nonspecific striatal damage⁷³. This protocol has been applied to larger animals, such as non-human primates, for the development of gene therapies⁷⁴. Even more promising is the recent development of a transgenic primate model that develops an aggressive form of Huntington's disease. This model could fulfil the need for a large animal model of the disease⁷⁴, despite the lack of efficiency of gene transfer during the protocol⁷⁵.

Table 2 | **Animal models used for proof-of-concept studies for rare neuromuscular diseases presented to the COMP**

Model (year of description)	Method of generation	Phenotype and progression	Key factors for translational applicability
Familial ALS			
Transgenic mouse model ¹⁴¹ (1994)	Many possible missense point mutations in <i>SOD1</i>	Muscle weakness and impairments in leg extension appear within a few days, but age of onset varies with transgene copy number; duration of symptoms is about 1 month or longer (70 days)	Motor neurons are preferentially affected, but the pathogenesis is different from the human disease
Sporadic ALS			
Rat model of nerve damage ¹⁴² (1995)	Axotomy in neonatal rats, sciatic nerve axotomy in Sprague Dawley rats	Compression or chemical injury leading to loss of neuronal cells after 3 days; development of neuropathological features of ALS	Well-characterized reversible model
Spontaneous motor neuron death: <i>pnm</i> mouse model ⁶³ (1991)	Missense mutation in the <i>Tbce</i> gene	Progressive degeneration of motor axons and death a few weeks after birth; however, the pathophysiology of motor neuron death in these animals is not the same as in human ALS	Limited phenotype, inaccurate progression of the disease
Transgenic mouse model of spinal muscular atrophy ¹⁴³ (2000)	Mutation in the <i>SMN</i> gene	Degeneration of motor neurons and pathological changes in the spinal cord and skeletal muscles	Same symptomatology as in human ALS
Huntington's disease			
Transgenic R6/2 mouse model ⁶⁶ (1996)	Overexpression of exon 1 of the human <i>HTT</i> gene	Rapid and progressive neuropsychomotor phenotype; decreased survival and classic histopathology including atrophy of the striatum and the presence of intranuclear aggregates	Partially relevant disease phenotype
Transgenic YAC128 mouse model ⁶⁸ (2005)	Introduction of YAC containing the entire human <i>HTT</i> gene with 128 CAG repeats	Clinical signs of Huntington's disease resemble the disease in humans: for example, motor deficits (at 6 months of age), premature death (at 12 months of age) and loss of striatal volume owing to selective atrophy and neuronal loss	Same pathogenesis as in human disease
Rat model of cognition and memory dysfunction ¹⁴⁴ (1996)	Selective inhibition of the cholinergic system <i>in vivo</i>	Long-term neurochemical deficit at cholinergic nerve terminals, leading to degeneration of neurons	Neurodegeneration is not truly progressive
Rat model of direct excitotoxicity ⁷² (2006)	Injection of excitatory amino acids (quinolinic acid) into the striatum	Axon-sparing striatal lesions, leading rapidly to hyperkinesias, impaired motor skills and deficits in spatial maze learning; no dyskinesia or chorea-like movements	Heterogeneous phenotype between animals
Myasthenia gravis			
Rat model of experimental autoimmune myasthenia gravis ¹⁴⁵ (1975)	AChR ₁₆₋₆₇ conjugated to KLH obtained from eel (<i>Torpedo californica</i>) injected to Lewis rats	Autoantibody responsible for self-mediated destruction or modulation of the neuromuscular junction; similar to the disease in humans; positive inhibition by pyridostigmine	Relevant disease phenotype
Mouse model of experimental autoimmune myasthenia gravis ¹⁴⁶ (1994)	Synthetic peptide sequences of human AChR α -subunit injected into BALB/c mouse	Impairment of neuromuscular transmission after antibody self-mediated destruction or modulation of the neuromuscular junction	Relevant disease phenotype
Mouse model of experimental autoimmune myasthenia gravis ¹⁴⁷ (1997)	Myastheniogenic peptides: Lys262–Ala207 injected into BALB/c mouse	Immunomodulatory effects transferred into naive mice by inoculation with splenocytes from previously treated mice, leading to impairment of neuromuscular transmission	Relevant disease phenotype
Canine model ⁷⁸ (2007)	Spontaneously acquired autoimmune AChR-antibody-positive myasthenia gravis	Similarities to human myasthenia gravis: natural occurrence of the disorder, clinical presentation, diagnostic (AChR autoantibodies) co-morbidity and shared environment with humans and dogs (as pets)	Relevant disease phenotype
α-sarcoglycanopathy			
<i>Sgca</i> -null male mouse model ⁸³ (1998)	Homologous recombination and embryonic stem cells lacking <i>Sgca</i> gene	Phenotype very similar to the human condition, with a severe pattern of regeneration–degeneration cycles	Relevant disease phenotype
γ-sarcoglycanopathy			
<i>Sgcg</i> -null mouse model ¹⁴⁸ (1998)	<i>Sgcg</i> ^{-/-} knockout mouse	Progressive muscle hypertrophy and weakness with age; symptoms of dystrophic muscles in mice over 1 year of age	Relevant disease phenotype

Table 2 (cont.) | Animal models used for proof-of-concept studies for rare neuromuscular diseases presented to the COMP

Model (year of description)	Method of generation	Phenotype and progression	Key factors for translational applicability
Calpainopathy			
Transgenic Δ ex2–3 mouse model ⁸⁶ (2003)	Homologous recombination to produce knockout mice (<i>Capn3</i> ^{-/-} mice)	Significant global atrophy starting at 3 months and persisting later on in females, accompanied by pathological changes characteristic of muscular dystrophy (area of necrosis and regeneration in muscles)	Relevant disease phenotype
Knock-in transgenic mouse model ⁸⁷ (2007)	Complete disruption of <i>Capn3</i>	Complete inactivation of CAPN3 expression; mice are viable and fertile, and display normal activity; global atrophy increases with age, reaching a significant level at 9 months of age	Relevant disease phenotype

AChR, acetylcholine receptor; ALS, amyotrophic lateral sclerosis; CAPN3, calpain 3; COMP, Committee for Orphan Medicinal Products; HTT, huntingtin; KLH, keyhole limpet haemocyanin; PMN, progressive motor neuropathy; SGCA, sarcoglycan- α protein; SGCG, sarcoglycan- γ protein; SMN, survival of motor neuron; SOD1, superoxide dismutase 1; TBCE, tubulin-specific chaperone E; YAC, yeast artificial chromosome.

Neuromuscular junction disorders. Myasthenia gravis is an autoimmune disease in which antibodies directed against the nicotinic acetylcholine receptor (AChR) lead to neuromuscular transmission defects, culminating in weakness and fatigability⁷⁶. GBS is an acute autoimmune-mediated neuropathy that leads to peripheral nerve demyelination and axonal damage, whereas LEMS is a disorder characterized by neuromuscular transmission with pathogenic circulating antibodies directed against voltage-gated calcium channels.

Animal models of experimental autoimmune myasthenia gravis (EAMG) can be induced in many mammals (rabbits, guinea pigs, rats and mice) by immunization with AChR purified from the electric ray *Torpedo californica*⁷⁷. Interestingly, rats develop both the acute and chronic phase of EAMG, even though the acute phase in rats (7–11 days after immunization) is not comparable with the human pathology, which shows changes over remission and exacerbation phases.

In mice, the strain chosen could lead to differences in the development of the disease, so consistency is recommended when choosing the animal model. The primary pathological event in both mice and humans is the loss of muscle AChR. Regarding the availability of this model and the number of additional genetic manipulations possible (mutant mice, transgenic mice, immune response mice and cytokine gene knockout mice), the murine model is useful for studying the immunopathogenesis of myasthenia gravis⁷⁷. Sponsors applying for OMP designation have widely used this mouse model and have extrapolated some results in adoptive transfer experiments, underlying the potential of the tolerance induction approach.

The canine model of spontaneous acquired autoimmune myasthenia gravis⁷⁸ has also been presented to the COMP as a suitable preclinical model for illustrating the potential efficacy of a peptide vaccine (TABLE 2). This natural model shares many similarities with the human disease, including clinical presentation, diagnostic similarities (AChR autoantibodies) and co-morbidity⁷⁸. A primate model involving the passive transfer of myasthenia gravis has also been described in the literature and provides further insights into the immunology of the disease⁷⁹.

To date, no animal models have been used in OMP applications in preclinical studies for GBS or LEMS, but animal models have been described in the literature. For GBS, a rabbit model of neuropathy was developed based on immunization with gangliosides — the same antigenic target that is thought to cause the human form of the disease⁸⁰. For LEMS, one interesting model is the passive transfer of LEMS to rodents by chronic injection of plasma, serum or purified IgG taken from patients, which replicates the hallmark electrophysiological symptoms of LEMS⁸¹. There is also a direct model of immunization, which is useful for characterizing the precise antigen–antibody interaction that is responsible for the autoimmune response. However, this model shows less severe presynaptic inhibition than animals with passively transferred LEMS or patients with LEMS⁸².

Sarcoglycanopathy. With an underlying genetic defect affecting the sarcoglycan complex, sarcoglycanopathies lead to the destabilization of the entire complex and secondary deficiency of sarcoglycan proteins (SGCs). The condition presents early in life and is characterized by limb girdle muscular dystrophy. There are four different types of sarcoglycanopathy (α -, β -, γ - or δ -sarcoglycanopathy), depending on the sarcoglycan gene affected.

Two animal models of sarcoglycanopathy (SGC α and SGC γ) have been presented to the COMP (TABLE 2), and are derived from autosomal recessive genetic defects. In these models, a marked degeneration and regeneration of muscle fibres is initially observed, leading to impaired muscle function throughout the lifespan of the animals⁸³. As these models reflect the human disease phenotype, they seem to be appropriate for preclinical and proof-of-concept studies. Indeed, in one such study the possibility of treating an *Sgca*-null mouse model with gene therapy was examined⁸⁴.

Calpainopathy. Patients with calpainopathy (also known as limb girdle muscular dystrophy 2A) have normal dystrophin and sarcoglycan levels; however, calpain 3 (CAPN3) levels are undetectable in muscles. The age of onset varies as widely as the symptoms, which affect the limb girdle muscles. It is unclear how the loss of CAPN3

leads to this particular form of muscular dystrophy, but it has been assumed that the protein is rapidly degraded, with subsequent impairment of the cytoskeleton and sarcomere assembly in muscle cells⁸⁵.

Few animal models have been presented to the COMP for calpainopathy (TABLE 2), but *Capn3*^{-/-} mice described in the literature show similar characteristics to the human disease⁸⁶. Moreover, this mouse model responded to gene therapy, exhibiting an increase in muscle mass and tetanic force⁸⁷.

Ophthalmological diseases

Ophthalmological diseases that have been designated as orphan conditions have heterogeneous causes, including inherited genetic defects (for example, Leber's congenital amaurosis (LCA) and Stargardt's disease), autoimmune syndromes (for example, keratoconjunctivitis) or neovascular impairments (for example, retinopathy of prematurity (ROP)). Consequently, products that are designated with orphan status in this therapeutic area encompass a broad range of therapeutic strategies, including gene therapy, peptides that induce immune tolerance and inhibitors of neovascularization. Animal models for these ophthalmological diseases are summarized in TABLE 3 and discussed below.

Retinal degeneration. Orphan diseases that are characterized by retinal degeneration include retinitis pigmentosa, LCA and Stargardt's disease. Their aetiology is closely linked to one or more underlying genetic defects that result in photoreceptor dysfunction. Not all of the genetic causes of retinal degeneration have been identified, but mutations defining distinct medical entities have been described (for example, in Stargardt's disease).

Many animal models have been described or developed for the broad spectrum of retinal degenerative diseases, but several have not been used for the development of orphan drugs. In a recent literature review, 29 different animal models of syndromic retinitis pigmentosa were described⁸⁸. It is noteworthy that many of the genes involved in retinitis pigmentosa are similarly altered in humans and in animals⁸⁹. Natural and transgenic animal models for these pathologies exist, with the former mimicking genetic defects and producing a similar phenotype, including the death of both rod and cone receptors of the retina and the retinal pigment epithelium.

Transgenic animals often display a less relevant phenotype but provide valuable insights into the molecular mechanisms involved and the disease pathophysiology. A good example is the ATP-binding cassette subfamily A member 4 (*Abca4*)-knockout mouse model of Stargardt's disease⁹⁰, in which both electrophysiological and morphological signs of retinal degeneration are observed. Another model for Stargardt's-like disease (the *E_mut*^{+/-} mouse model, in which five base pairs are deleted in *Elovl4* (elongation of very long chain fatty acids protein 4))⁹¹ has been proposed in the literature and is a viable option for developing new therapeutic approaches.

Animal models in which retinal degeneration naturally occurs have been widely used to study retinitis pigmentosa. Historic models include *rd* (retinal degeneration) mice and RCS (Royal College of Surgeons) rats (a model of inherited retinal degeneration), but these are no longer recommended owing to their limitations (that is, time of onset of the disease and different physiological background)^{92,93}. Recent models such as *rd10* mice⁹⁴ (for retinitis pigmentosa) and *rd12* mice⁹⁵ (for LCA) exhibit a delayed but stable phenotype over their lifespan, which is of added value when studying age-related human conditions such as retinitis pigmentosa. In addition to these natural models, transgenic rodents (for example, the P23H rat model of autosomal dominant retinitis pigmentosa) are useful in assessing the efficacy of trophic factors as a therapeutic option⁹⁶.

Recent positive results have been published for the treatment of RPE65-associated LCA⁹⁷ using gene therapy, and it is interesting to note the extensive proof-of-concept studies carried out before initiating the Phase I trial. Along with studies in the *Rpe65*^{-/-} mouse model (which is not representative of rod loss in humans)⁹⁸, studies in Briard dogs enabled the follow-up of many parameters such as electroretinography (ERG), retinal immunocytochemistry, histopathology and restoration of vision⁹⁹. Briard dogs suffer from severe, early visual impairment similar to that seen in humans. These results, obtained and presented at the time of orphan designation with this model, were reflected in subsequent successful clinical trials^{100,101}.

Ocular autoimmune diseases. Among the ocular autoimmune diseases, two have been designated as orphan conditions: non-infectious chronic uveitis and keratoconjunctivitis. Chronic non-infectious uveitis and atopic keratoconjunctivitis have an idiopathic aetiology; however, inflammatory processes occurring during the disease are linked to immunological perturbations.

The relevance of animal models of these diseases is dependent on both pathophysiological and immunological components. A broad range of animals sensitized against allergens have been used in proof-of-concept studies. In these simulated models of immunological disease, the clinical features are closely linked to the specific genetic background of the model¹⁰².

Animal models of experimental autoimmune uveoretinitis (EAU) in multiple strains and species have been developed using specific immunization protocols with the following retina-specific autoantigens: S-antigen, antigen interphotoreceptor retinoid-binding protein (IRBP; also known as RBP3), S-arrestin (also known as retinal S-antigen), rhodopsin, recoverin and phosducin¹⁰³⁻¹⁰⁵. The guinea pig was the first model described in the literature in pharmacological studies for this condition¹⁰⁴. However, sponsors have preferentially chosen rodent models to develop novel therapeutic approaches for ocular autoimmune diseases. The choice of the animal model is important, as few mouse strains are susceptible to EAU, and parameters such as animal strain, susceptibility to antigen, genetic background and the antigen itself can influence the manifestations observed¹⁰⁶. After active immunization,

Table 3 | Animal models used for proof-of-concept studies for rare ophthalmological diseases presented to the COMP

Model (year of description)	Method of generation	Phenotype and progression	Key factors for translational applicability
Retinitis pigmentosa			
rd mouse model ⁹² (1924)	Heterozygous deficiency in cyclic GMP PDE subunit	Early and rapid (<10 days) retinal degeneration and ERG perturbation until 2 months of age	Spontaneous disease in the model
rd10 mouse model ⁹⁴ (2002)	Missense homozygous mutation in the <i>Pde6b</i> gene (β -subunit of rod PDE) of rd10 mouse	Retinal degeneration with sclerotic retinal vessels at 4 weeks of age; delayed phenotype	Relevant disease phenotype
Transgenic <i>Rpe65</i> ^{-/-} mouse model ⁹⁸ (2008)	Mutation in <i>Rpe65</i>	Early loss of cone photoreceptors; altered expression of ECM constituents and cytoskeletal proteins; no loss of rod cells	Stable phenotype over animal's lifespan
RCS rat model ⁹³ (1938)	Mutation in MERTK; mutation in human gene causes retinitis pigmentosa	Phagocytosis of affected photoreceptors and degeneration by the third week of age, leading to blindness at 3 months	Spontaneous disease in the model
Transgenic P23H rat model ⁹⁶ (2002)	Heterozygous P23H mutation in rhodopsin gene	Photoreceptor degeneration and abnormal rod function, measured at day 21; progressive disease for several months	Relevant disease phenotype
RPE65-deficient Briard dog model ⁹⁹ (1999)	Mutation in retinal epithelium-specific protein	Vision defects begin at 5 weeks of age, affecting mainly rod cells and leading to loss of vision similar to that observed in human disease	Relevant disease phenotype
Stargardt's disease			
Transgenic <i>Abca4</i> ^{-/-} mouse model ⁹⁰ (1999)	Homozygous mutation in <i>Abca4</i> gene	Similar phenotype in both mouse and human disease; electrophysiological and morphological signs of retinitis pigmentosa are produced	Relevant disease phenotype
Non-infectious chronic uveitis			
Rat model of experimental autoimmune uveoretinitis ¹⁰³ (1992)	Synthetic peptides derived from rhodopsin sequences mixed in complete Freund's adjuvant	Total destruction of the photoreceptor cell layer, and increase in specific antibody titres	Well-characterized model
Rat and mouse models of experimental autoimmune uveoretinitis ¹⁴⁹ (2003)	Interphotoreceptor retinoid-binding protein (IRBP) in complete Freund's adjuvant	Ocular inflammation and photoreceptor destruction appear shortly after injection, with recognizable symptoms in 5–7 days; persistence from few weeks to several months	Relevant disease phenotype
Rat model of experimental autoimmune uveoretinitis ¹⁰³ (1992)	S-arrestin (also known as retinal S-antigen)	Suitable for adoptive transfer experiments (transfection of T cells from immunized rats to naive animals)	Well-characterized model
NOD2-deficient mouse ¹⁵⁰ (2008)	Natural model with genetic mutation background	Responsible for familial granulomatous uveitis and Blau syndrome; an autosomal dominant form of uveitis, arthritis and dermatitis	Relevant disease phenotype
Keratoconjunctivitis			
Mouse model of experimental allergic conjunctivitis ¹⁰⁹ (2009)	Intraperitoneal injection of allergen	Model of a common ocular problem; mice sensitized and challenged with allergens show characteristics similar to those seen in human allergic conjunctivitis	Preferred species for models of ocular allergy
Retinopathy of prematurity			
Rat model of neovascularization ¹¹⁰ (1998)	Corneal epithelium removed (mechanical or chemical burn)	Corneal and limbal epithelial injuries lead to CNV formation; neovascularization appears 7–14 days after the event; analysed by corneal photography or immunochemistry	Closely approximates the human condition
IGF1-knockout mouse plus transgenic VEGF mouse model ¹¹³ (2001)	Homozygous deficiency: <i>Igf1</i> ^{-/-} mice	Retinal blood vessels grow more slowly in <i>Igf1</i> ^{-/-} mice — a pattern very similar to that seen in premature babies with retinopathy of prematurity; VEGF acts as an angiogenic agent	Allows measurement of inhibition of neovascularization
Neovascular glaucoma			
Rat model of neovascularization ¹¹⁵ (1999)	Eye epithelium injured (mechanical or chemical burn)	Injuries to the anterior chamber epithelium of the eye lead to CNV formation; neovascularization appears 7–14 days after the event, followed by high intraocular pressure	Not ideal for studying corneal neovascularization but might be useful when limbal stem cell deficiency and post chemical-burn states are involved

ABCA4, ATP-binding cassette subfamily A member 4; CNV, choroidal neovascularization; COMP, Committee for Orphan Medicinal Products; ECM, extracellular matrix; ERG, electroretinography; IGF1, insulin-like growth factor 1; NOD2, nucleotide-binding oligomerization domain containing 2; PDE, phosphodiesterase; RCS, Royal College of Surgeons; rd, retinal degeneration; RPE65, retinal pigment epithelium-specific protein 65 kDa; VEGF, vascular endothelial growth factor.

animals classically show signs of ocular inflammation leading to the destruction of the photoreceptor cell layer. These pathological features are similarly observed in the human disease. After disease onset, examination of the retina shows histological modification and an increase in specific antibody titres.

The best known and best characterized model of EAU is a mouse model induced by immunization with IRBP in complete Freund's adjuvant, but studies have noted its limitations and more relevant models have been generated¹⁰⁷. The resulting ocular inflammation ranges from mild inflammation of cells to panuveitis and complete destruction of the retina. T cell activation is the main event underlying the expression of the disease and it is worth noting that clinical features vary depending on the route of presentation of the retinal antigen¹⁰⁷.

Indirect sensitization is possible with adoptive transfer experiments in naive rats using long-term CD4⁺ T cell lines cultured *in vitro* with a specific antigen or isolated from the spleens of rats already exposed to the antigen¹⁰⁸. The animals develop a severe intraocular inflammation, which could therefore represent a relevant model for evaluating tolerance induction. Models of atopic keratoconjunctivitis include BALB/c mice actively immunized with allergens¹⁰⁹.

Ocular neovascularization. ROP and neovascular glaucoma (NVG) are ocular neovascularization diseases for which therapies have been granted orphan status by the COMP. In NVG, normal drainage canals within the eye are physically blocked by the growth of new vessels. This condition is often caused by proliferative diabetic retinopathy or central retinal vein occlusion. It may also be triggered by other conditions that cause ischaemia of the retina. In ROP, the normal growth of retinal vessels stops and abnormal vessels begin to grow. The process of normal retinal vascularization is believed to occur in response to physiological hypoxia, which is impaired as observed in premature babies (<30–32 weeks old).

The development of both normal and abnormal blood vessels in the retina has been observed in different animal models. Because angiogenesis is a multistep process that is regulated by many growth factors and extracellular events, there are many targets that could be modulated with therapeutic potential. The development of various experimental animal models for ocular neovascularization is therefore useful for understanding the mechanism and pathogenesis underlying these processes as well as the potential efficacy of investigational therapeutics.

The pathogenesis of ROP is complex; however, low oxygen levels are clearly implicated. The rat model of neovascularization with the removal of the corneal epithelium is a well-characterized model and is considered to be suitable for preclinical studies¹¹⁰. In addition, oxygen-induced retinopathy in rats, using high or low oxygen exposure, is accepted as the most clinically relevant model¹¹¹. This rat model of ROP is preferred over other rodent models as it closely mirrors the human condition, producing a pattern of pathology that is reminiscent of human ROP and making it attractive for evaluating anti-angiogenic compounds¹¹².

However, rat genotypes are difficult to manipulate, and therefore mice have been preferentially used for genetic models of ROP. For example, retinal neovascularization is inhibited in mice in which the gene encoding insulin-like growth factor 1 has been knocked out¹¹³, with a pattern similar to that seen in premature babies with ROP.

Feline models also exist; however, as the pathophysiological features observed in these models do not resemble the human condition, the results cannot be extrapolated¹¹⁴.

Different models in which high intraocular pressure affects the anterior segment of the eye also exist for NVG in various animal species. In rats, an eye injury (for example, by chemical burn) results in neovascularization of the anterior chamber, leading to an obstructive high intraocular pressure (IOP)¹¹⁵. Subsequent proliferation of conjunctival tissue triggered by the loss of corneal stem cells exacerbates the corneal lesions. This therefore has true clinical correlation with limbal stem cell deficiency and post-chemical burn states. The model does not recapitulate the pathophysiology of NVG observed in humans and so its translatability is limited; nevertheless, it has been useful in the development of anti-angiogenic molecules for ophthalmological indications and has been presented to the COMP.

There are several other animal models of NVG. Reliable models in which angiogenesis is induced by inflammation include a surgical feline model¹¹⁶, a primate model in which NVG is induced with vascular endothelial growth factor 165 (VEGF165)¹¹⁷ and a primate model in which NVG is reproducibly induced in the eyes of cynomolgus monkeys by the occlusion of retinal veins¹¹⁸. Primate models are a more accurate representation of human anatomy and yield valuable information; however, cost and ethical considerations are major limitations of such models¹¹⁹. Laser retinal and/or choroidal neovascularization can be applied to monkeys, rats, rabbits and mice, which induces reproducible photocoagulation and has been described in the literature¹¹⁸.

Discussion

Proof-of-concept studies of candidate therapeutics in animal models aim to evaluate their efficacy (using clearly defined biomarkers) and to confirm and/or clarify the anticipated mechanism of action. Most orphan drug applications submitted to the COMP have included such studies as a basis for initiating clinical trials in patients with the orphan disease in question.

Out of the multitude of animal models in the three therapeutic areas discussed above, only a few are considered excellent by some experts in this field of research. These include the GAA-knockout mouse model for Pompe's disease, the *Sgca*-null mouse model for α -sarcoglycanopathy and the RPE65-deficient Briard dog model for LCA. In general, this reflects the fact that the underlying genetic defect in these animal models is analogous to the human disease, and the biological effects and symptoms in these models effectively recapitulate those observed in the human disease, so data from proof-of-concept studies can be translated into the clinical setting. For other disease models, this may not

be the case. For example, in the field of ophthalmology the utility of rodent models is limited because of key differences such as the rarity of cone photoreceptors in rodent retina.

Choice of species. The vast majority of animal models presented to the COMP, and in our literature review, are rodents (mice and rats). The advantages of rodents include the fact that they are inexpensive to keep, their generation time is short and they have large litters. The strains are highly inbred, providing uniform conditions in which experiments can be readily reproduced and statistical significance achieved if a clear outcome is used; additionally, although the lifespan of rodents is short, it is typically possible to study disease onset and the end stages of disease. Furthermore, transgenic technologies have enabled the creation of many monogenetic disease models in rodents either overexpressing transgenic forms of proteins or knockout models that are deficient in disease-related genes.

Nevertheless, there are limitations associated with the use of rodent models of disease, including their size (for example, the evaluation of an ocular implant is difficult) and pathophysiological parameters (for example, immunological responses differ between rodents and humans). Furthermore, even if the model closely resembles the human condition, some factors can limit the translatability of preclinical OMP evaluations to clinical trials. A classic example is ALS, for which various mouse models of the disease have been presented to the COMP (TABLE 2). The SOD1 mouse model is well characterized and has been widely used, but it is based on a single causative factor that only closely reflects a small proportion of patients with ALS (~20% of patients with familial ALS, and ~2% of patients with ALS overall). In such cases, it is recommended that additional animal models are used to test the efficacy of new therapeutic agents to help prioritize those with the most potential¹²⁰. These other models should cover other aspects of the disease so that an integrative approach may be used when evaluating the overall outcome of preclinical studies. In addition, lack of methodological rigour in the use of such models may have been a factor contributing to the failure of clinical trials of agents that appeared promising in preclinical studies, as discussed further below¹²¹.

ERT provides another example in which it is useful to test a candidate therapy in several animal models and species. ERT is the standard of care for several LSDs, such as Gaucher's, Fabry's and Pompe's disease as well as type I, II and IV MPS. However, one of the main limitations of ERT is its lack of distribution to some key disease-affected tissues. For example, in type I, II and IV MPS the distribution of recombinant enzymes and correction of the pathology is inefficient in tissues such as the bone, cartilage and heart¹²². Consequently, improvements to this aspect of ERTs have been sought. Enzyme delivery has been investigated in neonatal mice in which the blood–brain barrier is not completely formed, so that distribution to the CNS is facilitated, hence providing proof of concept for therapies that are designed to act in the CNS¹²³. Other complementary

strategies (such as relaxation of the cellular tight junctions, intracerebroventricular administration or chemical modification of the drug) might allow, in a later stage of development, drug delivery to the CNS over an intact blood–brain barrier. In addition, a mouse model deficient in the mannose receptor, which has a role in the transfer of lysosomal enzymes across the blood–brain barrier, has been developed to evaluate a new delivery system with the ability to cross into the CNS¹²⁴.

Immune responses to ERT often occur, and the mechanisms behind these reactions have been studied in rat models¹²⁵; however, in pharmacological studies it is worth assessing the onset of the antibody response in large animals, which more closely parallel the humoral immune response in humans. Ideally, more than one animal species¹²⁶ should be used to enhance the degree of validity of the results from animal models of disease⁵. Indeed, owing to marked differences in the immune system across species, the use of different models can improve the predictability of immunotoxicity and long-term efficacy for therapies such as ERT — for which the immune system could have an important influence. Therefore, to obtain knowledge regarding the long-term immunotoxicity of these therapies, tolerance induction protocols in ophthalmological and neuromuscular diseases may be performed in non-human primates¹²⁷.

Improving experimental design. Additional experimental designs could be used to increase the likelihood of preclinical studies translating successfully into clinical trials. For instance, studies comparing survival between experimental models and a control group of non-affected animals could be conducted to study the external validity of the animal models used, by assessing which variables potentially affect the outcome of such studies (that is, the extent to which the results obtained using an animal model can be generalized across patients with rare diseases), hence reducing the number of unknown variables in these studies¹²⁸. In addition, if a standard-of-care therapeutic for the human disease exists, it would be useful to conduct parallel studies in which the standard of care is compared directly with the investigational therapeutic. Validated parameters for use as efficacy readouts are crucial and must be justified regarding the model chosen. The disease and patient population subgroup appropriate for a particular therapy can also be refined using well-characterized preclinical models. For example, as noted above, different mouse models can parallel the various subpopulations of patients affected with retinitis pigmentosa.

Rigorous application of statistics in the non-clinical setting is also of utmost importance, not only to provide an accurate analysis of results but also to improve experimental design to increase the chances of obtaining results that are robust. As an example, current guidelines for ALS recommend using at least 48 mice for a single preclinical study¹¹¹. Other aspects are related to the control of all variables that may limit statistical significance. Finally, in terms of designing clinical trials for small patient populations, the EMA has published guidelines to help with trial design, hence facilitating clinical trials for rare diseases¹²⁹.

Advanced therapies. Apart from the classic pharmacological approaches, the COMP has gained substantial experience in reviewing applications for gene therapies and cell therapies, which are new prospects for treating genetic diseases. However, to fully understand and treat — using advanced therapies — defects that are characterized by isolated genes or pathways in rare diseases such as Huntington's disease or sarcoglycanopathy, authentic (gene-orthologous) animal models are vital considering that, for ethical or practical reasons, preliminary evaluations of such therapies are not possible in humans¹³⁰ without any proof of concept and preliminary safety results.

Many rare genetic diseases are good candidates for gene therapy strategies, in which the aim is to replace a deleterious mutant allele with a functional one, but progress in the field has been hampered by challenges such as achieving a sufficient duration of gene expression and the safety of gene transfer strategies. Nevertheless, the first gene therapy was approved in Europe in November 2012, marking a significant advancement for the field. The therapy — alipogene tiparvovec (Glybera; UniQure) — is indicated to treat lipoprotein lipase (LPL) deficiency in patients with severe or multiple pancreatitis attacks, despite dietary fat restrictions. Glybera uses an AAV vector to deliver functional copies of the *LPL* gene into muscle cells to enable production of the enzyme.

In the meantime, testing gene therapies in mouse models has also led to interesting results in AIP¹³¹, Pompe's disease¹³², calpainopathy⁸⁷ and α -sarcoglycanopathy⁸⁴. The conditions induced in mouse models of these diseases are uniform and therefore enable comparison with wild-type animals to assess the effectiveness of the deficiency correction. For instance, in Pompe's disease, correction of the genetic defect is effective in mice, in terms of GAA expression as well as complete clearance of glycogen stored in the diaphragm as well as in cardiac and skeletal muscle, but further development is needed to achieve persistent and tissue-specific expression of the impaired enzyme¹³².

Other end points investigated in these preclinical studies include the efficacy of gene transfer protocols, the level of enzymatic and functional correction of the disease, trials with different routes of administration and persistence of gene expression. These end points are relevant parameters for evaluation in early studies¹³³ and provide valuable data before advancing to testing in larger animals. However, transgenic rodent models have some limitations, as only short-term experiments can be performed because of the mean lifespan of these animals. Also, the general genetic background of these species is defined by inbreeding, making them more 'homogeneous' in comparison with the genetic background of humans. Finally, knockout rodent models do not always faithfully represent the human disease.

Preclinical studies of advanced therapies in larger animal models complement studies in murine models as they possess different features that are relevant for drug action, such as the size of the animal (which is important in transplantation, for example) and a longer course of the disease, before advancing to pivotal clinical studies. Many large animals display naturally occurring disease and reproduce molecular pathways that are consistent with

the human condition. These outbred models also more accurately mimic complex pharmacokinetic and pharmacodynamic interactions than murine models owing to their heterogeneous genetic background, and they are good models for extended pharmacological studies. Their size enables surgical manipulation and their longevity permits the assessment of long-term exposure and risks¹³⁴.

Simplistically, we can consider two ways of using large animal models: first, wild-type animals (particularly in sheep and non-human primates) can be used to improve gene transfer techniques; and second, authentic animal models of disease can be used to evaluate the impact of the treatment on meaningful surrogate or clinical end points. Some genetic disorders that occur in large animal models are classified as rare diseases. Many models have been described for LSDs, particularly in cats and dogs, and they are useful for addressing scale-up uses for gene therapy. Dogs are also used to assess the efficacy of candidate treatments for LCA; some of these treatments have been successfully translated to small clinical trials¹³⁵.

The eye is particularly suitable for gene therapy, as it is small and easily accessible, various routes of gene delivery can be used, the blood-brain barrier in the retina limits leakage into the circulation, and many rodent and large animal models of human diseases are available. For instance, replacement of the *RPE65* gene using an AAV vector has been reported to be effective in a rodent model of LCA⁹⁸, and corrected vision was maintained for a long time (at least 3 months) in the Briard dog model of spontaneously induced LCA¹³⁶. Disease caused by the *RPE65* mutation (which is found in ~10% of patients with LCA overall) is particularly suitable for gene therapy because patients have a preserved retinal morphology despite having severe and early vision impairment, as observed in dogs. The success of gene therapy in Briard dogs — for LCA and for retinitis pigmentosa — has shown that predictability can be achieved. Also, in neuromuscular diseases, large animals can be clinically normal for the first few months of life before disease onset (in contrast to rodents, which display symptoms of disease at a very early stage), providing models that more accurately mimic the course of the human disease.

With regard to cell therapies, in our experience of assessing OMP applications we have identified various problems associated with their preclinical testing in animal models. One important parameter to consider in choosing an appropriate species is the lifespan of the model. Ideally, long-term follow-up of the intended therapy is needed to fully evaluate efficacy. Indeed, the limitations of rodent models are particularly apparent in evaluating cell therapies, especially for the induction of graft tolerance. Rodents have been poor predictors of efficacy, and the guidelines from the EMA recognize that only non-human primate models have predictive value for clinical translation¹³⁷. As stated above, the rodent immune system does not reflect the complex human immune response to graft rejection. Large animal models such as pigs, which more closely resemble human biology in some respects, could be an adequate model. However, non-human primates would be the preferred models as their immune system has immunological memory that

often results in cross-reactivity and heterologous immunity to exogenous cell antigens^{138,139}, as observed in the human immune system. The difficulty here is the lack of primate models with the relevant disease in which to test these new therapeutics. Nevertheless, for all models it is important to describe the techniques used to identify transplanted cells, as well as to trace cell movements and tissue reconstruction in transplantation experiments.

Conclusions

The animal models presented in this article are available for proof-of-concept studies in metabolic, neuromuscular and ophthalmological rare diseases, and some have enabled the development of much-needed therapies such as ERTs. Nevertheless, there is a continuing need to develop more effective models to facilitate drug development and clinical trial design for rare diseases.

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Competing interests statement

The authors declare no competing financial interests.

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