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Gene therapy in monogenic congenital myopathies

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1. Introduction to monogenic congenital myopathies (MCM)

Current treatment options for patients with monogenetic congenital myopathies (MCM) ameliorate the symptoms of the disorder without resolving the underlying cause. However, therapies are being developed where the mutated or deficient gene target is replaced. Thousands of clinical trials have been undertaken relating to gene therapy, with around 9% focused on monogenetic diseases such as Duchenne muscular dystrophy (DMD) and limb girdle muscular dystrophy (LGMD)¹. Preclinical findings in animal models have been promising, as illustrated by studies of a potential treatment for X-linked myotubular myopathy (XLMTM) in canine and murine models². We will therefore discuss the prospective applications and approaches of gene replacement therapy, using these disorders as examples.

Both limb girdle muscular dystrophy type 2C and Duchenne muscular dystrophy are part of a subclass of myopathies known as dystrophies, diseases where muscle degeneration is accompanied by replacement with fatty or connective tissue. DMD is caused by X chromosome linked genetic mutations leading to the absence of membrane-anchored dystrophin protein, the centerpiece of the large dystroglycan complex that plays a pivotal role in sarcolemma stability during muscle contraction³(Table 1). The symptoms are visible as early as 2–3 years of age, a progressive decrease in striated muscle function, starting from proximal muscle such as legs and pelvis and eventually involve the whole body. Most patients are wheelchair-dependent starting from early teen. The average life expectancy is around 25 years (<http://www.nlm.nih.gov/medlineplus/ency/article/000705.htm>), with respiratory failure and cardiac complications the highest causes of mortality.

Congenital centronuclear myopathies are inherited muscle diseases where the nucleus is located in the center of the muscle fiber instead of the periphery. X-linked myotubular

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myopathy (XLMTM) is the most common centronuclear myopathy, affecting an estimated 1 in 50 000 male births (Table 1).^{4,5} The disease is due to a mutation on the long-arm of the X chromosome, usually inherited by hemizygous boys from an asymptomatic carrier mother.⁶ This mutation causes a deficiency of the protein myotubularin⁷. Myotubularin has been identified as a phosphoinositol phosphatase and may be critical to normal excitation-contraction coupling and remodeling of the sarcoplasmic reticulum in muscle.⁸ When XLMTM patients are first born, they typically exhibit hypotonia and may be blue due to respiratory insufficiency.⁶ The disease is often fatal in the first year of life and long-term survivors may require ventilatory support.⁹ Affected boys are particularly susceptible to infection and respiratory dysfunction is the leading cause of death.¹⁰

Although there are differences between the symptomatic presentations of these diseases, there are some shared difficulties to consider when design gene therapies. High vector titers may be required to reach an effective dose,¹¹ increasing the chance of adverse effects in patients. In addition, the need to treat respiratory muscles as well as the heart in DMD and XLMTM may complicate delivery. Improvements in delivery methods¹² and in vector characterization to increase efficiency may address this problem.¹³ Vector modification may also ensure more efficient delivery to the muscle and improve safety by reducing off-target delivery to organs like the liver.^{11,14} Tissue-specific promoters is another strategy to secure tissue-specific transgene expression. Immune response is a major concern, particularly in genetically-null patients who may have antibodies against the gene product produced by the treatment¹⁵ and immunosuppression before and during treatment may have to be considered. There are also challenges specific to each disease. For example, the large size of the dystrophin gene limits the choice of vector to be used in treatment. Overexpression of γ -sarcoglycan in LGMD patients may exacerbate the condition.¹⁶ Significant wasting in XLMTM patients leaves very little muscle to treat and, due to the young age of the patients, selecting an appropriate and reproducible outcome measure may prove difficult. We will be discussing new developments that address these concerns, including modifications of the vector and the combination of gene therapy with other approaches.

2. Gene therapy

2.1. What is gene therapy

Gene therapy is defined as the introduction of nucleic acids, including DNA, RNA and their analogues into cells of living organism to treat diseases³¹. This occurs through modified expression of genes of interest to trigger alterations of certain biological functions. Gene therapy targets living cells, primarily because cell's intrinsic gene expression machinery is indispensable to mediate the production of therapeutic molecules, including protein, shRNA and microRNA.

Since classical gene therapy acts on native tissues, the abundance of target cells largely determines the effect of gene therapy. This is especially true in congenital myopathies. In the advanced stage of diseases, such as DMD and XLMTM, surviving myocytes are so limited that even if the function of individual myofibers were fully restored, there would be no appreciable functional improvement on tissue level. The advent of stem cell technology, especially the discovery of induced pluripotent stem cells (iPSCs)^{32,33}, has the potential to

overcome this hurdle. Pluripotent stem cells may be able to replenish tissue loss through their indefinite self-replicating potential and capacity to be converted into nearly all cell types within the body. The advantages of combining stem cell therapy with gene therapy have been demonstrated in several animal studies, in which vectors were administered *ex vivo* and modified donor cells were later engrafted into native tissue^{34–36}.

Various gene therapy strategies target gene expression and regulatory network at different levels. For example, genetic sequence can be permanently inserted into genome for long-term expression, using retrovirus or lentivirus³¹. With the development of genome modification tools³⁷ such as clustered regularly interspersed short palindromic repeats (CRISPR) enzymes and Transcription activator-like effector nuclease (TALEN), the technical barrier of *in situ* editing eukaryotic genomic DNA has been substantially lowered. These techniques hold the potential to seamlessly restore the genome to a disease-free state without the introduction of foreign genetic elements, eliminating the widely shared concern of increased tumorigenic risk. Alternatively, the therapeutic genetic sequence can be designed to persist within cells as a stable episome, allowing maintenance of long-term expression without genomic integration³¹.

Introduced nucleic acids can be further divided into protein-coding and non-coding sequence. While protein-coding sequences serve as templates for protein production, non-coding nucleic acids function to modulate epigenetic processes controlling gene expression. A good example is RNA interference, in which microRNA (miRNA)³⁸ or small interfering RNA (siRNA)³⁸ bind to mRNA molecules to halt protein translation and mediate mRNA degradation.

2.2. What will be administered

Based on the mechanism of action, gene therapy can be categorized into gene addition, gene correction or gene subtraction. In gene addition, exogenous genetic sequence is introduced into cells. Gene correction alters the diseased loci. Attenuating or silencing the expression of single or a network of genes is known as gene subtraction.

The root causes of diseases dictate the gene therapy strategy, which differs dramatically among diseases (Table 2). Genomic mutations sometimes interrupt the reading frame of the protein-coding genes, leading to the absence of proteins, as occurs with dystrophin³⁹ and myotubularin⁴⁰ in DMD and XLMTM. Consequently the focus of gene therapy has been devoted to supplement a protein-coding sequence to replace the defective gene. Alternately, the mRNA splicing event can be modified to bypass the mutated region, restoring the reading frame with a truncated but functional protein product⁴¹. In other cases, where the disease is caused by pathogenic overexpression, it is imperative to silence the gene expression⁴². One such example is facioscapulohumeral muscular dystrophy (FSHD), which is caused by the overexpression of the myopathic DUX4 gene¹⁸.

2.3. Advantages of gene therapy in treating congenital myopathies

The goal of gene therapy in treating congenital myopathies is two-folds. For some monogenic diseases, the current technology is adequate to completely restore the genetic defect in somatic cells, representing a cure that is unachievable by any other methods.

Recent findings in canine and mouse models of XLMTM are good examples, where a single treatment recovered animals with a severe monogenic disease to normal function². For myopathies with complex genetic makeup, a more realistic goal is to delay disease progression and preserve muscle function in order to maintain life quality.

There are several advantages associated with gene therapies in comparison to conventional pharmacotherapies.

- Complete rectification of the abnormal genetic code

There are currently very limited therapeutic options that effectively target congenital myopathies. Most available drugs are largely symptom-alleviating agents with transient effect. In contrast, gene therapy is designed to target the root genetic cause and thus represent a potential cure for some monogenic diseases.

- High specificity

Conventional pharmacotherapy utilizes natural or synthetic small molecules aiming to alter certain biological functions. However, it is difficult to identify high-specificity molecules that only interact with the molecule of interest. As a result, pleiotropic effects of these agents are the main source of undesired adverse effects. Gene therapy functions to modulate the production of native proteins and is therefore more specific and effective.

- Long-term duration of efficacy

Certain vectors, such as lentivirus, retrovirus or AAV³¹, demonstrate persistent effects leading to extended phenotypic correction/disease remission. This is in stark contrast to conventional drugs, requiring repetitive dosing to reach a steady-state drug concentration for stable effects.

Gene therapy clinical trials for congenital myopathies—Ongoing clinical trials are mainly targeting diseases with defective dystrophoglycan complex, including DMD, BMD and LGMD. Local delivery of full-length dystrophin plasmid to patients' radialis muscle results in detection of dystrophin mRNA and protein in 6 out of 9 patients⁴⁵. Encouraged by this study, various gene therapy strategies have been employed in clinical trials aiming to restore dystrophin expression, including read-through agents (Ataluren/PTC124, Arbekacin Sulfate/NPC14 and Gentamicin), exon skipping oligonucleotides (AVI-4658, Drisapersen, Pro044/045/053, SRP4045/4043 and NS-065/NCNP-01) and virus mediated delivery of protein encoding genes, such as minidystrophin and Follistatin. Mendell *et al.* reported delivery of truncated minidystrophin gene (NCT00428935) elicited dystrophin specific T cells, without direct visualization of the protein in muscle, suggesting immune response to be a major hurdle for successful gene therapy¹⁵. The Phase 2a study of the read-through agent ataluren reported 61% patients demonstrated increase of dystrophin expression after a course of 28 days treatment (NCT00264888)⁴⁶. Intramuscular injection of the exon skipping agent AVI-4658 (NCT00159250) resulted in 17% increase of the mean dystrophin signal, reaching 22% to 32% of the healthy control⁴⁷. For LGMD, AAV packaged alpha and gamma sarcoglycan are now being tested in clinical trials. Local injection of AAV1.tMCK.hSGCA (NCT00494195, NCT01976091) led to persistent α -sarcoglycan expression up to 6 months

and augment muscle fiber size^{43,48}. A trial with AAV1- γ -sarcoglycan vector in LGMD 2C patients has completed though result has not yet been disclosed (NCT01344798). The ongoing clinical trials are summarized in the table 3.

3. Vector toolbox

A critical element of gene therapy is the vector, the vehicle that facilitates the transfer of genetic material. Due to the size and negative charge of ribonucleic acid, shuttles are needed to carry the cargo across biological barriers to reach target cells. The vector toolbox is composed of viral vectors and non-viral vectors. Viruses have naturally evolved sophisticated machinery to target cells with high efficiency, making them the ideal tool for gene transfer. A summary of the advantages and disadvantages of commonly used viral vectors is outlined in Table 4.

AAV is the preferred viral vector for many ongoing clinical trials, largely due to the fact that AAV efficiently targets post-mitotic parenchymal cells, such as neurons and skeletal myofibers, which are usually impermeable to other vectors⁵⁰. Unlike integrating viral vectors, AAV exists as episome, reducing the likelihood of insertional mutagenesis while maintain long-term transgene expression. Another advantage of AAV is that the vector genome can be pseudotyped with alternative capsids. The viral capsid largely determines tissue tropism, gene transfer efficacy and the vector's dose-dependent toxicity. For example AAV 1, 6, 9 transduce muscle with high efficiency⁵¹. This array of available serotypes allows the vector to be tailored to different applications. Though many natural AAV variants have been identified, efforts are being devoted to engineer synthetic viral capsids to address specific clinical challenges. Mutating tyrosine residues (Y445F and Y731F) in the AAV6 capsid can improve the skeletal muscle gene transfer⁵². Moreover, retention of AAV vector in liver has been a problem of systemic infusion. Vectors that "de-target" liver have been created through randomly mutating the surface-exposing region of AAV9 capsid (AAV9.45 and AAV9.61)⁵³ or by engineering a chimeric capsid with AAV2 and AAV8 (AAV2i8)⁵⁴. Both strategies redirected the vector away from liver while maintaining high transduction efficiency to skeletal muscle.

Viral vectors have been widely used in ongoing gene therapy clinical trials¹. Despite their differences, however, the use of any viral-based vector carries with it certain risks, including tumorigenicity, immunogenicity and limited cargo space. Most of these risks can be minimized by non-viral vectors. On the other hand, the main drawbacks of non-viral vectors, including the capacity to cross various biological barriers and stability, have been largely addressed by late breakthroughs. For example, material science has provided various lipid-based and polymer-based DNA vectors, many of which have been tested in clinical trials⁵⁵. Nucleic acid chemistry evolution has resulted in the development of nucleic acid analogues, such as 2'-O-methyl-phosphorothioate (2'OMe) and morpholino phosphorodiamidate oligonucleotide (PMO). While retaining the same nucleobase to enable Watson-Crick base pairs with natural nucleotides, ribonuclease-resistant moieties have replaced natural ribose ring and backbone phosphodiester linkage. These modifications lead to increased molecular stability against enzyme degradation. Direct infusion of analogue oligonucleotides is associated with efficient targeting⁵⁶⁻⁵⁹. 2'OMe and PMO have been employed as antisense

oligonucleotide (AON) to mediate exon skipping in DMD treatment (Table 5). Both Drisapersen (2'OMePS AON to exon 51) and Eteplirsen (PMO morpholino to exon 51) have also demonstrated restored dystrophin expression and even mild clinical improvement as measured by 6 minute walk^{60,61}.

4. Routes of delivery

The success of gene therapy is largely determined by the efficient delivery of vectors to target tissues directly determines the success. Effective delivery approaches include direct injection, locoregional perfusion and systemic delivery.

4.1. Direct injection

Direct injection into the target tissue is commonly used to determine the efficacy of a potential new therapy^{2,62–64}. This method ensures that the desired organ receives the necessary therapeutic dose and restricts the treatment to that organ, reducing off-target effects. However, distribution may be limited, even within the injected muscle⁶⁵. For example, XLMTM dogs treated by AAV8-*MTM1* injection into the cranial tibialis of the hindlimb show improvements in the strength, size and histopathology of the treated limb² but show a continued progression of the disease including muscle weakness, impaired ambulation and early death^{2,66}. This limited distribution is therefore disadvantageous when the disease affects multiple systems or the entire body, as is often the case with congenital myopathies. Also, direct treatment of organs like the heart or diaphragm may require invasive surgery or complicated techniques^{67,68}, which can be difficult in chronically ill-patients.

4.2. Locoregional perfusion

Locoregional perfusion, where a limb is isolated before intravascular infusion under high pressure, is another approach to gene therapy delivery. Despite the use of high pressure, it is a safe, relatively painless option for human patients¹² and has been used successfully in animal models, including the XLMTM dogs, where there was widespread clinical improvement^{2,69,70}. However, vector titers may be reduced due to the low permeability of the vascular endothelium⁷¹ and, like direct injection, isolated locoregional perfusion does not address treatment of the cardiorespiratory system.

4.3. Systemic delivery

In systemic delivery, a potential gene therapy vector is introduced to the entire body. This is of particular importance in congenital myopathies where cardiorespiratory failure is the leading cause of death but there are effects of the disease throughout the body.^{72,73} As with locoregional perfusion, the vascular endothelium could hinder distribution to the skeletal muscle and cardiorespiratory systems most affected by disease.^{71,74} Systemic dosing also increases the likelihood of off-target gene delivery, which may require the use of additional safety measures like tissue-specific promoters. Finally, higher doses may be required for systemic delivery, due to circulating antibodies and filtration by the liver. However, modifications of the vector or immunosuppression can address these problems.^{75–77}

5. Preclinical disease model systems

Reliable model systems are indispensable for the critical transition from bench to bedside. It is required by the FDA the potency and toxicity of vectors be validated on several levels of preclinical models, before entering clinical trials. The following section summarizes model systems at different level from cell culture to animal models.

5.1. Cell culture models

Cell culture models can be used to test the potency and cellular toxicity of a biological therapy. There are several advantages, such as intricate manipulation of experiment conditions and considerably lower costs. Early phase vector validations are usually carried out in relevant cell cultures. Immortalized mammalian human embryonic kidney (HEK) 293 cells and C2 myoblasts were employed to validate the vector expressing a truncated dystrophin protein⁷⁸. Engineered HEK293 cells over-expressing pathogenic DUX4 gene has been utilized to confirm the efficacy of RNAi vector⁴². Cells derived from diseased tissues, such as myoblasts isolated from animal models or human patients, have been utilized to test potency for vectors developed for DMD⁷⁹, Pompe disease⁸⁰ and myotonic dystrophy⁴⁴.

Due to physiological differences between animal models and human patients, it is not rare therapies that are effective in diseased model animals failed to show benefits in clinical trials⁸¹, including DMD⁸². Moreover, mutations may vary among patients, which requires vector personalization, such as oligonucleotides for exon skipping therapy⁸³. Consequently a personalized human cell culture system is highly desirable for potency testing.

The emergence of human iPSC offers a novel cell culture platform to meet both these criteria. Unlimited disease relevant cells with specific patient mutations could be generated. More importantly, these cells demonstrate disease-associated phenotypes reflecting disease severity⁸⁴. As a result, correction of these disease-associated phenotypes can be measured as efficacy. Moreover, testing vectors on these personalized cells will not only demonstrate the presence of the transgene product⁸⁵, but also enables quantified measurement of biological activities, as required by the FDA for gene therapy products.

5.2. Animal models

While *in vitro* models allow for the testing of potential therapies in human cells, the ability to adequately recreate the complexities of the human body is limited. As such, preclinical testing in an animal model remains the gold standard for investigating the efficacy and potential toxicity of a putative treatment.

Non-mammalian models such as zebrafish have been invaluable to the understanding of disease mechanisms within a complex organism, particularly in monogenetic neuromuscular disorders.^{86,87} However physiological and phenotypic dissimilarities with humans limit their translational power^{88,89} (Table 5).

Small mammalian models like rodents are often used in preclinical assessments of efficacy and toxicity. With extensive research into study of the murine genome,⁹⁰ mice in particular

are a powerful tool in the study of monogenetic disorders and knockdown or knockout mouse models have been developed to study musculoskeletal disease.

However the small size of the mouse as well as anatomical and phenotypic differences make them less than ideal candidates for the preclinical assessment of gene therapies.⁹¹ Therefore, a larger animal model like a dog, where organ size more closely approximates that of humans, may be more suitable. Methods of functional assessment developed for use in the clinic have also been successfully adapted for use in dogs.²² Many naturally-occurring musculoskeletal diseases, similar to those seen in human patients, have been identified and characterised⁹² (Table 5) facilitating the use of that dog model for the pre-clinical assessment of potential treatments.^{2,93,94}

AAV8-mediated delivery of myotubularin in the XLMTM dog provides a successful example of gene therapy in preclinical practice. Due to the gene's small size, a full-length canine *MTM1* cDNA was carried by the muscle-tropic AAV8. This was packaged with a human desmin promoter to ensure muscle specific expression. Dogs were treated by direct injection and by locoregional hindlimb perfusion, where systemic effects were observed, likely due to leakage. Treated dogs have near normal muscle strength, with the marked improvements in respiratory function and increased survival for dogs treated intravascularly. Indeed, these animals continue to survive more than two years after treatment and continue to thrive and breed. The size of the dog has allowed for the inclusion of many at other relevant assessments at multiple time points including neurological scoring, MRI, EMG and gait testing as we investigate potential outcome measures for translation of this therapy to human patients.

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Table 1

A comparison of some of the monogenic congenital myopathies presently under study

	Duchenne muscular dystrophy (DMD)	X-linked myotubular myopathy (XLMTM)	Facioscapulohumeral muscular dystrophy (FSHD)	Myotonic Dystrophy (DM)	Limb-Girdle muscular dystrophy (LGMD) 2C and 2D
Inheritance	Single gene mutation on the X chromosome	Single gene mutation at q28 on the X chromosome ¹⁷	Autosomal dominant, contraction of D4Z4 repeat on chromosome 4q35 and toxic gain of function of the DUX4 gene ¹⁸	Autosomal dominant. DM1 CTG triplet repeats expansion of DMPK gene locates on chromosome 19. ¹⁹ DM2 CCTG tetranucleotide repeat expansion of ZNF9 gene on chromosome 3 ²⁰	Autosomal recessive. Single gene mutation on chromosome 13 and 17 (2C and 2D, respectively)
Molecular biology	Deficiency of the protein dystrophin	Deficiency of the phosphatase myotubularin ¹⁷	Toxic protein product of DUX4 ¹⁸	Malfunctioned DMPK and ZNF9 proteins	Deficiency of gamma and alpha Sarcoglycan (2C and 2D, respectively).
Clinical symptoms	Weakness of the skeletal muscles; Respiratory insufficiency in teens; Cardiac dysfunction; Leading cause of death is cardiorespiratory failure	Weakness of the skeletal muscles ¹⁰ ; Wheelchair dependence ¹⁰ ; Respiratory insufficiency at birth ²¹ ; No cardiac phenotype; Leading cause of death is respiratory dysfunction ²²	Initial weakness of facial, scapula and humeral muscle, progressively involving other muscles; Sparing respiratory muscle.	Muscle wasting and myotonic; Heart conduction block; Cataract; Infertility.	Muscle wasting primarily involve proximal muscle such as hip and shoulder
Demographics	Presentation around 2–3 years of age; Average life expectancy of 25 years.	Affects 1 in 50 000 live male births ⁴ ; Presentation typically at birth ²³ ; Average life expectancy of 29 months ⁹ .	Affects 12/100,000 ²⁴	Affects 1/8000 people worldwide, Type 1 most common in most countries. ²⁵	Up to 68% of individuals with childhood onset and ~10% with adult onset ²⁶
Histology	Increased fibre size variability; Cycling of fibre regeneration and degeneration.	Centrally located nucleus ²⁷ ; Variably-sized myofibers with an abnormally large number of small fibers; Organelle abnormality and “necklace fibers” ²⁸ .	Non-specific fiber necrosis, increased variation in fiber size, internal nuclei, fiber type variability, connective tissue and fat proliferation. Mononuclear cell infiltration. ²⁹	Fiber atrophy, internal nuclei, pyknotic nuclear clumps, lipofuscin accumulation, increased fiber size variation ³⁰	Variation of fiber diameter, fiber degeneration and regeneration, split fibers, ring fibers

Table 2

The different strategies for various monogenic myopathies

Gene therapy strategy	Diseases
Gene addition	DMD ³⁹ , XLMTM ² , LGMD2D ⁴³
Gene correction	DMD ³
Gene subtraction	FSHD ⁴² , Myotonic dystrophy ⁴⁴

Table 3

Ongoing gene therapy clinical trials for congenital myopathy

NCT Number	Conditions	Interventions	Phases
NCT02354781	DMD	rAAV1.CMV.huFollistatin344	Phase 1/Phase 2
NCT01519349	BMD	rAAV1.CMV.huFollistatin344	Phase 1
NCT02255552	DMD	eteplirsen injection	Phase 3
NCT01918384	DMD	NPC-14	Phase 2
NCT02376816	DMD	rAAVrh74.MCK.micro-Dystrophin	Phase 1
NCT01247207	DMD/BMD	Ataluren	Phase 3
NCT01557400	DMD/BMD	Ataluren	Phase 3
NCT01826487	DMD	Ataluren	Phase 3
NCT02090959	DMD	Ataluren	Phase 3
NCT02329769	DMD	PRO044	Phase 2
NCT01826474	DMD	PRO045	Phase 1/Phase 2
NCT01910649	DMD/BMD	Drisapersen	Phase 2
NCT01957059	DMD	PRO053	Phase 1/Phase 2
NCT02310906	DMD	SRP-4053	Phase 1/Phase 2
NCT02500381	DMD	SRP-4045/SRP-4053	Phase 3
NCT02081625	DMD	NS-065/NCNP-01	Phase 1
NCT01976091	LGMD2D	scAAVrh74.tMCK.hSGCA	Phase 1/Phase 2

Table 4

Advantages and disadvantages of the various types of viral vector

Viral Vector	Advantage	Disadvantages
Retrovirus	<ul style="list-style-type: none"> • Stable integration • Allowing to be pseudotyped 	<ul style="list-style-type: none"> • Only infect dividing cells. • Size limit of 8kb • Insertional mutagenesis associated tumor risk • Low titers⁴⁹
Lentivirus	<ul style="list-style-type: none"> • Stable integration for persistent transgene expression; • Infect both dividing and non-dividing cells; • Allowing to be pseudotyped with the VSVG (vesicular stomatitis virus G), making concentration easier. 	<ul style="list-style-type: none"> • Possibility of insertional mutagenesis;
Adenovirus	<ul style="list-style-type: none"> • Large cargo space (36kb adenoviral genome) • High titer; • Permissiveness to non-dividing cells; • Persists as non-integrating episome⁴⁹. 	<ul style="list-style-type: none"> • Strong immune response associated toxicity; • Transient expression due to immune response;
Adeno-associated virus	<ul style="list-style-type: none"> • Easy to concentrate; • Availability of numerous serotypes makes it possible to choose a vector with a degree of selectivity for the desired target cell type; • Transducing non-dividing cells; • Mostly remain non-integrating episome • Naturally non-pathogenic 	<ul style="list-style-type: none"> • Limited cargo space around 4kb³¹;

Table 5

Some of the treatments currently under clinical trial for DMD (Abbr: SC, subcutaneous; IV: intravenous)

Drug Name	Description	Clinical trial number	Delivery Route
GSK2402968 (Pro051)/Drisapersen	2'OMePS AON to exon 51	NCT01803412	SC
Pro044	2'OMePS AON to exon 44	NCT01037309	SC/IV
Pro045	2'OMePS AON to exon 45	NCT01826474	SC
AVI-4658 (Eteplirsen)	PMO morpholino to exon 51	NCT02255552	IV

Table 6

Available mouse models for monogenic congenital myopathies.

DMD mouse models		
	Mutation	Phenotype
<i>Mdx</i> mouse ⁹⁵	<ul style="list-style-type: none"> Spontaneous point mutation in exon 23 	<ul style="list-style-type: none"> Similar but milder muscle phenotype compared to human patients
<i>Mdx52</i> mouse ⁹⁶	<ul style="list-style-type: none"> Exon 52 deletion 	<ul style="list-style-type: none"> Absence of shorter isoforms Dp260 and Dp140
<i>Mdx/Utraphin double knockout mouse</i> ⁹⁷	<ul style="list-style-type: none"> Utrophin knockout on the basis of <i>Mdx</i> 	<ul style="list-style-type: none"> Severe phenotype with early deterioration
LGMD 2C mouse models		
	Mutation	Phenotype
γ -sarcoglycan null mouse ⁹⁸	<ul style="list-style-type: none"> Exon 2 deletion 	<ul style="list-style-type: none"> Muscular dystrophy Progressive but mild Gait abnormalities Reduced activity Increased serum CK Pseudohypertrophy of the diaphragm
XLMTM mouse models		
	Mutation	Phenotype
XLMTM knockout mouse ⁹⁹	<ul style="list-style-type: none"> Deletion of exon 4 causes an absence of myotubularin 	<ul style="list-style-type: none"> Centronuclear myopathy Severely affected Skeletal muscle wasting, resulting in pronounced and progressive weakness Shortened lifespan
<i>MTM1</i> p.R69C mutant mouse ¹⁰⁰	<ul style="list-style-type: none"> C to T point mutation in exon 4 	<ul style="list-style-type: none"> Milder phenotype

Table 7

Available dog models for monogenic congenital myopathies.

DMD dog models		
	Mutation	Phenotype
Golden Retriever muscular dystrophy ¹⁰¹	<ul style="list-style-type: none"> X-linked mutation 	<ul style="list-style-type: none"> Dystrophin deficient Muscle hypertrophy and weakness Contracture Esophageal dysfunction due to enlargement of the tongue Cardiac phenotype
LGMD 2C dog models		
	Mutation	Phenotype
SG-deficient Boston terrier ¹⁰²	<ul style="list-style-type: none"> Mutation unknown 	<ul style="list-style-type: none"> Reduced α-sarcoglycan; β- and γ-sarcoglycan absent Muscular dystrophy
SG-deficient Chihuahua ⁹²	<ul style="list-style-type: none"> Mutation unknown 	<ul style="list-style-type: none"> α-, β- and γ-sarcoglycan absent Muscular dystrophy
SG-deficient Cocker Spaniel ⁹²	<ul style="list-style-type: none"> Mutation unknown 	<ul style="list-style-type: none"> Reduced α- and β-sarcoglycan; γ-sarcoglycan absent Muscular dystrophy
XLMTM dog models		
	Mutation	Phenotype
XLMTM dog ^{103–105}	<ul style="list-style-type: none"> Missense mutation in exon 	<ul style="list-style-type: none"> Myotubularin deficient Centronuclear myopathy Severely affected Skeletal muscle wasting, resulting in pronounced and progressive weakness Shortened lifespan

Table 8

Comparison of cell culture, small animal and large animal models

	Cell culture	Small animal	Large animal
Cost	Cheap	Relatively inexpensive	Very expensive
Mutations	<ul style="list-style-type: none"> • Harbors patient specific mutations • Easy to engineer specific mutations 	Created through the knockdown or knock out of related genes	Often naturally-occurring mutations that mirror human disease
Phenotype	Cellular phenotype	May differ from human phenotype	Often similar to human disease
Size	N/A	Small size	<ul style="list-style-type: none"> • Larger size • Organ size closer to that of humans • Able to adapt test originally developed for use in the human clinic, increasing translational power
Lifespan	N/A	Shorter lifespan may make assessing long term effects difficult	Longer lifetime allows for extended study of a single organism
Reproduction	<ul style="list-style-type: none"> • Primary cells have limited culture time • iPS cells theoretically can be propagated indefinitely 	Reproductive fecundity allows for multiple replicates for more fully powered experiments	Relatively slow reproduction with smaller litter size can limit study
Expressivity	Primary cells from patients with distinct genetic background	Genetically very similar to wildtype outside of loci of interest	Some variation in genetic background, even when using true littermate controls
Immunological	N/A	Immune response may differ from that seen in humans	Immune response may differ from that seen in humans