

From a bispecific monoclonal antibody to gene therapy: A new era in the treatment of hemophilia A

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The treatment of hemophilia A has progressed amazingly in recent years. Emicizumab, a bispecific-humanized monoclonal antibody, is able to improve coagulation by bridging activated factor IX and factor X. Emicizumab is administered subcutaneously and much less often compared to factor VIII products. It has low immunogenicity, does not require dose adjustment, and can be administered regardless of the presence of factor VIII inhibitors. Thrombin generation assays but not factor VIII activity are indicated to guide and monitor the treatment. Emicizumab has enabled the conversion of patients with severe forms into patients with milder forms of hemophilia A. It has reduced the number of bleeding episodes compared to both on-demand and prophylactic substitution therapy and has an excellent safety profile. Gene therapy can elevate factor VIII plasma levels for many years after a single treatment course, could offer long-term protection from bleeding episodes, and minimize or eliminate the need for substitutive treatment with factor VIII concentrates. Gene therapy can provoke an immune response, manifested by an increase in common liver enzymes, that require immunotherapy. Long term monitoring is necessary to identify possible adverse effects. Future objectives are: the development of an ideal viral vector, the possibility of its re-administration, the use of gene therapy in hemophilic children, and determining whether it can be successfully used to induce immune tolerance to factor VIII *ceteri paribus*. The future will determine the place of each type of treatment and group of patients for which it is indicated.

Key words: adeno-associated viral vector, emicizumab, hemophilia A, lentiviral vector, valoctocogene roxaparvovec

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INTRODUCTION

Hemophilia A is a rare X-linked hereditary disorder¹. The patients with hemophilia A have a low production of coagulation factor VIII (FVIII) or a dysfunction of it². Hemophilia patients are prone to bleeding (including spontaneously) more frequently in the joints (hemarthrosis) and muscles (muscular hematomas). However, hemorrhages can have other locations too, and cerebral ones (often post-traumatic) are particularly serious. Hemorrhages frequently affect the same joint or the same joints, repeatedly; that leads over time to synovitis and severe arthropathies, with a tendency to ankylosis, often in non-functional positions. Even subclinical forms of hemarthrosis can lead to the appearance and development of hemophilic arthropathy³. Hemophilic arthropathy causes chronic pain, requires joint remodeling and contributes to the decrease in the patients' quality of life. Point-of-care ultrasonography is useful for the study of joint damage, such as acute hemarthrosis and early hemophilic arthropathy³. Arthropathy requires complex therapy, led by a multidisciplinary team⁴. Some patients even require joint replacement³. The substitution treatment is indicated in order to prevent hemorrhagic episodes and their complications. Primary hematological prophylaxis is now considered to be the gold standard for the management of hemophilia patients⁴.

The treatment of patients with hemophilia A has evolved over time, from fresh frozen plasma transfusion, and cryoprecipitate to FVIII concentrates (in the 1970s), purified FVIII (ref.^{5,6}), recombinant FVIII (in the 1990s) and prolonged half-life recombinant FVIII products^{5,7}. Among replacement therapies, those bioengineered using XTEN fusion technology seem to be the most promising, according to the results from phase 1/2 studies⁸ (the polypeptide XTEN can be produced using *E. coli*, is less immunogenic, and is potentially biodegradable) (ref.⁹).

The disadvantages of therapy with FVIII products are intravenous administration (even to those with difficult venous access), frequent injections (especially in the case of standard FVIII products) and the risk of the appearance of anti-FVIII antibodies (inhibitors) (ref.¹⁰) in about 30% of patients, especially at the beginning of substitution treatment with FVIII concentrates¹¹. These limitations contribute to the decrease of patients' compliance and quality of life¹⁰. The presence of anti-FVIII alloantibodies strongly decrease or abolish the effect of human FVIII products¹². In real clinical practice, by-passing agents [activated prothrombin complex concentrates (aPCC) or recombinant activated factor VII (rFVIIa)] are frequently used and for a long time in patients with congenital hemophilia A and inhibitors and in acquired hemophilia, mostly as first choice. Porcine FVIII could be used to obtain hemostasis in patients with hemophilia

A with inhibitors in whom human FVIII products have proven to be ineffective. A recombinant porcine factor VIII (rpFVIII) product can be an option in these patients as on-demand therapy, including in those undergoing prophylaxis with emicizumab¹¹. Patients with inhibitors use by-passing agents (aPCC or rFVIIa) for the treatment of hemorrhagic episodes and immune tolerance induction therapy – the last with the purpose of eradicating the inhibitors, which is very expensive.

Overcoming the inconveniences related to long-term therapy with standard FVIII products can be achieved by using recombinant FVIII products with prolonged half-life, products that mimic FVIII activity (eg emicizumab), obtaining a coagulation rebalance by acting on natural anticoagulants [tissue factor pathway inhibitor (eg concizumab, PF-06741086) or antithrombin (eg fitusiran)] (ref.¹³), or by using gene therapy, which induces the endogenous production of FVIII (ref.¹⁰). Novel non-factor therapies (such as emicizumab – a FVIII mimetic bispecific antibody or Mim8 – a next-generation FVIII mimetic human bispecific antibody) augment or bypass the hemostatic pathway⁵. Emicizumab mimics the function of activated FVIII (ref.⁶), is administered subcutaneously, as prophylactic treatment of patients with severe hemophilia A, and has at least similar efficacy to FVIII replacement therapy⁷. Other investigational pipeline therapies suppress specific natural anticoagulant pathways (ie, anti-TFPI antibodies or siRNA antithrombin) (ref.⁷). Researchers and clinicians wonder today if the new therapeutic agents that target anticoagulant proteins in the coagulation cascade will be able to replace FVIII therapy⁶. Gene therapy is a higher step because it is potentially curative⁵.

EXPLORATION OF COAGULATION IN PATIENTS UNDER PROPHYLAXIS WITH EMICIZUMAB

The chromogenic assay with bovine source reagent is indicated to determine the level of FVIII in plasma of patients with hemophilia A undergoing prophylactic treatment with emicizumab¹⁴. The bleeding episodes of patients with acquired hemophilia A can be more commonly treated with aPCC or rFVIIa, but rpFVIII may also be an option. They also require a chromogenic FVIII assay¹⁵ using non-human reagents¹⁶ to determine the blood level of FVIII if they are under prophylaxis with emicizumab, because chromogenic FVIII assays underestimate the plasma level of rpFVIII (ref.¹⁵). Clinicians who use emicizumab must know that it interferes with haemostasis tests, especially those based on activated partial thromboplastin times (APTT) (ref.¹⁶). APTT screens showed a consistent shortening in persons treated with emicizumab. APTT-based assays are not indicated for the measurement of coagulation factors or inhibitors in subjects treated with emicizumab¹⁷.

But FVIII activity level is less able to predict the potential risk of bleeding episodes, because there are inter-individual differences in pro- and anticoagulant factors, which contribute to hemostatic balance. In addition, FVIII activity assays are not suitable for monitoring the prophylactic

treatment with emicizumab administered to patients with hemophilia A (ref.¹⁸). The French group BIMHO made proposals for biological monitoring of subjects during prophylactic treatment with emicizumab¹⁶. Thrombin generation assays provide more accurate information regarding the hemostatic balance as a whole and are indicated to guide and monitor the treatment of people with hemophilia A (ref.¹⁸). The parameters of thrombin generation improved and correlated with the clinical status of 11 children with severe form of hemophilia A with inhibitors prophylactically treated with emicizumab during a median period of 36 weeks. Thrombin generation may more accurately reflect the hemostasis balance than rotational thromboelastometry in children prophylactically treated with emicizumab¹⁹.

TREATMENT WITH EMICIZUMAB

Pharmacokinetic data have shown that recombinant FVIII products with standard half-life are not able to achieve zero bleeding in each patient with hemophilia A. This is an important argument for switching from standard half-life to extended half-life FVIII products. But not all subjects receiving prophylaxis with extended half-life FVIII products achieve zero bleeding²⁰. One-third of the interindividual variation in FVIII clearance in individuals with severe forms of hemophilia A depends on the subject's age, blood levels of von Willebrand factor and blood group. The other two thirds of inter-individual variation in FVIII clearance could be explained by powered studies on omics and phenotypic heterogeneity. The clinical response is variable from one subject to another, a fact observed in patients when plasma FVIII activity is mimicked by fixed subcutaneous doses of emicizumab²⁰.

A solution to replace the prophylactic substitution of FVIII is emicizumab (ACE910) – a bispecific-humanized monoclonal antibody able to improve coagulation by bridging activated factor IX and factor X (ref.²¹). It activates factor X and allows the coagulation cascade to continue²². Thus, it functionally substitutes the absent activated FVIII (ref.²¹).

The mean maximum blood concentration after a single dose of 1 mg/kg emicizumab was $7.11 \pm 1.77 \mu\text{g/mL}$ and it had a mean terminal half-life of 26.7 (± 4.3) days, in a phase I clinical trial that included 16 healthy Chinese males. It was found that there is no difference regarding emicizumab pharmacokinetic profiles between Chinese, Caucasian, and Japanese individuals²³.

The dose of emicizumab is determined according to body weight, without the necessity for coagulation monitoring. This assumes a dose-concentration-response relationship, which includes an acceptable variability due to factors other than body weight. A systematic review analyzed 15 studies that included 140 volunteers and 467 subjects with hemophilia A with and without inhibitors, children and adults, to whom emicizumab was administered. It was found that it has a dose-linear pharmacokinetics. The variability between subjects of trough concentrations was 32% and was similar across various

subgroups, such as the presence or absence of FVIII inhibitor, age group and the interval between doses²⁴. Monitoring of blood emicizumab concentrations is a solution for a more cost-effective dosing²⁴. Phase III HAVEN 1 study analyzed blood samples from 112 subjects with hemophilia A with inhibitors treated prophylactically with 1.5 mg/kg once-weekly subcutaneous emicizumab. The blood concentration of emicizumab was maintained at values ≥ 50 $\mu\text{g/mL}$ during the study. In addition, FVIII-like activity was over 20U/dL, and the peak height of thrombin generation was over 100 nM; both parameters correlated with emicizumab blood concentrations. Thus, people with severe clinical forms were converted into subjects with average clinical forms. The plasma level of APTT value was normalized²⁵.

Among the advantages of emicizumab are the easier route of administration – subcutaneously (abdomen, upper arm, and thigh), its low immunogenicity²⁶, and the fact that doses do not need to be adjusted¹⁶. Emicizumab must not be activated by thrombin and is not inactivated by activated protein C, but a small amount of activated factor IX is necessary to initiate emicizumab-mediated hemostasis. Fibrin formation and fibrinolysis appear to be similar in the case of emicizumab prophylaxis or activated FVIII-mediated hemostasis²⁷.

Emicizumab managed to reduce the number of hemorrhagic events compared to both on-demand and prophylactic substitution therapy. It has an excellent safety profile; only rare cases of thrombotic microangiopathy and thrombotic event are reported²¹. It is subcutaneously delivered and much less often compared to factor VIII products.

There is little information on the effectiveness of emicizumab administered to children, but factors that can contribute to the decrease in the efficacy of the treatment are known: immunologic naivety, lower production of vitamin K dependent proteins, and higher clearance of emicizumab²⁸. But emicizumab administered prophylactically for 18 months protected a child with severe hemophilia A and high titer inhibitors from bleeding, after treating him with recombinant-activated factor VII for a spontaneously occurring spinal epidural hematoma²⁹.

Until recently there were no standardized guidelines regarding the concomitant administration of haemostatic agents (dose and laboratory monitoring) during surgical interventions in patients with hemophilia A with inhibitors who have received prophylactic treatment with emicizumab³⁰. In patients under non-factor prophylaxis treatment, some minor surgical procedures do not require additional haemostatic treatment, others can be performed with few low-dose administrations of FVIII concentrates or bypassing agents. Major surgery requires additional FVIII or bypassing agents in association with emicizumab³¹. Patients with hemophilia A with inhibitors who present with bleedings or require surgery, including orthopedic interventions, have an indication for adding bypassing agents to emicizumab^{4,13}. The second therapeutic option of choice for them is recombinant activated factor VII (ref.¹³). There are reports according

to which surgery can be successfully performed in these patients³⁰. Other researchers also claim that thrombotic microangiopathy and thrombosis events have occurred in the case of simultaneous administration of emicizumab and high, repeated doses of activated prothrombin complex concentrate (ie > 100 U/kg/d for ≥ 24 h). This is why a professional organization in the United Kingdom developed the first guideline for the therapy of hemorrhagic episodes in hemophiliacs treated with emicizumab. German professionals added recommendations regarding the optimization of the management of patients with hemophilia A treated prophylactically with emicizumab³². The practical German guidance recommends adequate laboratory tests to monitor blood emicizumab levels, FVIII replacement and inhibitors; doses higher than 100 U/kg for > 24 h of activated prothrombin complex concentrate should not be used to avoid the high risk of thrombotic events and/or thrombotic microangiopathy³³. Experts believe that by increasing thrombin generation, prothrombotic mutations co-segregating with FVIII gene mutation may trigger thrombotic events in hemophilia A patients who have acquired thrombogenic factors (e.g. venous catheters) (ref.³⁴).

To date long-term data on the effect of emicizumab on joint health, FVIII inhibitor development and thrombotic events risk are not available³⁵. Experts believe that until the long-term effects of emicizumab become known, patients with hemophilia A with inhibitors should continue to receive treatment for the induction of immune tolerance³⁶. The Atlanta protocol for immune tolerance induction, applicable to patients with hemophilia A with inhibitors, provides doses of 50–100 IU/kg of FVIII three times a week in combination with emicizumab³⁷.

Emicizumab has significantly changed the quality of life of hemophilia patients, including those with inhibitors, the direct consequence of the important decrease of the annual bleeding rate and, in most of them, the absence of bleeding. The association of FVIII products is necessary, however, during acute bleeding or surgery¹¹. The quality of life of 241 adults with hemophilia A after 24 weeks of prophylactic treatment with emicizumab was assessed using the Haem-A-QoL questionnaire. The results showed greater improvements than the previously reported 10-point reduction in physical health and 7-point reduction in total score³⁸.

However, emicizumab is not a cost-effective therapeutic option at a willingness-to-pay threshold of \$50,000 per quality-adjusted life-year in hemophilia A patients with severe clinical forms without inhibitors³⁹.

Patients treated with emicizumab, as well as those receiving replacement and other non-replacement medicines, must be monitored in the long term to detect possible adverse effects, whether they are predicted or unexpected and rare, as an expression of transparent pharmacovigilance⁴⁰.

CLINICAL TRIALS WITH EMICIZUMAB

Emicizumab, was approved by the FDA in November 2017 to be used as a prophylactic treatment of hemophilia A patients with anti-FVIII inhibitors⁴¹, in those with congenital hemophilia A without inhibitors – in October 2018 (ref.⁴²) and for all age groups since 2019, but it should not be used in newborns with severe forms of hemophilia A and acute bleeding⁴³. It is able to reduce the annualized bleeding rate to almost zero, as was found in different clinical trials, independent of the presence or absence of inhibitors²².

Based on a multicriteria decision analysis, emicizumab had greater efficacy compared to short half-life FVIII and extended half-life FVIII products and contributed to increasing the quality of life of patients with moderate to severe forms of hemophilia A compared with replacement therapy in Greece². Eighteen patients with or without factor VIII inhibitors were treated prophylactically with emicizumab for up to 5.8 years. Annualized bleeding rates for hemorrhagic episodes treated with coagulation factors were lower than pre-emicizumab rates or remained zero in all patients. Bleeding episodes were less severe and the time until they stopped was shorter⁴⁴.

A number of 401 pediatric and adult persons with hemophilia A with/without FVIII inhibitors were included in the phase 3 of HAVEN 1–4 studies and treated prophylactically with emicizumab. Over a median follow-up period of 120.4 weeks, the model-based treated annualized bleed rate was 1.4, in subjects of all ages and independent of the presence or absence of inhibitors. The product was well tolerated. Adverse effects: three thrombotic microangiopathies and 2 thromboembolic events, all occurred in the case of using the combination of emicizumab with activated prothrombin complex concentrate; in addition, a myocardial infarction and a venous device occlusion⁴⁵. Only 3 out of a total of 400 patients developed neutralizing antidrug antibodies⁴⁶. A review found that one subject developed antidrug antibody to emicizumab, from over 600 treated patients⁴⁷. An important message from the authors of the HAVEN 3 trial is that the total treated bleed rate was lower in patients with hemophilia A without inhibitors during emicizumab prophylaxis compared with FVIII prophylaxis⁴⁸. In the phase 3 HAVEN trial, 4 patients aged over 12 years with severe congenital hemophilia A or hemophilia A with FVIII inhibitors were prophylactically treated with emicizumab s.c. 6 mg/kg every 4 weeks for 24 weeks or more; patients from the expansion cohort previously received four weekly loading doses of 3 mg/kg. Emicizumab showed clinically significant bleed control and was well tolerated⁴⁹. Emicizumab proved to be highly effective in prophylactic administration in the 88 children with hemophilia A and inhibitors from the phase 3 trial HAVEN 2. Nasopharyngitis and injection-site reactions were the most frequent secondary events; the children did not present thrombotic events. Antidrug antibodies with neutralizing potential were detected in 2 patients who also had decreased emicizumab plasma concentrations⁵⁰.

A review on off-label use of emicizumab in patients with acquired hemophilia A included 33 subjects. All of them had a clinical response to emicizumab. A stroke occurred in a patient who received emicizumab concurrently with activated recombinant factor VII for a surgical intervention⁴².

The removal of central venous access devices was performed in ten male pediatric patients with severe form of hemophilia A who were on prophylactic treatment with emicizumab without administration of factor VIII concentrate or bypassing agent. There were no significant hemorrhages and no patient returned to the hospital in the week following the intervention⁵¹.

AKATSUKI is an ongoing multicenter trial, with patients with hereditary hemophilia A with inhibitors, immediately after immune tolerance induction (ITI) therapy, and who are prophylactically treated with emicizumab. The aim is to analyze the adverse effects (especially thromboembolic events), the number of episodes of bleeds requiring substitutive treatment, the number of patients achieving a partial response to ITI treatment, the blood FVIII level immediately after ITI, to analyze the quality of life and measure the time to achieve the disappearance of factor VIII inhibitors⁵².

In the UNEBI Study, the researchers will enroll 60 patients with hemophilia A with inhibitors under prophylactic treatment with emicizumab and bypassing agents, who will be monitored for 3 years regarding global coagulation function. The degree of improvement in the maximum coagulation rate will be analyzed using clot waveform analysis, before and after administration of fixed-dose of bypassing agents⁵³.

GENE THERAPY

The third generation gene-editing technology CRISPR/Cas9 is extremely useful for gene insertion and gene therapy, including in hemophilia A (ref.⁵⁴). Hemophilia A can become a phenotypically curable disease if liver-directed gene therapy is used⁵⁵. But gene therapy may not offer a permanent cure for hemophilia A (ref.⁵⁶). This treatment can elevate FVIII plasma levels for many years and minimize or eliminate the need for substitutive treatment with FVIII concentrates⁵⁷. Gene therapy could offer long-term protection from bleeding episodes after a single treatment course²⁶.

Gene therapy uses a functional gene copy that encodes FVIII (ref.⁵⁸). It is packaged inside a recombinant adeno-associated vector or lentiviral vector^{57,58}. Currently, the most used viral vector is the adenovirus because the gene delivered by it does not integrate into the subject's genome and has a low immunogenicity⁵⁹. But this type of vector also has disadvantages: it has a predominantly episomal nature in the nucleus of hepatocytes and there is often a pre-existing immunity against it⁶⁰. The genome of adeno-associated virus does not replicate during cell division⁶¹, so it is predictable that the plasma level of FVIII encoded by it will decrease over time, especially in

children, in which the hepatocytic division is higher. The realization of an adeno-associated viral vector containing a codon-optimized F8 cDNA allowed obtaining a persistent transgenic expression for three years, accompanied by plasma levels of FVIII activity of 52.3% (ref.⁶²). HIV-derived lentiviral vectors integrate into the hepatocyte chromatin and persist even after the duplication of the hepatocyte genome, a fact that contributes to the long-term expression of FVIII, especially in children, where hepatocytes replicate more frequently. Lentiviral vectors protected against phagocytosis have recently been created. They selectively target the liver and spleen, increase the gene transfer in hepatocytes and contribute to increasing the plasma activity of transgenic FVIII (ref.⁶⁰).

After a single dose, these particles reach liver cells, where the viral vector uncoats and delivers the DNA fragment to the nucleus of the hepatocyte. Genetic elements that are associated with the genes are involved in the appropriate expression and secretion of FVIII protein into the plasma⁵⁸. An ideal gene therapy should be based on minimizing the amount of vector administered and decreasing the number of adverse events without reducing the efficacy of the protein expressed⁶³. It is considered that even an increase in plasma FVIII levels of $\geq 5\%$ of normal value leads to a significant improvement of the bleeding phenotype in patients with severe clinical forms of hemophilia⁶⁴.

Ideally, the method of inserting the gene encoding FVIII into hepatocyte using the viral vector must be safe, predictable, effective, and provide a durable FVIII expression. The long-term monitoring of the subjects remains to establish the long-term safety and effectiveness of the method⁶⁵.

The results of preclinical studies suggest that gene therapy can even contribute to the eradication of pre-existing anti-FVIII antibodies, induce immune tolerance, and is able to provide long-term therapeutic FVIII expression with the aim of preventing bleeding episodes⁶⁶.

Immune responses can appear against the viral capsid, the vector's nucleic acid, other elements that contaminate the vector, excipients, or the transgene product encoded by the vector⁶⁷. An immune response to the vector capsid can be manifested by an increase in common liver enzymes⁵⁸. The presence of toxicity correlates with the T-cell response to the viral capsid, which justifies the need for immunosuppression⁶⁸. Therefore, patients must be monitored for a long period of time and if they show signs of toxicity, immunosuppressive treatment is indicated⁵⁸. The physiopathological explanation of the increase in transaminases after the administration of the viral vector is not fully known. Indeed, they return to normal values using corticosteroid therapy, in many patients⁶². Patients rarely require immunosuppression, and if they do, it is usually short-lived. Before applying gene therapy, liver function must be evaluated and the liver must be kept healthy. Immunomodulatory strategies, if necessary, must be available and adequate, to contribute to the sustainable preservation of gene expression⁵⁸.

For now, there are limits in the application of gene therapy. For example, the eligibility criteria for these trials

require the exclusion of key patient groups, such as children, adolescents, those with liver or kidney dysfunction and those who have had inhibitors against FVIII in their history or pre-existing neutralizing adeno-associated viral antibodies⁶⁹. Furthermore, the number of CD8⁺ T cells, an indicator of cellular immunity, must be maintained over a long period of time, and must be checked periodically⁶¹.

But this therapy has not yet been widely applied for several reasons: long-acting FVIII products and therapies that mimic the action of FVIII are available; the risks are unknown and long-term monitoring of patients is necessary; the therapeutic effect decreases over time (after the use of adeno-associated viral vector) and one of the challenges is the possibility of reintroducing the vector; lack of experience regarding the discovery and management of adverse effects; there is no expertise regarding the analyzes necessary to establish the cost-effectiveness ratio⁴⁹.

CLINICAL TRIALS USING GENE THERAPY

A multicenter, phase 3 study, used valoctocogene roxaparvovec (AAV5-hFVIII-SQ) – an adeno-associated virus 5 (AAV5) containing a FVIII complementary DNA driven by a liver-selective promoter. A number of 134 subjects over 18 years of age, without preexisting anti-AAV5 antibodies or inhibitors in their history, and who had been prophylactically treated with factor VIII concentrates, were included. They received a single dose of 6×10^{13} vector genomes of AAV5-hFVIII-SQ per kilogram of body weight. The mean FVIII activity plasma level at weeks 49 through 52 had increased by 41.9 IU/dL. After four weeks from the infusion, the mean annualized rates of FVIII concentrate used and treated bleeding events had reduced by 98.6% and 83.8%, respectively. Serious adverse events were presented by 16.4% of the participants. Alanine aminotransferase levels increased in 85.8% of patients and were treated using immunosuppressive therapy. Other frequent adverse effects were headache, nausea, and increases in aspartate aminotransferase levels⁷⁰.

In a phase 1–2 trial, an investigational adeno-associated viral vector (SPK-8011) was used to introduce the gene encoding FVIII into hematocytes in 18 men with hemophilia A. Eight subjects had a total of 33 adverse events during a median monitoring period of 36.6 months, of which 17 events were vector-related; two patients lost their factor VIII expression due to an anti-AAV capsid cellular immune response that did not respond to immunosuppressive treatment; the other 16 kept their FVIII expression; twelve patients had an average level of FVIII activity of $12.9 \pm 6.9\%$ of the normal value at 26 to 52 weeks when they were not receiving glucocorticoids. The annualized bleeding rate decreased by 91.5%, so that in the 16 subjects who had a sustained expression of FVIII, the prophylactic substitution therapy could be abandoned⁷¹. Indeed, the studies that were done with different gene constructs inserted into hepatocytes using a viral vector resulted in a plasma level of FVIII sufficient to significantly reduce the number of bleeding episodes and it was possible to

abandon prophylactic substitution in most hemophiliacs with severe disease⁷².

A number of 15 adults with severe hemophilia A who received a single infusion of AAV5-hFVIII-SQ at various dose levels were monitored for several years. After 3 years, seven of them had a median plasma level of FVIII of 20 IU/dL; they had no hemorrhagic episodes and did not use exogenous FVIII; two participants had plasma FVIII level below 1 IU/dL. Two years after infusion, six subjects had a plasma FVIII level of 13/dL; the median annualized rate of bleeding episodes was 0, and the median use of FVIII was significantly reduced (up to 0.5 infusions per year). The participants who received 4×10^{13} or 6×10^{13} vector genomes/kg of the gene therapy had an important, significant reduction in annualized rates of bleeding events and gave up prophylactic substitution with exogenous FVIII, which was no longer necessary. In addition, no cases of the occurrence of inhibitors, thrombotic events, deaths or long-term changes of functional liver tests were reported⁷³.

CONCLUSIONS AND FUTURE DEVELOPMENTS

Several years of real-world experience with FVIII products with extended duration of action, emicizumab, and other agents in development will be necessary to establish with certainty their place, based on efficacy, safety and cost, in the prevention of bleeding and its complications⁵⁶.

Future clinical trials are necessary to define the optimal dosing and scheduling of bypassing agents (or FVIII products) to be used in combination with emicizumab in case of hemorrhagic events or surgery, and the establishment of a standardized assay of thrombin generation to monitor the coagulation status during emicizumab prophylaxis³⁴.

A multidisciplinary team is essential to manage major surgery in patients on prophylaxis with emicizumab³¹.

Studies with gene therapy using an adeno-associated viral vector have proven a significant increase in plasma levels of endogenous FVIII during sustained periods, important reduction of bleeding episodes, decrease in the need for exogenous FVIII substitute and so far a positive safety profile⁶⁹.

It is necessary to apply practical strategies for the use of gene therapy in hemophiliac children (74), remove pre-existing neutralizing antibodies against adeno-associated viral vectors, develop practical ways to re-administer the viral vector, and analyze the possibility that gene therapy can be used effectively to induce immune tolerance to FVIII and the disappearance of inhibitors⁷⁵.

Search strategy and selection criteria

Strategy research aimed at analyzing the efficacy and safety of emicizumab and gene therapy in patients with hemophilia A. Articles published in PubMed and Web of Science databases between January 1, 2000 and June 30, 2022 were searched, using the terms “hemophilia A”, “emicizumab” and “gene therapy”.

ABBREVIATIONS

AAV5, adeno-associated virus 5; AAV5-hFVIII-SQ, valoctocogene roxaparvovec; aPCC, activated prothrombin complex concentrates; APTT, activated partial thromboplastin time; CRISPR, clustered regularly interspaced short palindromic repeats; Cas9, CRISPR-associated protein 9; FVIII, coagulation factor VIII; cDNA, complementary deoxyribonucleic acid; FDA, Food and Drug Administration; Haem-A-QoL, Haemophilia Quality of Life Questionnaire for Adults; ITI, immune tolerance induction; rFVIIa, recombinant activated factor VII; rp-FVIII, recombinant porcine coagulation factor VIII; TFPI, tissue factor pathway inhibitor.

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