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Gene therapy for hemophilia: Current status and future perspectives

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Abstract

Hemophilia is an inherited bleeding disorder caused by the lack of a protein necessary for blood clotting. Gene therapy for hemophilia involves the introduction of a healthy gene into the patient's cells to produce the missing protein.

There are two main types of gene therapy for hemophilia: ex vivo gene therapy, which involves the extraction of cells from the patient, introduction of the healthy gene into these cells in the laboratory, and reintroduction of the modified cells into the patient; and in vivo gene therapy, which involves the direct delivery of the healthy gene into the patient's body using viral vectors.

However, there are still challenges in the large-scale implementation of gene therapy, including safety and long-term efficacy, and the costs associated with the technology.

Keywords: Gene therapy; Hemophilia; Bleeding disorder; Hemophilia A and B

1. Introduction

Gene therapy has emerged as a promising treatment option for hemophilia and is being actively studied in clinical trials. It has been shown to be effective in reducing the frequency and severity of bleeding episodes in patients with hemophilia. Some objectives that are set are:

- Reduce or prevent the frequency and severity of bleeding episodes in patients with hemophilia.
- Improve the quality of life of patients with hemophilia, reducing the need for prophylactic treatment and improving the ability to carry out activities of daily living.
- To provide a safer and more effective alternative to current treatments for hemophilia, such as the infusion of clotting protein concentrates or the use of hemostatic agents.
- To investigate the feasibility of gene therapy as a long-term treatment option for hemophilia, with the possibility of a single dose providing lasting benefit.

It is important to note that gene therapy for hemophilia is still in an investigational phase and there are challenges that need to be addressed before it can be implemented on a large scale. However, gene therapy offers a promising treatment option for hemophilia patients and continues to be the subject of study and development.

2. History of hemophilia

The first references suggestive of hemophilia date back to the 2nd century BC, with descriptions in Babylon of males who died after circumcision. However, the first modern description of hemophilia is believed to date from 1803 when the American physician John Conrad Otto described a hereditary bleeding disorder in several families in which only

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males were affected with transmission through unaffected females. The word "hemophilia" seems to have been first documented in 1828 by the German physicians Johann Lukas Schönlein and Friedrich Hopff, who described the condition in their thesis "On Hemophilia, or the Hereditary Predisposition to Fatal Bleeding"^[1].

In 1820, the pattern of genetic transmission from unaffected women to their male children was described for the first time. Hemophilia has been called "the disease of kings" as several members of the European royal family were affected by the condition. The famous Queen Victoria of England (1837-1901) was a carrier of hemophilia B and inherited it from her son Leopold, who had frequent bleeding and died of a brain hemorrhage at age 31. The condition was extended to other royal families in Germany, Spain and Russia through the queen's daughters^[2].

2.1. Why does hemophilia occur?

Hemophilia usually runs in families. The term "inherited" means that the disorder is passed from parent to child through genes. In hemophilia there are alterations in the genes that determine the production of coagulation factors VIII or IX. These genes are located on the X chromosome of which women have two and men only one. A man who has the gene responsible for the production of FVIII or FIX on the altered X chromosome will develop the disease, while a woman must have an alteration on both chromosomes for it to develop, a complex situation that rarely occurs. For this to occur, it is required that the father have hemophilia and the mother be a carrier; in some cases of equal rarity, some women who only have one X chromosome (Turner Syndrome)^[3]. If a woman has the altered gene on only one of her X chromosomes, she is a "carrier" of hemophilia^[4]. Carriers sometimes have certain symptoms, without fully developing the condition, and can pass the faulty gene on to their children. Figure 1 exemplifies the type of inheritance in hemophilia. On the other hand, there are cases where there is no family history of hemophilia, and this is due to a change in the genes (mutation) that affect the function of a coagulation factor. More than 1,000 mutations with no family history have been described and up to a third of hemophilia cases may be secondary to this type of mutations in coagulation factors VIII and IX.^{1,4} On rare occasions, hemophilia can be acquired^[4].

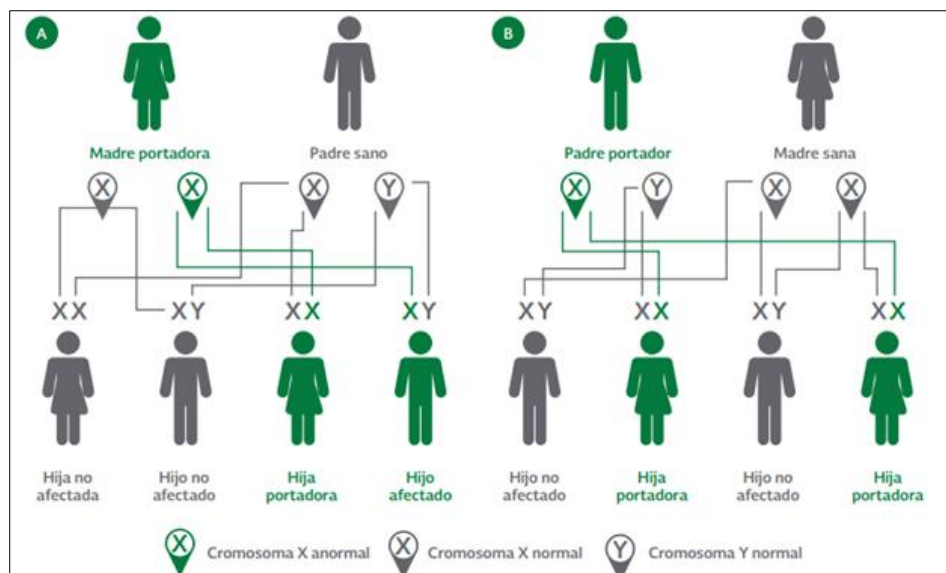


Figure 1 Inheritance in hemophilia^[4].

2.2. Molecular basis of hemophilia

Hemophilia A and B are transmitted in a recessive manner linked to the X chromosome, so the disease occurs mostly in males.^[5]

Hemophilia A: It is a monogenic disease caused by pathological variants in the F8 gene that encodes the factor VIII protein.

The gene consists of 26 exons that give rise to a 9 kb mRNA, including 7,053 coding nucleotides. More than 2,000 variants of the F8 gene have been described, with nonsense mutations and splice mutations being the most frequently associated with severe hemophilia A. Approximately 45% of the pathological variants of severe hemophilia A correspond to intron 22^[5].

Factor VIII is a 2,332 amino acid protein that circulates in plasma non-covalently bound to von Willebrand factor (vWF), which acts as a carrier molecule, ensuring its protection against proteolytic degradation.

The role of factor VIII in coagulation is the activation of factor X, acting as a cofactor of factor IX, thus increasing its proteolytic activity 20,000 times to generate thrombin

Hemophilia B is exclusively associated with pathogenic variants in the F9 gene, which consists of 8 exons, giving rise to a 2.8 kb mRNA. More than 1000 variants have been described, the majority being isolated nucleotide substitutions (73%), with mutations that generate a stop codon in severe cases⁸. Factor IX is a chymotrypsin belonging to the family of serine proteases that is activated after undergoing specific proteolysis by factor XI or the tissue factor/factor VII complex and, once activated, acts as a cofactor for factor X to generate thrombin (fig. 2) ^[5].

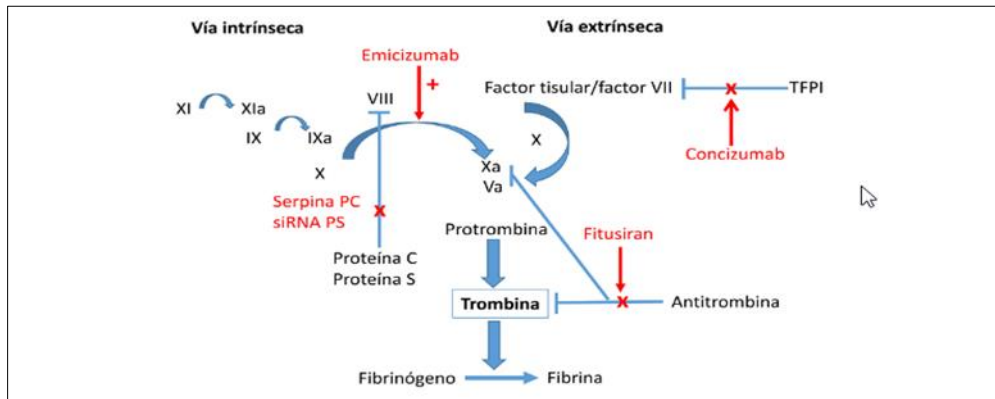


Figure 2 "Coagulation cascade and mechanism of action of novel non-replacement agents in hemophilia." ^[5]

2.3. What is gene therapy?

Human gene therapy is defined as the treatment of disorder or disease through transfer of engineered genetic material into human cells, often by viral transduction ^[6]. In contrast to other diverse therapies for the X-linked bleeding disorder hemophilia that are currently in clinical development, gene therapy holds the promise of a lasting cure with a single drug administration. Near-to-complete correction of hemophilia A (factor VIII deficiency) and hemophilia B (factor IX deficiency) have now been achieved in patients by hepatic in vivo gene transfer. Gene therapy has the potential to revolutionise treatment for patients with haemophilia and is close to entering clinical practice ^[6, 14, 16].

2.4. Hemophilia: ideal target of gene therapy

Hemophilia A and B are monogenic disorders that were felt to be ideal targets for initiation of gene therapy ^[7].

Monogenic disease: ideal target for gene therapy, providing a working copy of the disease-causing gene ^[8].

- Hemophilia lends itself to correction by gene therapy.
- The bleeding phenotype is responsible for a wide variety of coagulation factor levels.
- An absolute correction is not necessary.
- The size of the cDNA encoding the factor is within the packaging capacity of AAV vectors.

2.5. Historical evolution of gene therapy in hemophilia

Recombinant, replication-defective viral vectors were the first molecular tool enabling efficient, nontoxic gene transfer into human somatic cells. Retroviruses and adeno-associated virus (AAV) have shown the most clinical promise, and we will limit our discussions to these vectors.

The identification of a genome packaging signal and the creation of a producer cell line paved the way for design and facile production of vectors capable of undergoing reverse transcription and DNA integration but lacking replication potential. The γ -retroviral vectors developed in the 1980s and early 1990s were the first to be shown to deliver genes into repopulating HSCs. C-type retroviruses were also adapted for efficient gene transfer into primary T lymphocytes. These vectors were used in first-generation clinical trials designed to deliver a normal copy of a specific defective gene into the genome of T cells or HSCs from patients with immunodeficiencies or cancer Fig.3^[9].

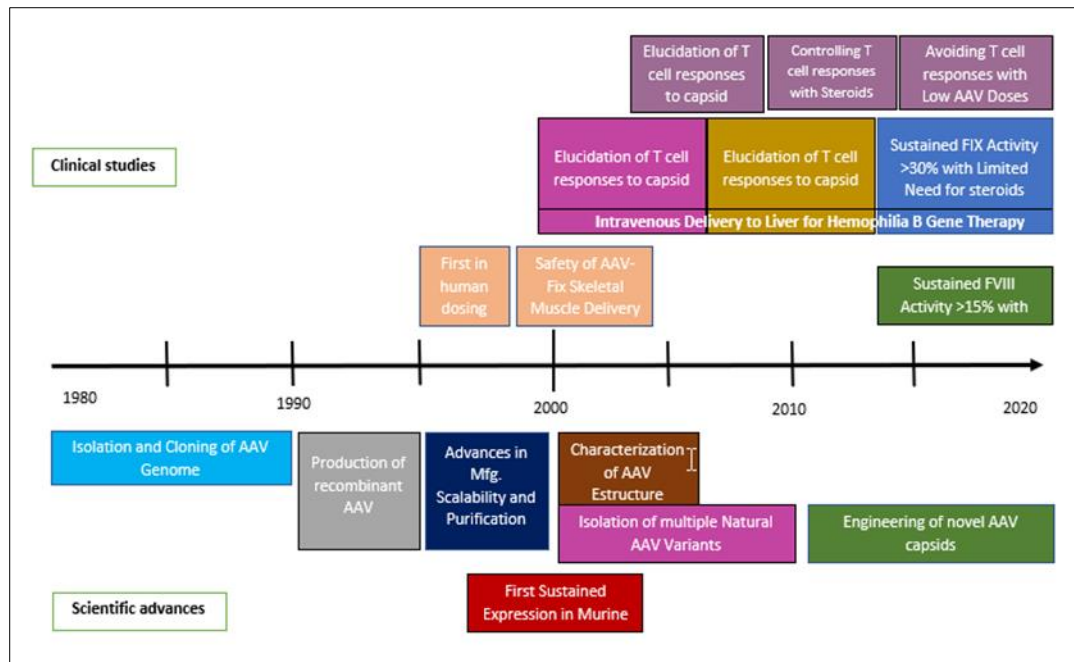


Figure 3 Historical evolution of gene therapy in Hemophilia. ^[9]

2.6. AAV gene therapy strategies

This strategy aims to deliver a gene product to compensate for loss-of-function mutations.

Adeno-associated virus (AAV) was first discovered from laboratory adenovirus (AdV) preparations in the mid-1960s ^[10]. They were discovered as contaminants of adenovirus productions. They are very small non-enveloped viruses, their capsid has a diameter of 20-25nm. Its genome is a 4.7 Kb2 single-stranded linear DNA (ssDNA). They are naturally replication defective and require the cell to be co-infected with another virus to complete its viral cycle ^[10]. They are widely used for gene therapy, as they largely do not integrate and can be transduced to various tissues, where they stimulate long-term expression of the transgene^[11].

In the early 2000s, the discovery of a new family of naturally occurring primate AAV serotypes and variants greatly expanded the capsid toolbox for much more widespread transgene delivery after intravascular injection. Several ongoing gene replacement clinical trials take advantage of these newer capsids and have shown promising therapeutic results, including trials for hemophilia A and hemophilia B (directed to the liver) ^[10].

2.7. Vector design in hemophilia A and B

Hemophilia A: they use AAV serotypes with different capsids as vector, they code for a human FVIII, with deletion of the B domain and codon optimization, under the control of a liver-specific promoter (Valactogene roxaparvovec, SPK-8011 Giroctogene fitelparvovec). These considerations prompted efforts to develop novel approaches for treatment of hemophilia using gene therapy, which has the potential for lasting treatment and even curing of the disease. ^[12,19].

Hemophilia B: Using several AAV serotypes with different capsids as vector, they code for the codon-optimized Padua variant of human FIX, under the control of a liver-specific promoter. (Etrsnacogene dezaparvovec, Fidanacogene elaparvovec, Verbrinacogene setparvovec). (eleven) In clinical development, the first work on the use of gene therapy in hemophilia B, called "Stage I clinical trial of gene therapy for hemophilia B" was developed in 1993, ^[13] without having results in a short time. However, it was not until 2011 that the results of 6 patients were published. Six men with severe hemophilia B (FIX activity, <1% of normal values) were enrolled according to a protocol approved by relevant ethics boards and regulatory agencies, after providing written informed consent. All but one of the participants received regular prophylaxis with FIX concentrates (two or three times per week) prior to gene transfer (Figure 4). Participant 4 was receiving targeted prophylaxis (about once a week), that suited his sporting activities and the associated risk of traumatic injury ^[14]. Factor IX is a critical clotting protein present in human plasma. Patients suffering from Hemophilia B are variably deficient in the content or activity of this protein and must receive repeated scheduled injections of intravenous Factor IX to survive ^[15].

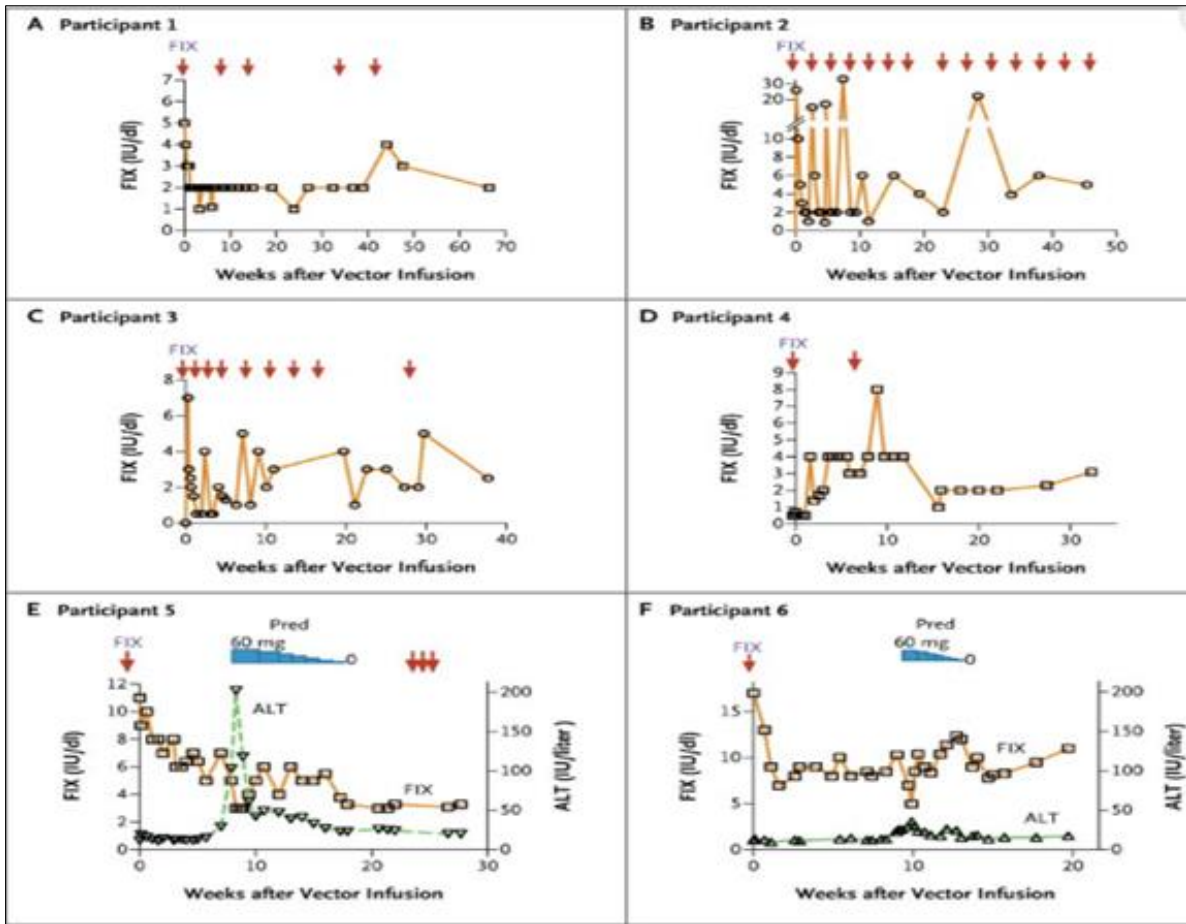


Figure 4 Factor IX (FIX) Activity after Peripheral-Vein Infusion of the Adenovirus-Associated Virus (AAV) Vector in the Six Study Participants (A) [14].

Participant 2 had a null mutation in the FIX gene and participant 6 had a promoter mutation; consequently, none had FIX protein expression.

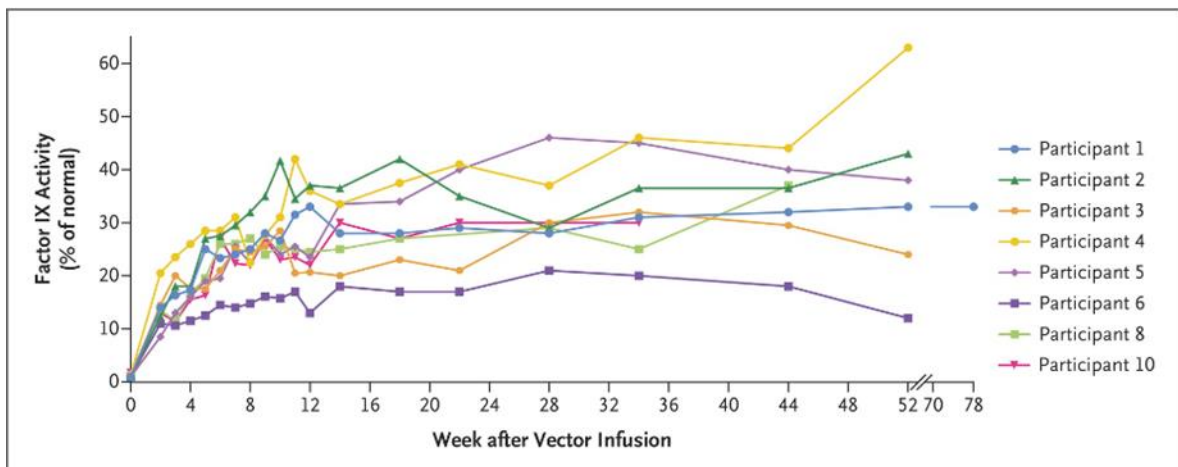


Figure 5 Factor IX Activity after One Peripheral Infusion of SPK-9001 in the Eight Participants Who Did Not Have an Adeno-Associated Viral Capsid-Directed Immune Response [14]

The other four participants had missense mutations, resulting in normal plasma levels of FIX antigen, but less than 1% clotting activity. All participants had modest levels (>5 relative units) of anti-AAV2 IgG antibodies prior to gene transfer.

New results were subsequently published where A total of 14 men with hemophilia B (factor IX level $\leq 2\%$ of normal), 18 to 53 years of age, were enrolled at four institutions. A total of 10 participants received an infusion of SPK-9001. Among the 4 participants who did not receive an infusion, 2 were found to be ineligible due to hepatitis C virus (HCV)-related liver fibrosis, and 2 had the delayed infusion (see Supplementary Results section in the Supplementary Appendix). Vector-derived factor IX coagulant activity was observed within 1 week after vector infusion. Steady-state factor IX-R338L expression was achieved within 14 weeks of vector infusion. Among all participants, the mean (\pm SD) vector-derived factor IX coagulant activity was $33.7 \pm 18.5\%$ of normal (Figure 6). Vector-derived factor IX coagulant activity was observed within 1 week after vector infusion. Steady-state factor IX-R338L expression was achieved within 14 weeks of vector infusion. Among all participants, the mean (\pm SD) vector-derived factor IX coagulant activity was $33.7 \pm 18.5\%$ of normal.

2.8. Annualized bleeding rate and use of exogenous factor IX

Among all participants, the annualized bleeding rate decreased significantly after vector administration (mean rate, 11.1 event years [range, 0 to 48] before vector administration vs 0.4 event year [range, 0 to 4] after administration; $P=0.02$; $W=28$) (Figure 5). Participant 3, who had extensive hemophilic arthropathy at baseline, reported four hemarthroses in two joints (one in the left ankle and three in the right knee) in the 52-week follow-up of the study (Figure 6). Participant 8 received two doses of factor for central catheter removal(mediport) which was deemed no longer necessary due to cessation of factor use (Figure 8). No other participants had bleeding or used factor.

The data on the reduction in the annualized bleeding rate are noteworthy given the completion of prophylaxis after vector infusion and the degree of baseline hemophilic arthropathy represented among the participants (mean number of target joints, 1.8 ± 1.9).

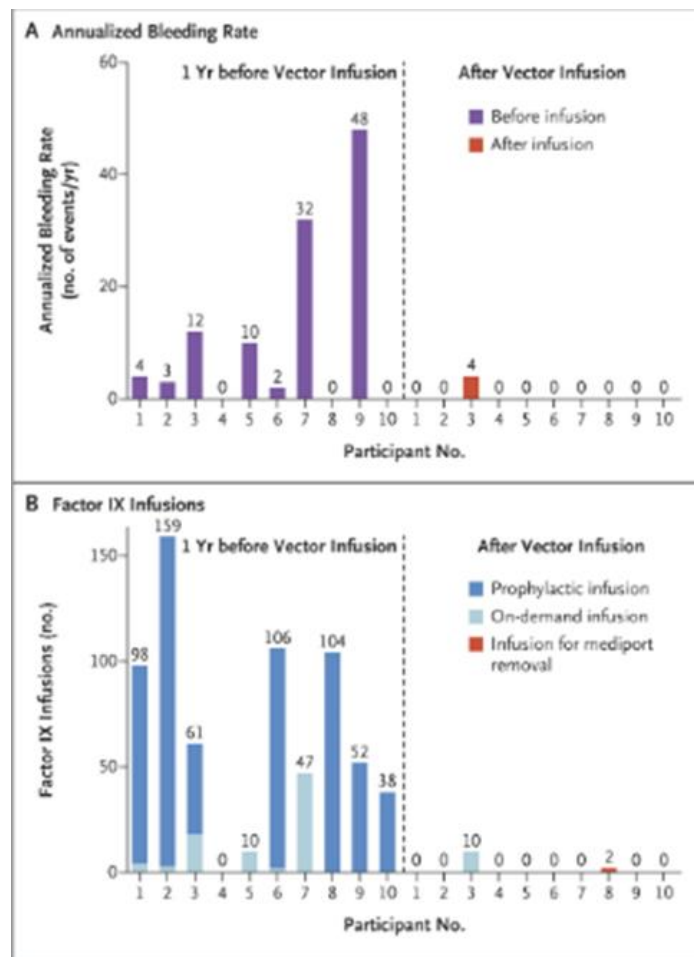


Figure 8 Comparison of Annualized Bleeding Rate, Number of Exogenous Factor IX Infusions, and Use of Factor IX Concentrate in the 52 Weeks before Screening and after Vector Infusion. ⁽¹⁴⁾

Concurrent with the reduction in bleeding, factor consumption was significantly reduced (mean, 2908 IU/kg [range, 0 to 8090] before vector administration vs. 49.3 IU/kg [range, 0 to 376] after administration; $P=0.004$; $W=45$), as was the number of infusions (mean, 67.5 [range, 0 to 159] before vector administration vs. 1.2 [range, 0 to 10] after administration; $P=0.004$; $W=45$) (Figure 7). During the cumulative follow-up interval of 492 weeks, a 100% reduction in the use of exogenous factor IX was observed in 8 of 10 participants and a 91% reduction in the 1 participant who reported bleeding after vector infusion (Figure 7). Taken together, this equated to a reduction in factor IX concentrate use of 1,

After 3 decades of clinical trials, repeated proof-of-concept success in hemophilia A and B gene therapy has now been demonstrated. Current hemophilia clinical gene therapy efforts are largely focused on the use of Systemically administered recombinant adeno-associated viral (rAAV) vectors for the addition of the F8 or F9 gene. With multiple ongoing trials, including licensing studies in hemophilia A and B, many are cautiously optimistic that the first AAV vectors will gain regulatory approval within approximately 1 year. While backed optimism suggests that gene therapy's goal of disrupting the paradigm of hemophilia care may soon be achieved, several salient questions have emerged from clinical trials that need answers to unlock the full potential of gene therapy for patients with hemophilia.

In conclusion, we found that a single intravenous infusion of SPK-9001 resulted in a sustained level of factor IX coagulant activity of approximately 30% that consistently allowed termination of prophylaxis, prevented bleeding, and almost completely eliminated the need for prophylaxis. exogenous administration. factor in 10 men with hemophilia B. This early success requires confirmation in a larger cohort and long-term monitoring for safety and efficacy. After 3 decades of clinical trials, repeated proof-of-concept success has now been demonstrated in hemophilia A and B gene therapy [14, 17]. In recent years, the number of clinical trials in which adeno-associated virus (AAV) vectors have been used for in vivo gene transfer has steadily increased. The excellent safety profile, together with the high efficiency of transduction of a broad range of target tissues, has established AAV vectors as the platform of choice for in vivo gene therapy [14, 18]. Research is at present also focusing on the tissue factor pathway inhibitor (TFPI), the main inhibitor of the onset of the coagulation cascade, which has been shown to regulate the severity of a wide range of hemorrhagic and coagulation disorders. Multiple animal studies have shown that TFPI inhibition can reduce bleeding in the context of hemophilia [20].

3. Conclusion

Gene therapy offers the possibility of transforming the lives of patients with hemophilia, achieving a functional cure of the disease with a single infusion and possibly a total cure with gene editing techniques.

The latest results of clinical trials with higher levels of the deficient factor, maintained for a longer time and with a good safety profile have changed the perspective of the hematologist and the patients. Even so, improvements are necessary to increase the number of patients who are candidates for it, as well as protocols for the proper management of toxicities or loss of response.

Giving as a response to beneficial potentials, for example; A single infusion, clinically relevant expression of FVII and FIX, potentially within the normal range, clinical trials with response durability of at least 4 years in HA and 8 years in HB, reduction of bleeding events and prophylactic treatment, and improvement in quality of life.

However, there are currently limitations such as; Infusion-related effects (mild), high variability in reaching factor levels and FVII decline over time in HA, immune response to capsid, impaired liver function, and need for immunosuppressive medication (reactive or prophylactic), patients with ac. Prior anti-AAV and children excluded from most trials, unknown long-term durability of treatment and adverse effects due to integration, and an impossibility of re-dosing with rAAV

Given the complexity and potential complications of gene therapy administration, it is the responsibility of health professionals to ensure their prescription, administration, and follow-up.

Compliance with ethical standards

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Disclosure of conflict of interest

There are no conflicts of interest among the authors who have contributed to this manuscript.

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