Examining the Effectiveness of Gene Therapy as A Therapeutic Remedy for Hemophilia Patients: A Review

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Hemophilia is a genetic disorder affecting one in every 5500 males in the US, and around 200,000 individuals are affected. Due to the condition being caused by a recessive allele on the X chromosome, males are significantly more likely to contract it. Around 4 of every 5 hemophilia patients are male. Hemophiliac patients have thus far been treated with factor or non-factor treatments. Representative therapy such as Emicizumab®was used in the non-factor treatment, which did not add any additional factors to rectify the defect, while the factor treatment injected the previously mentioned factors directly into the vein. However, with the use of gene therapy, hemophilia can be cured, and its defects can be fixed by adding, removing, or changing specific genetic information through the use of recombinant adeno-associated virus (rAAV) vectors.

Keywords: Hemophilia, genetic disorder, recessive allele, X chromosome, factor/non-factor treatment, rAAV vectors

Introduction

Hemophilia is a rare genetic disorder caused by a deficiency or lack of certain blood clotting proteins causing patients to bleed more and for much longer when cut compared to people without hemophilia. There are often two commonly known types of hemophilia, hemophilia A and B, with a rare type, hemophilia C. Each type of hemophilia is related to a deficiency or absence of a specific blood clotting factor - Factor VIII, Factor IX, and Factor XI, respectively. Patients experiencing hemophilia experience frequent nosebleeds, bleeding gums, swollen and stiff joints, bruises, coughing or vomiting blood, and/or blood in stool or urine.

Hemophilia is a recessive disorder that is genetically carried through the X chromosome. Given this, males (who only have one copy of the X chromosome) are more likely to be affected as compared to females (who have two copies of the X chromosome). The condition affects around one in every 5,000 males and the United States currently has around 200,000 affected people [1]. Additionally, only around one in 5 hemophilia patients admitted to treatment centers are female [2]. The median years [1,2]. Overall, with proper treatment, the life expectancy for those suffering from severe hemophilia is 63 years compared to the all-cause mortality median life expectancy which is 75 [3].

As of 2023, there is no cure for hemophilia, but treatments are constantly being developed to improve the quality of life of affected patients and scientists are working towards eventually finding a cure. Currently, there are two distinct categories in which these treatments fall into: Factor replacement therapies, where the missing factor (VIII, IX, or XI) is injected directly into the affected patient's vein, and non-factor replacement therapies, where the defect is corrected without the addition of factors. However, rising in popularity and administration is a non-factor replacement therapy called Emicizumab®(Sold as Hemlibra, manufactured by Genentech) that works to mimic the efficacy of Factor VIII by spatiotemporal relocation of Factor IX and X. This is favorable because it works in conjunction to the body's natural response to clotting a wound. Emicizumab®is given by an injection under the skin and a non-inhibitor variation was approved by the Food and Drug Administration (FDA) in 2018.

The current standard of treatment for hemophilia patients is not sufficient as frequent clotting factor injections are needed. Many adults don't continue prophylactic therapy after childhood, mainly due to the frequent injections needed, instead opting to look for less frequent regimens [4] which offer a longer lasting treatment. One of these regimens includes gene therapy. However, due to its high cost due to its relatively new development, many patients are unable to access this level of treatment.

In order to treat or prevent diseases, doctors use a medical technique called gene therapy, which involves modifying a person's DNA. The basic concept of gene therapy is to introduce, delete, or replace particular genetic information within an individual's cells in order to treat a hereditary condition or rectify a genetic insufficiency. Gene therapy is often done in two ways: with viral vectors, where genetically engineered viruses are modified to carry the desired gene to the specified location of the deficiency; or with non-viral methods such as electroporation, where an electric current is induced to create temporary pores in cells *ex vivo* to deliver the gene or direct injection of the therapeutic gene [5]. As of 2023, FDA has approved the use of

gene therapy to treat Hemophilia B and the treatment of gene therapy for Hemophilia A is in clinical trials.

Regarding the use of viral vectors in gene therapy administration, a prominent type of viral-assisted gene therapy is recombinant adeno-associated virus (rAAV) gene therapy involving the modification of adeno-associated viruses (AAVs). rAAV gene therapy refers to the use of recombinant DNA created by combining genetic material from two different sources to alter their characterization. Adeno-associated viruses are used as vectors to transfer genes into an affected patient's cells. The first step to creating rAAV vectors is to modify the AAV to make a vector that cannot cause disease while inserting the desired gene into its genome to ensure that the vector is safe for human use. The vector is then used to deliver the gene into the patient's cells. Once introduced into the body the rAAV vector makes its way into cells and releases the therapeutic gene.

To conclude, hemophilia is a rare genetic disorder caused by a deficiency in certain clotting factors, the variation in which results in the three types of hemophilia. There is no definite cure for hemophilia, but treatments are being developed to either mitigate symptoms, such as DDAVP®(manufactured by Ferring Pharmaceutical) and Emicizumab®, or replace missing clotting factors, such as factor replacement therapy using rAAV vectors. Research is being conducted to better understand any potential negative side effects and make gene therapy for hemophilia safe and accessible for the general populace. This literature review will be covering the importance of treating hemophilia, the development of gene therapy, alternative therapies to gene therapy along with their benefits and drawbacks, benefits and challenges within gene therapy, the results of clinical trials using gene therapy as well as a summary of current gene therapies in use.

Significance

Patients suffering from hemophilia report poor quality of life scores which is further exemplified by the fact that most patients who suffer from hemophilia suffer from the severe form of it. Along with continuously suffering from joint issues, bleeding, and the high levels of wariness patients have to exert to prevent cuts and bruises, many patients often suffer from extended stays in hospitals and reported a poorer quality of life. The same is true for patients who frequently visited doctors for their condition[6].

Finding a permanent or near-permanent solution to hemophilia is essential as patients not only suffer from the detrimental symptoms of hemophilia itself but experience other comorbidities. Possible comorbidities of hemophilia include cardiovascular disease, disorder of joints, stroke, thrombosis, osteoporosis, and renal disease. These conditions paired with hemophilia can result in a severely reduced quality of life compared to the average person. For example, cardiovascular disease can result in breathlessness, dizziness, and swollen limbs[7]. Repeated bleeding into joints caused by hemophilia can result in long-term deformation of joints, potentially developing hemophilic arthropathy and disability[8]. Joint diseases can lead to loss of basic movement as well as bone spurs[9] with hemophilic arthropathy being the most common complication of hemophilia. This often results from repeated bleeding into a joint leading to cartilage and synovium breakdown[10]. Therefore, it is imperative that a cure or long-term solution be found to effectively treat this debilitating genetic disorder.

Development of Gene Therapy

The evolution of gene therapy began around 70 years ago with the initial discovery of the shape of DNA being a double helix structure[11] . In 1973, researchers discovered a method that allows for the transfer of genetic material between one organism to be implanted or expressed in another. An *Escherichia coli (E. coli)* bacterium received genetic material after DNA was spliced into a plasmid carrier, a DNA structure that can multiply without integration into the chromosome. The bacterium copied the foreign DNA and preserved the genetic material of the original organism when it reproduced. The first gene therapy clinical trial incorporating new viral vector technology was conducted in 1990, making use of gamma retrovirus technology with the first AAV vectors used in a human patient in 1995 for the treatment of cystic fibrosis[11]. A clinical trial using gamma retrovirus raised some concerns about how safe genetic injections were since 4 out of 9 that were treated in the trial developed leukemia. This study showed the importance for improved viral vectors to be used in genetic technology. A more detailed understanding of gene therapy and its mechanism is found in Figure 1[12].

The two main types of gene therapy are: gene addition and gene editing. Gene editing can be further broken down into gene silencing and gene correction[13]. Gene therapy that involves the restoration or addition of a faulty or missing gene is known as "gene addition." In order to bypass the genetic condition, this method inserts a functioning copy of a gene into the cell or inserts a different gene. The extra gene enables the body to produce proteins that may be used to control or treat a hereditary illness[14]. Gene addition has the ability to restore faulty or missing genes by adding a working gene to the affected cells[15]. However, it can be expensive and while - as a whole - gene therapy is safe, the process is still relatively new and more information regarding the therapy is coming to light.

By 'silencing' or inhibiting particular genes linked to particular diseases, gene silencing is a relatively new therapy method that makes use of the body's natural processes to control disease[16]. Gene silencing makes fixing adverse side effects easy since gene silencing has a regulated, reversible effect that has benefits. Additionally, it is very precise and particular, thus the outcomes are predictable. Research into gene silencing is advancing quickly. However, along with the normal associated

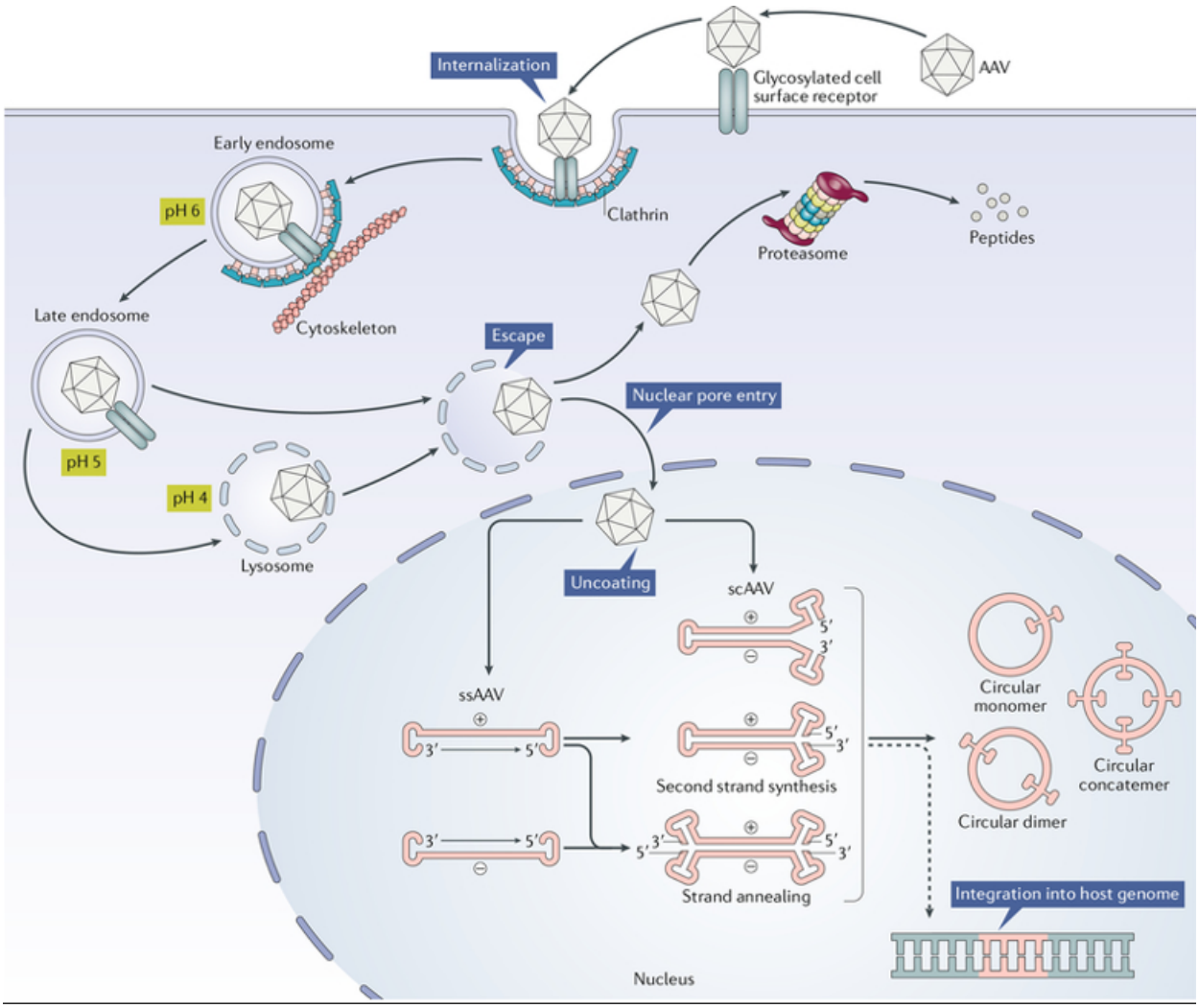

Fig. 1 Mechanism of vector replication within a host cell. Adapted from Wang et al., 2019.

risks with gene therapy, liposome encapsulated SiRNA has the possibility to be toxic to the host and cause a host of severe immune responses[17]. Scientists are currently working to mitigate these potential negative side effects to make gene silencing a safe treatment for hemophilia patients.

Gene editing involves correcting, introducing, or deleting a DNA sequence for certain genes to be expressed or silenced[18]. In order to remove current DNA and insert replacement DNA, gene editing is carried out utilizing enzymes called nucleases that have been specifically designed to target a particular DNA sequence[19].

Hemophilia is ideally suited for gene therapy since even a small increment (∼5% of normal) of blood factor levels can result in a significant improvement when measuring how much patients with severe hemophilia bleed[20]. As well as this, one-time therapeutic treatment can provide an effective treatment for up to eight years in most patients and up to ten years in a few[21]. Doses can vary depending on frequency of treatment, severity of hemophilia, or multiple other factors relating to the type of gene therapy.[22,23]

Alternative methods of treating Hemophilia

As previously mentioned, non-factor replacement therapies exist for hemophilia which help prevent bleeding or help improve blood clotting without using factor replacement therapy. These therapies include: Emicizumab®, DDAVP®or Amicar®(manufactured by Akorn Pharmaceuticals). Emicizumab®(Hemlibra), a treatment for hemophilia A, helps patients avoid bleeding episodes. It is often referred to as a mimetic

since it combines Factor X and Factor IX to mimic Factor VIII allowing the blood to clot. Emicizumab®is administered by a subcutaneous injection, as opposed to factor replacement therapy, in which the missing factor is delivered directly into a patient's vein (referred to as an infusion). In 2017 and 2018, the FDA authorized Emicizumab®for the treatment of hemophilia A with inhibitors and hemophilia A without inhibitors[24], respectively.

However, there are some potential drawbacks to Emicizumab®. The most significant is that the coagulation defect is only partially corrected, leaving patients who have received the treatment at risk of bleeding issues in circumstances like trauma or invasive procedures. Emicizumab®cannot be viewed as a treatment option for severe Factor VIII insufficiency as monotherapy because supplementary intravenous Factor VIII or bypass therapy may be required in some cases[25]. The most frequent adverse medication responses are injection-site reactions. Other frequent adverse medication responses that have been documented are headache (15%), arthralgia (15%), pyrexia (6%), diarrhea (6%), and a slight chance of rhabdomyolysis[26]. Although much less frequently, patients on Emicizumab®may experience spontaneous or traumatic bleeding, needing treatment with additional hemostatic medications including recombinant Factor VIIa (rFVIIa) and activated prothrombin complex concentrates (aPCC)[26].

Another potential non-factor replacement treatment option for hemophilia is the use of DDAVP®which is a synthetic version of a natural diuretic hormone, vasopressin. It can be used in patients with mild hemophilia for nose and mouth bleeding, joint and muscle bleeds, as well as before and after surgery. Injectable and nasal spray versions are both available. DDAVP®is used as a treatment for mild hemophilia A and von Willebrand diseases[24]. DDAVP causes the release of von Willebrand's antigen from the platelets and the cells that line the blood vessels where it is stored. Von Willebrand's antigen is the protein that carries factor VIII. This increase in von Willebrand's antigen and factor VIII helps to stop bleeding[27,28].

General use of DDAVP®comes with the possibility of low levels of sodium, flushing, swelling, seizures, weak breathing, and feeling lightheaded. In addition to this, it is recommended to stay away from using DDAVP®if you have kidney disease, low blood sodium levels (hyponatremia)[24] or if you have heart problems and have had a coronary artery clot. Many of the side effects that can occur with the IV form of DDAVP can be decreased by changing the dose or switching to the nasal form of desmopressin if possible[27,28].

Benefits of Gene Therapy

Gene therapy provides the opportunity to cure someone of a disease which may have previously been thought to be incurable using other methods of treatment[29]. In addition, modern gene therapy has advanced to a stage where only one dose needs to be given, which could be more appealing compared to other treatments that require multiple doses, to provide long lasting effects and a better quality of life. Furthermore, a lesser-known benefit of gene therapy is that the positive effects of the gene therapy will be passed down to the offspring. This is due to the fact that removing a faulty gene from a parent prevents the possibility of it being passed down generations. We can also expect more frequent and massive changes to gene therapy as technology advances over the next few decades, potentially opening the doors to provide the cure to more conditions which seem incurable with our current technology.

Benefits of gene therapy specific to hemophilia include: (1) a need for only a single infusion; (2) clinically relevant factor VIII and IX expression, even at levels that are within the normal range; (3) long-lasting treatment, at least 4 years for hemophilia A and at least 8 years for hemophilia B; (4) a reduction in the number of bleeding episodes; (5) no need for prophylaxis; and (6) a measurable improvement in quality of life[30].

The main treatment for hemophilia is standard half-life therapy where dosage varies from three times a week to everyday, depending on the patient. Clotting factors come in two different varieties: plasma-derived and recombinant. Plasma is used to create plasma-derived factors. DNA technology is used in the creation of recombinant factor products in a lab[31]. Although there are still plasma derived FVIII medicines on the market, about 75% of people with hemophilia use recombinant FVIII products. A major limitation with general short half-life therapy used for hemophilia is the need for factor replacement injections to be administered multiple times a week, since half-lives range between 6 hours to 24 hours depending on the patient and even less in younger children[32]. Gene therapy seeks to induce the body to manufacture the missing clotting factor naturally, which would reduce or eradicate the need for clotting factor injections and decrease the frequency of bleeding which is what makes it superior to other methods of treatment[33].

Studies summarized by Miesbach et al. 2022[23] combine phase-1 gene therapy trials used for dose determination and phase-3 trials used for the purpose of including a larger sample size. All studies summarized showed a significant decrease in the median number of bleeding episodes. A decrease in bleeds from 8.5 to 0.3 for hemophilia A and a decrease from 15.5 to 1.5 for hemophilia B[23].

Challenges and Potential Solutions to Gene Therapy

In general, gene therapy is well tolerated. However, numerous studies have documented adverse effects due to infusions; for instance, up to 12 hours after gene therapy, 5 of 18 patients experienced symptoms like vomiting, fever, and myalgia; these symptoms persisted for up to 72 hours after outpatient treatment[23,24] . Additionally, there is a chance that a patient can

develop deep vein thrombosis due to abnormally high Factor VIII levels[23].

Moreover, elevated liver enzymes, particularly elevated alanine transaminase (ALT), are a typical side effect of gene therapy that can reduce or eliminate the therapeutic effect. In phase-3 trials, this occurs more frequently in gene therapy for hemophilia A (89%)[34] compared to gene therapy for hemophilia B (17%)[23?] . This is frequently caused by an erratic immune response triggered by T cells to transduced liver cells that have capsid fragments of the viral vector on their surface. This might also cause a brief and asymptomatic increase in transaminases. To date, all elevations in liver values have been effectively treated with short-term immunosuppressive medication, such as glucocorticoids. However, the same therapeutic effect from the gene therapy was no longer restored in some patients which highlights the importance of monitoring liver enzymes to initiate immunosuppressive treatment as soon as possible which can significantly contribute to the preservation of factor expression. To highlight other potential hazards that could go unnoticed, there needs to be more research into the effects of this therapy as it interacts with the human immune system.

Additionally, a patient with a history of previously treated hepatitis C infection reported developing hepatocellular carcinoma during regular follow-up nearly a year after receiving gene therapy for hemophilia B. This raises questions regarding a possible causal association between gene therapy and the development of cancer[23]. However, a histopathological analysis showed no connection to gene therapy. Five individuals in a trial on gene therapy for hemophilia A had liver biopsies taken 2.6–4.1 years after the treatment, and the results showed no aberrant histopathological abnormalities in the liver; the vector DNA was present in episomal forms and did not integrate into the genome. In light of the small, but potential, danger of cancer, it is crucial to regularly follow-up, especially with regard to liver health and registration in national and international registers like The World Federation of Hemophilia (WFH) Gene Therapy Registry.

Gene therapy is an expensive treatment often costing around $1 million and $2 million per dose[35]. This steep cost is often due to the research and development that constitutes developing a new gene therapy. Moreover, the patient base for traditional pharmaceutical drugs is much larger than for gene therapy. This means that the amount each patient has to pay will be significantly more if pharma companies plan to earn a profit off the therapy. However, efforts are being made in an attempt to reduce the cost of gene therapy and make it more accessible to the general population. A technique called cell-free amplification is being developed which greatly reduces the time it takes for purification and can make the entire process of producing the therapy much faster and, as a result, cheaper[36].

Clinical trials for Gene Therapy

A trial by Nathwani et al., 2011[37] on the intravenous administration of rAAV gene therapy for hemophilia was shown to produce the first successful results on patients suffering from hemophilia B. Even after a period of 8 years, consistently increased FIX activity in the region of 2–5% was demonstrated in the dose cohorts. A further study by Miesbach et al., 2017[38] 6 years later confirmed similar findings with the lower dose cohort having an average increase of 4.4 IU/dL in FIX activity and the higher dose cohort having an average increase of 6.9 IU/dL in FIX activity.

The introduction of the Padua variant of the FIX gene, which has five- to 10-fold higher activity and was initially found in familial thrombophilia. The molecular regulation of activation, inactivation, and cofactor dependence is similar to FIX wild type, but the faster rate of factor X activation leads to hyperactivity and significantly higher factor levels. 54 patients with severe hemophilia B participated in a phase-3 trial by Drygalski et al., 2019[39], the results of which were recently published. The researchers found that mean FIX activity of 39.0 IU/dL at 6 months and 36.9 IU/dL at 18 months could be achieved, independent of previous anti-AVV antibodies, up to a certain threshold value.

In 2017, the first successful trial results for hemophilia A using gene therapy were released (BMN-270)[40]. Over the course of a year, factor VIII activity was consistently normalized in six out of seven patients in the high-dose group (mean, 93 percent $\pm$ 48). This resulted in the stabilization of hemostasis and a relatively significant decrease in annual factor VIII use, from 5286 IU/kg to 65 IU/kg. However, an increase in alanine aminotransferase of up to 1.5 times the upper limit of the normal reference range was seen. Additionally, results have been released from a phase III trial that followed 134 patients for a year. Mean factor-VIII activity rose by 41.9 IU/dL by week 52 in 132 HIV-negative participants[41].

Lifetime of the Part

Current gene therapies for hemophilia include Roctavian and Hemgenix for hemophilia A and B respectively. Hemophilia A represents around 60% of all cases[42] which Roctavian can treat as a onetime therapy with a single dose of intravenous infusion. Using a viral vector containing a gene for factor VIII, gene expression is increased in the liver resulting in an overall blood increase of factor VIII. In a multinational study consisting of men previously treated for severe hemophilia A between the ages of 18-70, Roctavian's effectiveness was proven as mean annual bleeding reduced by 54% (5.6 annual bleeds per year down to 2.6)[42]. Hemgenix uses the same mechanism as Roctavian, utilizing viral vectors to carry the necessary gene for factor replacement into the liver using intravenous infusion. However, treatment response to Roctavian has the possibility of decreas-

	Treatment method	Type of hemophilia treated	Dosage	Cost (Millions of dollars)	Annual bleed reduction (bleeds per year)	Incidence of increased liver enzymes
Roctavian	Viral vector	Hemophilia A	6×10^{13} vector genomes per kg	$1.96	-54%	91%
Hemgenix	Viral vector	Hemophilia B	2×10^{13} vector genomes per kg	$2.95	-64%	∼10%

Table 1 Summarizing Roctavian and Hemgenix [42–49]

ing over time while Hemgenix has been shown to have a more consistent treatment response and lower incidences of liver inflammation[43]. Hemgenix has also shown similar efficacy with a 64% reduction in annual bleeding rate, however the cost is much higher than Roctavian at around $2.95 million compared to Roctavian's $1.96 million[44].

Conclusion

To conclude, gene therapy has the ability to cure and prevent genetic diseases that were previously thought to be incurable, offering an opportunity to explore a relatively new field of research. Gene therapy has now reached the point where some conditions only require one dose, which provides a significant advantage to other methods of treatment that may require multiple injections or doses over time opening the possibility to injection-site complications. Due to these benefits, among others, gene therapy is widely considered to be a potential long-term cure to hemophilia as it introduces functional clotting factor genes to reduce or eliminate the need for regular factor replacement injections and provide a better quality of life for hemophilia patients.

However, there are some downsides that are associated with gene therapy such as the cost, along with the fact that scientists are not sure of its long-term effects due to the fact that it is a relatively new field. Despite this, gene therapy has the ability to revolutionize global health and medicine due to around 1 in 50 people being affected by a genetic disorder.

For the future, scientists and researchers will need to focus on reducing or eliminating the adverse side effects that can be caused by gene therapy for some patients. These side effects can be relatively mild, such as vomiting or fever, or more severe such as deep vein thrombosis. This may deter patients from opting for gene therapy over other therapies, fearing that it may result in severe unintended complications. Eliminating these side effects can make gene therapy safer and more suitable for a larger majority of the population, improving global health. As well as this, efforts need to be made in order to reduce the cost of gene therapy, allowing it to be more accessible and affordable to a larger percentage of the population. Being able to treat more people genetically against hemophilia safely also reduces the proportion of the population that passes down hemophilia to their offspring.

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