Gene Therapy in Duchenne Muscular Dystrophy

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Gene therapy allows for the modulation and correction of specific problem genes which are mutated in severe pathologies. The term “gene therapy” is loosely defined by many sources. The FDA defines it as “products whose effects are transferred through transcription/translation of genetic material via administration as nucleic acids, viruses, or genetically engineered microorganisms”. Duchenne muscular dystrophy (*DMD*) is a severe X-linked recessive muscle-wasting disease that results from mutations in the *DMD* gene on chromosome 21. Due to its poor survivorship, many interventions are being researched to improve outcomes for patients with this disease; gene therapy is an emerging field in this regard.

Keywords: gene therapy ; Duchenne muscular dystrophy ; gene replacement ; micro-dystrophin ; GALGT2 ; antisense oligonucleotides (ASOs) ; CRISPR/Cas9

1. Introduction

Typically, gene therapy is categorized into two broad types: somatic and germ-line therapy. In somatic therapy, genetic material is inserted in target cells, but the resulting changes are not passed along to the next generation; in contrast, germ-line gene therapy involves the transfer of therapeutic or modified genes in target cells to subsequent generations [1]. The concept of gene therapy was first conceptualized and trialed in 1928 with Frederick Griffith’s “Griffith experiment” [1]. Griffith’s trial focused on the transformative qualities of bacteria, demonstrating that bacteria could be injected with pathogenic genetic properties, which enhanced their virulence [2]. These trials laid the groundwork which was further explored by Joshua Lederberg and his team in 1947. Ledergberg, who won a Nobel prize in 1958 for his work in bacterial genetics, discovered the transformative and conjugative properties of bacteria [1]. Watson and Crick’s discovery of DNA structure in 1953 and Marshall Nirenberg’s discovery of the “triple code of DNA” in 1961 were subsequent and important breakthroughs in genetic therapy. Following these discoveries, the area of genetic therapy has seen numerous advancements. In the mid-1990s, scientists began to see increased success and potential with the use of viral vectors for genetic therapy [2]. This led to trials in animals and humans which yielded variable responses [2]. Adenoviral vectors, retroviral vectors and naked plasmids are currently the most popular vectors used in genetic therapy as they correlate with the most ideal results [1]. Today, cancer is also the most researched field with regard to gene therapy; however, other fields like orthopedics also have extensive ongoing trials [1]. Pediatric orthopedics is a subset of orthopedics that focuses on pathologies involving children aged 0–18 years old. In pediatric orthopedics, gene therapy is an area of great promise. Drug trials are currently ongoing for the treatment of severe conditions like Duchenne muscular dystrophy (*DMD*), osteogenesis imperfecta (OI), spinal muscular atrophy (SMA) and osteosarcoma, with a few of these drugs reaching the point of commercial use.

Duchenne muscular dystrophy (*DMD*) is a severe X-linked recessive muscle-wasting disease that results from mutations in the *DMD* gene on chromosome 21 [3]. The *DMD* gene forms dystrophin protein, an integral muscle structure and integrity component. Without sufficient dystrophin, muscles are exposed to increased damage leading to progressive muscle wasting and loss of muscle function. *DMD* is a pediatric-laden disease with a diagnosis usually occurring in children aged 3–5 [3]. The disease complications of *DMD* typically progress during childhood, leading to mortality in young adults in their early 20s [3]. Due to the nature of the disease, *DMD* care can often be complex, and treatment usually involves a multi-disciplinary team (MDT) treating cardiorespiratory, endocrine, orthopedic and rehabilitative complications. Due to its poor survivorship, many interventions are being researched to improve outcomes for patients with this disease; gene therapy is an emerging field in this regard.

2. Gene Replacement Therapy

The goal of GRT is to transfer corrective material into cells to alleviate disease symptoms. Corrective material in these cases can be delivered via viral/non-viral delivery systems. In *DMD*, GRT is not aimed at correcting specific mutations but

rather restoring muscle function via the injection of truncated or muscle-protective enzymes [4]. To this avail, many gene replacement therapies/treatments aimed at perfecting truncated/muscle-protective enzymes to be used in *DMD* treatment are currently ongoing.

2.1. Microdystrophin Targeting

Most of the trialed GRTs in *DMD* focus on the *micro-dystrophin* gene. The *micro-dystrophin* gene is a truncated version of the *dystrophin* gene. Because of its altered form, the gene produces dystrophin proteins which are a third of their expected size. These proteins have a decreased capacity to strengthen and protect muscle fibers, leading to their injury. *Micro-dystrophin* targeting trials range from phase 1 to phase 3 and have expected finish dates from 2023 to 2028. Currently, three drugs are being developed for GRT targeting *micro-dystrophin* in *DMD*: SRP-9001 by Sarepta Therapeutics, PF-06939926 by Pfizer and SGT-001 by Solid Biosciences [4]. SRP-9001 is transmitted using vector rAAVrh74 and uses promoter MHCK7 to enhance cardiac dystrophin expression [4]. SRP-9001 first showed promise in Study 102, a two-part phase 2 placebo-controlled trial. The first part of the trial involved SRP-9001 meeting the biological outcome of micro-dystrophin protein production, the second part had *DMD* patients present with statistically significant scores on the North Star Ambulatory Assessment (NSAA) when treated with SRP-9001 compared to the external control group [4]. These results led to SRP-9001 initiating the EMBARK study whose primary outcomes were to see if basepoint NSAA could be changed after 52 weeks of use. In addition to the EMBARK study, SRP-9001 also finished the 1-year phase ½ trial (NCT03375164) with promising results [5]. The trial involved SRP-9001 administration via a peripheral limb vein with daily administration of prednisolone started 1 day before SRP-9001 administration and tapered off for 30 days after [5]. The trials showed SRP-9001 to be well tolerated with minimally adverse effects while eliciting improvements in baseline NSAA scores and system creatine kinase levels [5]. SRP-9001 is also currently undergoing a phase 1 open-label trial called ENDEAVOR which is expected to finish in 2024 [4]. PF-0693992, unlike SRP-9001, uses AAV9 instead of rAAVrh74 as a vector. Its safety following IV administration is currently being tested in a phase 1b open-label clinical trial. Pre-trial data for the study presented PF-06939926 to have an acceptable safety profile with treatment-related side effects thrombocytopenia, dehydration and acute kidney injury all resolving within 15 days of presentation [4]. In the same phase 1b open-label trial, PF-0693992 also resulted in an NSAA score of +1 in the experimental group compared to the −4 seen in the control cohort group. This, similar to what was seen in SRP-9001, prompted an ongoing phase 3 study for PF-0693992 which is examining basepoint NSAA scores after 52 weeks of use [4]. SGT-001 contains a neuronal nitric oxide domain which aids it in preventing ischemia-related muscle injury. It is currently being evaluated in the phase 1⁄2 trial IGNITE *DMD*. IGNITE *DMD* is a trial aimed at testing SGT-001's effect on SV95C, an assessment of post-administration peak ambulatory performance in *DMD* patients five years old or older [6]. The study involves patients receiving one IV shot of SGT-001 and being subsequently followed for about 5 years [7] (NCT03368742). So far, the study has been promising, with IGNITE *DMD* patients demonstrating average improvements in SV95C scores of 8.8–9.5% compared to baseline, 23.9–24.6% compared to natural history and 26.0–26.7% compared to the control patients [6]. These patients have also expressed stable and increased micro-dystrophin as well as continued localization of B-sarcoglycan and nNOS compared to their baseline results [4].

2.2. GALGT2 Targeting

Although *micro-dystrophin* targeting is the mainstay of gene replacement therapy in *DMD*, success has also been seen in trials targeting the *GALGT2* gene. One such study which finished in 2021 has since progressed to a phase ½ trial due to its promising results. The *GALGT2* gene encodes for the GALGT2 enzyme which glycosylates α-dystroglycan in skeletal muscles, increasing dystroglycan-binding proteins like dystrophin. Targeting of this gene has proven useful and could be a future alternative to *micro-dystrophin* gene targeting for *DMD* treatment. In 2018, an AAVrh74-mediated *GALGT2* GRT trial under the control of a Muscle Creatinine Kinase (MCK) promoter finished with positive results [4]. In the trial, no organ damage was seen at 1 and 3 months after drug administration, and widespread positive transduction was seen with mice injected with the drug. These results were positive and show promise in the area of *GALGT2* targeting.

3. Antisense Oligonucleotides

As the term "gene therapy" is a bit loosely defined, the use of antisense oligonucleotides (ASOs) in *DMD* is occasionally debated. The general definition for this therapy is the treatment of disease via the transfer of genetic material into cells [8]. As stated by the YU lab, although they act on genetic diseases, ASOs are not considered gene therapy because they act on RNA, not DNA [9]. Other studies, however, acknowledge ASOs as a form of genetic therapy, and thus, their inclusion is ambiguous. In *DMD*, ASOs are used as a method of skipping exons via binding to a region of mRNA; this is especially beneficial in *DMD* as the majority of *DMD* mutations are located between exons 43 and 53, allowing for widespread application of treatments [4]. There are a few FDA-approved ASO treatments available; these include Eteplisern,

Casimersen and Golodirsen, all developed by Sarepta Therapeutics, and Vitolarsen, developed by Nippon Shinyaku in collaboration with the National Centre of Neurology and Psychiatry [4]. Eteplisern was the first FDA-approved drug for *DMD*. The drug is a Phosphorodiamidate morpholino oligomer (PMO) which binds to the complementary exon 51 on preMRNA dystrophin, causing exon skipping during the dystrophin mRNA splicing process [4]. Despite the specificity of its function, it applies to a wide range of patients as 13–14% of *DMD* patients have mutations that benefit from skipping exon 51 [4]. In July 2011, a 12-participant study showed that the drug did not elicit any serious disadvantages while restoring dystrophin levels; the success of this study was followed up upon FDA request, leading to a phase 3 trial called PROMOVI, which similarly demonstrated Eteplisern's ability to restore dystrophin levels despite a lack of statistically significant restoration [4]. Following these results, Eteplisern was approved for public use; however, ongoing confirmatory studies to establish its efficacy are still being run by Sarepta Therapeutics [4]. Golodirsen is another PMO developed for the treatment of *DMD*. It facilitates the skipping of exon 53 in the dystrophin gene, which assists in the treatment of around 7.7% of *DMD* patients. It first garnered attention in a phase ½ trial which showcased its use resulting in increased dystrophin production in the skeletal muscles of patients [4]. These results led to its accelerated approval by the FDA. Despite its approval, Golodirsen carries the risk of renal toxicity, as seen in some animal studies involving the drug. Because of this, the FDA mandated renal function monitoring when this drug is used [4]. Casimersen is a PMO that facilitates the skipping of exon 45 in the *DMD* gene, which benefits around 8.1% of all *DMD* patients [4]. It was approved by the FDA following its promising results of dystrophin increase in a double-blind placebo-controlled study (NCT02500381). Similar to Golodirsen, however, this drug was shown to elicit renal toxicity in non-clinical studies, leading to the FDA mandate of renal monitoring when the drug is taken [4]. Sarepta Therapeutics is currently conducting two confirmatory studies for the drug at the moment. Viltolarsen is a PMO that allows the skipping of exon 53, similar to Golodirsen. Like Golodirsen and Casimersen, Viltolarsen requires renal function monitoring while being taken. In trial NCT02740972, mandated for the drug's accelerated approval process, Viltolarsen yielded statistically significant results for the primary endpoint "time to stand" as well as secondary motor-related functional outcomes [4]. NS Pharma is currently conducting phase 2, 3 and 4 clinical trials for the drug. Apart from ASOs of PMO origin, exon-skipping trials involving the 2OMePS (Drisapersen) chemistry and locked chirality stereo pure ASO structures also exist. Clinical trials involving the latter of the two have not been published yet; however, research into the efficacy of 2OMePS oligonucleotides is extensive [10]. Despite chemical similarities to the drug Nusinersen (2OMOE) used in the treatment of spinal muscular atrophy (SMA), Drisapersen appears to lack a sufficient therapeutic index to drive adequate levels of dystrophin to compensate for its dose-related toxicities [10]. Trials for Drisapersen were almost successful in gaining the drug FDA approval; however, these tries were denied due to concerns about the safety around extensive injection site reactions that continued after cessation of the drug. Due to its similar chemistry with Nusinersen, the method of drug administration used for Drisapersen (subcutaneous) vs. intrathecal (Nusinersen) could be potentially related to the negative effects seen upon the use of the drug [10]. Despite their extensive usefulness in the field of *DMD* treatment, PMO ASOs are limited in their immunogenicity and sensitivity to degradation [4]. An approach to combat these shortcomings is the infusion of ASOs into a U7 snRNP molecule before subject administration. U7 is a uridine-rich ribonucleoprotein that is small in size, is concentrated in the nucleus and does not produce an immune response [4]. ASO infusion with U7 snRNP molecules is currently only useful in the treatment of dystrophin exon 2 duplications. In these cases, the ASOs are delivered using scAAV9 vectors leading to wild-type mRNA and asymptomatic patients [4]. Currently, this combination is being tested in trial NCT04240314 for safety and efficacy.

4. CRISPR/Cas9 Therapies

Since its discovery about a decade ago, clustered regularly interspaced short palindromic repeat, CRISPR-associated (CRISPR/Cas9) has been a groundbreaking tool in the field of precision medicine [11]. The CRISPR/Cas9 system is currently used to precisely edit mutagenic genes at specific genomes. As *DMD* is mainly caused by point mutations (30% of patients) and exon deletions (70%), the increasing precision and functionality of CRISPR/Cas9 treatments present the tool as a potential remedy to some presentations of the disease [11]. Currently, clinical trials related to CRISPR/Cas9 and *DMD* do not exist; however, the current sentiment is that clinical implementation of these trials is close to feasibility, with around 35 non-clinical CRISPR-related studies existing as of 22 October 2021 [11]. Currently, experimental trials for CRISPR/Cas9 are focused on two main areas, mediating single- and double-strand DNA breaks. Double-strand breaks involve inducing specific breaks in the DNA leading to the restoration of dystrophin expression in the cells of *DMD* patients [11]. The main issue with double-stranded DNA breaks is their high mutagenic potential and tendency to lose genetic information. Additionally, if there is imprecision with double-stranded DNA techniques, genetic mutations and INDELs can occur [11]. On the contrary, single-stranded DNA techniques are used to avoid genetic damage that can occur from the use of double-stranded break techniques [11].

In sum, many genetic interventions exist/are being trialed for the treatment of *DMD*. The two main areas focused on in these therapies are antisense oligonucleotides (ASOs) and gene replacement therapies (GRTs) [4]; other therapies like CRISPR editing also exist but have not heralded the success of the previous methods.

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