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Review Article

Study of Hemophilia A Gene Therapy: Current and Next Generation

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ABSTRACT

Introduction: Hemophilia includes a group of X-linked bleeding disorders caused by clotting factor deficiencies. The disease primarily affects males and causes chronic pain, joint deformities, reduced mobility, and increased mortality. Current treatments need to be introduced frequently Although it is a shift coefficient, the appearance of allontel (inhibitor) reduces them.efficiency. New treatment methods have been developed to get rare factors of coagulation and prevention Appearance of inhibitor. Areas covered: This article reviews the characteristics and pathophysiology of the disease.Focus on hemophilia A and current treatments, especially ongoing clinical trials related to hemophilia A Gene replacement therapy. Expert opinion: Gene replacement therapy allows safe, durable, and stable transgene expression... It is important to improve the specificity of the virus structure and improve the decrease in treatment doses.Minimization of cell stress, guidance of detailed protein response and obtained protein loss Liver cell product. Next generation genetic treatment,Transjen increases coagulation factors' synthesis and secretion, efficiency, safety,and The durability of genes of genes in hemophilia A and other blood coagulation disorders.

INTRODUCTION

1.1. Group of hemophilia and genetic hemorrhage

Hemophilia is classified as a group of genetic X Bleeding disorder after absence or Defects of the important factors of coagulation cascade[1].Patients with hemophilia compromise trombin Eradication and the formation of fibrin coagulation, it leads to bleeding Episode, most of the time in the joints (Hemarthosis). The 2 main types of hemophilia (A and B) relate to deficiency or dysfunction of the specific clotting factors VIII or IX, respectively, with the severity depending on the level of clotting factor activity. Hemophilia affects mainly males, and hemophilia A accounts for the large majority of cases (~80%), affecting approximately 1 of every 5000 live-born males. Hemophilia B Hemophilia A is common in about 1 in 5 people, with an incidence of about 1 in 30,000 births [2, 3]. More than 1 million people worldwide suffer from hemophilia, including more than 30,000 in the United States (US) [4].

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Prevalence is higher in whites than in blacks and Hispanics [5]. A meta-analysis of national registries from six countries, including Australia and the UK [6], showed that the prevalence of hemophilia A is 17.1 cases per 100,000 men with any severity, and 6.0 cases per 100,000 men with severe hemophilia A only. Although these estimates are higher than previously reported, the prevalence still characterizes hemophilia as a rare disease according to definitions used in the US (<200,000cases) and the European unions (<5 cases/10,000 persons) [6].

1.2. Hemophilia A – Disease Characteristics and Symptoms

The most common of the two main types of hemophilia is: Hemophilia A, caused by reduced activity of plasma clotting factor VIII (FVIII) by mutations in the F8 gene, which codes for this protein. The severity of bleeding episodes tends to directly correlate with plasma FVIII concentration, while 5%–40% of normal is considered mild, 1%–5% of normal is moderate, and less than 1% of normal is considered severe [1,7]. Bleeding is more important for sweet hemophilia Probably not after injury or surgery, not. Bleeding is rare. Medium bleeding hemophilia Usually observed after damage, but voluntary bleeding Sodes can also occur without obvious reasons. In severe hemophilia, patients experience recurrent spontaneous bleeding with arthropathy, muscle and soft tissue hemorrhages, and other life-threatening bleeding (e.g., intracranial hemorrhages), as well as excessive bleeding during and after surgery or injury. Recurrent arthropathy leads to hemophilic arthropathy, which is characterized by hypertrophic synovitis, progressive cartilage deterioration, chronic pain, severe deformity, and limited mobility [1, 7-10].

1.3. Hemophilia A - The burden of the disease

Hemophilia A is a deadly chronic condition which Irons The important clinical, psychological and economic burden On patients and attentive people, they affect Life [11-15]. Currently, hemophilia therapy is recommended Related bleeding episodes in patients with serious illness Prevention by replacing the products of the coagulation factor It is given intravenously 2 or 3 times a week [16]. However, approximately 30% of patients develop neutralizing alloantibodies to FVIII (inhibitors), which is a serious complication of this treatment [17]. Patients with hemophilia who develop inhibitors have poorer health-related quality of life, greater clinical burden, and higher resource utilization compared with patients who do not develop inhibitors, as well as more annual bleeding, joint bleeds, pain, and hospitalizations [18,19]. Further, the high frequency of the treatment regimen creates a significant burden for the patient, caregiver, and healthcare system. Patients with non-severe hemophilia also suffer considerable morbidity and an increased mortality risk [20,21]. Even patients with mild haemophilia the average number of bleeding episodes ranges from 0.44 to 4.5 per year, which seriously affects their quality of life [22]. Male haemophiliacs have a lower life expectancy than the general male population, even after treatment-related improvements [23–25]. In developed countries, the reduction in life expectancy for people with hemophilia A is 30% and for people with severe hemophilia A it is 37%.[6]

1.4. Hemophilia A – pathophysiology of the disease

Hemophilia A is caused by a lack of the essential blood clotting factor FVIII, encoded by the F8 gene located on the X chromosome. The severity of bleeding depends on the plasma level of FVIII, which depends on the specific mutation. The F8 gene is a large gene consisting of 26 exons. In severe hemophilia A, FVIII activity is almost completely cancelled. In the most frequent cases (~45%), the cause is: A large inversion in intron 22 of the F8 gene [26]. Point mutations The causes of hemophilia include 85% missense mutations,

which can cause quantitative or qualitative changes in protein biosynthesis, secretion, activity, or clearance. In some cases, exonic alterations can adversely affect mRNA splicing: a further 15% are nonsense mutations, and a small proportion (5%) are large and small deletions and insertions, as well as intron 1 inversions [27,28]. FVIII is synthesized from hepatic and extrahepatic sources. It is probably of endothelial origin. Extrahepatic sources include: Kupffer cells, monocytes and monocyte-derived macrophages Within the hematopoietic system. The liver is the main source, and although hepatocytes are the most common cell type that compose the liver, hepatic sinusoidal endothelial cells are the major source of liver-derived FVIII [29-31]. Once in the bloodstream, the FVIII heterodimer forms a tight, noncovalent complex with von Willebrand factor (vWF), the FVIII carrier protein produced and secreted by vascular endothelial cells. The half-life of FVIII in the absence of VWF is only 2 h, but 12 h when bound to VWF [32]. The 2,332 amino acid FVIII protein contains six domains (A1-A2-B-A3-C1-C2) and circulates in the form of a 90–200 kDa heavy chain (A1-A2-B) and an 80 kDa light chain (A3-C1-C2). When the coagulation cascade begins, in the presence of thrombin and activated factor X, vWF dissociates. Serine proteases cleave and release FVIII. Domain B plays a potential regulatory role [27]. The activated form of FVIII (FVIIIa) binds to factor IXa of the factor X activator complex and promotes the proteolytic conversion of factor X. (Xa) in the presence of calcium ions and phospholipids [33-36]. High FVIII activity increases the risk of stroke, while low activity has a negative effect on bone metabolism [37].

1.5. Haemophilia A – outcome measures

The primary goal of treatment is to reduce the number of bleeding episodes in patients with haemophilia A. Therefore, the annual bleeding rate (ABR) has become the primary outcome measure in haemophilia treatment trials [38]. However, ABR is an essentially communication patient Enter what the patient records the occurrence of bleeding If there is an event, their places, their seriousness, and spare Random event. For the subjective nature of this measurement Factor coagulation activity considered to be more Specific and objective main final points are proposed As a more objective measurement of the evaluation of therapeutic efficiency [39]. FVIII active level (endogenous and plasma level) Used to determine for a long time) Expressive risk of bleeding Prikania of the whole severity of the disease [39]. Advent Bio engineer therapy FVIII (see the section. 1.6) However, a discrepancy is observed in the in vitro activation profile of FVIII when measuring these molecules: a conventional one-step clotting test (partial activation) compared to a chromogenic assay [40]. This discrepancy has also been observed in a gene therapy clinical trial program with a single-step assay: reported approximately 1.6-fold higher FVIII activity compared to a chromogenic assay [41]. Thus, in the context of gene therapy, uncertainty has arisen regarding the correlation between FVIII expression levels across the full range observed and bleeding prevention depending on whether a one-stage or chromogenic assay is used. Thus, both FVIII activity (by either assay method or both) and ABR have been primary outcome measures.

1.6. Hemophilia A – Current and Future Treatment Options

The current standard of care for hemophilia A is prophylaxis, which aims to increase FVIII levels to levels sufficient to prevent bleeding episodes and reduce the incidence of hemarthrosis and subsequent joint disease through regular intravenous infusion of exogenously derived FVIII concentrates [42-45]. It is desirable to maintain a minimum FVIII level of 3% to 5% to prevent bleeding, as even occasional clinical and

subclinical bleeding episodes can lead to progression of joint disease throughout the patient's life. To provide effective bleeding prophylaxis in patients with hemophilia A, frequent intravenous infusions of FVIII concentrates (every 2–3 days) are necessary due to the relatively short half-life of FVIII in the circulation. The use of exogenous FVIII concentrates typically results in an ABR of 2–5 [46]. However, the burden of frequent administration and the difficulty of achieving and maintaining therapeutic FVIII levels have prompted the development of therapies with longer half-lives. Modified product modified with improved drugs Exercise characteristics (for example, fusion composition protein FVIIIII-FC or Glykol polyethylene bonds provide a smaller frequency. Inject (3-5 days or once a week Patient) [47] And the ability to target a higher level of depression. However, these strategies are limited from 1.3 to 1.5 times. Expanding FVIII life to resolve the recombination Blood protein is mainly adjusted by its interaction VWF [48 --51]. The various restrained half-life recombinants FVIII products have improved the ABR, with values going from 1.2 to 1.9 and pivotal clinical studies showing a program Sive decrease in the ABR during the extension phases [52]. These news The products gain popularity and in 2019, 28% of people with severe hemophilia have in the United States received from extended half-life factor products, 7.1% were pre-triggered non -factors and a decreasing proportion of Patients (64.0%) continued to be treated with the standard half Life factor products [53]. Unfortunately, when replacing elements, treatment is accompanied by the development of inhibitors neutralizing (alloantibodies) against the infusion concentrates. Inhibitor development occurs approximately in 30% of patients with severe hemophilia A and 13% of patients with non-severe hemophilia A [56 58]. Pathological physiology The development of inhibitors is considered to be a genetic and involvement. Environmental factor [59,60]. Large F8 gene deletion It is the most powerful prediction factor that the production of FVIII is almost or not. Fviii It is likely to be an immunity and is likely to be an association- It is related to the development of inhibitors compared to Missense Mutual [61].

One of the treatment options for managing bleeding episodes in patients who have developed inhibitors is the administration of bypassing agents (such as recombinant activated factor VII or activated prothrombin complex concentrates that contain activated serine proteases) [61-63]. A new bispecific antibody (emicizumab) was recently approved in both the United States and Europe for the prevention of bleeding in all patients with hemophilia A, regardless of whether they have inhibitors [64,65]. Emicizumab recognizes both activated factor IX (FIX) and factor X and mimics the activity of the cofactor FVIIIa. Thanks to its long half-life and subcutaneous administration, emicizumab has significantly improved the treatment of hemophilia A, regardless of the presence of FVIII inhibitors [66-68]. Another non -factor Fitusran (Treatment has been developed such as RNA interference Treatment is introduced subcutaneously once a month) Anti -trap antibody inhibitor monoclonal antibody, Like a combination and marstashimab [69 - 74]. These investments The GATIONAL agent also provides the benefits of the subcutaneous Delivery to monthly dosage or several months. Another approach to combating inhibitors is to try to... ITI uses a repeated dosing regimen of FVIII (40–300 IU/kg) at intervals of 1–3 days [61]. Success rates of current ITI protocols range from 60–80%, and pre-ITI anti-FVIII titers correlate with prognosis, but success rates vary widely depending on several factors, including age at ITI onset, race/ethnicity, FVIII genotype, and ITI historical inhibitor peak [75]. IIT is expensive and compliance with treatment regimens is a challenge for both patients

and caregivers [61]. Treatment with emicizumab, which mimics activated FVIII, is a possible option for patients who develop inhibitors. Not a candidate for IIT, studies on its use alone or in IIT. Combination with FVIII in IIT is under development [76]. Alternative IIT FVIII research strategies are based on: development of new technologies including gene therapy, regulatory T cell therapy and transgenic plants to induce oral tolerance [77].

2. Gene Therapy for Hemophilia

2.1. Review of Gene Therapy

The modification and transfer of genetic material to compensate for abnormally mutated genes is called gene therapy. The goal of gene therapy is to treat or even prevent genetic diseases by inducing long-term expression of the transferred gene at therapeutic levels [78,79]. Hemophilia is a hereditary disease whose genetics are well understood, making it an ideal target for gene therapy. Furthermore, since the severity of the bleeding phenotype is relatively insensitive to the plasma levels of blood clotting factors, precise control is necessary. The greatest limitation of currently available therapies is their short therapeutic half-life, which requires frequent infusions, leading to intensive efforts to develop more effective gene therapy strategies [71, 80-85]. Two types of vectors are most commonly used in current gene therapy strategies: lentiviral vectors are used for ex vivo gene transfer into hematopoietic and other stem cells [83,86], and adeno-associated viral (AAV) vectors are typically used for in vivo gene transfer into post-mitotic cells [78,87]. Lenti-Virus vector is very difficult to manufacture, clinical stub Dying using these vectors It is difficult to generate the amount of vector required In -body delivery [39,88]. Wild (WT) AAV is a small single-DNA virus of Palvovirus Family, a non -sympathy Genic and duplicate deficiency so that it cannot be guided disease. Ongoing clinical trials of hemophilia gene therapy use recombinant AAV (rAAV) vectors to deliver clotting factor genes directly into hepatocytes in the liver, converting the hepatocytes into protein biofactories that produce and release the transgene products into the blood circulation. After transduction of target cells, the therapeutic rAAV gene sequences are found primarily as concatameric episomes with low levels of integration into the host genomic DNA (87, 89). AAV has a wide range of naturally existing serotypes, each having distinct organ/cell tropism [90]. Hybrid serotypes can also be manufactured to increase the vector efficiency. A The first clinical research on hemophilia used the first generation Aav-serotype, aav2, this is the best feature and majority. He carefully studied serum format. Additional serum type AAV5, AAV8, and AAV110 are tested. Soryosal format AAV8 effectively converts the gene into the liver and promotes it. Even in intravenous introductions, high expression of genes [87.92]. AAV5 is the most excellent vector sulfur. Opal from the viewpoint of the capside structure, others [91] has more than 80 % of the serum type that is usually used. [91] One of the restrictions on AAV vector is that they are limited. Packaging container (~ 4.7 kg base [KB]) [93]. Therefore, early Research on genetic hemophilotherapy was conducted on the hemo Philia B [94] using the smallest transgen F9 [94]. Similar clinical studies were slow to start for hemophilia A because the F8 transgene is ~7 kb and F8 has a poor expression profile [95,96]. An AAV-based gene-transfer the approach to address the size constraints was recently developed by removing the FVIII B-domain (referred to as B-domain deleted [BDD]) to reduce the size of the FVIII expression cassette [96]. In addition, the relatively poor FVIII expression profile can be improved 10-fold by codon optimization (i.e. engineering the codon to improve gene expression and protein translation on the basis of the host codon bias) of human FVIII wt cDNA [97]. In

2017, BioMarin Pharmaceutical successfully applied this construct using a codon-optimized AAV5 vector encoding a BDD human FVIII vector (AAV5-hFVIII-SQ) [98]. For clinical visualization See Section 3, a study required for races of hemophilia A.

2.2. Limitations and risks associated with gene therapy for hemophilia

Gene therapy targeting hemophilia involves intravenous injection of the F8 transgene within the viral capsid (Figure 1). Intravenous administration results in preferential targeting of the transgene to the hepatocyte due to the architecture of hepatic capillaries [99]. Once the host cell recognizes the AAV capsid by its glycosylated surface once it binds to the receptor, the virus is internalized by clathrin-mediated endocytosis and transported through the cytoskeleton to the cytosol net. AAV must leave the endosome at the optimal time to avoid lysosomal degradation and promote degradation transport to the nucleus and subsequent detachment due to conformational changes in pH-sensitive endosomolytic viral proteins (100). Virus reversal rehearsal Genome RAAV lead extension molecules or Molecular recombination for training (that is, consciousness) Survive circulating genome Nuclear [101]. Main restrictions of genetic therapy based on AAV The approach is that AAV episomagenom is not reproduced. Cell division. Important points that need to be considered when using This approach is a potential loss of factor expression, Degusal results for liver growth Hepatocytes of young patients [71]. Unfortunately, repeat After the first dose, the administration is contraindicated Capsid tump protein. But perhaps in the case of Vector RAAV Integrate into animal genome [102], The effect of livestock. It is important to consider the potential for rare but possible occurrences of genotoxicity.[103]

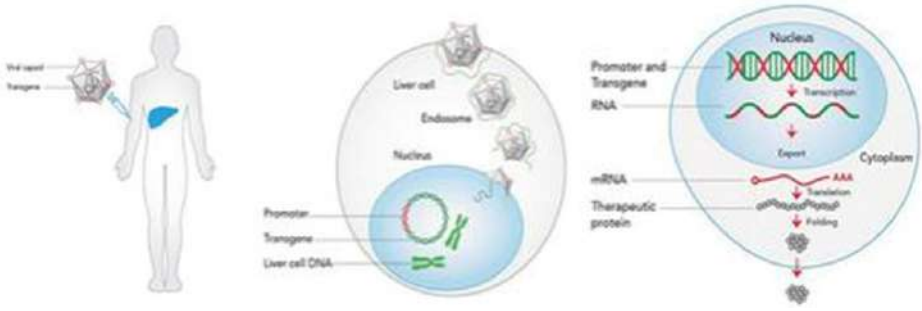


Figure 1. **Flow chart of targeted gene therapy.** Interesting Transgen is packed in a recombinant virus vector and introduced to the subject. Vector It is considered by various types of cells by the endociissis of the olganella called the end -some. The vector is released from the endosome, attaches to the nuclear membrane and delivers its genomic cargo into the nucleus. The vector genome contains a tissue-specific promoter so that it is transcribed only in the target cell type (e.g., hepatocytes). The host transcriptional machinery transcribes the transgene into mRNA, which is transported out of the nucleus and translated into the protein of interest.

The main challenges of gene therapy in general include large-scale production and costs of vectors, quality control and standardization of testing, and immunological barriers to rAAV gene delivery. Difficulties in removing cellular and viral contaminants from rAAV particles as well as empty AAV capsids, lack of standardization, and inherent batch variation of vectors potencies influence production costs [101]. Basic limitations and risks of gene therapy for hemophilia are detailed below.

2.2.1. AAV-neutralizing antibodies

Pre-existing neutralizing antibodies specific for various AAV serotypes (various degrees of serology cross-reactive), which may neutralize the vector and therefore reduce the efficacy of treatment, are common in the population due to natural infection with wild type AAV during childhood [92,100,104-106]. The prevalence of

anti-AAV antibodies varies from 20% to 70% depending on the specific AAV serotype in subjugated and conquered populations [39,87,91,107]. The subject's population is small LATION has previously indicated an antibody to existing AAV5. Skillly, the result of the low intersection of AAV superiority is low Antibody [92]. Earlier evaluation of existing illness rates The immunity to AAV in the general group is complicated, However, the fact that clinical tests are performed to detect rebellion. ABC antibodies have not been standardized yet [39,87,107,108].

In addition to pre-existing immunity, delayed cellular immune responses to the AAV capsid usually occur 4–12 weeks after vector injection and can lead to destruction of transduced cells and loss by cytotoxic T lymphocytes. Therapeutic efficacy (i.e., reduced longevity over time). Induction of cytotoxic T lymphocytes prevents effective regeneration Dosage increases, reducing long-term therapeutic efficacy [61,79,109]. In addition to the capsid tumpled, another AAV vector Ingredients such as stimulating CPG patterns, It may affect the immune response [61,110-113] Some therapies based on AAV2, AAV8, or other similar serotype vectors produced in mammalian cells induce a cellular immune response against the AAV capsid by targeting the transducer in hepatocytes causing loss of transgene expression, which may be controlled with immunosuppressant therapy [114]. Ongoing research may determine whether such cellular immune responses can be circumvented by producing AAV vectors in more distantly related species such as insects. Some studies have demonstrated that pre-existing AAV neutralizing antibodies may interfere with and limit vector transduction. No therapeutic effect (see section 2.2.3) [115], other No evidence of transgene loss or cell-mediated immunity was reported (116). Transduction efficacy did not appear to be observed in clinical trial programs for hemophilia B. Depends on pre-existing anti-AAV5 antibody titers, which are commonly observed. In the current clinical trials, subjects with pre-existing anti-AAV antibodies were not excluded. Capsid neutralizing antibodies [91,115,117,118]. The effects of neutralizing antibodies can be overcome by strategies such as changing the AAV serotype or increasing the vector dose to replace the antibodies, however, serotype modification of AAV may be ineffective due to cross-reactivity of some neutralizing antibodies. Other potential strategies include incorporation of empty capsids, reduction of titer using 1 or plasmapheresis, alteration of AAV capsids, and isolated delivery of AAV to limited areas to reduce systemic exposure. However, all of these strategies compromise the expected therapeutic effect [119]. Another approach is an attempt to prevent development T -cell adaptation immunity and immune reaction ability AAV CAPSID. For example, salt -resistant nanoparticles. SULFACT's Rapamycin was recently developed for guidance It improves immunity resistance, and thus the expression of transgene. After the initial dose and adaptation of the AAV vector Antibodies and T cells for AAV caps, Solving mouse AAV vector re -introduction And inhuman primates [120,121]. Most current research on gene therapy for hemophilia is aimed at patients who do not have pre-existing anti-AAV capsid neutralizing antibodies. Thus, these antibodies prevent widespread use of currently available gene therapies for the hemophilia population. Furthermore, because AAV-neutralizing antibodies can be produced after AAV-based gene therapy, patients should not receive multiple doses of the same AAV serotype (87).

2.2.2. Inhibitors of transgenic products

To date, the emergence of FVIII or FIX inhibitors in clinical studies of AAV gene therapy has not been reported. The F8 and F9 genotypes of these participants have not yet been reported. In

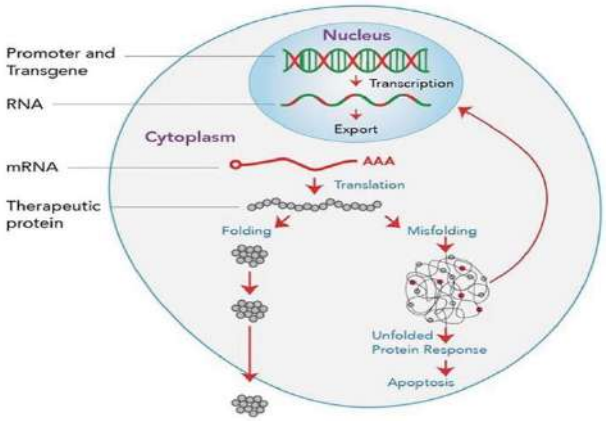
However, clinical research currently contains only patients. With people who had more than 150 days of expo due to exchange factors A person excluded from the history of inhibitors. therefore, Participants in these research probably have F8 and F9. Gene type associated with low risk of inhibitor formation. It is unclear whether untreated patients may develop inhibitors following gene therapy [84, 95]; therefore, clinical trials are underway to evaluate individuals with active inhibitors to determine whether gene therapy can induce tolerance and eradicate the inhibitors (92).

2.2.3. Hepatotoxicity

Clinical studies of both hemophilia A and B report asymptomatic transient increases in alanine transaminase (ALT) levels that can be controlled with a tapering course of glucocorticoids [98,122,123]. This typically mild toxicity may be related to viral particle trafficking, uncoating, and the DNA damage response induced by the vector DNA [98]. Several studies have shown that elevated ALT levels coincide with a detectable T cell response to the AAV capsid, but results have been inconsistent: in some patients, ALT levels increase without a capsid response [124], whereas in others, a capsid response occurs without an elevation in ALT levels [94, 125]. The increase in ALT levels observed after AAV-based gene therapy is vector dose dependent but is independent of the AAV capsid or genome Configuration, Transgenery -precomotor or manufacturing method [96]. Biomarine Phase 1/2 Phase 1/2 Phase Genetic treatment phase (Section 3.2.1), the increase in ALT was not linked to the general public. Lost the activity of FVIII immune response or Tivirus cells Cap side peptide [92,126]. However, most ALT elevations peak at 1.5‑2 times the upper limit of normal and may or may not be associated with hepatocyte loss (92). The National Medical and Scientific Advisory Committee on Hemophilia The National Hemophilia Foundation recommends biopsy to determine hepatocyte death or damage, T-cell cytotoxicity, and congenital conditions. To consider safety, efficacy, durability, and variability of response, investigate immune response and inflammation, FVIII/FIX expression and distribution, and evidence of residual intracellular AAV capsids in at least a subset of clinical trial subjects. [127,128] These endpoints contribute critically to our understanding of long-term safety and efficacy of treatment, as well as hepatocyte turnover, and may facilitate the detection of differences in AAV serotype tropism [92].

2.2.4. Tumorigenesis

Proviral DNA is typically maintained in the episomes of transduced cell nuclei. Thus, the risk of genomic insertional mutagenesis after AAV-mediated gene transfer is low, which is consistent with the fact that humans are frequently infected with wild-type AAV, but AAV infection is not associated with wild-type AAV tumorigenesis [96].



However, AAV genomes are rarely integrated into the host, although extensive sequencing studies have demonstrated that such integration does indeed occur in the liver [129,130], and several recent studies have found an association between hepatocellular carcinoma and wild-type AAV [131-133]. There is further evidence supporting the absence, the risk of insertional mutagenesis in both animal and human models Hemophilia patients have been treated with AAV to rule out this possibility Given the risk, further studies treating more patients with the disease are needed [84].

2.2.5. Cellular stress

The number of synthetic and other complex biological functions performed by the liver makes it highly sensitive to endoplasmic reticulum (ER) stress [134,135]. Because transgene expression is restricted to a subset of cells, some individual cells may produce an overload of FVIII, inducing cellular stress. ER is a place where the new protein is folded And it was secreted. For example, overload of the ER function is caused More demands for folding or accumulation of protein The enlarged or accidentally curved protein leads to a detailed protein. Answer (UPR) [136], cell stress index Answer [137,138] (Fig. 2). If cells are produced too much, cell stress will be induced Protein or protein is not processed correctly I change the direction or accidentally curl. This cell stress attracts UPR Make more esoteres to improve The squirrel turned around and was wrong. When cellular stress is too great, the UPR can lead to cell death via apoptosis. The UPR upregulates genes in the nucleus, activating downstream signaling cascades that result in translation arrest and protein degradation, reducing the protein load in the ER [139]. The UPR is a coordinated cellular mechanism that regulates protein synthesis and secretion in the ER [140]. It acts as an adaptive signaling pathway that prevents the accumulation of misfolded and unfolded proteins in the ER, thereby minimizing oxidative stress [141,142]. The UPR involves three inositol-containing transmembrane ER stress sensor proteins. It requires transcription factor 6-activated kinase and double-stranded RNA-like ER kinase-activated protein kinase. Induction of the UPR can be measured by assessing the activity of ER luciferase response element reporter splicing X-box binding protein. 1 and upregulation of immunoglobulin-binding protein (BiP), also known as Grp78 (145). Grp78/BiP is a central regulator of ER stress due to its role as a major ER chaperone with anti-apoptotic properties and its ability to control the activation of transmembrane ER stress sensors via a docking mechanism [141,146-148]. Chronic activation of the UPR and accumulation of unfolded proteins in the ER can lead to apoptotic cell death. Hepatocytes The purpose of gene treatment to obtain fviii proteins Do not express the natural expression of FVIII or VWF [31]. Increase the risk of stressful ER with super extractions FVIII [84] of these cells. SUPER EXPRESSED FVIII tends to be wrong Lumen ER folding to activate UPR, Cell damage or apoptosis. An increase in the UPR correlates with decreased FVIII expression when assessed using an in vitro cell expression system and decreased plasma FVIII concentrations in vivo after gene transfer with a viral gene therapy vector (40).Interestingly, the biosynthesis of porcine FVIII constructs containing the A1 and ap-A3 domains is 10- to 100-fold more efficient than human FVIII, providing higher levels of expression and secretion efficiency (149, 150). Expression of human FVIII activates the UPR to a greater extent than expression of porcine FVIII [145].

2.2.6. In vivo gene editing

Targeted genome editing techniques that use programmable nucleases (e.g., zinc finger nucleases, transcription activator-like effector nucleases, clustered regularly interspaced short palindromic repeats system [CRISPR]/CRISPR-

associated protein 9 [Cas9]) to correct gene mutations at the genome level [151]) may provide a more durable treatment for hemophilia [102,152,153]. Zinc finger nuclease (ZFN)-based genome editing studies were first investigated in patients with hemophilia B, where a normal F9 transgene was placed in intron 1 of albumin under the control of the endogenous albumin locus promoter. However, the program was discontinued (NCT02695160). Recent discoveries have demonstrated that in vivo genome targeting is possible. A human transgene at the Alb locus using CRISPR/Cas9 produced human FVIII in the liver, ameliorating the severe hemophilia A phenotype in mice [153]. Such genetic approaches effectively translated to humans may provide more permanent solutions for hemophilia A patients.

2.3. Summary

To date, the most serious problem associated with liver-targeted AAV administration is liver toxicity, accompanied by loss or decrease in transgene expression (96). As a vector dose and optimal transgene expression can influence whether to induce an immune response against the AAV vector and transgene product [84,154,155]. However, the exact pathophysiological mechanisms of loss of transgene expression and hepatotoxicity are unclear [96]. The underlying mechanisms are likely complex and may involve other factors, such as the UPR [95]. Although there is evidence for the existence of memory CD8+ T cell targeting AAV capsid responses, it is still poorly understood, loss of transgene expression and hepatotoxicity do not always correlate with T cell responses, so much more is needed to achieve robust long-term expression of foreign genes. Currently, children are not eligible to receive gene therapy directed at hemophilia, as the FVIII and FIX expression cassettes are present episomally and therefore not replicated during cell division, which may result in diluted expression when treating patients whose livers are still developing [84]. Studies in adults found stability Transgene expression is required for at least 10 years for FIX, a 50% decrease in FVIII expression occurred between years 1 and 2, and continued to decrease until the end of the third year [92]. Long-term goals of investigational gene therapies for hemophilia include extending the duration of transgene expression Potential treatment for patients with pre-existing anti-AAV antibodies Neutralizing antibodies or inhibitors of FVIII and FIX [61, 78, 96]. FVIII (ET3) Increased human honey molecule The secretion ability is a potential solution to acquire. Expression of sustainable introduction gene [149,157]. Furthermore, that's right It is necessary to collect long -term data on security and vibration. Efficiency sustainability [158]

3. Current Clinical Studies of Gene Therapy Hemophilia A

3.1. Timeline of Gene Therapy Clinical Trials Hemophilia A

Table 1 shows the timeline progression of active gene therapy studies for patients with Hemophilia A by reported chronology ClinicalTrials.gov (sorted by study start date). A total of 16 clinical studies of gene therapy for hemophilia A are registered with ClinicalTrials.gov.

3.2. Overview of gene therapy clinical trials for hemophilia A

Gene therapies for hemophilia A developed by eight companies are currently being evaluated in clinical studies, as summarized in Table 2 and the sections that follow.

3.2.1. BioMarin Pharmaceutical

BioMarin Pharmaceutical is conducting five clinical trials evaluating the efficacy of different gene therapy regimens in severe hemophilia A using AAV5-hFVIII-SQ (baloctocogen), a roxaparvovec, which contains amino acid 14 and replaces the human SQ binding sequence in the B domain.

Company/pro duct	Study Phase	ClinicalTrial.g ov Identifier Number	Study Start Date	Estimated Primary Completion a	Estimated Study Completion Date
BioMarin/Roct avian	Phase 1/2	NCT0257679 5	Aug 2015	Mar 2024	Mar 2024
Spark/ SPK8011	Phase 1	NCT0300353 3	Jan 2017	May 2020	May 2020
UCL/AAV2/8-HLP-FVIII–V3	Phase 1	NCT0300183 0	Jun 2017	Dec 2020	Jun 2025
Pfizer-Sangamo/SB-525	Phase 1/2	NCT0306120 1	Jun 2017	Jul 2024	Jul 2024
BioMarin/Roctavian	Phase 3	NCT0337091 3	Dec 2017	Dec 2022	Sep 2023
BioMarin/Roctavian	Phase 3	NCT0339297 4	Mar 2018	Dec 2022	Mar 2024
Takeda-Shire/ TAK754	Phase 1/2	NCT0337017 2	Mar 2018	Sep 2021	Sep 2024
BioMarin/Roctavian	Phase 1/2	NCT0352071 2	Apr 2018	Jun 2025	Jun 2025
Pfizer-Sangamo/SB-525	Phase 3	NCT0358711 6	Jul 2018	Sep 2021	Sep 2021
Spark/ SPK8011	Observationa l	NCT0343252 0	Aug 2018	Dec 2022	Dec 2022
BayerUltragenix/BA Y 2599023	Phase 1/2	NCT0358829 9	Nov 2018	May 2022	Jul 2026
Spark/SPK8016	Phase 1/2	NCT0373458 8	Jan 2019	May 2020	May 2020
BioMarin/Roctavian	Phase 3b	NCT0432309 8	Jun 2020	Dec 2021	Dec 2025
Shenzhen/YU VAGT-F801	Phase 1	NCT0321703 2	Jun 2020	May 2022	Jun 2022
Pfizer-Sangamo/SB-525	Phase 3	NCT0437005 4	Jul 2020	Aug 2022	Nov 2026
Expression Therapeutics	Phase 1	NCT0441841 4	Feb 2021	Apr 2025	Apr 2039

Product	Study number/title (ClinicalTrial.gov identifier number)	Status
BioMarin Pharmaceutical		
Valoctocogene roxaparvovec (Roctavian, previously known as valrox and BMN-270)AAV5-hFVIII-SQ	Study BMN270-201: A Phase 1/2, Dose-Escalation, Safety, Tolerability, and Efficacy Study of Valoctocogene Roxaparvovec, an Adenovirus-Associated Virus VectorMediated Gene Transfer of Human Factor VIII in Patients With Severe Hemophilia A (NCT02576795)EudraCT Number: 2014–003880-38	Phase 1/2 study – active, not recruiting 15 subjects Results published [98,126,159,160]

Study BMN 270–203: A Phase 1/2 Safety, Tolerability, and Efficacy Study of Valoctocogene Roxaparvovec, an Adeno-Associated Virus Vector-Mediated Gene Transfer of Human Factor VIII in Hemophilia A Patients with Residual FVIII Levels 1 IU/dL and preexisting Antibodies Against AAV5 (NCT03520712)	Phase 1/2 – enrolling 10 subjects planned	Study BMN 270–301: A Phase 3 Open-Label, Single-arm Study to Evaluate the Efficacy and Safety of BMN 270, an Adeno-Associated Virus Vector-Mediated Gene Transfer of Human Factor VIII in Hemophilia A Patients with Residual FVIII Levels 1 IU/dL (NCT03370913)
Phase 3 – active, not recruiting 134 subjects planned [160]	Study BMN270-302: Phase 3 Study To Evaluate Efficacy/Safety of Valoctocogene Roxaparvovec an AAV Vector-Mediated Gene Transfer of hFVIII at a Dose of 4x1013vg/kg in Hemophilia A Patients with Residual FVIII Levels 1 IU/dL Receiving Prophylactic FVIII Infusions (GENEr8-2) (NCT03392974).	Phase 3 – active, not recruiting 40 subjects planned
Study BMN 270–303: A Phase 3b, Single Arm, Open-label Study to Evaluate the Efficacy and Safety of BMN 270, an Adeno-Associated Virus Vector-Mediated Gene Transfer of Human Factor VIII, with Prophylactic Corticosteroids in Hemophilia A Patients (GENEr8-3) (NCT04323098)EudraCT Number: 2018–004616-21	Phase 3b – not yet recruiting 20 subjects planned	University College London (UCL)
		AAV2/8-HLP-FVIII-V3
Study UCL 13/0076: GO-8: Gene Therapy for Hemophilia A Using a Novel Serotype 8 Capsid Pseudotyped Adenoassociated Viral Vector Encoding Factor VIII-V3 (NCT03001830)	Phase 1 – recruiting 18 subjects planned	Spark Therapeutics
		SPK-8011rAAV-LK03
Study SPK-8011-101: Gene Transfer, Dose-Finding Safety, Tolerability, and Efficacy Study of SPK-8011 (a Recombinant	Phase 1 – recruiting 30 subjects planned	Study SPK-8011-LTFU: A Multi-Center Evaluation of the Long-Term Safety and Efficacy of SPK-8011 (Adeno-Associated Viral Vector with B-

Adeno-Associated Viral Vector with Human Factor VIII Gene) in Individuals with Hemophilia A (NCT03003533)		Domain Deleted Human Factor VIII Gene) in Males With Hemophilia A (NCT03432520)
Observational study, long term follow-up – enrolling by invitation 100 subjects planned	SPK-8016	Study SPK-8016-101: Dosefinding Study of SPK-8016 Gene Therapy in Patients with Hemophilia A to Support Evaluation in Individuals with FVIII Inhibitors (NCT03734588)
Phase 1/2 – active, not recruiting 30 subjects planned	Pfizer/Sangamo Therapeutics	
	SB-525PF-07055480 Giroctocogene fitelparvovec	Study SB-525-1603: A Phase 1/2, open-label, adaptive, dose-ranging study to assess the safety and tolerability of SB-525 (PF-07055480) (recombinant AAV2/6 human factor 8 gene therapy) in adult subjects with severe hemophilia A (Alta Study) (NCT03061201)

Phase 1/2 – recruiting 13 subjects planned	Study C0371004: An Open-Label, Non-investigational Product, Lead-in Study to Evaluate at Least 6 Months of Prospective Efficacy and Safety Data of Factor IX or Factor VIII Prophylaxis Replacement Therapy in the Usual Care Setting of Moderately Severe to Severe Adult Hemophilia B Subjects (FIX:C 2%) who are Negative for Nab To AAV Vector-Spark100 and Moderately Severe to Severe Hemophilia A Adult Subjects (FVIII:C 1%) who are Negative for Nab to AAV Vector Sb-525 Capsid (AAV6), Prior to the Respective Therapeutic Ph 3 Gene Therapy Studies (NAB Protocol) (NCT03587116)EudraCT Number: 2017–001271-23	Phase 3 – recruiting 250 subjectsThe data obtained from this 6-month lead-in study will serve as the control group for the subsequent Phase 3 study.

Study C3731003: Phase 3, Open-Label, Single-Arm Study to Evaluate the Efficacy and Safety of PF-07055480 (Recombinant AAV2/6 Human Factor VIII Gene Therapy) in Adult Male Participants With Moderately Severe to Severe Hemophilia A (FVIII:C 1%) (AFFINE) (NCT04370054)EurdaCT Number: 2019–004451-37	Phase 3 pivotal – recruiting 63 subjects planned	Bayer/Ultragenix Pharmaceutics
		BAY 2599023 (DTX 201)
Study 19,429: A Phase 1/2 Open-label Safety and Dosefinding Study of BAY2599023 (DTX201), an Adenoassociated Virus (AAV) hu37-mediated Gene Transfer of B-domain Deleted Human Factor VIII, in Adults With Severe Hemophilia A (NCT03588299)EudraCT Number: 2017–000806-39	Phase 1/2 – recruiting 30 subjects plannedResults presented at Scientific Meeting: [165]	Takeda/Shire (Baxalta)
		TAK-754 (previously SHP654 and BAX 888)

Study 201,501: A Global, Open-Label, Multicenter, Phase 1/2 Study of the Safety and Dose Escalation of BAX 888, an Adeno-Associated Virus Serotype 8 (AAV8) Vector Expressing B-Domain Deleted Factor VIII in Severe Hemophilia A Subjects Administered a Single Intravenous Infusion (NCT03370172)EudraCT Number: 2015–005576-22	Phase 1/2 – active, not recruiting 12 subjects	Shenzhen Geno-Immune Medical Institute
		YUVA-GT-F801
Study GIMI-IRB-17007: Lentiviral FVIII Gene Therapy for Hemophilia A (NCT03217032)	Phase 1 – not yet recruiting 10 subjects planned	Expression Therapeutics
		CD34+ hematopoietic stem cells transduced with CD68ET3 lentiviral vector

(called HSQ and currently the most commonly used) Clinical trial for severe hemophilia A). A phase 1/2 dose-escalation clinical trial (BMN270 study) 201, NCT02576795) evaluating the effect of one single dose of several doses with 3 years of follow-up was conducted in 15 men with severe hemophilia A [98,126]. Overall, the treatment substantially reduced the ABR, allowing subjects that received 4 × 1013 or 6 × 1013 vector genomes (vg)/kg body weight of AAV5–hFVIII-SQ to discontinue prophylactic FVIII use. During the 3-year follow-up after the single administration, none of the participants had developed inhibitors, thromboses, or showed persistent changes in liver-function tests, and none died [126]. An additional year of data (cutoff 8 April 2020) on this cohort was presented at the World Federation of Hemophilia (WFH) for the 6 × 1013 vg/kg cohort as well as 3 years of data for the 4× 1013 vg/kg cohort. The 6 subjects in the 6 × 1013 vg/kg AAV5–hFVIII-SQ cohort who had received FVIII prophylaxis prior to the AAV5–hFVIII-SQ treatment exhibited remarkable and long-lasting reductions in bleeding episodes that required an FVIII infusion. Mean cumulative TEA over 4 years after AAV5–hFVIII-SQ treatment was 0.8, indicating a 95% reduction from the year before the study (mean baseline ABR = 16.3, median = 16.5). After 4 years, the mean ABR for the 6 subjects was 1.3 (median = 0). In this group FVIII use was reduced overall by 96% over the 4-year study period, from a baseline mean of 135.6 infusions/year to a mean of 5.4 infusions/year. Of the 7 subjects in this cohort, 6 (86%) had no bleeding episodes during the fourth year. None of the seven subjects currently require FVIII prophylaxis. Six subjects in the 4×10^{13} vg/kg AAV5–hFVIII-SQ cohort also demonstrated a long-term reduction in bleeding events. FVIII infusions are required. All 6 participants were able to discontinue FVIII preventive treatment. The mean cumulative ABR over 3 years after treatment was 0.9, indicating a 95% reduction from the year before AAV5-hFVIII-SQ treatment (mean ABR = 12.2, median = 8.0), and 5 of 6 participants had no bleeding at target joints at the 3rd year of observation. In the third year, the average ABR was 0.5 (Mediana = 0), no bleeding case occurred in 4 out of six. Subject 5 in 6 composition organizations declares non -distributed Bridge bridge. In this group, the number of FVIII infusions reduced by 96% from 142.8 infusions/dose over 3 years. Initially 5.7 infusions/year after 3 years. Mean FVIII activity levels at the end of the evaluation period for both dose cohorts confirm the reduction in ABR Number of FVIII infusions. At the end of the study period, all subjects continued to produce their own endogenous FVIII. The mean FVIII production in the group was 6 × 1013 vg/kg In the A 4 × 1013 yds/kg cohort, the mean (median) FVIII activity was 24.2 (16.4) IU/dl (chromogenic substrate assay) and 35.4 (23.4) IU/dl (one-step assay). In the B 4 × 1013 yds/kg cohort, the mean (median) FVIII activity was 9.9 (7.9) IU/dl (chromogenic substrate assay) and 14.9 (12.3) IU/dl (one-step test) assay). Treatment with AAV5-hFVIII-SQ is currently being tested in additional phase 1/2 and phase 3 clinical studies (Table 2). A multinational phase 3 study of AAV5-hFVIII-SQ dose-based 6 × 1013 vg/kg (GENEr8-1, study BMN 270-301, NCT03370913, n = 134 participants) aimed at comparing efficacy Transition from AAV5 – hFVIII-SQ to the current standard of care, i.e. prophylactic FVIII therapy, is ongoing. Recruitment is complete, and data from at least 1 year of follow-up show an 84% reduction in mean ABR and a 99% reduction in mean annual FVIII infusion rate. One year after treatment, the mean FVIII expression level was 42.9 IU/dL. Biomarine is also a subject of 1/2 phase. (N = 10) Existing AAV5 antibody using 6x13 AAV5 -HFVIII -SQ dose VG / kg (BMN research 270 --203, NCT03520712). In addition, bio -marine leads the

other two. Research: Evaluation of AAV's serum positive Includes severe hemophilia A and non - intervention research The purpose is to establish the basic characteristics of people. Hemophilia A. Due to differences between Phase 1/2 and Phase 3 trials, the treatment duration of AAV5-hFVIII-SQ is

3.2.2. University College London (UCL)

The University College London study is an ongoing Phase 1/2 open-label clinical trial evaluating treatment with an AAV vector expressing a 17 amino acid peptide containing six N-linked glycosylation motifs derived from the B domain of human FVIII. A liver-specific transporter (AAV8-HLP-hFVIII-V3; GO-8, Study UCL 13/0076, NCT03001830) was used in this study. A relatively low dose of AAV8-HLP-FVIII-V3 was used in this study compared with other related FVIII gene therapies. Preliminary results published in 2018 [162] showed that all 3 participants had FVIII activity below 5% and one participant had normal levels of procoagulant activity (FVIII:C). There were cases of spontaneous bleeding that was reduced or prevented during the preliminary observation period. No grade 3 or higher adverse events were reported during the first 47 weeks after treatment (162).

3.2.3. Spark Therapeutics

Spark Therapeutics is also evaluating the efficacy of reduced-size FVIII cassettes for FVIII production in patients with hemophilia A. Two different designs, SPK-8011 and SPK-8016, are being evaluated. SPK-8011 (rAAV-LK03 vector) is a recombinant AAV vector containing a codon-optimized human FVIII gene controlled by a liver-specific promoter. SPK-8016 is a gene therapy developed in-house. Three clinical studies, 2 evaluating SPK-8011 and 1 evaluating SPK-8016, are ongoing [163]. In the open-label, non-randomized phase 1/2 study of SPK-8011 in subjects with hemophilia A (data cutoff was May 3, 2021, NCT03003533), a single dose of SPK-8011 (dose cohorts ranged from 5×10^{11} to 2×10^{12} vg/kg) was administered to a total of 18 subjects in 4 cohorts: $5X10^{11}$ (N= 2), $1X10^{12}$ (n= 3), $1.5X10^{12}$ (n= 4), and $2x10^{12}$ (n= 9) [164]. Sixteen participants demonstrated robust expression of FVIII. 91.5% discontinued basic prophylaxis and demonstrated a reduction in AEP and a 96.4% reduction in annual FVIII infusions. Two participants lost FVIII expression, likely as a result of a capsid-based immune response. The second SPK-8011 clinical trial will monitor the safety and efficacy (for 5 years) of a single dose of SPK-8011. Approximately 100 men with hemophilia A enrolled in a previous study sponsored by Spark SPK-8011 (SPK Study) 8011-LTFU, NCT03432520). Spark Therapeutics is also conducting an open-label, non-randomized Phase 1/2 dose-finding study of SPK-8016 (Study) SPK-8016-101, NCT03734588) in adult men with severe hemophilia A who are not developing a FVIII inhibitor. Safety, efficacy, and tolerability of SPK-8016 in adult males without clinically severe hemophilia A The developed FVIII inhibitor will be evaluated in part 1, and data from part 1 will be used to design and select doses for parts in adult males who developed FVIII inhibitors. Preliminary data from 4 participants show that FVIII is stable and sustained (range 5.9% to 21.8%) over 52 weeks in the cohort 5×10^{11} vg/kg, with an annualized infusion rate of 98% and an ABR reduction of 85%.

3.2.4. Pfizer/Sangamo Therapeutics

Pfizer is promoting an investigational new drug, giroctocogene fitelparvovec (SB-525 or PF-07055480; originally developed Sangamo Therapeutics, but transferred to Pfizer Phase 3 clinical trials. Giroctocogene fitelparvovec is a recombinant AAV vector encoding the human FVIII gene, from which the B domain has been deleted. Giroctocogene Fitelparvovec was studied in an open-label phase 1/2 (Alta study, study SB-

525-1603, NCT03061201) 11 men treated in 4 phases Doses: 9 × 1011 vg/kg (n = 2), 2 × 1012 vg/kg (n = 2), 1 × 1013 vg/kg (n = 2) and 3 × 1013 vg/kg (n = 5). Updated results Presented at the 2020 World Federation of Hemophilia World Congress Held in June 2020, here is what 2020 has in store: Five participants in the cohort giroctocogene fitelparvovec 3 × 1013 vg/kg demonstrated a sustained increase in FVIII activity levels (median 64.2%), there were no bleeding events, and no FVIII infusions were required. giroctocogene fitelparvovec Generally well tolerated, with only one participant in the cohort At the highest dose (3 × 1013 vg/kg), the only patient who experienced serious treatment-related adverse events, namely hypotension (grade 3) and fever (grade 2), occurred within 6 hours after infusion (completely dissolved in the body 24 hours). Of the five participants in the cohort who received the 3 × 1013 vg/kg dose, four were treated with oral corticosteroids for elevated liver enzymes (ALT), which completely resolved with treatment. Pfizer is currently also recruiting subjects for a preliminary phase 3 study (NAB protocol, study C0371004, NCT03587116). No investigational drug will be administered (only standard alternative treatments), and that data provides a baseline for the topic. Pivotal phase 3 study (AFFINE study, study C3731003, NCT04370054). Phase 3 studies will primarily evaluate ABR over 12 months, with secondary endpoints including steady-state FVIII activity levels, annualized FVIII infusion rates, annualized FVIII consumption, cause and site of ABR, and changes in joint health. This is an ongoing study, with the first participants receiving one dose in October 2020.

3.2.5. Bayer / Ultragenix Pharmaceutical

Bay 2599023 Bayer is developed in cooperation with Ultragenyx Pharmaceutical. Bay 2599023 - Vector AAV FVIII coding with a remote B domain which is controlled specific promoter and improvement, optimized for transgenics expression. An ongoing Phase 1/2 dose-ranging study (Study 19,429, NCT03588299) is evaluating the safety, tolerability, and initial efficacy of three ascending doses of BAY 2599023 in patients with severe hemophilia A previously treated with FVIII agents. Preliminary data presented at the American Society of Hematology Annual Meeting (December 2020) showed that BAY 2599023 demonstrated a 5x1013 efficacy in three dose cohorts (0.5 x 1013, 0.5 x 1013, 1 x 1013 and... Several patients (groups 2 and 3) who had all been on FVIII prophylaxis prior to gene therapy Treatment, prophylaxis was discontinued approximately 6 weeks after Post-gene transfer, participants reached FVIII levels ≥15 IU/dL, and no spontaneous bleeding was reported. Both SUBJECTS of cohort 3 has risen Alt level (1.5 times the top> 1.5 times Normal restrictions) and corticosteroids. the study Currently, I am registered as a subject (up to 30 qualified adult subjects).

3.2.6. Takeda /Shire

Takeda genes for hemophilia include otu pipeline 754 (formerly known as SHP654 and BAX 888), this is Vector AAV Semo Following 8 to express FVIII by deleting domain B for hemophilia A Clinical research (research 201.501, NCT03370172) is active However, there is no recruitment.

3.2.7. Shenzhen Institute of Genetic Immunology

In 2017, Shenzhen Institute of Genetic Immunology recorded: Clinical Study of Autologous Hematopoietic Stem Cells and Mesenchymal Stem Cells Modified by Lentiviral FVIII Gene (Study) GIMI-IRB-17007, NCT03217032), but since June 2021, the study has remained in a non-recruiting state.

3.2.8. Therapeutic Expression

Expression Therapeutics registered a hematopoietic stem cell transplantation gene

therapy clinical trial (ET3-201 study) incorporating a lentiviral vector encoding a highly expressed FVIII ET3 transgene for the treatment of severe hemophilia A (NCT04418414), but since June 2021, the study has remained in a non-recruiting state.

4. Second generation gene therapy, ASC618

4.1. ASC618 design

Applied Stem Cells (ASC) Therapeutics, Inc. We developed a construct called ASC618 (AAV2/8 HCB-ET3-LCO BDD FVIII viral vector), which is an AAV2/8 hybrid vector encoding BDD codon-optimized hFVIII (hFVIII BDD) with a liver-targeted synthetic promoter (Figure 3). Compared to other clinic tested gene therapy constructs, ASC618 has the shortest vector genome. The design includes a liver-specific, codon-optimized (LCO) and controlled bioengineered hFVIII (ET3) BDD. A synthetic liver combinatorial promoter bundle (HCB). ASC618 is designed to express hFVIII protein for the treatment of severe and moderate hemophilia A. ASC618 is supplied frozen in the form of a viral vector in individual bottles and is administered by a single intravenous infusion. Rational and empirical design strategies have been applied To produce ASC618, a minimum and very powerful AAV-FII-FII vector incorporating 2 unique elements: 1) a minimal liver- HCB promoter carried out (146 pb) to minimize the packaging size and allow higher protein expression levels; and 2) a New bio-conted fviii molecule, and 3, with 10 to 100 times Increased biosynthesis, expression and efficiency of secretion Compared to the standard HFVIII transgenes (called HSQ; The HFVIII BDD protein which contains the acid 14-Amino SQ linker sequence derived from man instead of domain b) Currently used in most hemophilia AGENE therapies. Expression Therapeutics/Emory University characterized the HCB-ET3-LCO construct in a mouse model of hemophilia A and licensed it for further therapeutic development to ASC Therapeutics. The ASC618 construct utilizes a chimeric human/porcine FVIII molecule, ET3, to enhance vector efficiency. ET3 is a human BDD FVIII protein bioengineered with elements of the porcine A1 and A3 domains (91% human, 9% porcine) [86,145,149,157,166,167]. A new bioengineered molecule, FVIII ET3 (previously known as HP47), was developed from recombinant porcine FVIII BDD [149,150]. Recombinant porcine FVIII (rpFVIII, Obizur®) was first developed by Emory University and the FDA approved this treatment for acute bleeding in patients with acquired hemophilia A [168,169]. Numerous optimization efforts have been carried out by studying the pharmacological properties of the FVIII protein by comparing the FVIII sequences of different ancestral species (ancestral sequence reconstruction approach). FVIII protein option Designed with excellent characteristics compared to Modern biology HFVIII, improved activities, stability, Possibility of live synthesis and reduced anti -clinical inhibition Medicinal antibody [170]. RPFVIII and ET3 molecules do not interact much. Resident's chaperon, so it is unlikely to be caused. UPR, they are much more effectively secreted than other FVIIIs Structure [145 149,150,171]. In ET3, ET3 is higher, higher expression The RPFVII sequence is replaced by A1 and AP-A3 The area of the recombinant FVIII. It's a small sequence Changes (~ 9 %) are wonderful from 10 to 100 Fold the improved live synthesis [149].

4.2. Liver-specific codon optimization

Compared to standard genome-wide codon optimization strategies, tissue-specific codon optimization strategies can enhance FVIII transgene expression in certain cell types (e.g., hepatocytes). All current rAAV-FVIII product candidates in clinical trials utilize codon-optimized transgenes. In traditional codon optimization strategies, the codon usage bias of an organism is derived from the whole genome

cDNA, which is assumed to represent the transfer ribonucleic acid concentration in individual cells. In fact, the transfer RNA concentration in individual cells varies greatly between tissues and cell types [172]. Using this novel codon optimization strategy, Doering and colleagues [166] investigated tissue/cell type specific codon usage tables for codon optimization in liver-targeted AAV gene therapy to further improve BDD expression. hFVIII (HSQ) and ET3. When transfected into HepG2 cells, ET3-LCO was expressed as either myeloid codon-optimized or native non-codon-optimized related [166]. The effect of hepatic codon optimization was also confirmed in vivo in a hemophilia A mouse model, where ET3-LCO resulted in a 3- to 4-fold increase in expression compared with myeloid codon-optimized and non-codon-optimized ET3 [166].

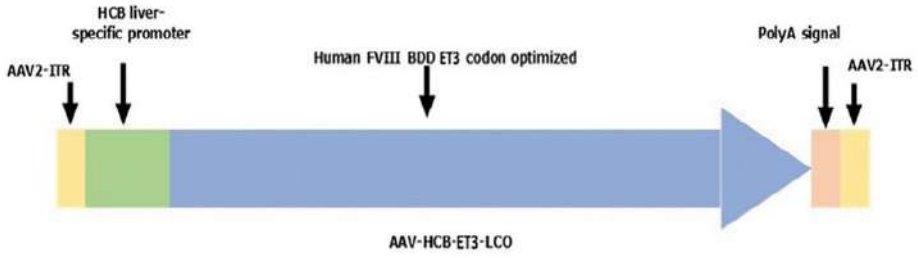


Figure 3. Schematic diagram of the ASC618 expression cassette. The ASC618 construct consists of an AAV8 vector encoding a codon-optimized hFVIII gene that has been minimized by B domain deletion (BDD). The construct is designed for specific expression in liver cells.

4.3. HCB promoter

The optimal genome capacity of rAAV vectors is approximately 4.7–4.9 kb [93,157,173]. rAAV-FVIII vectors Typically, this ideal vector genome length is exceeded due to the following reasons:

Large transgene size and the requirement for non-coding viral and gene regulatory control elements result in poor transgene packaging and delivery. At a minimum, the rAAV-FVIII genome must contain:the promoter, FVIII transgene, polyA signal, and the inverted rAAV terminal repeats framing both sides of the cassette. Some require 4664 bp for the inverted terminal repeats and FVIII transgene. out of the available 4900 bp. So, the promoter, polyA signal, and other required sequences must fit into the remaining 246 bp. Doering and his colleagues [166] addressed this limitation using both random and rational in silico combinatorial design approaches by: Creating a synthetic promoter that is more compact than existing promoters and is able to consistently generate strong expression. Activating hepatocytes while maintaining comparable or higher transcription yields. After three successive rounds of design/optimization, they identified a 146 bp synthetic promoter that HCB controls FVIII production by 20-fold. Less than 100 bp shorter while establishing the reference HLP promoter. The HCB assays involved transient transfection of a human hepatocellular carcinoma cell line HepG2 and hydrodynamic injection of naked plasmid DNA encoding the corresponding AAV genome into mouse models of hemophilia A (166).

4.4. Comparison of ASC618 with other Investigational products

Enhanced ET3 transgene biosynthesis provides significantly better therapeutic potential than standard hemophilia A gene therapy based on preclinical studies (Figure 4). Comparison of the effects of ASC618 transgene with ASC618 transgene Standard transgene for FVIII activity. Three transgenes, AAV2/8-HCB-HSQ-LCO, AAV2/8-HCB-ET3-LCO and AAV2/8-HLP-V3co, were administered intravenously at a dose of 1 × 1011 vg/kg in a mouse model of hemophilia A (n = 4/group). Plasma FVIII activity was measured within 16 hours. The ASC618 transgene

produced significantly greater FVIII activity than the standard and control transgenes (Fig. 6 C from Brown et al. [166] Reproduced with permission from the publisher. ET3 and HSQ were tested in C57Bl/6 mouse models, cynomolgus monkey models, and a humanized mouse liver model (FRG-KO). In all 3 models, the ET3 transgene-containing vector produced higher FVIII levels than the HSQ transgene-containing vector [174].

In the C57Bl/6 mouse model, AAV2/8 HCB-ET3-LCO at doses of 5×10^{10}, 5×10^{11}, and 5×10^{12} led to stable expression of human FVIII with mean ET3 FVIII levels reaching 50% (0.5 IU/mL), 300% (3 IU/mL), and 350% (3.5 IU/mL) of normal, respectively. In contrast, treatment with AAV2/8 HCB-HSQ-LCO at doses of 5×10^{11} and 5×10^{12} vg/kg produced HSQ factor VIII expression levels that were 7-fold and 3-fold lower, respectively, and no HSQ expression was detected at a dose of 5×10^{10} vg/kg. In cynomolgus monkey experiments, the trend was similar: AAV2/8 HCB-ET3-LCO at 5×10^{11} vg/kg induced expression levels of nearly 30% (0.3 IU/mL) of normal. In the humanized FRG-KO liver model, ET3 treatment at 3×10^{12} vg/kg induced mean human FVIII expression levels reaching 480% (4.8 IU/mL) of normal, compared to only approximately 30% after HSQ treatment. Furthermore, in the model, administration of FRG-KO human hepatocytes, ASC618 led to high ET3 mRNA expression as assessed by RNAscope analysis. In all three models, security investigations have been performed, including: Clinical observations; measurement of food intake, body weight, and body temperature; and evaluation of liver enzymes and overall pathology showed no toxicity. Thus, ASC618 was well tolerated in animal models and demonstrated the potential to provide a therapeutic benefit to patients at reduced vector doses [174].

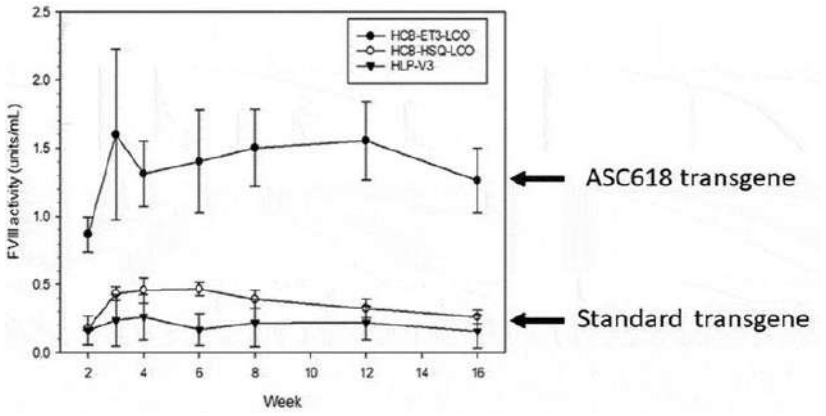


Figure 4. Delivery of tissue-optimized transgene cassettes by AAV vector.

5. Expert opinion:

Hemophilia is a well-studied target for gene therapy. Preclinical and clinical data indicate that gene therapy may improve patients' quality of life by inducing sufficient FVIII synthesis and secretion and normalizing coagulation factor activity, however, certain limitations prevent currently available gene therapies from being a definitive treatment for all patients. The duration of treatment and long-term safety are influenced by many factors, including the development of neutralizing antibodies against AAV, inhibitors of the transgene product, hepatitis, toxicity, cell stress and potential for tumor formation. To extend the duration of treatment, intensive preclinical and clinical research is focused on the causes and mitigation of cellular stress in hepatocytes caused by post-translational folding of the FVIII protein.

A comprehensive assessment of demographic, genetic and other individual factors is also required to understand the significant variability in FVIII activity observed in treated patients. Next generation gene therapy is expected to improve FVIII synthesis and secretion while limiting the development of neutralizing antibodies against AAV and the development of cellular stress. This goal can be achieved through transgenic engineering strategies that maximize transgene expression while minimizing potential post-translational cellular stress. Causes apoptosis of transfected hepatocytes. Preclinical Studies of human-pig chimeric constructs in wild-type mic In humanized liver mice and non-human primates, FVIII synthesis is increased 10-100-fold, Reduced cellular stress. Clinical studies on a chimeric human-porcine FVIII transgene (ASC618, NCT04676048) will start soon and may confirm the results of preclinical studies showing that compared with the fully human-porcine FVIII transgene, the chimeric transgene allows the use of higher and lower doses of AAV, while providing sufficient serum FVIII levels and a longer therapeutic effect. Advances in the field of gene therapy require better technologies Understanding the target cells (e.g. physiology) Hepatocytes as FVIII biofactories. Identifying factors Transcription, translation, post-translation, and Protein secretion are essential to improve efficacy, safety, and especially longevity of gene therapy. Future gene replacement therapies will need to address the challenge of extending the persistence of expression transgenics and improve therapeutic outcomes in children. Current gene replacement therapies have limitations. Because transgenes do not replicate in cells, transgene expression is diluted and lost over time. This is especially relevant in children. ASC Therapeutics is currently pursuing a gene-editing program using a CRISPR/Cas9-based in vivo genome-editing. This method incorporates non-homo- logous end-joining that enables permanent chromosomal integration of a modified human B-domain–deleted FVIII at the albumin locus in liver cells to prevent the loss of AAV vector due to hepatocyte proliferation. Such an approach could revolutionize the treatment of hemophilia in young patients who are currently ineligible for standard gene therapy. Advances in understanding the mechanisms of transgene insertion into hepatocytes will greatly improve our understanding of liver-targeted gene therapy for other hepatic indications and diseases, including nonalcoholic fatty liver disease, alcoholic liver disease, and hepatitis. Creating safe, durable and stable therapies to replace or supplement missing or defective proteins for a wide range of conditions, as well as reducing the social and patient burden, is the ultimate goal of gene therapy and recent advances in this field. anticipates promising achievements in the near future.

Abbreviation

AAV, adenocythal virus. ABR, annual bleeding rate. Alt, Alanine Amino Transferase; BP, basic couple. CRISPR related to CAS9, protein 9 CRISPR, regularly grouped and repeats short Paralindrome. Erotic body; fviii, factor VIII; fixing, factor IX; HCB, liver Binary package; ITI, immune -resistant guidance; KB, kilo -based; love, AAV recombination; UCL, London University; UPR, detailed protein Answer; United States, the United States; VG, Vector genome; VWF, Von Villebrand Postponed delivery person; WT, wild type

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Declaration of interest

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