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Review

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Duchenne muscular dystrophy, one of the most complicated diseases for gene therapy

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Abstract

Gene therapy for Duchenne muscular dystrophy (DMD) is hindered by many pitfalls related in particular to the limitations of current technologies, the specificities of muscle and cardiac targets, and the disease itself, a chronic, multisystem, dystrophic and inflammatory disorder. Following RNA-based therapies, DNA gene transfer, mainly based on adeno-associated viral vectors, is now able to deliver therapeutic genetic sequences on a massive scale, and the first antisense and adeno-associated virus (AAV)-microdystrophin gene products are now reaching the marketing stage in Europe and/or in the US. However, only a subset of patients are eligible for those therapies. Many questions remain, such as the duration of the therapeutic effect, the burden of high doses of vectors, and the immunogenicity of viral capsids and therapeutic proteins, in the context of a disease-related inflammatory background. Evaluations of these treatments by the different biotech, pharma or non-for-profit sponsors also come up against the great clinical heterogeneity of patients. This review summarizes the significant progress made over the past three decades to optimize both the efficacy and safety of DMD gene therapies, as well as the remaining challenges, short-term prospects, and future directions such as more targeted vectors and combination therapies.

Keywords: AAV, antisense oligonucleotide (ASO), DMD, microdystrophin, immune rejection, duchenne

INTRODUCTION: NUMEROUS HURDLES TO OVERCOME

Duchenne muscular dystrophy (DMD) is certainly one of the most complex diseases to address with gene

therapy. It piles up a number of barriers and difficulties that decades of research have tried to overcome gradually. It is a rare disease. Its prevalence is currently around 1/5,000 boys worldwide[1]. There are rare cases of female patients or complications for female carriers of recessive mutations present on the X chromosome (OMIM#300377, Xp21.2-p21.1). The relative rarity of the disease hampers large-scale clinical trials.

DMD (MIM #310200), the largest human gene, spans 2.24 million base pairs. Mutations in this gene are the primary cause of the disease. It contains multiple (seven) gene promoters, which drive the expression of different mRNAs and protein isoforms (ranging from 40 kDa to the full-length 427 kDa) in a tissue-specific and time-dependent manner[2]. Clinical manifestations of DMD include progressive muscle degeneration and waisting, typically becoming apparent between the ages of 2 and 5 years. Loss of ambulation often occurs during the early teenage years, followed by the need for respiratory assistance in the early 20s and premature death in the subsequent decade, due to respiratory and cardiac complications[3]. Clinical consequences and pace of disease course may vary depending on the mutation site and the genomic (such as in-frame versus out-of-frame) alterations, which complicate the constitution of clinically homogeneous trial cohorts. Other confounding factors in the assessment of the progression of muscle pathology, with or without treatment, need to be taken into account, such as the initial motor improvement seen in young patients due to age growth and development, which confound statistical interpretations. The first gene-targeted therapeutic trials were based on the 6-min walk test, which proved to be poorly sensitive and highly variable, especially in children[4,5]. Today, the authorities mainly require clinical assessment scores such as North Star Ambulatory Assessment (NSAA), which is not much more sensitive over short follow-up periods.

The disease is multisystemic, affecting various tissues where the protein or its isoforms are expressed: skeletal muscles, vascular and visceral smooth muscle, the heart, endocrine tissues, and the retina, as well as central and peripheral neurons. Therefore, it is essential to ensure the distribution of vectors and their expression throughout the body, even if the priority should be given to the life-threatening skeletal muscles including respiratory muscles and the heart, which together represent 40% to 50% of the body's mass[6].

Dystrophin is a structural, cytoskeletal membrane protein that plays a critical role as a mechanical link between the extracellular matrix and contractile proteins, providing protection against force-related fiber damage. To achieve this protective function, it is essential not only to target a large number of cells but also to ensure robust and widespread expression of dystrophin. The expression must be sufficient to protect the entire length of muscle fibers from membrane disruptions caused by the mechanical stress of contractions[7].

DMD is a chronic disease, which necessitates that molecular therapies remain effective throughout the patient's lifetime. This can be achieved either through a single administration or repeated administration of the therapeutic vector, which, if viral, induces an immune response akin to a vaccine, preventing secondary injections and even first-line treatment in seropositive patients. DMD is also a slowly progressive disease, posing challenges for clinical trial design. Trails often require long follow-up periods and highly sensitive clinical endpoints to achieve statistical significance. Consequently, such trials are typically limited to patients with sufficient motor abilities. Additionally, DMD is a developmental disorder with potentially deleterious consequences from the fetal period, which we do not know if post-natal treatment can reverse[8].

Skeletal muscles are regenerative tissues, and DMD is characterized by a succession of necrosis and regeneration cycles, which raises questions about the persistence or the dilution of the transduced vectors over time. The condition involves dystrophic changes, including histological rearrangements and the

invasion of fibrotic and fatty tissues (by transdifferentiation of muscle cells, activation of fibro-adipogenic progenitors, or chemoattraction of non-muscular cells)[9], which all create physical and physiological barriers that impede the distribution and efficacy of vector particles. To maximize therapeutic outcomes, intervention must be timed carefully-early enough to prevent irreversible remodeling, yet not so early as to encounter the dilutive effects linked to both regenerative cycles and the rapid muscle growth of children, where muscle mass can increase up to 20-fold after birth. Additionally, the transduction efficiency may be influenced by patient age, as vector biodistribution in skeletal muscles seems to be more favorable at earlier ages[10].

DMD is a chronic inflammatory disease, which is an important limiting factor. Mononucleated cell infiltrates (activated cells that can phagocytose foreign particles) can hinder the ability of vector particles to reach muscle fibers. These infiltrates also pose a risk for inflammatory/immunological overreactions to the administration of vector particles, which are mainly viral. The risk of a robust innate immune response is increased due to the high doses to be administered, not only against the vector but also against previously unrecognized dystrophin epitopes. These immune responses may vary across patients[11]. In particular, patients with deletions in the most immunogenic areas of dystrophin, who are "naïve" to this protein, would be at risk of immunological rejection[12]. This concern is amplified by the fact that dystrophin is expressed throughout the body, especially in vital tissues such as the respiratory muscles, heart, and blood vessels. While a humoral response may not be problematic - since dystrophin is an intracellular protein and thus inaccessible to antibodies - a cellular immune response must be avoided at all costs.

GENE THERAPIES OF DMD

Gene therapy for DMD is complicated by the complex genotype-phenotype relationships associated with the disease. Thousands of different mutations in DMD have been found in DMD patients, including those with Becker muscular dystrophy (BMD), a milder form of the disease. The majority of these mutations (60%-70%) are deletions, 5%-15% are duplications (20% in BMD), and 20% are point mutations (10% in BMD). Deletions and duplications predominantly occur in hotspot regions between exons 45-55 and 3-9, affecting 47% and 7% of patients, respectively[2,13,14]. While some trends in genotype-phenotype correlations have been observed, other factors - such as genetic modifiers, inflammation, and fibrosis invasion - may also influence disease progression, making therapy evaluation particularly challenging. The introduction of molecular therapies would further add to this variability[15].

Several therapeutic technologies have been approved or are under development to target specific dystrophin mutations at the DNA or (pre)-messenger RNA levels. A first targeted therapy conditionally approved by the European Medical Agency (EMA) for DMD was Ataluren, based on pharmacological readthrough of premature stop codons during protein translation. Although it has not yet been approved by the US Food and Drug Administration (FDA), the EMA recommended in June 2024 that the conditional authorization granted to Ataluren in 2014 not be renewed due to insufficient evidence supporting the medicine's effectiveness[16].

RNA-based therapies

At the pre-messenger RNA level, the conversion of out-of-frame (leading to DMD) to in-frame deletions (associated with milder forms) can be achieved by exon skipping (with oligonucleotides or U7snRNPs expressing an antisense sequence) or by exon deletions (with genome editing) leading to partially functional BMD-like truncated dystrophins. This treatment strategy is mutation-dependent and each individual exon-skipping strategy is only applicable to a small subset of the DMD patients. Nevertheless, collectively, with multiple medicinal products, the exon skipping strategy could potentially treat ~60% to up to 90% of

DMD patients[17,18,19].

Approved RNA-based therapies

Several antisense oligonucleotide (ASO) chemistries have been developed and approved by the FDA and by the Japanese authority (vitolarsen). The current marketed products target mutations amenable to skipping exons 51 (eteplirsen), 45 (casimersen), and 53 (golodirsen and vitolarsen). However, these therapies have not yet been approved by the EMA. ASOs are small modified RNA sequences (20-30 nucleotides) that can be systemically delivered and specifically bind to a target exon during pre-mRNA splicing, preventing the inclusion of the exon into mRNA. Exon skipping can address certain deletions, duplications, and point mutations. However, due to the turnover of both ASO and the transcript and protein, repeated treatments are required[14,18]. More importantly, the ability of these therapies to produce truncated dystrophin remains very limited, with levels typically ranging from 0.4% to 6% of normal dystrophin expression, which are not sufficient for the biological protection of muscle fibers (estimated at 20% to 40%). These numbers are based on numerous studies conducted in both animal models and DMD and BMD patients[20,21]. However, the reliability of these figures is not guaranteed, as they may be influenced by factors such as the functionality of the dystrophin produced, individual variations, and species-specific specificities.

Future directions

Efforts are increasingly focused on developing more efficient chemistries that enhance biodistribution, extend pharmacokinetics (to reduce injection frequency), and target additional exons to expand the pool of eligible patients[18]. The first ASO exon-skipping drugs were limited by low myocardial efficacy and suboptimal pharmacokinetics. To address these limitations, a new chemical approach based on tricyclo-DNA antisense molecules with better bioavailability has been designed[22] (NCT05753462) and is currently being evaluated in DMD patients, with results pending. Additionally, a novel retargeted ASO, AOC1044, a phosphorodiamidate morpholino oligomer (PMO) conjugated to a truncated anti-transferrin antibody to enhance muscle cell uptake, has recently shown promising efficacy, with up to 25% restoration of dystrophin production compared to normal levels and up to 80% reduction in Creatine Kinase (CK) levels in a phase 1/2 clinical trial[23](NCT06244082).

Duplicated exons, including exon 2, one of the most commonly duplicated exons, are also eligible for exon skipping. Skipping of duplicated exons potentially restores a full-length (and thus fully functional) dystrophin. This approach is currently being investigated using an adeno-associated virus (AAV) vector carrying four copies of a modified U7 small nuclear RNA that contains antisense sequences targeting the splice donor (2 copies) and splice acceptor (2 copies) of the DMD exon 2[24] (NCT04240314).

However, the recruitment of patients with specific genetic subtypes within this already rare disease presents a bottleneck for drug development, which is further complicated by the individual variability in clinical scores over time. Additionally, to optimize statistical power and avoid bias, trial designs should prioritize matching comparative groups based on reliable, data-driven prognostic factors. Recent studies suggest that trial designs should avoid overemphasizing balance in genotype classification[25]. This requires, for instance, to include a natural history/baseline study to monitor clinical and biological evolution for at least 6 months before enrolling DMD patients. Part of the genotype effect may already be captured through baseline functional status, reducing the confounding influence of genotype. This is crucial for any type of therapeutic approach to DMD.

DNA-based therapies

Genome editing

Recently, genome editing has been proposed to restore the reading frame at the DNA level. CRISPR/Cas gene editing may restore full-length dystrophin expression after a single treatment in dystrophic rodent and dog models, although additional work is required to demonstrate the feasibility and safety of this approach in humans. It has several drawbacks such as: (I) very low efficiency in deleting multiple exons, (II) lack of efficiency in delivering the CRISPR-Cas system in vivo, (III) possible need for repeated treatments, and (IV) off-target related safety concerns[26]. In a first-in-man trial, a 27-year-old patient with DMD was treated with a dose of 10^{14} viral genomes (vg)/kilogram(kg) of body weight of an AAV of serotype 9 containing dSaCas9 (Staphylococcus aureus Cas9 in which the Cas9 nuclease activity has been inactivated). Eight days after infusion, the patient sadly died after an initial mild cardiac dysfunction and pericardial effusion, followed by acute respiratory distress syndrome and cardiac arrest. Given the timelines, genome editing is probably not in question as it did not have enough time to take place. A postmortem examination showed severe diffuse alveolar damage, probably elicited by an innate immune reaction to the high-dose AAV. Transgene early expression was minimal, and there was no evidence of AAV serotype 9 antibodies or effector T-cell reactivity in the organs[27].

Replacement gene therapy

Since DMD is a monogenic disease, gene replacement therapy is one of the most logical and promising treatment options. The first gene therapy clinical trial was completed in 2004. It showed that local intramuscular administration of a low dose (600 μg) full-length dystrophin plasmid in 9 Duchenne and Becker patients allowed only very low and local (along the needle track) expression of dystrophin[28]. Intravascular delivery in isolated limbs (referred to as hydrodynamic limb vein (HLV)-HLV- injection) of the plasmid showed a much higher muscle transfection efficiency (up to 40% of transfected limb muscles) in primate, and in mouse (*mdx*) and dog (*Golden Retriever Muscular Dystrophy*) DMD models[29]. Safety of the locoregional HLV delivery of increasing volumes of saline buffer was asserted in a series [limb-girdle muscular dystrophy 2A, Emery-Dreifuss muscular dystrophy, autosomal recessive autosomal dominant Limb Girdle Muscular Dystrophy (LGMD), LGMD, and BMD] of volunteers in lower[30] and upper[31] limbs. However, certain limits were imposed on the volume administered (not exceeding 20%-40% of the limb volume to be administered) due to the internal pressure within the muscle tissues and the theoretical risk of compartment syndrome in already weakened muscles. This limitation on the injected volume posed a barrier to the use of plasmids in DMD.

Viral vectors may transduce skeletal muscle much more efficiently and more broadly. A few of them can accommodate the full-length, 14 kb dystrophin coding sequence together with the associated promoter and regulatory sequences, but they are either ineffective (lentiviruses) or too inflammatory (e.g., poxvirus) to be considered[32,33]. As of today, AAVs outperform other vectors in delivering genes to skeletal muscles and the heart. Moreover, they can infect both dividing and nondividing cells and enable relatively long-lasting and stable gene expression. However, the dystrophin cDNA exceeds the capacity of AAVs (~5 kb).

Microdystrophin gene therapy

The initial discovery that truncated dystrophins retain functionality[34] provided the rationale for using minimized versions of dystrophin compatible with the capacity of AAVs. These highly modified, smaller molecular weight versions of dystrophin, known as microdystrophins (~150 kDa compared to normal 427 kDa dystrophin), contain over half of the normal dystrophin amino acid sequence but are designed to preserve essential functions of the protein. Microdystrophins could theoretically be applied to all DMD patients irrespective of their mutation[35].

AAVs come in the form of various serotypes that differ in tissue tropism and transduction efficiency that may also be species-dependent[36]. AAV8, AAV9, AAVrh74 (highly similar to AAV8), and Myo-AAV are currently used in clinical trials for DMD. The sarcolemmal dystrophin is organized into highly focal and nonmobile nuclear domains of ~80 μm in size[37], although we cannot exclude the existence of a diffusible cytoplasmic fraction that could be below the level of detection, as well as the contribution of mobile nuclei. Therefore, any AAV gene therapy should transduce as many myonuclei as possible to avoid an ineffective mosaic microdystrophin expression.

As of June 2024, the FDA has approved one product, Elevidys (AAVRh74-hMD1), developed by Sarepta/Roche, for patients aged 4 years and older[38]. The EMA is currently reviewing the application for patients aged 4 to 7 years only. Long-term and extension studies are ongoing (NCT05881408, NCT04626674, NCT05096221, NCT06128564, NCT0596735). Another product developed by Pfizer was recently dropped after missing the primary endpoint in a phase 3 trial (NCT05689164, NCT02907619, NCT05429372). The death of two patients during this study certainly influenced the sponsor's decision. This product uses an AAV9 vector carrying a different version of microdystrophin[12]. Based on public releases and communications at congresses, we know that Solid Bioscience has also decided to stop the development of its AAV9-microdystrophin (the third version of truncated dystrophin) and switched to a second-generation vector, SGT-003 (NCT06138639) based on Myo-AAV vector with expected reduced immunogenicity and improved efficiency. Additionally, two other sponsors are pursuing the clinical development of two AAV8-microdystrophin vectors. Genethon has completed the dose-escalation phase of its seamless clinical program with an AAV8-hMD1 (the same microdystrophin version as Sarepta) (Eudra-CT2020-002093-27) and Regenxbio is completing the dose-escalation phase of a very similar vector that includes a small sequence added to the C-terminal part of the microdystrophin to improve part of its function (NCT05693142, NCT06491927). Both of these programs have now entered into the pivotal phase based on promising functional data.

The degree of functionality and therapeutic impact of microdystrophins in humans remains unclear. The retained portions of the protein and the level of expression may influence its function. Chamberlain *et al.*[39] and Boehler *et al.*[40] provide a detailed discussion of critical microdystrophin elements. Additionally, the quantity of microdystrophin expressed may contribute to some aspects of clinical benefit[39]. All sponsors are using different tissue-specific promoters (MHCK7, MHC, K8, spc5.12, and spc2.12)[12]. Therapeutic doses generally exceed 10^{14} vg/kg except for Genethon (3×10^{13} vg/kg). However, uniform and head-to-head titrations should be performed to accurately assess the differences in viral loads injected. Large-scale production methods also vary. Some use plasmid-transfected HEK293 cells of different strains, while others (such as Solid Biosciences) use an Herpes Simplex (HSV)-based process. Purification methods may also differ[41]. Overall, these variations may result in differences in transduction efficiency, functional benefit, and safety.

AAVs transduce differentiated fibers but not satellite cells as initially reported. Kwon *et al.*, however, reported that satellite cells can be transduced with AAV vectors and can undergo gene editing to restore the dystrophin reading frame in a sensitive Cre/lox-based dual-reporter *mdx* mouse model. Interestingly, higher AAV transduction of satellite cells was seen in *mdx* mice compared to Wild Type (WT) mice, supposedly because of the constant need for regeneration and a higher number of activated satellite cells in *mdx* mice[42].

Safety and durability of AAV-based gene therapy

AAV integration into the host genome

AAV are thought to rarely integrate into the host genome and it is possible that, with successive cycles of degeneration/regeneration (which may occur naturally throughout life), the therapeutic transgene will fade with time due to some dilution effect. Whether this really happens and how long it takes remain unknown, at least in humans. To avoid the risk of gradual loss of episomal transgenes and microdystrophin expression, transgene expression must exceed the threshold (yet unclear) necessary. This has been reported in dystrophic animal models[43,44]. Additionally, the "dilution" effect associated with muscle growth in very young patients suggests that repeated administration of any DMD gene therapy will likely be needed. However, the required interval between treatments and how to circumvent the presence of circulating AAV neutralizing antibodies and the potential T cell response induced by the primary treatment remain to be determined. Nonetheless, studies in Golden Retriever Muscular Dystrophy (GRMD) dog models have shown that long-term (lifelong, > 10 years) clinical benefits[45,46] can still be expected.

Anti-AAV immunity

As seen in the general population[47], many DMD boys may have been exposed to AAVs, potentially resulting in pre-existing immunity to the AAV serotype used for gene therapy[48,49]. Consequently, these patients are currently excluded from receiving gene therapy. To address this limitation, immunosuppressive strategies are being developed[50,51]. For instance, prior administration of IdeS (Imlifidase), a bacterial protease that cleaves human antibodies[52] (NCT06241950), is being investigated in DMD. Systemic AAV gene delivery, with high doses of ~10^{13} to 10^{14} vector genomes/kg, has been shown to elicit the activation of the innate immune response to the vector in large animal models and humans, including neuromuscular patients[41]. An adaptive T lymphocyte cell (CTL)-mediated immune response following AAV transduction could lead to the clearance of transduced cells. However, this response is not expected to be detrimental, as healthy skeletal muscle cells exhibit low MHC-I expression, which reduces the T-lymphocyte-mediated reaction[53]. In inflammatory contexts, such as in DMD patients, MHC-I antigens are overexpressed, and muscles contain activated T cells, which would make DMD muscle tissue more susceptible to T-cell immune responses[54]. Additionally, given the high viral load used, a dose-dependent innate immune response eventually initiates adaptive immune responses against both the viral capsid and the transgene. Strategies like CpG depletion from rAAV vector constructs[55] and enhancing vector production by artificially increasing CpG methylation could partially alleviate this anti-AAV innate immune response. Empty capsids may also be used as decoys for anti-AAV neutralizing antibodies, which means that pure full-capsid batches may not necessarily be more effective than mixed preparations[56]. On the other hand, using different AAV strains may offer the possibility of selecting a suitable vector for patients who are seropositive for a certain strain (and therefore, non-eligible for therapy as they would neutralize the injected vector), provided there is no cross-reactivity.

Anti-transgene immune rejection

Recently, five cases of strikingly similar suspected unexpected serious adverse reactions (SUSARs), including life-threatening myositis, were reported in DMD boys aged 7-9 years, who were recruited into three separate microdystrophin gene therapy trials (NCT04281485, NCT04626674, Eudra-CT2020-002093-27). The three AAV products used in these trials contained different microdystrophin transgenes, each under a distinct muscle-specific promoter, and were packaged in different serotypes (AAV9, AAV8, AAVrh74). The kinetics of these SUSARs was consistent with transgene expression, and laboratory findings suggested a cytotoxic T-cell immune response against dystrophin. Notably, all the participants had similar DMD mutations, which pointed to epitopes located within exons 8-11 containing hinge 1 and the beginning of the spectrin-like repeat domain, representing non-self epitopes

for the patients[12]. This observation resembles that in the boy reported by Mendell injected locally and who had a deletion of exons 3-17[57] (of note, none of the 9 patients of the pioneer plasmid study where bearing "at risk" deletions[28]). In response to these findings, the four AAV clinical trials have been amended so that patients with genomic deletions that significantly overlap with transgene sequences are currently excluded from dosing[12]. Interestingly, various immunomodulatory treatments - such as pulsed-dose steroids, intravenous immunoglobulins, plasmapheresis, and tacrolimus) - were used to manage the SUSARs across the different programs, with a resolution of symptoms occurring within 3 months and tapering of the immunosuppression. However, determining the optimal duration of immunosuppression to achieve permanent tolerization of the transgene in "at-risk" patients will require further studies, given the particular complexity of human immune responses. In this respect, the management of severe immunological events reported by the three microdystrophin gene therapy sponsors may provide some guidance. Alternatively, re-designed "de-immunized" epitopes without compromising their functionality are being explored in *mdx* mice, but translating this approach to the more complex human immune context will require careful attention[58].

Level of microdystrophin expression

It is clear that a major clinical goal is to achieve sufficient microdystrophin expression throughout the skeletal and cardiac muscles. However, very little is known about the impact of microdystrophin overexpression. Cardiac toxicity has been reported in transgenic mice with 100-fold overexpression of microdystrophin[59]. Recently, Hart *et al.*[60] reported the accelerated onset of dilated cardiomyopathy, heart failure, and death in dystrophic mice following AAV-induced overexpression of two of several microdystrophin variants. Further studies are needed to better characterize the potential cardiotoxicity elicited by overexpressed microdystrophin in the heart.

While microdystrophin gene therapy may prove to be a game-changer, it is not expected to fully restore function in skeletal and cardiac muscles. The therapy is theoretically limited to shifting the phenotype from DMD to a mild BMD phenotype, even with high levels of expression. This is supported by current trials, where blood creatine kinase levels - an indicator of skeletal and cardiac muscle damage - seem to drop to BMD levels, but not to those of healthy controls[57].

Alternative transgenes

Full-length or quasi-dystrophin

Researchers are developing dystrophin versions that are closer to the full-length protein, using combinations of AAV vectors and leveraging the ability of AAV genomes to undergo intermolecular concatemerization. A few studies have reported the use of dual AAV vectors for therapeutic correction in DMD, and even a triple-vector system has been used to reconstitute the full dystrophin coding sequence through a trans-splicing system, though with very low efficiency[61,62]. Full-length dystrophin can also be generated using a triple AAV combination based on split inteins - small polypeptides that self-assemble and undergo a protein trans-splicing reaction. This triple-vector combination improved muscle histopathology and function in a mouse model of DMD[63]. However, the translational potential of dual or triple AAV vectors for human patients needs further investigation. For instance, homologous recombination efficiency requires further optimization to reduce the total load of AAV vectors. Additionally, the formation of aberrant products resulting from AAV Inverted Terminal Repeats (ITR) concatemerization requires in-depth evaluation. Vectors that can efficiently transduce satellite cells would be particularly useful for promoting muscle regeneration. Lastly, the quasi-dystrophin produced by this approach may bear a higher

risk of inducing an anti-transgene immune response, which must be prevented.

Non-dystrophin genes

To bypass any anti-transgene immune response, genes that are constitutively expressed are being investigated. Utrophin, a functional paralog of dystrophin that is naturally expressed in muscles, has been proposed as an immunologically safer therapeutic gene alternative. Utrophin is a ubiquitous protein, expressed at the sarcolemma of embryonic muscles and progressively replaced by dystrophin at the muscle membrane after birth. In adult skeletal muscle, utrophin is confined to the neuromuscular and myotendinous junctions, as well as blood vessels, and is only expressed at the sarcolemma of regenerated myofibers. Utrophin is highly homologous to dystrophin and can recruit most of the components of the dystrophin-associated protein complex[64]. Despite efforts, pharmaceutical stimulation of utrophin to compensate for the absence of dystrophin has not yet been successful. Therefore, utrophin gene therapy remains a promising alternative. Given the size of its coding sequence, truncated versions of micro-utrophin are being designed, with consideration given to the differential binding capacity and suitable folding of utrophin and dystrophin[65].

Another potential candidate is Cytotoxic T cell GalNAc transferase (GALGT2), an enzyme normally distributed, like utrophin, at the neuromuscular junctions of myofibers postnatally. GALGT2 glycosylates the dystrophin-associated glycoprotein a-dystroglycan. The delivery ofrAAVrh74-GALGT2 has been shown to successfully treat DMD mice[66] and, to a lesser extent, DMD dogs[67]. Encouraging functional data were obtained in a phase 1/2 clinical trial assessing the safety and efficacy of GALGT2 gene therapy (NCT03333590)[68]. However, the clinical development of this program was discontinued by the sponsor[69].

AAV-microdystrophin, utrophin, or GALGT2 gene therapy would be potentially applicable to all DMD patients regardless of their mutation. Active clinical development programs and approved gene-based drugs are shown in Figure 1.

A range of challenges remain in fully optimizing AAV dosage, immune control and large-scale manufacturing. A more potent expression cassette could reduce the required viral dose to achieve the therapeutic effect. However, according to the nuclear domain theory, stronger expression cassettes with lower vector amounts would not align with the need to transduce a maximum of nuclei along the muscle fibers. Natural variant-based AAVs lack specificity and often accumulate in the liver, with the concomitant risks of hepatotoxicity and general toxicity due to high dosage[70]. One potential solution is lowering the dose by enhancing vector specificity through capsid modification. High-throughput screened or computationally designed AAV capsids that more specifically target skeletal muscle to lower treatment doses are being developed[71]. A recent variant, LICA1, demonstrates comparable muscle transduction to other myotropic AAVs, with significantly reduced liver targeting and, not to underestimate, large-scale production yield capacity[72], which could help reduce the heavy burden of manufacturing costs. Nevertheless, while myotropic AAVs represent attractive second-generation vectors, they still require careful safety assessment.

CONCLUSION

The perfect molecular therapy, administered in very low doses and targeting motor function, circulation, and digestive tracks, as well as cognitive skills, does not exist yet. Nevertheless, emerging combination therapies show promise in addressing the specific needs of each organ. The remaining hurdles (immunological issues, persistence and optimization of therapeutic effects for complete disease remission) are being investigated separately and possible solutions are under consideration. Another key obstacle

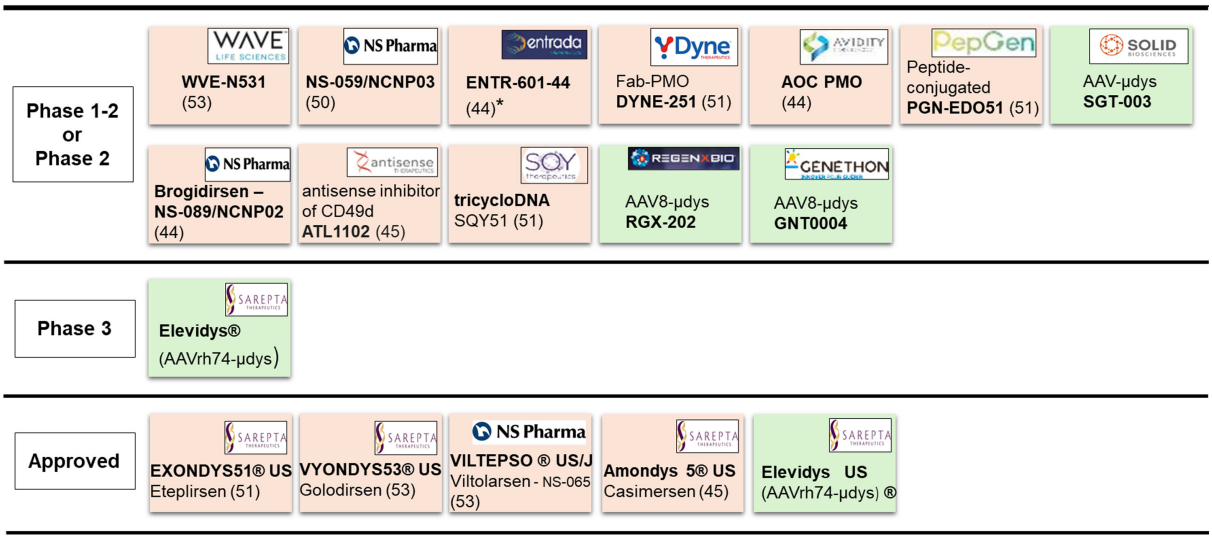

Figure 1. Current pipeline of gene-based therapies. Positioning of the antisense (beige) or gene-replacement (green) therapies, according to the stage of development (left column). Bold: drug name including commercial name (®); US: approved in USA; J: approved in Japan; Logos: name of the sponsor; Number in parentheses: targeted exon; *: clinical hold by FDA; µdys: microdystrophin; Fab-PMO: antibody-conjugated Phosphorodiamidate Morpholino Oligomers. Two other studies carried out in China (NCT06114056 relative to an AAV-microdystrophin, and NCT06594094 relative to a CRISPR-gene editing approach) are not indicated here. PMO: Phosphorodiamidate morpholino oligomer; AAV: Adeno-associated virus; FDA: Food and drug administration.

remains: the development of an economically viable model that guarantees access to these expensive combination biological treatments for all patients[73], including non-ambulant individuals with DMD, BMD, rare female DMD patients, and affected female carriers. Part of the answer relies on scientific and technological progress.

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Authors' contributions

The author contributed solely to the article.

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