Thrombosis Research 231 (2023) 99–111

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Review Article

Treatment of congenital coagulopathies, from biologic to biotechnological drugs: The relevance of gene editing (CRISPR/*Cas*)

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ARTICLE INFO

Keywords:
Hemostasis
Inherited coagulopathies
Hemophilia A
Hemophilia B
Factor V deficiency
Plasma-derived
Recombinant factors
Advanced therapies
CRISPR/*Cas*

ABSTRACT

Congenital coagulopathies have, throughout the history of medicine, been a focus of scientific study and of great interest as they constitute an alteration of one of the most important and conserved pathways of evolution. The first therapeutic strategies developed to address them were aimed at restoring the blood components lost during hemorrhage by administering whole blood or plasma. Later on, the use of cryoprecipitates was a significant breakthrough as it made it possible to decrease the volumes of blood infused. In the 1970′ and 80′, clotting factor concentrates became the treatment and, from the 1990's to the present day, recombinant factors –with increasingly longer half-lives– have taken over as the treatment of choice for certain coagulopathies in a seamless yet momentous transition from biological to biotechnological drugs. The beginning of this century, however, saw the emergence of new advanced (gene and cell) treatments, which are currently transforming the therapeutic landscape. The possibility to use cells and viruses as well as specific or bispecific antibodies as medicines is likely to spark a revolution in the world of pharmacology where therapies will be individualized and have long-term effects. Specifically, attention is nowadays focused on the development of gene editing strategies, chiefly those based on CRISPR/*Cas* technology. Rare coagulopathies such as hemophilia A and B, or even ultra-rare ones such as factor V deficiency, could be among those deriving the greatest benefit from these new developments.

Hemostasis comprises a series of well-conserved mechanisms capable of preventing blood loss following a vascular lesion through clot formation [1]. The hemostatic balance achieved during the healing phase is also able to restore blood fluidity by means of fibrinolysis [2,3].

A disruption of these mechanisms usually results in a congenital coagulopathy, a hereditary condition characterized by an alteration in

Abbreviations: AAV, adeno-associated virus; AdV, adenoviral vector; Alb, albumin; APC, activated protein C; aPCCs, activated prothrombin complex concentrates; aPTT, activated thromboplastin time; AT, antithrombin; BDD-FVIII, B-domain deleted FVIII; *Cas*, CRISPR-associated protein; CRISPR, clustered regularly interspaced short palindromic repeats; EPCR, endothelial protein C receptor; FFP, fresh frozen plasma; FIX, factor IX; FIXa, activated factor IX; FV, factor V; FVa, activated factor V; FVII, factor VII; FVIIa, activated factor VII; FVIII, factor VIII; FVIIIa, activated factor VIII; FX, factor X; FXa, activated FX; FXI, factor XI; FXII, factor XII; FXIIa, activated factor XIIa; FXIIIa, activated factor XIII; GGCX, gamma glutamyl carboxylase; HDR, homology-directed repair; HEK293, human embryonic kidney cells; HIV, human immunodeficiency virus; iPSCs, induced pluripotent stem cells; ITI, immune tolerance induction; iRNA, interference RNA; KO, knockout; LNP, lipid nanoparticles; LVs, Lentivirus; LVV, lentiviral vector; mESC, mouse embryonic stem cell; MSC-EVs, extracellular vesicles derived from mesenchymal stem cells; NHEJ, non-homologous end joining; NGG, PAM sequence where "N" is any nucleotide followed by two guanin nucleotides (G proteins); NOD/SCID, non-obese diabetic mice or in mice with severe combined immunodeficiency; PAI-1, plasminogen activator inhibitor-1; PAM, protospacer adjacent motif; PBMNC, peripheral blood mononuclear cells; PC, protein C; PLCs, placenta-derived MSCs; Pre-K, prekallikrein; PT, prothrombin time; PWCs, patients with coagulopathies; rtPCR, real time polymerase chain reaction; SCNT, somatic cell nuclear transfer; sgRNA, single-guide RNA; SpCas9, Streptococcus pyrogenes Cas9; ssODN, single-stranded oligodesoxynucleotides; TALEN, transcriptor activator-like effector nucleases; t-PA, tissue plasminogen activator; TF, tisular factor; TFPI, tissue factor pathway inhibitor; u-PA, urokinase plasminogen activator; vWF, von Willebrand factor; WT, wild type; ZFN, zinc-finger nucleases; ZFP, zinc-finger protein; ZPI2A, inactivating mutant ZPI.

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https://doi.org/10.1016/j.thromres.2023.10.001
Received 17 July 2023; Received in revised form 9 September 2023; Accepted 2 October 2023
Available online 6 October 2023

blood clotting. Such an alteration is often due to a deficiency in some component of the coagulation process and may result in a state of hypocoagulability, typically leading to hemorrhages, or hypercoagulability, caused either by an alteration in the systems governing the restoration of homeostasis or by mutations of some clotting factor such as factor V (FV) Leiden [4]. Coagulopathies include rare diseases such as hemophilia A and B and ultra-rare ones such as FV deficiency.

The treatment of the various congenital coagulopathies (Fig. 1) has in the recent past been based on the administration, either prophylactically or on demand, of biological drugs such as plasma-derived factors or biotechnological medicines such as recombinant factors. For coagulopathies where no specific plasma-derived or recombinant product is available, the treatment of choice is based on fresh frozen plasma (FFP) or a combination of several clotting factors [5]. At the same time, various non-factor replacement therapies have either been developed or are under development to overcome the current limitations of factor replacement treatment.

Moreover, in an era marked by a rising interest in the different kinds of advanced therapies, *i.e.*, gene therapy, cell therapy and immunotherapy, and personalized and individualized pharmacology, significant efforts are being directed to the development of advanced therapies for congenital coagulopathies. Specifically, gene therapy, with its increasing efficacy levels [6], could bring about a paradigm shift in the treatment of coagulopathies. Gene editing, based mainly on CRISPR/*Cas* strategies [7,8], is gaining increasing attention as a tool to address both rare and ultra-rare coagulopathies.

This study provides an in-depth analysis of the potential implementation of these new therapies set to herald a new era marked by individualized pharmacology. In it, treatments will be based on the restoration of the genetic defect present in the patient, with medium- or long-term effects, and on regulating procoagulant and anticoagulant hemostatic processes to restore the homeostasis of the coagulation cascade. Hemophilia A and B and factor V deficiency will be at the center of the analysis, with the main focus being placed on the CRISPR/*Cas* gene editing system.

1. Hemostasis, a well-conserved and tightly regulated mechanism

Hemostasis is a clear example of a multiregulated physiological pathway comprising both procoagulant and anticoagulant processes, which come together to maintain homeostasis in the coagulation system. The success of this process depends on the interaction between the vascular endothelium, the platelet system, the coagulation cascade and the fibrinolytic system [2].

Hemostasis may be broken down into primary and secondary hemostasis [9,10]. Primary hemostasis occurs within seconds of a blood vessel injury and its aim is to form a white or platelet thrombus. This requires the coordinated action of platelets, the endothelium and adhesion proteins, with platelets playing an essential role in the formation and maintenance of the blood clot [11,12]. Secondary hemostasis (also known as the coagulation cascade) is a more complex process directed at transforming soluble fibrinogen into fibrin, which is mediated by a series of proteins known as clotting factors. Lastly, fibrin is responsible for forming a stable red thrombus made up of fibrin fibers and blood cells (white blood cells, red blood cells and platelets) [11,12]. The clotting cascade comprises two interconnected pathways: the extrinsic pathway (tissue factor pathway) and the intrinsic pathway (contact activation pathway) [12].

The extrinsic pathway of coagulation is activated following a vascular injury where the blood comes into contact with the cells of the endothelium, which express tissue factor (TF), the major initiator of the coagulation cascade. TF binds to inactivated factor VII (FVII), which becomes activated factor VII (FVIIa). Both factors form the TF-FVIIa initiation complex which, in turn, activates factor IX (FIX) when TF levels are low, and factor X (FX) when TF levels are high. Activated FX (FXa) interacts with activated FV (FVa) in the phospholipids of the cell membrane to unleash the subsequent stages of the process, which are common to both pathways [10,13]. On the other hand, the intrinsic pathway is activated when the plasma comes into contact with a surface other than the endothelial basement membrane or the collagen fibers of the connective tissue located in the vicinity of the vascular lesion. Collagen fibers are the basis for the formation of the initiation complex, together with other components and factor XII (FXII). FXII transforms

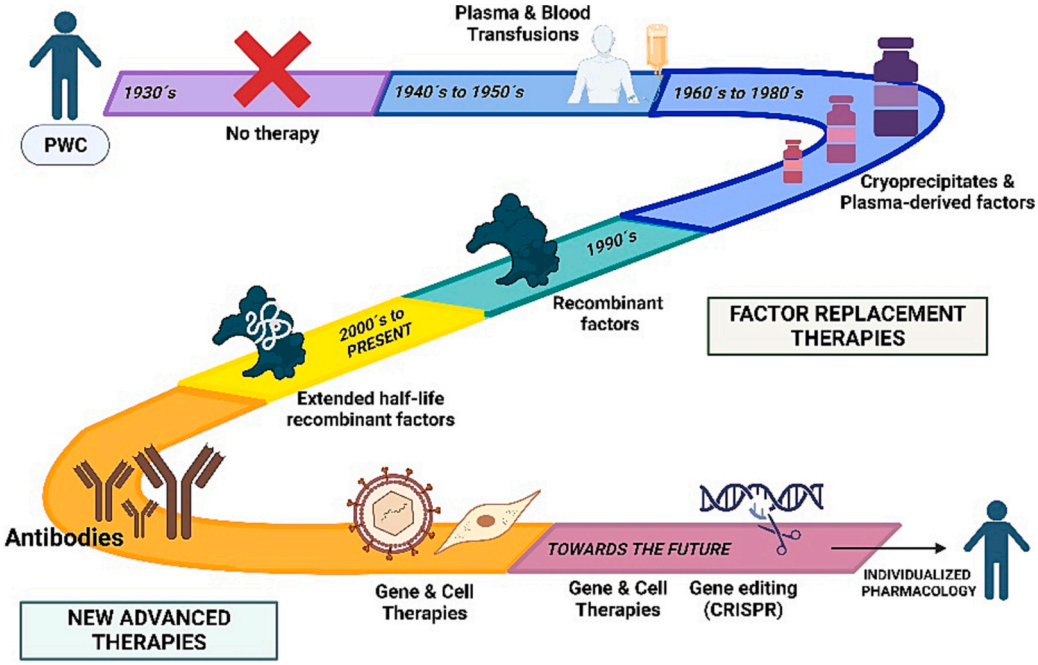

Fig. 1. Evolution of therapeutic strategies for the treatment of patients with coagulopathies (PWCs).
CRISPR: Clustered regularly interspaced short palindromic repeats.

prekallikrein (Pre-K) into kallikrein, which is responsible for activating FXII itself. In the presence of Ca^{2+}, activated FXII (FXIIa) activates factor XI (FXI), which activates FIX which, in turn, binds to activated factor VIII (FVIIIa). In the presence of Ca^{2+} and phospholipids, activated FIX (FIXa) and FVIIIa form the tenase complex (first amplification complex), whose main function is to activate FX. Subsequently, FXa binds to FVa to form the prothrombinase complex, which catalyzes the conversion of prothrombin into thrombin in the presence of phospholipids and Ca^{2+} [13]. In addition, there are certain coagulation cascade inhibitory proteins such as activated protein C (APC), antithrombin (AT) and the tissue factor pathway inhibitor (TFPI), which are also responsible for homeostasis of the coagulation cascade [9]. Lastly, thrombin activates fibrinogen promoting its polymerization and leading to the production of fibrin polymers, stabilizing by activated factor XIII (FXIIIa), which interact with the platelet clot that was formed during primary hemostasis, increasing its stability and plugging the vascular lesion [13]. Moreover, apart from its role in the activation of some coagulation factors (FXI, FXIII, FVIII and FV), thrombin is also a crucial component of hemostasis as a result of its interaction with numerous factors and components of blood plasma. Once the hemorrhage has ceased and the endothelium has recovered, fibrinolysis is essential to complete the hemostasis process as dissolution of the clot is a prerequisite for successful healing [10]. The process is activated by thrombin, which promotes the synthesis and release of the tissue plasminogen activator (t-PA) in endothelial cells; and the urokinase plasminogen activator (u-PA), responsible for mediating the transformation of plasminogen into plasmin. Inhibitors such as the plasminogen activator inhibitor-1 (PAI-1) or α2-antiplasmin, α2-antimacroglobulin and the C1 esterase inhibitor also play an important role in the regulation of fibrinolysis [14].

2. From normal function to congenital coagulopathies

Although disruptions of the coagulation process are typically attributable to a genetic cause, they may also be due to an acquired condition [15,16]. Any one of the factors participating in the hemostatic process may experience a genetic alteration due to a change in DNA composition caused by different kinds of mutations. Congenital coagulopathies, with the exception of von Willebrand disease, may be rare diseases such as hemophilia A and B or ultra-rare diseases such as FV deficiency.

2.1. Hemophilia A

Hemophilia A is a congenital coagulopathy with an incidence of 1 per 6,000 live births associated with a deficiency of FVIII [17]. This coagulation factor, which comprises six protein domains, participates in the intrinsic coagulation pathway and is synthesized in hepatic sinusoidal endothelial cells and at the level of the vascular endothelium. It is stabilized by von Willebrand factor (vWF) and is activated by binding to thrombin through its arginine residues, which results in the cleavage of its B domain. Moreover, together with FIXa, FVIIIa forms the tenase complex along the intrinsic pathway that activates FX. Its inactivation by removal of its A2 domain is mediated by protein C (PC) [18–20]. FVIII is encoded by the FVIII gene (*F8*) located in the X sex chromosome. Hemophilia A is therefore a monogenic X-linked disease. Multiple mutations of *F8* have been described, nearly half of the most severe ones associated with either an inversion of intron 22 or an inversion of intron 1 [21]. These kinds of mutations decrease or altogether inhibit the production of FVIII. Missense *F8* mutations have also been reported, which affect FVIII's activity and biosynthesis [22].

The severity of hemophilia A is determined by the patient's FVIII plasma concentrations, with a clear correlation having been identified between FVIII levels and the patients' symptoms. Thus, mild hemophilia A corresponds to the presence of 5–40 % of FVIII in plasma, moderate hemophilia A to 1 and < 5 %, and severe hemophilia to <1 % [18,23]. Clinical signs are related with spontaneous or trauma-related bleeds. The disease typically involves recurrent bleeds (hemarthrosis) into the joints, which, in the long term, result in chondral degeneration because of the degradation of the synovial capsule caused by the presence of blood [24,25].

The diagnosis of the disease is based on the measurement of prothrombin time (PT) and activated partial thromboplastin time (aPTT), which are prolonged in patients with hemophilia A. At the same time, a specific analysis of FVIII levels and functionality must be carried out, as well as a quantification of FVIII antigen concentrations. Moreover, molecular tests should also be performed to detect the potential mutation causing the defect in *F8* [26–29].

2.2. Hemophilia B

Hemophilia B is also a monogenic X-linked hereditary hemorrhagic disorder. However, unlike hemophilia A, it is caused by mutations in the FIX gene (*F9*), which produce alterations in FIX. The incidence of hemophilia B is 1 per 30,000 live births [30]. FIX, which comprises four domains and is produced in hepatocytes, can be defined as a vitamin K-dependent coagulation factor. FIX is activated to FIXa in the presence of Ca^{2+} and catalyzed both by FXIa and by the TF-FVIIa complex. Together with FVIIIa it forms the tenase complex, which activates FX [31]. The gene coding for FIX is formed by eight exons and is 34 kb long. Most mutations occurring in *F9* are missense mutations, which could lead to the hypothesis that the condition's severity phenotype is lower than that of hemophilia A. In hemophilia B, large deletions and insertions represent only 7 % of all cases [32,33].

As in the case of hemophilia A, there is a strong correlation between the levels of functional FIX present and the patient's clinical symptoms. The disease phenotype is also classified as mild when plasma FIX levels are between 5 and 40 %, moderate when they are between 1 and < 5 % and severe when they are <1 % [23,34]. Clinical signs are similar to those observed in hemophilia A although some authors consider this condition to be less serious on account of its milder symptoms, the lower requirements of exogenous factor and the lower incidence of joint involvement [34,35].

The diagnosis of the disease is based on the measurement of PT and aPTT, on assays aimed at measuring the functionality of FIX, and on the quantification of the FIX antigen levels of present; in addition to molecular diagnostics [29,36,37].

2.3. Factor V deficiency

Factor V deficiency, or Owren's disease [38], is an autosomal recessive condition associated to mutations of the FV gene (*F5*) [39]. Its very low incidence (1 to 9 per million live births) [40] makes it an ultra-rare disease [41].

In humans, *F5* is located at region q24.2 of the long arm of chromosome 1. It is a very large gene, of about 80 kb in size, which contains 25 exons and 24 introns, exon 13 being the largest, accounting for 42 % of the size of the total exon count, and the worst preserved as compared with other species. Its mature messenger RNA, which encodes a protein made up of around 2224 amino acids, is approximately 6.8 kb in size. In its inactive form, approximately 330 kDa in weight, FV comprises six domains (A1-A2-B-A3-C1-C2). The A1 and A2 domains constitute the heavy chain and the B domain, entirely codified by exon 13, corresponds to the posttranslational region. The A3, C1 and C2 domains form the light chain of the molecule [38,42–44]. FV is mainly synthesized in the liver, 80 % by hepatocytes, and 20 % by megakaryocytes and platelets. With a half-life ranging between 12 and 36 h [45], the protein is secreted to the bloodstream, where most of it circulates in free form, while a small part is stored in the platelets' alpha granules [46]. FV actively participates in the common coagulation pathway and collaborates with FXa to form the prothrombinase complex. It also performs an anticoagulant role, taking part in the inactivation of the tenase complex and the TF pathway [38,47].

FV deficiency is characterized by an alteration in the coagulation cascade, associated with low or undetectable levels of FV [38], whose bifunctional activity plays a very important role in the clotting process [41,48]. Over 250 mutations have been described in *F5*, most of them affecting exon 13, which codes for FV's B domain [49]. Clinical manifestations of bleeding are highly heterogeneous, episodes ranging from very mild to very severe [38]. Such events, which usually start at an early age, are at times limited to mucosal bleeding but can also involve life-threatening hemorrhages [50]. Unlike what has been observed in hemophilia A and B, no correlation has been found between circulating FV levels and the patients' symptoms [29]. The disease is mild when circulating FV levels are higher than 10 %, moderate when they are below 10 % and severe when they are below 1 % [38].

The diagnosis of the disease is based on a study of patient's family history and the measurement of PT and aPTT, and of functional FV levels. Molecular studies are also typically performed to identify the type of mutation giving rise to the condition and to confirm or rule out the existence of consanguinity, which is very common in patients with this deficiency [29,40].

3. From classical pharmacology to the new therapies

The first curative approaches (Fig. 1) to coagulation alterations date back to the 1930's and were basically empirical. Later on, strategies were developed to restore the blood components lost following hemorrhage through administration of whole blood or plasma. Subsequently, the advent of plasma-derived cryoprecipitates constituted an important step forward as it reduced the volumes to be infused: In the 1970s and 80s, intermediate- and high-purity coagulation factor concentrates gained great popularity [51]. Ever since the 1990s, recombinant factors, with increasingly longer half-lives, have been the treatment of choice for some coagulopathies. The development of recombinant products marked a turning point in the transition from biological to biotechnological drugs.

The emergence of new advanced (gene and cell) therapies at the beginning of this century, however, has revolutionized the therapeutic landscape of congenital coagulopathies [6,26,52].

3.1. Plasma-derived and recombinant factors

Plasma-derived and recombinant factors spurred a paradigm shift in the treatment of congenital coagulopathies. The first plasma-derived products were manufactured based on plasma fractionation, purification and viral inactivation. Administered either prophylactically or on demand, they for a long time remained the prototypical biological drugs in clinical practice, specifically in patients with hemophilia A and B. However, results were far from promising since the non-performance of viral inactivation of the products resulted in a high percentage of hemophilic patients, particularly those with hemophilia A, becoming infected with the human immunodeficiency virus (HIV), which led to a considerable death rate among this patient population [53,54].

The new recombinant DNA techniques pioneered at the end of the 1980's led to the development of recombinant coagulation factors, which were the forerunners of the new biotechnological drugs. Their highly specific activity, their efficacy and, particularly, their safe pharmacological profile, have made these factors the current treatment of choice for some congenital coagulopathies, mainly hemophilia A, and B [55–57]. In the course of time, new extended half-life FVIII and FIX were been developed, which afford patients a better quality of life thanks to longer dosing intervals, all the while promoting therapeutic adherence [57–59].

3.2. Fresh frozen plasma and factor concentrates

A certain group of congenital coagulopathies, for which no specific treatment with a purified exogenous factor is available, are currently being treated with FFP obtained from whole blood donations that are frozen to −30 °C. FFP has been shown to correct factor deficiencies, reverse de effect of warfarin and to be effective in correcting ATIII deficiency and treating immunodeficiencies and even thrombotic thrombocytopenic purpura [60–62]. As no plasma-derived or recombinant factor is currently available for the treatment of FV deficiency, treatment of these patients is typically based on infusion of FFP [29,63]. However, continued administration of FFP could be associated with side effects such as acute pulmonary injury, the development of neutralizing antibodies (inhibitors) or, in some cases, mild or even severe anaphylactic reactions [38].

In addition to the above-mentioned products, other clotting factor concentrates are available, such as Octaplas® [40,64], an intravenously administered preparation with a quantitatively and qualitatively balanced composition of several coagulation factors. Octaplas® has been subject to enveloped virus inactivation, which boosts its safety profile. Being made up of controlled levels of various coagulation and fibrinogen factors, the product provides for an optimal dosing protocol, typically more effective than that associated with FFP [41].

Hemophilia A and B are currently treated with plasma-derived and recombinant FVIII and FIX factors, respectively [65]. For FV deficiency, a new recombinant FV concentrate is under development [66,67].

3.3. New advanced therapies

The new advanced therapies comprise gene therapy, cell therapy and regenerative medicine. More recently, immunological strategies based on the use of monoclonal antibodies against cancer and other diseases, among them congenital coagulopathies, have also come to be considered advanced therapies. These advanced therapies have prompted a major transformation in pharmacology. They have opened up new therapeutic possibilities involving new, personalized treatments offering the promise to cure or at least delay the progression of disease.

Gene therapy involves the modification of the genetic material of a cell to correct a mutation causing a disease and produce the protein whose deficiency causes the pathological condition. This strategy can be implemented *ex vivo* (by using *ex vivo* genetically modified cells that are subsequently administered to the patient) or *in vivo* (by administering the patient a gene-delivery vector) [68]. These vectors may be viral or non-viral [69].

Adeno-associated virus (AAV) vectors are considered the most effective viral vectors and, as such, are the ones in widest use. However, they are the ones resulting in a higher number of adverse events [70]. Lentivirus (LVs) [71] and oncolytic adenoviruses [72] are also in common use, albeit to a lesser extent. The choice between different vectors depends on how safe they are for a particular patient and on more technical criteria such as their genome packaging capacity, cellular tropism and potential for genomic integration [73].

Although AAV vectors are the ones achieving the best results in patients with hemophilia A and B, such results have at times been overshadowed by, at times severe, hepatotoxicity-related side-effects [74]. AAVs, which belong to the Parvoviridiae family, may infect any kind of cells and can preserve the stability of the transgene in tissues of low replication potential such as the liver. Although AAV vectors are easy-to-manage, highly effective non-integrating vectors, they present with low packaging efficiency and high levels of capsid immunogenicity [75]. Use of LVs in congenital coagulopathies has been gaining great acceptance mainly because of their integrative potential, which is associated with a limited mutagenic risk, and a low immunogenic effect [76].

Cell therapy uses either living cells or extracellular vesicles derived mainly from mesenchymal stem cells (MSC-EVs), which are one of the central mediators of the therapeutic functions of MSCs [77]. The cells may be obtained from the patients themselves (autologous cell therapy) or from a human donor (allogeneic cell therapy) [78]. The greatest advances of cell therapies in the context of congenital coagulopathies have been related to the use of induced pluripotent stem cells (iPSCs)

[79–81].

Immunotherapy is a highly innovative therapy that has been applied to a large number of conditions, including infections, cardiovascular and oncologic conditions as well as congenital coagulopathies [82,83]. In the case of cancer, the idea is to get the patient's immune system to target primary and metastatic cancer cells [84,85]. In the case of hemophilia, the aim is to neutralize the immune system so as to decrease the production of inhibitors against an administered exogenous factor [86,87].

Around 30 % of patients with severe hemophilia develop inhibitors (neutralizing antibodies), which render exogenous factor-based treatment ineffective. In these cases, immune tolerance induction (ITI) protocols have been used to decrease the inhibitor titer by administering high doses of the factor that has triggered the immune response [88,89]. Patients with irreversible inhibitors have been administered FVII and activated prothrombin complex concentrates (aPCCs) [90]. More recently, the first bispecific antibody (emicizumab) which interacts with FIXa and FX was approved. Emicizumab mimics the function of FVIIIa and allows progression of the coagulation cascade [83]. Currently, other specific antibodies such as befovacimab, marstacimab and concizumab are being developed, which are targeted against TFPI [91–93]. Another antibody under development is Mim8, whose function is similar to that of emicizumab [94].

Other interesting non-factor replacement alternatives are currently being developed, such as interference RNA (iRNA)-based drugs like fitusiran, which limits the synthesis of AT in hepatocytes, rebalancing hemostasis and driving an increase in thrombin production. The drug is now undergoing clinical trials to determine its safety and efficacy in patients with hemophilia A and B with or without inhibitors [95,96]. On the other hand, clinical trials are underway on the so-called serpins (serine protease inhibitors). Serpins include serpinPC, a drug targeted at PC [97]; the protein *Z*-dependent proteinase inhibitor (ZPI), which interacts with FXa and FXIa inactivating mutant ZPI (ZPI2A) [98]; and protease nexin-1, which is one of the most effective inhibitors of thrombin [99].

4. Gene editing (CRISPR/*Cas*), the key to future treatments

Gene editing makes it possible to alter the sequence of the genome to repair or to produce a mutation. In addition to CRISPR (clustered regularly interspaced short palindromic repeats), other gene editing strategies have been developed [100]. One such strategy is represented by the transcriptor activator-like effector nucleases (TALEN). These are type III effector proteins secreted by pathogenic bacteria, which bind to the *Fok*I domain (restriction endonuclease) as a dimer, generating a DNA double strand break [101]. Another gene editing strategy is that of zinc-finger nucleases (ZFN), formed by the DNA binding domain of the zinc-finger protein (ZFP), which must also bind to the *Fok*I domain to cleave the DNA [102]. The latter strategy is being tested as a potential treatment for congenital coagulopathies such as hemophilia [103].

The CRISPR/*Cas* strategy (Fig. 2) is, however, the one with the greatest therapeutic potential, as it is a simple yet highly precise and effective gene editing tool whose cost is lower than that of other systems [104]. It has been described as an adaptive defense mechanism used by certain bacteria and archaea against viral infections [105]. The system comprises genes coding for nucleases as *Cas* (CRISPR-associated protein), which differ according to the bacterial or archaeal species involved; and CRISPR sequences formed by small DNA-integrated viral sequences originating from the viruses that previously infected those cell lines. Such viral sequences are separated by a series of regularly interspaced short palindromic repeats, which give this strategy its name. Infection by the virus is followed by activation of the defense mechanism, which involves the transcription and translation of the corresponding *Cas* nuclease gene and the transcription of the various viral sequences integrated in the DNA, giving rise to the so-called crRNA. If the bacteria or archaea have been previously infected by the virus, one

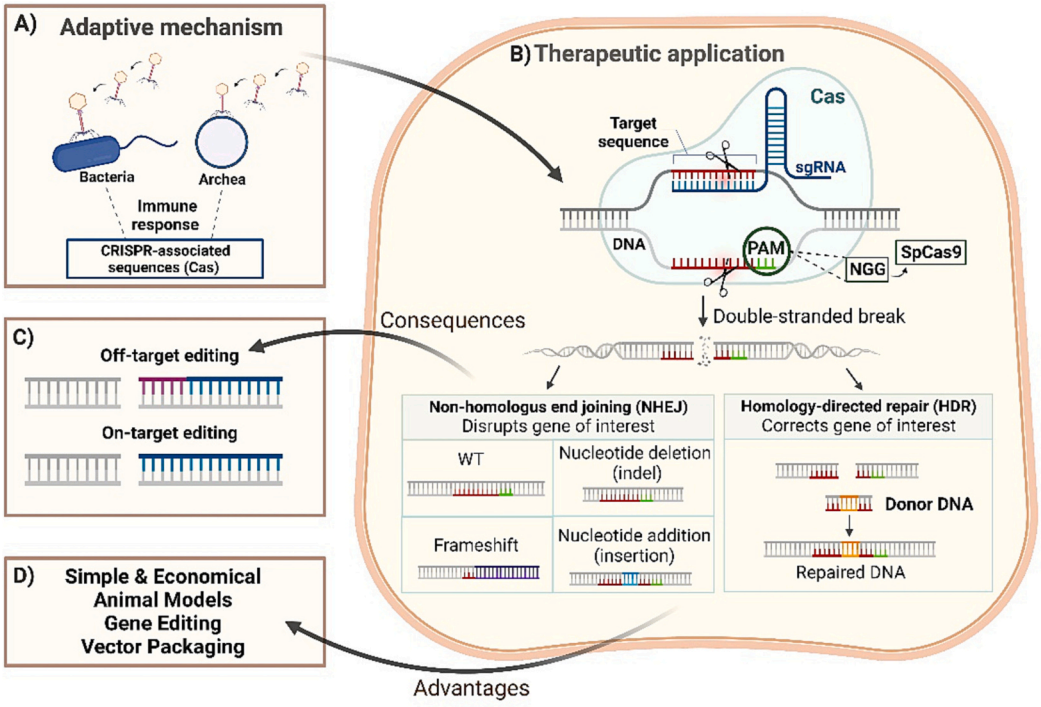

Fig. 2. CRISPR gene editing strategy.
(A) Primitive bacterial adaptive mechanism against viral infection. (B) Therapeutic application of the CRISPR/*Cas* strategy: double DNA strand breaks to generate NHEJ or HDR. (C) Off- and on-target effects of gene editing. (D) Advantages of CRISPR/*Cas*-based gene editing. *Cas*: CRISPR-associated protein. CRISPR: Clustered regularly interspaced short palindromic repeats. sgRNA: single-guide RNA. PAM: Protospacer adjacent motif. NGG: PAM sequence where "N" is any nucleotide followed by two guanin nucleotides (G proteins). SpCas9: *Streptococcus pyogenes Cas9*. WT: wild type.

of the crRNAs will be complementary to the genome of the new infective virus, which means that the crRNA will bind by complementarity to the viral genome and form a complex with the *Cas* nuclease. This complex is stabilized by a different RNA molecule (tracrRNA), which is produced by the bacteria or archaea themselves. Once the crRNA/tracrRNA/*Cas*/ viral genome has been formed, the *Cas* nuclease makes a double strand break in the viral genome, thus interfering with the virus' biological cycle. The protospacer adjacent motif (PAM) domains located in the various crRNA molecules (and in the viral genome) are very short sequences that are specific to each type of *Cas* nuclease and whose presence is indispensable for the formation of the complex and for mediating the double strand break and eliminating the virus.

This biological strategy was proposed as a potential laboratory technique that could eventually be applied in clinical practice. In essence, the strategy is based on the use of a guide RNA that binds to the target DNA of the cell through base complementarity and collaborates with an endonuclease capable of cleaving the double strand [106]. The CRISPR/Cas strategy offers a simple and economical alternative for the development of treatments; for the generation of animal disease models or for editing of the genetic material of cells that will later be transplanted into the patient [107–110]. Efforts are also underway to test the potential *in vivo* use of CRISPR/Cas [7].

5. New developments on the application of CRISPR/*Cas* to the treatment of congenital coagulopathies

5.1. CRISPR/Cas and hemophilia A

The majority of *in vitro* studies carried out using the CRISPR/*Cas* strategy in the context of hemophilia A have been performed with patient-derived iPSCs. Their aim was to find a way to correct the mutations causing the disease (Table 1).

The first of these studies, performed by Park et al. [111], used the CRISPR/*Cas* strategy to repair disease-causing chromosomal *F8* intron 1 and 22 inversions in the iPSCs of patients with severe hemophilia A. A year later, in 2016, the same authors used the CRISPR/*Cas* technique to analyze and correct structural chromosomal variations (inversions and translocations), restoring the normal DNA sequence in these iPSCs [112,113]. The idea was to apply the procedure to diseases such as hemophilia A, fragile X syndrome, Hunter syndrome or Friedreich's ataxia.

Other *F8* corrective strategies have been proposed [114], including a genomic correction strategy to restore the expression of FVIII in iPSCs from a patient with severe hemophilia A and a large deletion in the B domain (from exon 8 through exon 22). These authors used CRISPR/*Cas*-mediated homologous directed repair (HDR) to restore the B domain, with the assistance of the human elongation factor 1 alpha promoter (EF1α). A very high correction frequency (81.8 %) was achieved [97].

Additionally, some authors proposed a universal CRISPR/*Cas*-based approach to modify the multiple mutations observed in the iPSCs of patients with hemophilia A by means of a targeted insertion of *F8* in the human H11 locus. Correction frequency stood above 60 %, with no off-target effects and secretion of adequate levels of functional FVIII [115]. At the same time, a CRISPR/*Cas*-based strategy was also used in the iPSCs of patients with hemophilia A to insert the cDNA of FVIII into exon 1 of the mutated locus of *F8* [109]. In the same vein, Hu et al. [116] used iPSCs from a patient with severe hemophilia A who presented with *F8* intron 1 inversion. A plasmid was used to deliver the CRISPR/*Cas9* system together with *F8* exon 1 and the alpha-1 anti-trypsin promoter, and NHEJ-mediated gene editing was performed. Following the correction, iPSCs were differentiated to hepatocytes that expressed functional FVIII.

Other studies have proposed the use of placenta-derived mesenchymal stem cells (PLCs), known to produce endogenous FVIII. Using LV vectors and the CRISPR/*Cas* strategy, Ramamurthy et al. [117] demonstrated the feasibility of a specific insertion of a transgene (FVIII),

Table 1
Results of applying CRISPR/*Cas*-based gene editing to the treatment of congenital coagulopathies.

Disease	Type of study	Methodology	Study findings	Reference number
Hemophilia A	*In vitro*	iPSCs	NHEJ/cDNA mutation correction	[109] [111] [112] [113] [114] [118]
			HDR mutation collection	[115]
			Multiple mutation correction	[116]
		PLCs	LVV *vs* CRISPR/*Cas 9*	[117]
	In vivo	Mouse	HDR iPSC infusion	[118]
			CRISPR-bearing AAV vectors	[119] [120] [121]
			LNP-mediated AT editing	[7]
			Animal model	[122]
		Rat	Animal model	[123]
Hemophilia B	*In vitro*	iPSCs	HDR mutation correction	[80] [124] [125] [127] [128]
		mESCs	Cell model + HDR correction	[126]
			AAVs *vs* AdVs	[110]
	In vivo	Mouse	iPSCs (mutation correction, Padua)	[131] [140]
			CRISPR-bearing AAV vectors (Padua, HDR, NHEJ, Alb, cDNA)	[130] [132] [134] [135] [136]
			CRISPR-bearing AdV vector	[137]
			iPSCs + AAVs	[133]
			HDR mutation correction	[138] [139]
			Animal model	[141]
		Rat	Padua correction	[129]
		Porcine	Animal model + HDR mutation correction	[142]
Alterations in the vitamin-K metabolism	*In vitro*	KO cells	KO cell model γ-glutamyl carboxylase	[144] [145] [146]
			KO cell model *VKORC1* and *VKORC1L1*	[147]
Platelet disorders	*In vitro*	iPSCs	Enhancement of thrombopoiesis by inhibiting *NOTCH4*	[148]
	In vivo	Mouse	KI *STXBP5 cell* model	[149]
Xenotransplants	*In vivo*	Porcine	Bioartificial organ generation	[150] [151]
Tissue factor	*In vitro*	KO cells	Cell model (cancer, haploinsufficiency)	[152] [153]
	In vivo	iPSCs Zebra fish	Animal model (TFPI)	[154]
Factor V	*In vitro*	IPSCs	Point mutation correction	[158]
		KO cells	KO cell model + NHEJ mutation correction	[8]
	In vivo	Zebra fish	Animal model	[160]
Related factors and proteins	*In vitro*	HEK293 cells	FII, VII, IX & X overexpression	[108]
		KO cells	ADAM17/ADAM19-deficient cell model	[155]

(*continued on next page*)

Table 1 (*continued*)

Disease	Type of study	Methodology	Study findings	Reference number
			Thrombomodulin-deficient cell model	[156]
	In vivo	Medaka	FXIII-deficient cell model	[157]

iPSCs = induced pluripotent stem cells; NHEJ = non-homologous end joining; HDR = homology-directed repair; PLCs = placenta-derived mesenchymal stem cells; LVV = lentiviral vector; AAV = adeno-associated virus; LNP = lipid nanoparticles; AT = antithrombin; mESC = mouse embryonic stem cell; AdV = adenoviral vector; Alb = albumin; KO = knockout; TFPI = tissue factor pathway inhibitor; HEK293 = human embryonic kidney cells; F = factor.

which minimizes the potential risks associated with the semi-random genomic integration inherent in LV vectors. The results obtained with these vectors were even more favorable with respect to the gene expression obtained, which was more robust and longer-lasting.

As regards *in vivo* studies, the majority of these have been performed in mouse models. These studies typically use iPSCs from patients with severe hemophilia A presenting with a frameshift mutation in the B domain. A corrective gene editing strategy using single-stranded oligodesoxynucleotides (ssODN) with CRISPR/*Cas9* provided a very effective *in situ* correction. Differentiation of the corrected iPSCs to endothelial cell progenitors and infusion of the latter into mice with hemophilia A resulted in a high correction frequency, the rescue of the disease phenotype, and a higher survival rate [118].

Another strategy consisted in the use of AAV vectors as vehicles for inclusion of the various components of the gene editing system. The results obtained were very promising as the disease phenotype was eliminated in a hemophilia A mouse model from which the promoter region and *F8* exon 1 were absent. Expression of functional FVIII was reestablished and aPTT was normalized [119].

Chen et al., [120] described an *in vivo* CRISPR/*Cas*-based gene editing strategy combined with non-homologous end joining (NHEJ). This methodology achieved permanent chromosomal integration of a B-domain deleted FVIII (BDD-FVIII) at the albumin locus in liver cells. C57BL/6 mice were intravenously injected with two vectors: AAV8-Sa*Cas9*-gRNA, targeted at albumin's intron 13, and AAV8-BDD-F8, responsible for insertion of the region of the *F8* gene that codes for BDD-FVIII into the albumin locus, which led to the expression of functional FVIII in the mice's liver. This dose-dependent FVIII expression remained stable for at least seven months, with no significant hepatic toxicity.

Zhang et al. [121] reported similar results using a double-cut donor, which achieved a 10 to 20-fold increase in the efficacy of hepatic gene editing, with full restoration of plasma FVIII activity in a hemophilia A mouse model. Thus, hydrodynamic administration of *Cas9*-sgAlb plasmids and a BDD-FVIII double-cut donor have laid the foundations for curing hemophilia by means of a NHEJ-mediated knock-in of BDD-FVIII in albumin introns following AAV-mediated administration of CRISPR/*Cas* components.

A more recent and groundbreaking strategy consisted in the use of lipid nanoparticles (LNP) carrying *Cas9* mRNA together with a single guide RNA targeted at AT in the mice's liver. Administration of LNP-mediated CRISPR/*Cas* inhibited the action of AT and produced an increase in thrombin levels, which resulted in the restoration of a normal phenotype in mice with hemophilia A and B, with no immune response against *Cas9* or hepatotoxicity reactions being observed [7].

CRISPR/*Cas*-based gene editing has also been used to obtain pathologic animal models. Han et al. [122], obtained a severe hemophilia A mouse model (F8I22I) by using two sgRNAs and the *Cas9* protein in mouse embryos to bring about the inversion of *F8* intron 22 in a 319 kb fragment of the gene. Shi et al. [123] for their part, developed a hemophilia A rat model using RNA guides that brought about the inversion of *F8* by generating a stop codon after the signal sequence.

5.2. CRISPR/Cas and hemophilia B

Although *in vitro* studies in the field of hemophilia B have been fewer in number than those in hemophilia A, the strategies used are likewise addressed to the use of iPSCs (Table 1). Morishige et al. [80] used gene editing to correct, in patient-derived iPSCs, a deletion in *F9* exon 2. The corrected cells were differentiated *in vitro* to hepatocytes, which were capable of expressing FIX. Similarly, He et al. [124] subjected iPSCs to homologous recombination which, following hepatic differentiation, resulted in the expression of functional FIX.

Recently, Tang et al. [125] were the first to use the CRISPR/*Cas9* strategy in conjunction with ssODNs to generate a human iPSC line, which was transduced with the *F9-Padua* mutation. The cells were subsequently differentiated to hepatocytes, expressing very high levels of FIX (364 %). In the same study, the authors used the CRISPR/*Cas9* strategy to insert a plasmid vector into the iPSCs of a patient with hemophilia B where *F9* cDNA contained the *F9-Padua* mutation. Following differentiation to hepatocytes, the cells also showed a four-fold increase in the procoagulant activity of FIX as compared with unedited cells.

Other types of cells, such as mouse embryonic stem cells, were also subjected to analysis [126] employed CRISPR/*Cas* to insert, into a previously-designed cell model, a nonsense mutation in a patient with hemophilia B. The authors demonstrated the higher efficacy of *nCas9*, a nickase variant of *Cas9,* delivered through HDR and a non-viral vector (pMrnF9). Recombination efficacy was higher and fewer off-target effects were observed, which makes this kind of strategy a potentially appropriate clinical tool to address hemophilia B [127].

Bergmann et al. [128] used an *in vitro* hemophilia B canine model (HEK293 cell line) with a point mutation in *F9*'s catalytic domain. A series of double-strand breaks were inserted into the mutation locus with a CRISPR/*Cas* strategy and the defective gene was corrected with HDR. A year later, Gao et al. [110], generated different hepatocyte cell lines with point mutations in *F9*'s catalytic domain and achieved effective multiviral transfection with an adenoviral vector (AdV) that carried the CRISPR/*Cas* system, an AAV that carried the modified donor, and another AdV that carried the whole assembly. The correction rates obtained were very high.

In vivo gene editing strategies for hemophilia B are typically based on mouse models, which have been used either to generate the hemophilia B model itself, inducing mutations by means of gene editing and AAV vectors, or to be applied in cell therapy protocols.

In this regard, FIX Padua has been used as a transgene and inserted with high specificity into the albumin locus of hemophilia B rat models to obtain therapeutic FIX concentrations at four weeks from initiation of treatment [129]. Wang et al. [130] used the CRISPR/*Cas* strategy to insert *F9* Padua into an AAV and injected the assembly into neonatal and adult FIX-deficient KO mice. This resulted in the expression of functional FIX expression at eight months post-injection.

A more recent study [131] used gene editing to transfect iPSCs from a patient with severe hemophilia B and insert the Padua transgene. Subsequently, the cells were differentiated to hepatocytes and intrahepatically infused into an FIX-deficient mouse model to restore the animals' normal bleeding phenotype.

The synergistic use of the CRISPR/*Cas* strategy together with AAV vectors currently represents the most promising therapeutic strategy for many conditions and, particularly, for hemophilia. Thus, Ohmori et al. [132] succeeded in reversing the hemophilic phenotype in a cohort of mice with hemophilia B by packaging the CRISPR/*Cas* system into an AAV8 both through HDR and NHEJ, and both in adult and younger mice. A subsequent study [133] using iPSCs obtained from peripheral blood mononuclear cells (PBMNC) and AAVs-*Cas9*-sgRNA through electroporation showed that hepatocytes differentiated from these iPSCs were able to secrete functional FIX. These hepatocytes were transplanted into non-obese diabetic mice or in mice with severe combined

immunodeficiency (NOD/SCID) resulting in much higher FIX antigen levels than observed in the control group.

In the same vein, Wang et al. [134] found that a two-AAV-based gene editing strategy using a lower dose of each vector directed at the liver to edit the genome resulted in higher efficiency levels. The strategy allowed a specific CRISPR/*Cas*-mediated integration of the therapeutic transgene within the albumin gene, a protocol that could be considered universal and applicable to other kinds of hereditary diseases. This strategy made it possible to rescue the clotting function in neonatal and adult mice with hemophilia B by a single injection of dual AAV vectors.

Various approaches have been tested with a view to mitigating the immunogenicity and hepatotoxicity resulting from the use of AAV vectors [135,136], such as the aforementioned dual viral strategy, which involves the use of different types of vectors, and the isolated use of non-AAVs such as the adenovirus [137].

Some authors also used HDR-based gene editing in the context of hemophilia B to reverse and correct the defective gene in mice affected with the disease [138,139].

It is a known fact that one of the greatest drawbacks of the CRISPR/*Cas* strategy is that every *Cas* recognizes its specific PAM, which may result in unspecific cuts in the DNA. In an effort to minimize this problem, Hiramoto et al. [140] employed a *SpCas9*-NG to reverse a point mutation in iPSCs obtained from a patient with severe hemophilia B (c.947 T > C; I316T) and achieved expression of functional FIX. These authors also generated the same point mutation in HEK293 cells and in knock-in mice, which brought about the production of functional FIX.

Lastly, some studies in the literature have published results that could open the door to new biotechnological strategies. By means of microinjection of the CRISPR/*Cas* system into C57BL/6 mouse zygotes, Wang et al. [141] succeeded in generating KO individuals as FIX-deficient models with an 85 % mutation efficiency rate. Attempts have also been made to generate FIX-deficient models in other species. Through a combination of CRISPR/*Cas* and somatic cell nuclear transfer (SCNT), Chen et al. [142] produced a FIX-deficient porcine model to test a potential therapy for hemophilia B whereby the mutation in the generated knockout individuals was corrected by a knock-in at the FIX locus. Correction of the mutation resulted in a partial improvement of the bleeding phenotype.

5.3. CRISPR/Cas in other coagulopathies

Given that many coagulation factors are vitamin K-dependent [143] any alteration in vitamin-K turnover may negatively impact the functionality of such factors. Thus, CRISPR/*Cas* gene editing has been used to obtain animal models with a deficiency of gamma glutamyl carboxylase (GGCX), a protease involved in vitamin-K metabolism [144]. Gene editing was also used to develop an animal model for fibrinogen deficiency, which is vitamin-K dependent [145].

Mention should also be made of GGCX-deficient cellular models, where exon 2 of the gene that codes for GGCX and disrupts the synthesis of vitamin K was removed [146]. Likewise, other models such as those deficient in the VKORC1 and VKORC1L1 proteins, which participate in the reduction of vitamin K, have been proposed [147].

As far as platelet function is concerned, familial platelet disorder is associated with the presence of the *RUNX1* gene, which results in a lower production of platelet precursor cells (megakaryocytes). The *NOTCH4* gene, which negatively regulates thrombopoiesis, has been identified as a target gene of *RUNX1*. A study applied CRISPR/*Cas*-based gene editing to iPSCs from a patient carrying an altered *RUNX1* gene in order to inactivate the *NOTCH4* gene. This resulted in an enhanced stimulus for the production of megakaryocytes. These findings could make a decisive contribution to the development of new pharmacological therapies for that kind of platelet disorders [148].

With respect to the thrombotic phenotype, Zhu et al. [149] developed a mouse model using CRISPR/*Cas*-based gene editing by mimicking a mutation in the *STXBP5* gene, which regulates platelet secretion. These authors observed a reduction in vWF levels and a decreased production of platelets, with an ensuing reduction in the severity of the thrombotic phenotype.

Application of gene editing to a porcine model resulted in promising results for the generation of bioartificial organs. The CRISPR/*Cas* strategy was used to obtain a knock-in by inserting a fusion of the gene encoding human FVII and human albumin. Nineteen pigs expressed both genes in the liver [150]. Another study, aimed at conducting a porcine xenotransplant in a human being, introduced modifications in several genes, among them the one coding for vWF, which is related to rejection in the context of lung xenotransplants. The modifications were performed by applying CRISPR/*Cas*-based gene editing to primary kidney cells, which bought about a disruption in the genes responsible for thwarting xenotransplantation [151].

Although TF is a molecule that participates in the coagulation cascade, it has also been associated with the aggressivity and progression of cancer. TF is secreted in the form of microvesicles produced by tumor cells. A KO human breast cancer cell model with TF- silencing demonstrated a decrease in the number of microvesicles secreted. This study revealed that TF could play an important role in the development of cancer treatments [152].

Another study analyzed the impact of TF in spontaneous bleeds using a CRISPR/*Cas* strategy to create a heterozygous mutation that caused a stop codon in a cell model. Pluripotent stem cells were generated that carried the mutated variant. The cells were differentiated to endothelial and vascular smooth muscle cells, the immunological phenotype of these cells being validated using indirect immunofluorescence for vWF. Evaluation of the iPSCs and other complementary tests demonstrated that TF haploinsufficiency contributes to disrupting the activation of the coagulation cascade [153].

As regards TFPI, a study was carried out on zebra fish, given its similarities with humans with respect to its hemostatic system. First of all, a rtPCR analysis demonstrated the presence of TFPI in the zebra fish. Subsequently, the CRISPR/*Cas9*-strategy was used to introduce a deletion in the gene that codes for TFPI, which brought about the inactivation of the protein. As a result, homozygous individuals experienced increased clotting activity (thrombotic phenotype) [154].

The CRISPR/*Cas* gene editing strategy has also been used to obtain the expression of clotting factors. For example, Feser et al. [108] achieved a CRISPR/*Cas*-based targeted overexpression of factors II, VII, IX, X and fibrinogen in embryonic kidney cells (HEK293).

Using the CRISPR/*Cas* tool, Lécuyer et al. [155] generated ADAM17 and ADAM10-deficient cell lines. Following infection of these cell lines with a meningococcal strain, an analysis was performed of the expression of the endothelial protein C receptor (EPCR), which is known to shed from endothelial cells during interaction with the pathogen. The authors were able to show that the shedding of the receptor was mediated by ADAM10, as no shedding of the receptor was observed in the EPCR-deficient cells generated. Protein C deficiency being associated with purpura fulminans, this finding is relevant as it suggests that EPCRs could harbor a defect caused by the presence of meningococcal bacteria.

In another study, Giri et al. [156] used a CRISPR/*Cas* strategy to delete thrombomodulin, a receptor of thrombin, in a population of endothelial cells. The result was a disruption of procoagulant (vWF), proinflammatory (NF-κB) and angiogenic (angiopoyetina-2) molecules. As regards other coagulation factors such as FXIII, Horimizu et al. [157], obtained a FXIII-deficient medaka model by applying CRISPR/*Cas*-based gene editing. The authors identified the orthologous human *F13* in that fish species and, although FXIII deficiency was seen to result in miscarriage, viable individuals were obtained with evidence of mendelian inheritance in heterozygous crosses. Individuals presented with FXIII deficiency and impaired fibrin polymerization.

With regard to FV, Nakamura et al. [158], reprogrammed iPSCs from the somatic cells of a patient with FV deficiency (<3 %) and FV antigen (<2 %) and applied CRISPR/*Cas* to correct a C > G missense mutation in exon 14, which was responsible for the condition. Differentiation of

these corrected iPSCs resulted in the expression of functional FV.

The multiple attempts made to obtain FV-deficient animal models have unfortunately yielded few successes as FV deficiency seems to play an important role in embryogenesis. In this connection, Cui et al. [159] used homologous recombination to generate a FV-deficient mouse model and found abnormalities in the yolk sac vasculature as well as an arrest of embryogenesis, with full-term neonates succumbing to a premature death.

Using CRISPR/*Cas*-based gene editing, Weyand et al. [160], generated a FV-deficient KO mouse model by deleting 49 base pairs in *F5* exon 4 of a zebra fish model, whose hemostasis presents with striking similarities with human hemostasis. Although embryogenesis of homozygous individuals did not undergo any alterations, these individuals did experience fatal hemorrhages in adult age.

Our research group has used CRISPR/Cas and NHEJ to obtain a FV-deficient cell model (in HepG2 cells) [8], generating a stop codon that mimics a mutation (c.3279G > A, p.Trp1093*) found in our patient in study [39]. Drawing on this FV-deficient cell model, the same technique was employed with a therapeutic purpose restoring the normal sequence of *F5* and the expression of functional FV. Our group is at present also working on the development of a FV-deficient mouse model (results in progress).

6. Conclusions

Hemostasis is a highly conserved and essential mechanism made up of procoagulant and anticoagulant processes that determine the homeostasis of blood clotting. Coagulopathies, particularly congenital ones, represent a disruption of these processes. Given their impact on health, these conditions have been the focus of major scientific attention throughout the history of medicine. The restoration of blood components following a bleeding episode by administration of whole blood or plasma was the first therapeutic strategy employed. Subsequently, cryoprecipitates and later on plasma-derived clotting factor concentrates became the treatment for some coagulopathies. Finally, recombinant coagulation factors represented the transition from biological to biotechnological drugs and the treatment of choice up to the present time.

Advanced therapies, which emerged at the beginning of the 21st century, have led to an about-face in pharmacology. They invite us to embark on a transition from classical to modern pharmacology where treatments will be individualized and longer-lasting. Gene therapy, cell therapy, immunotherapy and gene editing will open the door to a safe and effective treatment for many conditions including both rare (hemophilia A and B) and ultra-rare (FV deficiency) congenital coagulopathies. Specifically, gene editing (CRISPR/*Cas*) is regarded by many as the approach with the highest therapeutic potential.

The development of bispecific monoclonal antibodies has led to the development of non-factor replacement treatments, which mimic the action of some coagulation factors and allow the progression of the coagulation cascade. Certain antibodies act at the level of various anticoagulant proteins such as PC and AT, which participate in the homeostasis of coagulation.

Gene editing involves the use of iPSCs and gene transfer vectors, particularly AAV vectors. It is a technology that makes it possible to modify the DNA sequence in order to produce or correct a mutation. As compared with other CRISPR/*Cas* systems, gene editing offers a higher therapeutic potential due to its simplicity, precision, efficacy and lower cost.

The gene editing protocols developed for both hemophilia A and B consist in obtaining iPSCs from a patient's somatic cells, correcting them by means of CRISPR/*Cas*, differentiating them to adult endothelial or hepatic cells, and transplanting or infusing these cells, which now express the corresponding coagulation factor.

In vivo gene editing has recently been achieved through the transfection of cells with an AAV vector carrying the components of the CRISPR system. The goal is to reverse a severe phenotype of the disease.

Gene editing is also taking over from the classical procedures used to obtain KO animal models given its greater precision and, above all, the lower investment required in terms of time and cost. CRISPR strategies have been used in animal (mainly mouse) models for hemophilia A and B.

This strategy may also be applied to congenital coagulopathies that are directly or indirectly related to other coagulation factors or cofactors. Thus, cellular and animal models have been obtained with deficient levels of the enzymes participating in the metabolism of vitamin K, on which depends the functionality of several coagulation factors. Moreover, gene editing can correct alterations in the genes responsible for the functionality of platelets, acting on the thrombopoiesis pathway.

Gene editing, in conjunction with the use of iPSCs, has also obtained very promising results in the case of some ultra-rare coagulopathies such as FV deficiency. Many attempts have been made to create an appropriate model for this condition, but results have unfortunately been rather disappointing as FV seems to affect embryogenesis, which means that animal FV deficiency models cannot be viable. Our research group has succeeded in obtaining an FV-deficient cell model in HepG2 cells using NHEJ-mediated CRISPR/*Cas*. Using the said methodology, has been shown to reverse the mutation in this cell model, producing functional FV. At the present time, our laboratory is also about to complete work on an FV-deficient mouse model, using CRISPR strategy.

Although the treatment of some congenital coagulopathies is nowadays based on exogenous administration of plasma-derived or recombinant factor concentrates, as replacement therapy, or on the use of non-factor replacement therapies, the new advanced therapies open the door to a myriad of opportunities for a more effective and longer-lasting treatments.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This research was funded by the Association for Research and Cure of Factor V Deficiency (ASDEFAV), grant number ASDEFAV/2021-23, and by Complutense University of Madrid and Banco Santander, grant number CT63/19-CT64/19. Figures were designed with Biorender.com.

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