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Reviewing the Latest Treatments for Haemophilia

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Abstract:
Advancements in haemophilia treatment have been significant in the first 20 years of the 21st century. However, the progress started with the fractionation of plasma in 1946. The first concentrates were developed after discovering FVIII in frozen plasma cryoprecipitate and FIX in the supernatant in the early 1960s, leading to initial attempts at replacement therapy. Unfortunately, due to the lack of screening methods for viral pathogens, people with haemophilia (PWH) received contaminated concentrates, leading to infections with the hepatitis A virus, hepatitis C virus, and human immunodeficiency virus. Thankfully, by 1985, viral screening methods and virucidal techniques were developed, making concentrates safe. The introduction of chromatography steps with monoclonal antibodies in the production process led to increasingly pure products. However, the problem of immunogenicity of administered concentrates persists. The development of alloantibodies against FVIII in a significant percentage of PWH is a serious adverse effect of replacement therapy. The next major advancement came with cloning the F8 and F9 genes, which allowed the production of factor concentrates using recombinant DNA technology. The injected FVIII and FIX molecules have a relatively short circulating half-life in the plasma of people with haemophilia A and B, approximately 12 and 18 hours, respectively. Methods such as conjugating the factor molecule with fragment crystallizable of IgG1, albumin, or adding polyethylene glycol have been applied to prolong the plasma half-life and extend the interval between injections, especially for risk. The next frontier in haemophilia therapy is the development of durable and potentially curative treatments, such as gene addition therapy. Experiments in haemophilia B have shown promising long-lasting responses. However, the results of gene therapy for haemophilia A have not been as remarkable, and the durability of the treatment is yet to be demonstrated. The long-term safety, predictability, durability, and efficacy of gene therapy for haemophilia A and B remain open questions. Currently, only healthy adult PWH has been enrolled in gene therapy clinical trials, and further studies are needed before gene therapy can be widely applied to children and those with pre-existing antibodies against the delivery vector.

Keywords: replacement therapy, adverse events, pharmacokinetics, extended half-life concentrates, non-replacement therapy, gene therapy

Introduction:

Hemophilia A (HA) and B (HB) are rare genetic disorders that affect the blood's ability to clot. These disorders are caused by a lack of certain proteins in the blood. Hemophilia A is caused by a lack of a

protein called factor VIII, while a lack of factor IX causes Hemophilia B. These disorders occur due to a problem with a gene on the X chromosome. Males with an XY genotype are likely to have hemophilia, while females with an XX genotype are carriers of the disease. In some cases, females with the XX genotype may also experience symptoms of hemophilia. These women are generally mildly symptomatic, but in some cases, due to non-random inactivation of one of the XX chromosomes (lyonization), they can be more severely affected, especially with menorrhagia[1].

The range of FVIII concentration in the healthy population is quite wide, ranging from 64–197 IU/dL, and it also depends on the blood group: 55–150 IU/dL in group O and 71–186 in non-O. People with hemophilia (PWH) have FVIII or FIX concentration in plasma of less than 1 IU/dL for those with a severe form of the disease, 1–5 IU/dL for those with moderate hemophilia, and 5–40 UI/dL for patients with a mild form. Spontaneous bleeding in PWH occurs most commonly in the joints and skeletal muscles and rarely at the skin or mucosal surfaces as in patients affected by thrombocytopenia or those with platelet dysfunction. Bleeding following minor trauma or with surgical procedures may lead to severe bleeding at the injury site in PWH. There is a rough correlation between the severity of the disease and the types of bleeding observed in PWH. In severe hemophilia, frequent bleeding includes hemarthrosis, muscle hematomas, CNS hemorrhages, and hematuria, even in the absence of any apparent trauma. In moderately severe hemophilia, moderate trauma or minor surgery may trigger bleeding, and in mild disease, bleeding usually only occurs following severe trauma or major surgery[1].

HB is generally considered less severe than HA, but there is not complete agreement on this issue. We observed that arthropathy in Italy was less frequent in HB patients than in HA. The difference in genotypes of the two diseases seems to be the reason for this discrepancy. In severe HA, the inversion of intron 22 results in an extreme change of the F8 gene sequence and is the causative mutation in about 50% of the patients. In comparison, in HB, the small and complete deletion of the F9 gene represents only 17%, and missense mutations account for 55% of cases. Missense mutations represent about 75% of moderate and 16% of severe HA, respectively. The more severe manifestations of joint and muscle bleeding are cartilage damage and synovial inflammation and hypertrophy, developing into a chronic debilitating arthropathy. The muscle and skeletal derangement may soon cause a limitation of movements during daily activities, with a consequent decrease in patients' quality of life.[1]

The Replacement Therapy and Its Adverse Events

In 1948, the first treatment for hemophilia was introduced through the infusion of plasma from healthy blood donors to address the deficiency of a plasma clotting factor. Subsequently, in 1958, Nilsson M and Blowback B in Sweden successfully treated hemophilia A patients with a globulin fraction of human plasma purified by Cohn's fractionation method. Concurrently, the discovery of cryoprecipitate from a single blood donor's plasma in 1964 initiated a new era of hemophilia therapy. Although cryoprecipitate had low specific activity and necessitated storage at -20°C in plastic bags, each containing approximately 100 IU of FVIII, its use was laborious, with an estimated 14 to 21 bags required for a hypothetical dose of 20-30 IU/kg in a patient weighing 70 kg. The advent of lyophilized FVIII concentrates, derived from large plasma pools, brought about substantial advancements in production processes, particularly through immunoaffinity chromatography using monoclonal antibodies specific for FVIII. However, both concentrates from large plasma pools and single-donor cryoprecipitates were found to be contaminated by hepatitis viruses, with the latter presenting lower infection risks.

Subsequently, HIV transmission was observed in a considerable percentage of hemophilia A patients treated with commercial clotting factor concentrates, with a lower transmission rate among those treated with single-donor cryoprecipitates, potentially due to the known donors, typically a parent. The prevalence of HIV infection was significant among individuals with hemophilia in the United States and Italy. Before the conclusion of the last millennium, three significant advances in the manufacturing process positively influenced the viral safety of products used in hemophilia replacement therapy.[2]

1. Implementing more accurate screening of blood donors for infection by enveloped viruses.
2. Implementing various virucidal methods such as pasteurization, vapor heating, and heating in the lyophilized state at 80°C. Additionally, the use of solvent/detergent in the final steps of the manufacturing process eliminated lipid-enveloped viruses, but not naked viruses such as parvovirus, HAV, or transfusion-transmitted virus (TTV).[2]
3. Producing new concentrates through DNA-recombinant biotechnologies.

The first-generation recombinant FVIII concentrates were developed in Chinese Hamster Ovary (CHO) cells and contained human albumin. The second-generation rFVIII concentrates removed albumin from the culture media, while the third-generation rFVIII included the addition of a non-protein stabilizer to the final formulation.[3]

Transitioning from plasma-derived FVIII to FVIII concentrates for hemophilia A treatment increased to inhibitors against rFVIII, with reported incidences ranging from 33% to 52%. However, a study in Germany contradicted this finding, showing similar inhibitor rates for FVIII and FVIII. The immunogenicity of both FVIII and FVIII remains a significant, unresolved challenge in HA therapy. Conversely, the incidence of FIX inhibitors after treating hemophilia B with FIX or FIX concentrates is exceptionally low, about 3%, although it can lead to severe anaphylactic reactions[4].

Targets of replacement of hemophilia therapy:

The main goal of treating hemophilia is to prevent life-threatening bleeding and provide relief for muscle and joint bleeding. In the past, treatment was limited to on-demand therapy, which involved infusing concentrate after the bleeding had occurred. However, this approach did not fully prevent damage to joints or muscles, which would start a few hours after bleeding began. Studies have shown the benefits of prophylaxis, where treatment is started after bleeding has occurred (secondary prophylaxis), compared to on-demand therapy. Research has indicated that maintaining a certain level of factor (FVIII/FIX) in the blood can help prevent bleeding. For example, a study in the Netherlands found that a baseline FVIII level of at least 12 IU/dL could completely prevent joint bleeding, while another study from the US suggested that levels of 20% might be needed to prevent all joint bleeding. These findings highlight the importance of maintaining adequate factor levels to prevent bleeding and its associated complications. Continuous, early prophylaxis, known as primary prophylaxis, is effective in preventing joint damage in children with severe hemophilia A. In contrast, episodic on-demand treatment resulted in a higher bleeding rate[5]. Prophylaxis initiated after two or more episodes of joint bleeding but before the onset of joint disease, known as secondary prophylaxis, also proved beneficial in reducing bleeding frequency and preventing further joint and muscle damage. Importantly, studies have demonstrated that discontinuing prophylaxis in adolescents and young adults to switch to episodic treatment can lead to a decrease in quality of life and joint health due to increased bleeding frequency. This highlights the importance of maintaining prophylactic treatment to prevent complications associated with hemophilia[6].

The effectiveness of replacement therapy can be predicted by examining pharmacokinetics:

Replacement therapy is used to prevent fatal bleeding and the effects of bleeding on the joints and muscles. Some patients can recognize the symptoms that signal an oncoming bleed, known as "aura." However, it is better to prevent bleeding rather than treat it with on-demand replacement therapy, as joint inflammation can lead to further bleeding and result in severe arthropathy. Therefore, the prevention of bleeding, called prophylaxis, is thought to lead to better outcomes than on-demand therapy, especially if a minimum trough level is maintained in the plasma. Keeping the trough level of FVIII or FIX above 3–5 IU/dL is enough to reduce the number of bleeding events to one or two episodes every year[7].

The plasma concentration of standard half-life FVIII or FIX concentrates decreases after intravenous infusion in an exponential decay curve, with a half-life of 12–15 hours or 25–30 hours, respectively. There is a wide variation among patients in the response even to the same factor concentrate, and it is recommended to assess the pharmacokinetic (PK) characteristics of a particular concentrate in each patient to predict the dose and the interval between IV administrations. "One size doesn't fit all," and tailoring prophylaxis is the best way to ensure that each patient has the best and optimal outcome[8].

By conducting a pharmacokinetic study, the maximal concentration (Cmax) of the protein in the plasma just after the infusion and the in vivo recovery (IVR), defined as the ratio between the Cmax and the dose administered in IU/kg, can be determined to guide dosing. Other PK parameters include the area under the curve (AUC), clearance (Cl), half-life (HL), and volume of distribution (Vd). All of these parameters should be considered to determine the optimal dosing regimen and improve the efficacy of the concentrate in preventing can protect the patient during intense activities, including some sports; the HL can provide the patients an idea about how long until the next dose is needed. The most critical PK parameter to define the right dose is the Cl, which represents the amount of plasma freed of the concentrate after the infusion. The following formula explains the procedure for an accurate calculation of the dose for repeated treatments, according to three fundamental parameters[9]:

The interval between administrations is denoted as Tau, and CSS represents the concentration of infused clotting factor at the steady state. Continuous infusion is the most cost-effective method for delivering therapy, particularly during post-surgical treatment.Population pharmacokinetics (PopPK) offers a user-friendly approach to evaluating PK parameters, providing rough estimations based on the drug's performance in a large patient population. It may be useful for customizing treatment regimens but should be validated against standard individual PK. Additionally, genetic polymorphisms have been evaluated to understand their impact on FVIII individual PK parameters in hemophilia A patients.

The Extended Half-Life (EHL) Recombinant FVIII/IX Concentrates (FVIII/FIX EHL)

The prominent feature limiting the benefit of prophylaxis has been the need for frequent, repeated iv infusions every 2–3 days for FVIII and every 5–7 days for FIX concentrates, respectively. The pharmaceutical industry has developed some biochemical methods to increase the HL of concentrates to reduce the treatment burden on patients, especially young patients. The ability to prolong the plasma half-life and extend the interval between injections followed the application of methods to conjugate the factor molecule with the fragment crystallizable (Fc) of IgG1 or albumin or by adding polyethylene glycol (PEG), which has led to a significant extension of the half-life of concentrates, especially for FIX. Modifications were first applied to FIX[10]:

1. pegylation, the linkage of the molecule with PEG (Rebinyn/Refixia®, Novo Nordisk), which had been a successful procedure for many other drugs to extend their HL;

2. conjugation with recombinant albumin (Idelvion®- CSL Behring);
3. conjugation with Fc fragment (Alprolix®SOBI).

Non-Replacement Therapies

Despite the progress made with the availability of clotting factors, there are still unmet needs for the optimal treatment of People with Hemophilia (PWH). Currently available EHL FVIII/FIX products reduce administration frequency, but treatment still needs to be administered by IV infusion, which poses challenges associated with venous access, especially in young children. Immunogenicity of exogenously administered concentrates remains a significant adverse effect of replacement therapy. Alloantibodies against FVIII and FIX occur in about 25-35% and 3-5% of patients with severe Hemophilia A and B, respectively. Patients with inhibitors are not suitable candidates for prophylaxis, which can only be provided by expensive bypassing agents such as activated prothrombin complex concentrates (APCC) and activated recombinant FVII (FVII). Furthermore, inhibitor patients have to infuse bypassing agents frequently, including daily, to prevent bleeding, resulting in a significant treatment burden[12].

The coagulation system is a complex mechanism in which procoagulant and anticoagulant proteins maintain the correct balance of blood clotting. Any changes to this balance can lead to an increased risk of bleeding or blood clot formation. For hemophilia patients, the bleeding tendency is influenced by various factors that affect the natural balance between procoagulant and anticoagulant factors. New treatments have been developed to rebalance the hemostatic system in people with hemophilia. These novel agents work by either enhancing coagulation (such as emicizumab) or inhibiting anticoagulant pathways (for example, fitusiran and concizumab).[13]

Enhancing Coagulation with Hemlibra® (INN Emicizumab, ACE910)

Emicizumab-KYWH is a new humanized antibody that targets both FIXa and FX. It is produced using a complex biomolecular process where specific monoclonal antibodies for FIXa and FX are generated by CHO or human embryonic kidney cells. These cells are then transfected with plasmids carrying the genes for heavy and light chains of IgG. Emicizumab acts as an FVIIIa cofactor mimetic, binding FIXa and FX, and assumes a three-dimensional structure in the tenase complex, ensuring the balance of activation coagulation and FX activation for downstream reactions. The target activity of emicizumab is at sites of bleeding due to its dependence on phospholipids. When administered subcutaneously at a loading dose of 3.0 mg/kg initially and 1.5 mg/kg weekly afterward for 4 weeks, emicizumab completely prevents bleeding. Its pharmacokinetic profile is linear with a half-life of 4–5 weeks. An initial trial was conducted with 64 healthy subjects in escalating doses, showing that plasma emicizumab concentrations increased soon after subcutaneous injection. The half-life ranged from 28.3 to 34.4 days. The first global Phase III open-label clinical trial program called HAVEN was performed to assess different prophylactic regimens. The results showed a significant decrease in bleeding events in patients receiving emicizumab prophylaxis compared to the control group. However, some adverse events were observed, such as thrombotic microangiopathy (TMA) and cavernous sinus thrombosis in a few patients, particularly those with inhibitors to FVIII. The efficacy of emicizumab was demonstrated in hemophilia patients with inhibitors, with 86% of them reporting no bleeding, and in patients without inhibitors, the percentage of those not experiencing any bleeding was approximately 60%.Dampening the Anti-Coagulant Pathway Towards Equilibrium.[13]

Fitusiran

Fitusiran is a treatment that lowers the levels of antithrombin in the body, leading to improved blood clotting. It has been shown to lower antithrombin levels in both animal models and human patients with hemophilia A and B. Trials have demonstrated a decrease in antithrombin levels by 70% to 90% with monthly administration, leading to a significant reduction in bleeding events. However, the trials were temporarily suspended due to a case of sinus vein thrombosis in a patient with hemophilia A. After a thorough evaluation, dosing has now resumed with modified dosing regimens and additional therapies to manage breakthrough bleeding[14].

Concizumab

Concizumab is a humanized IgG4 antibody monoclonal that selectively targets the second Kunitz domain of tissue factor pathway inhibitor (TFPI). TFPI regulates the tissue factor pathway by inhibiting the initiation phase of the coagulation system, specifically by binding factor Xa and TF- FVIIa. Concizumab works by reducing TFPI levels, which in turn reduces overall blood clotting time. In a Phase 1 clinical study, it was found that after a single infusion, concizumab had a bioavailability of 93% and a half-life of 72 hours. In a Phase 1 trial, different doses of concizumab were administered to hemophiliac patients, and it was found to reduce bleeding tendency without any safety issues. Additionally, a Phase 2 study evaluated the efficacy of daily concizumab administration in hemophilia patients, showing that concizumab was still present in blood samples 43 days after administration. Overall, concizumab has shown promising results in reducing bleeding tendency in hemophiliac patients.In the Explorer 3 trial, concizumab was given subcutaneously in a placebo-controlled, multiple-dose, dose-escalation study. The study showed a decrease in free and total TFPI and a procoagulant effect that was dependent on the dosage. The safety and efficacy of concizumab were evaluated in the Explorer trial program, including the Explorer 4 and 5 trials, which focused on assessing concizumab's effectiveness in severe hemophilia A and B patients with and without inhibitors. The trial design involved dose escalation, with initial doses at 0.15 mg/kg (150 mg/kg/day) and a maximum of 0.25 mg/kg (250 mg/kg/day). Patients were monitored for 6 months, followed by an additional 12 months of observation in the extension phase. Explorer 4, which focused on patients with inhibitors, was a multicenter, open-label, randomized controlled trial. Explorer 5, focusing on patients without inhibitors, was a multicenter, single-arm, open-label phase 2 trial. In Explorer 4, concizumab at 0.5 mg/kg was initially administered, with doses then escalated to determine the minimal effective and safe dose for each patient. Patients were randomized to receive prophylaxis with concizumab or on-demand treatment with rFVIIa. The median annualized bleeding rate (ABR) was 4.5 for concizumab and 19.7 for rFVIIa. In the concizumab group, there were no significant differences between spontaneous and traumatic bleeds, with a spontaneous bleeding ABR of 2.3 (95% CI: 1.4–3.6). The trial observed 70 treated bleeding episodes in 23 patients (63.9%), with a median ABR of 4.5. Overall, treatment with concizumab appeared to be safe and well-tolerated, with a few adverse events (AEs) observed but no severe AEs or withdrawals due to AEs. Notably, no thromboembolic events or deaths were observed. On March 16, 2020, Novo Nordisk temporarily suspended the clinical trials of Concizumab due to thrombosis occurring in three patients. After modifying the breakthrough bleeding treatment protocol, the phase 3 trial was resumed on August 13, 2020[14].

The Gene Therapy

Significant advances in therapy for HA and HB over the past four decades have been achieved, as described in the preceding sections. Despite these innovations, gaps still exist in terms of efficacy, safety,

and patient administration ease.100 Breakthrough bleeding continues to occur with EHL products to treat HA and HB patients despite optimized regimens using PK to tailor the treatment regimen and with novel non-factor products, albeit to a lesser degree. The long-term safety of drug conjugates designed to extend the HL of FVIII/ FIX products remains a concern for some, as do the non- factor products, which are sometimes criticized as "not natural FVIII or FIX." All these products require repeated administrations either by the intravenous or subcutaneous route, and adherence to a treatment schedule is necessary. Besides, patients frequently voice their concerns and dissatisfaction with the available treatments for their hemophilia due to the imposed limitations in career and educational choices for the patient and employment choices for patients and caregivers, inability for patients to participate in recreational activities fully, and potential limitations in family life as well as the persistent concern regarding health insurance status. People with hemophilia may experience the inability to live with spontaneity in their life due to their hemophilia.101 Gene therapy offers the possibility to eliminate the need for repeated administration of a medication to prevent bleeding, enhance the quality of life, and provide the spontaneity people with hemophilia lack due to its single administration paradigm. This transformative effect has been realized by people with hemophilia who have undergone a liver transplant for other reasons (eg, HCV-related liver failure)[15].

The ultimate goal for hemophilia treatments should be a "functional cure" and "health equity".101 Gene therapy research has explored various mechanisms for transgene delivery, including non-viral techniques including chemical, electroporation, and polymer-based procedures; however, viral-mediated delivery (eg, lentiviral, adeno-associated, or gene editing) of a transgene encoding the information necessary for the recipient cells to express the FVIII or FIX protein to potentially cure the patients' hemophilia has been most commonly used. Recently, efforts have focused on the r-adeno-associated virus (rAAV) as a preferred delivery method, although three trials currently underway will utilize lentiviral gene delivery combined with autologous stem cell transplantation for patients with HA (NCT04418414, NCT03818763; and NCT03217032) and one trial for HB (NCT3961243). One trial for patients with HB is underway using a gene-editing approach (NCT3961243)

and 5 summarize the prior and current, ongoing trials using an rAAV to deliver the *F8* or *F9* transgene for people with HA or HB, respectively[15].

Any gene therapy's key attributes must focus on safety, effectiveness, predictability, durability, and ultimately, providing a meaningful impact for patients. A core set of outcome measures was described by Iorio et al 102. It included the frequency of bleeding events and the achieved FVIII or FIX level, the duration of transgene expression, alleviation of chronic pain utilization of healthcare resources, and mental health. To date, the data from the completed and ongoing clinical trials of gene therapy in HA and HB have demonstrated benefit in terms of reductions in bleeding frequency and the need for supplemental infusions of FVIII or FIX concentrates to treat breakthrough bleeding events; however, data to support the benefit in the other core outcome parameters are lacking.103 In terms of safety, open questions regarding the cause of and long-term implications of elevations in liver enzymes and the risks of insertional mutagenesis remain for clinicians and patients alike[15].

Furthermore, pre-existing antibodies to AAV represent a significant hurdle to overcome in approximately half of the patients, and dosing in children and the need for repeat dosing are also open questions. Nonetheless, many patients are eager to realize gene therapy's full potential to achieve a "functional cure" and health equity[16].

Conclusions

The development of hemophilia therapy has made significant progress in the first 20 years of the third millennium. This progress began with the fractionation of plasma in 1946. Over the past 75 years, the life expectancy and quality of life of people with hemophilia have improved dramatically. Screening methods for viral pathogens in blood and blood products and techniques to eliminate these pathogens have made concentrates safe. Cloning the F8 and F9 genes paved the way for factor concentrates developed using recombinant DNA technology. Innovative technologies have extended the circulating plasma life of injected FVIII and FIX molecules. The availability of rFIX EHL concentrates has improved adherence to the prophylaxis regimen for people with hemophilia B. However, the benefits of rFVIII EHL concentrates are less evident, despite being well accepted by patients. Tailoring the prophylaxis regimen using individual PK or PopPK due to large inter-patient variability remains an ambition for the community. Bleeding and adherence to the treatment regimen continue to be a problem for many patients. A new era of bleeding prevention began with the introduction of a bi-specific monoclonal antibody that mimics the effects of FVIII in the tenase complex. The excellent efficacy, subcutaneous route of administration, and long duration of effect of this antibody have been well-received by many people with hemophilia. Other non-replacement therapies are also in development, but the thrombotic risk of these novel agents requires careful evaluation. Several gene therapy trials are underway in people with hemophilia, but the long-term safety and efficacy remain to be established. The high cost of hemophilia treatments, including replacement therapy and non-factor therapies, as well as the potential very high costs for gene therapy, limit access to people in countries with high gross national income. Worldwide, about 70% of people with hemophilia do not have access to replacement therapy, which has led to reduced life expectancy and severe reductions in motor skills and the ability to perform daily activities. The World Federation of Hemophilia distributes coagulation factor concentrates and emicizumab in low- and low–middle-income countries through a humanitarian aid program, which has improved the lives of a limited number of people with hemophilia in the developing world.

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