The changing treatment landscape in haemophilia: from standard half-life clotting factor concentrates to gene editing

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Congenital haemophilia A (factor VIII deficiency) and B (factor IX deficiency) are X-linked bleeding disorders. Replacement therapy has been the cornerstone of the management of haemophilia, aiming to reduce the mortality and morbidity of chronic crippling arthropathy. Frequent intravenous injections are burdensome and costly for patients, consequently with poor adherence and restricted access to therapy for many patients worldwide. Bioengineered clotting factors with enhanced pharmacokinetic profiles can reduce the burden of treatment. However, replacement therapy is associated with a risk for inhibitor development that adversely affects bleeding prevention and outcomes. Novel molecules that are subcutaneously delivered provide effective prophylaxis in the presence or absence of inhibitors, either substituting for the procoagulant function of clotting factors (eg, emicizumab) or targeting the natural inhibitors of coagulation (ie, antithrombin, tissue factor pathway inhibitor, or activated protein C). The ultimate goal of haemophilia treatment would be a phenotypical cure achievable with gene therapy, currently under late phase clinical investigation.

Published **Online**
January 15, 2021
https://doi.org/10.1016/S0140-6736(20)32722-7

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Introduction

Haemophilia A and B are rare congenital X-linked coagulation disorders caused by factor VIII (FVIII) deficiency in haemophilia A, and factor IX (FIX) deficiency in haemophilia B.[1] In severe haemophilia (FVIII or FIX <1 international units [IU]/dL) there is spontaneous or post-traumatic bleeding, or both, primarily into joints and other tissues, some of which might be life-threatening or organ-threatening. The main observed morbidity is caused by repeated haemarthroses, which lead to a degenerative joint disease (haemophilic arthropathy), resulting in chronic pain and loss of function.[1] Prevention of bleeding episodes with replacement therapy has been the cornerstone of management for these disorders for the past decades to reduce mortality and chronic arthropathy.[1] Replacement therapy is done with FVIII and FIX concentrates delivered by intravenous injections, either episodically to treat acute bleeds, or according to prophylactic regimens to prevent bleeds.[1] Long-term prophylaxis started early in life has been proven to be highly effective in preventing joint damage[2,3] and life-threatening bleeds (ie, intracranial haemorrhage),[4] and is the standard of care.[5] The frequency of regular intravenous injections needed to attain and maintain adequate haemostatic concentrations of FVIII and FIX might impair adherence to treatment, which might lead to suboptimal treatment effectiveness.[6] Moreover, the high cost of treatment restricts access for most patients worldwide.[7]

Treatment optimisation has been the main objective over the last decade, with progress based on the finding of delayed but not abolished development of joint damage in large cohorts of patients regularly treated with standard prophylaxis.[6] Accordingly, prophylactic regimens have shifted from standardised, so-called one-size-fits-all strategies to the individualisation of regimens for the best outcomes both from the clinicians' and the patients' perspective.[8,9]

In recent years, with the first of these products licensed in 2014, new bioengineered FVIII and FIX molecules with enhanced pharmacokinetic profiles were developed. Some of these molecules are an improved form of previously used molecules, and some are products with an entirely new origin. Fusion and conjugation technologies (ie, fusion with albumin or the Fc fragment of immunoglobulins; conjugation with polyethylene glycol) have resulted in several extended half-life (EHL) FVIII or FIX products, which have been licensed to treat and prevent bleeding episodes in patients with congenital haemophilia A and B.[10–17] The pharmacokinetic improvement has been more substantial for FIX (3 to 5 times longer half-life) when compared with FVIII (1·5 to 1·8 times longer half-life). This difference is because all FVIII products require stabilisation in plasma from binding to von Willebrand factor (VWF), which creates a ceiling effect by linking the pharmacokinetics of FVIII products to the clearance of VWF.[18] These molecules are given less frequently, reduce the treatment burden, and can maintain higher plasma factor concentrations.[10]

The development of anti-FVIII or anti-FIX neutralising antibodies, known as inhibitors, in patients who are

Search strategy and selection criteria

Data for this Review were identified by searches of MEDLINE, Current Contents, PubMed, and references from relevant articles using the search terms "h(a)emophilia A", "h(a)emophilia B", "prophylaxis", "extended half-life", "emicizumab", "concizumab", AND "gene therapy". Abstracts and reports from scientific meetings related directly to previously published work were included to give the most updated information. The dates of the articles ranged from January, 2010, to October, 2020, and were in English. Clinical trials and pharmacological studies were included.

previously untreated is the main complication of replacement therapy,[1] even with EHL products;[19,20] with inhibitor incidence in these patients ranging between 25 and 40%. In fact, in the presence of inhibitors, particularly at high titres, haemostasis cannot be restored by factor replacement therapy. Thus, the treatment of acute bleeds requires the use of alternative haemostatic agents that can bypass the inhibitory effect of the antibodies.[1] Such bypassing agents (BPA), represented by recombinant activated FVII and activated prothrombin complex concentrate, have many drawbacks: their effectiveness is often suboptimal, inconsistent, and unpredictable; their use is associated with the risk of thrombosis; their haemostatic activity cannot be easily monitored; they are costly treatments; and prophylaxis is challenging and not always effective.[21]

To close this big therapeutic gap, several new molecules, all delivered subcutaneously, were developed and provide effective prophylaxis despite the presence of inhibitors.[22–26] A humanised bispecific monoclonal antibody (emicizumab) improves haemostatic function by mimicking the co-factorial function of activated FVIII.[27] Other novel therapeutics can rebalance haemostasis by targeting the natural inhibitors of the coagulation cascade: antithrombin, tissue factor pathway inhibitor (TFPI), and activated protein C.[25,26,28–30]

Finally, in this rapidly evolving treatment landscape, a functional cure can be found by gene therapy, with which a protective endogenous steady state production of FVIII and FIX can be established. The safety and efficacy of these approaches are under investigation in several clinical trials, which are still ongoing.[31–46]

Therapeutic goals in haemophilia: the role of long-term prophylaxis

Over the last decades, the goal of the management of patients with haemophilia has evolved from supportive care, to reducing the risk of spontaneous bleeds, to the elimination of all clinically evident bleeds, including those related to physical activity. These goals can be achieved with prophylactic replacement therapy with FVIII and FIX concentrates. The primary aim of regular long-term prophylaxis is to abolish recurrent joint bleeds and prevent chronic arthropathy. Early insight into prophylaxis came from the observation that patients with moderate and mild haemophilia seldom experience joint bleeds,[47,48] and that the risk of joint bleeds in patients with haemophilia A receiving regular FVIII replacement increases with increased time spent with low residual plasma concentrations of FVIII,[49] with higher residual plasma concentrations required to prevent traumatic bleeding events.

Prophylaxis with standard half-life (SHL) products

Over the last three decades, a variety of prophylactic regimens have included SHL recombinant and plasma-derived products, and multiple studies have shown that any prophylactic regimen (even at low doses [10 IU/kg two times per week]) is superior to episodic treatment.[50,51]

The efficacy of prophylaxis has been established by evaluating bleeding, joint health outcomes, and health-related quality of life, irrespective of trough concentrations of FVIII or FIX,[52,53] or by targeting definite trough concentrations through pharmacokinetic-guided regimens.[54] Indeed, trough concentrations of 1–3 IU/dL have been considered the benchmark to ensure adequate bleed protection, with the time spent with less than 1 IU/dL inversely related to the risk of joint bleeds.[49] However, long-term follow up data from large cohorts of patients treated over decades on regular prophylaxis showed that those concentrations were not sufficiently protective for all patients and that the progression of joint damage was only delayed in time but not completely abolished,[6] suggesting the need for attaining and maintaining substantially higher trough concentrations.

Maintaining higher concentrations is challenging with SHL products, because of the short half-life of native FVIII (8–12 h) and FIX (18–24 h). For this reason, prophylaxis with these products is usually accomplished with three to four injections per week for haemophilia A and two to three injections per week for haemophilia B.[1] However, some new generation SHL recombinant FVIII products have been shown to provide effective prophylaxis with two or less injections per week in approximately 30% of patients.[55–58] This effectiveness has been attributed to refinements conferred to these new molecules through manufacturing, including the optimisation of relevant post-translational modifications that can affect the stability of the mature FVIII protein. The main characteristics of these molecules are reported in table 1. The various manufacturing processes that contribute to the optimisation of the mature FVIII protein include: the co-expression of a chaperone protein, heat-shock protein 70, that ensures a better viability of the mammalian cell lines made use of for recombinant protein expression;[55] the engineering of a covalent link between the FVIII heavy and light chains that preserves the FVIII molecule from premature degradation and confers a higher binding affinity for VWF;[56] the use of human cell line cultures that provide the mature protein with post-translational modifications and glycosylation structures that are more similar to those of human plasma FVIII, which might modulate clearance and degradation.[57]

Prophylaxis with EHL products

EHL products are defined as those bioengineered molecules that can have a prolonged plasma half-life at least 1·3 times that of SHL products.[59] The advent of EHL products has provided another step forward for prophylaxis optimisation, with better protection and fewer intravenous injections.[10] Indeed, the modified pharmacokinetic properties of either fusion-glycolylated or polyethylene-glycolylated FVIII and FIX molecules

	Brand name (manufacturer)	Molecular characteristics	Reference
BAY 81–8973 (BHK)	Kovaltry (Bayer Healthcare)	Full-length rFVIII with a high degree of N-terminal glycan sialylation; co-expressed in cell culture with human heat-shock protein 70, which enhances the viability of cell lines by inhibiting apoptosis and increases proper folding of the mature protein	Saxena et al (2016)[55]
Lonoctocog α (CHO)	Afstyla (CSL Behring)	Single chain rFVIII with heavy and light chain covalently fused into a single polypeptide that has an increased binding affinity for von Willebrand factor	Mahlangu et al (2016)[56]
Simoctocog α (HEK)	Nuwiq (Octapharma)	B-domain deleted rFVIII fully sulphated and with oligosaccharide and sialic acid content similar to plasma-derived FVIII	Lissitchkov et al (2017)[57]
Turoctocog α (CHO)	NovoEight (Novo Nordisk)	B-domain truncated rFVIII fully glycosylated at N-linked glycosylation sites and fully sulphated at Tyr1680, resulting in strong binding affinity for von Willebrand factor	Lentz et al (2018)[58]

BHK=Baby Hamster Kidney. CHO=Chinese Hamster Ovary. FVIII=factor VIII. HEK=Human Embryonic Kidney. rFVIII=recombinant FVIII.

Table 1: **Approved standard half-life rFVIII molecules (and cell lines) that have been effectively used twice a week for regular prophylaxis**

has translated to increased trough concentrations and fewer yearly injections for most patients when compared with previous prophylactic regimens with SHL products.[11–17]

The main characteristics of these modified molecules and investigated prophylactic regimens in adult and adolescent (12–65 years old) patients are summarised in table 2. As evident from pharmacokinetic variables, there has been a marked improvement for FIX products with a 3-times to 5-times prolongation of terminal half-life, and hence, the chance to maintain trough concentrations at more than 5%, with infusions every 7, 10, or 14 days.[11,13,15]

The pharmacokinetic improvement for FVIII molecules has been more modest, with a 1·4-times to 1·8-times prolongation of the terminal half-life. Accordingly, on average, patients with haemophilia A on EHL for FVIII are treated every 3–5 days,[12,14,16–17] although selected subgroups of patients could also benefit from a once-per-week dosing regimen.[12,17] The availability of EHL products has also offered the chance of more flexible treatment regimens, adjustable to the clinical and social needs of patients. These characteristics also contribute to easier treatment individualisation to improve adherence, which has a relevant effect on treatment outcome.[60]

	Median Annual Bleeding Rate (95% CI or IQR)	Proportion of patients without bleeds	Mean trough percent at steady state	Reference
Recombinant factor IX-Fc (Alprolix); Fc fusion				
50 IU/kg once per week*	3·0 (95% CI 1·0–4·4)	23·0%	1·0–3·0%†	Powell et al (2013)[11]
100 IU/kg once every 10 days‡	1·4 (IQR 0·0–3·4)	42·3%	1·0–3·0%†	Powell et al (2013)[11]
N9-GP (Refixia/Rebinyn); glycoPEGylation				
40 IU/kg once per week	1·04 (IQR 0·0–4·0)	45·0%	27·3%	Collins et al (2014)[13]
Recombinant fusion protein linking coagulation factor IX with albumin (Idelvion); albumin fusion				
35–50 IU/kg once per week	0·0 (IQR 0·0–1·87)	52·6%§	20·0%	Santagostino et al (2016)[15]
75 IU/kg once every 10 days	0·0 (IQR 0·0–1·78)	52·6%§	NR	Santagostino et al (2016)[15]
75 IU/kg once every 14 days	1·08 (IQR 0·0–2·7)	52·6%§	12·4%	Santagostino et al (2016)[15]
Recombinant factor VIII-Fc (Elocta/Eloctate); Fc fusion				
65 IU/kg once per week	3·6 (IQR 1·9–8·4)	17·4%	1·0–3·0%†	Mahlangu et al (2014)[12]
25–65 IU/kg once every 3–5 days¶	1·6 (IQR 0·0–4·7)	45·0%	1·0–3·0%†	Mahlangu et al (2014)[12]
Bax855 (Adynovi/Adynovate); random PEGylation				
45 IU/kg twice per week	1·9 (IQR 0·0–5·8)	39·6%	NR	Konkle et al (2015)[14]
N8-GP (Esperoct); glycoPEGylation				
50 IU/kg once every 4 days	1·33 (IQR 0·0–4·61)	40·0%	3·0%	Giangrande et al (2017)[16]
BAY 94–9027 (Jivi); site-specific PEGylation				
30–40 IU/kg twice per week‖	4·1 (IQR 2·0–10·6)	15·4%	NR	Reding et al (2017)[17]
45–60 IU/kg once every 5 days	1·9 (IQR 0·0–5·2)	44·2%	NR	Reding et al (2017)[17]
60 IU/kg once per week	3·9 (0·0–6·5)	37·2%	NR	Reding et al (2017)[17]

NR=not reported. *Median pharmacokinetic-guided dose used during the study (45 IU/kg one time per week). †Per protocol design. ‡Median pharmacokinetic-guided interval used during the study (once every 12·5 days). §Across all treatment groups. ¶Median dose interval was 3·5 days and median once per week dose was 77 IU/kg. ‖Patients not eligible for randomisation between the less frequent dose groups because of a more severe bleeding phenotype.

Table 2: **Different licensed extended half-life factor VIII and IX molecules, technologies, and doses for the treatment and prevention of bleeding episodes in patients with haemophilia A and B**

Moving from replacement to non-replacement therapy: a new framework for prophylaxis

Despite the great advantages of prophylaxis over episodic treatment, the restrictions of the route of administration and potential development of neutralising anti-FVIII and anti-FIX antibodies represent the main challenges with replacement therapy. Therefore, the rationale behind the design of new non-replacement therapies is to overcome the difficulties of intravenous delivery and to improve the effectiveness of therapies in all patients, regardless of the presence or absence of inhibitors.

The delivery of therapy through regular intravenous injections is a substantial burden for patients and caregivers. To administer intravenous injections frequently, central venous devices are often required, with associated risks from the surgical procedures and possible complications, such as infection or thrombosis.[61] This risk is particularly high in infants but can be an issue across all ages. The transition to a subcutaneous route of administration would have a substantial effect on treatment feasibility and adherence, thus greatly reducing the chance of breakthrough bleeds related to missed infusions.

	Number of patients	Studied regimens	Median Annual Bleeding Rate (95% CI)	Reference
Emicizumab (bispecific monoclonal antibody able to bind factor IXa and factor X)				
Haemophilia A with inhibitors (≥12 years old)	109	1·5 mg/kg once per week*	2·9 (1·7–5·0)	Oldenburg et al (2017)[22]
Haemophilia A with inhibitors (<12 years old)	65	1·5 mg/kg once per week*	0·3 (0·17–0·50)	Young et al (2019)[66]
Haemophilia A with inhibitors (<12 years old)	10	3·0 mg/kg once every other week*	0·2 (0·03–1·72)	Young et al (2019)[66]
Haemophilia A with inhibitors (<12 years old)	10	6·0 mg/kg once every 4 weeks*	2·2 (0·69–6·81)	Young et al (2019)[66]
Haemophilia A without inhibitors (≥12 years old)	84	1·5 mg/kg once per week*	1·5 (0·9–2·5)	Mahlangu et al (2018)[67]
Haemophilia A without inhibitors (≥12 years old)	35	3·0 mg/kg once every other week*	1·3 (0·8–2·3)	Mahlangu et al (2018)[67]
Haemophilia A with and without inhibitors (≥12 years old)	41	6·0 mg/kg once every 4 weeks*	2·4 (1·4–4·3)	Pipe et al (2019)[68]
Haemophilia A without inhibitors (<12 years old)	6	3·0 mg/kg once every other week*	1·3 (0·6–2·9)	Shima et al (2019)[69]
Haemophilia A without inhibitors (<12 years old)	7	6·0 mg/kg once every 4 weeks*	0·7 (0·2–2·6)	Shima et al (2019)[69]
Fitusiran (small interfering RNA able to inhibit the synthesis of antithrombin)				
Haemophilia A and B with and without inhibitors	34	80 mg once every 4 weeks	1·5 (not available)	Pasi et al (2019)[70]
Concizumab (monoclonal anti-TFPI antibody able to prevent the binding of TFPI to the factor VII-tissue factor complex)				
Haemophilia A and B with inhibitors	17	0·15 mg/kg once per day†	4·5 (3·2–6·4)	Shapiro et al (2019)[24]
Haemophilia A without inhibitors	36	0·15 mg/kg once per day†	7·0 (4·6–10·7)	Shapiro et al (2019)[24]

TFPI=tissue factor pathway inhibitor. *After a loading phase with 3·0 mg/kg once per week for 4 weeks. †With a stepwise increase to 0·20 and 0·25 mg/kg if three or more spontaneous bleeds occurred over 12 weeks.

Table 3: **Annual Bleeding Rates observed during prophylaxis with different regimes of non-replacement therapies in different patient populations in licensure clinical trials**

Prophylaxis in patients with inhibitors, especially in those with high titre antibodies, is challenging, with a reliance on BPA. Some prophylaxis regimens have been described but with widely varied efficacy rates.[62,63] For this reason, arthropathy prevention has been challenging in this patient population, with higher rates of overt joint damage observed earlier than their non-inhibitor peers[64] and poorer quality of life measures for both patients and caregivers.[65] New non-replacement agents do not contain FVIII or FIX proteins, hence they are not neutralised by pre-existing inhibitory anti-FVIII and anti-FIX antibodies, nor do they elicit a specific immune response against FVIII or FIX.

In addition to a subcutaneous method of delivery and similar effectiveness in patients both with and without inhibitors, a key feature of these new non-replacement therapies is a substantially longer duration of action that leads to a more stable steady state haemostatic effect, with treatment schedules that range between once per day to once per month injections with a low volume of drug.[22–25]

There has been more extensive research, more follow-up, and post-marketing experience for emicizumab, the first non-replacement therapy licensed for prophylaxis in patients with haemophilia A with and without inhibitors, that is absent for other drugs under investigation. This therapy is a humanised bispecific monoclonal antibody that binds and bridges activated FIX and factor X (FX), mimicking the function of activated FVIII.[27] This drug can be administered once every 7, 14, or 28 days, with similar effectiveness across different ages and bodyweights.[22,66–69]

Fewer data are available for the haemostatic rebalancing agents that are still under investigation, including fitusiran, a small interference RNA able to inhibit the synthesis of antithrombin,[23,70] or monoclonal antibodies that target TFPI,[24,25] the primary regulator of the tissue factor-factor VIIa complex.

Table 3 summarises the median Annual Bleeding Rates observed in different patient groups within clinical trials on new non-replacement therapies. With respect to anti-TFPI monoclonal antibodies, no data have been reported so far on the development programme of marstacimab (PF-06741086), which is currently recruiting patients for phase 2 (NCT03363321) and phase 3 (NCT03938792) clinical trials.

Non-replacement therapies: current limitations

The results of phase 3 trials with new non-replacement therapies showed good efficacy rates in bleed prevention in patients with and without inhibitors.[22,24,66–70] Nevertheless, there are some crucial aspects to take into account when the use of these drugs is considered.

The first aspect is breakthrough bleeding and perioperative management. Pharmacokinetic and pharmacodynamic studies done in patients treated with emicizumab, fitusiran, and anti-TFPI antibodies show that the extent of haemostatic correction provided by these molecules at the doses used for prophylaxis is, on average, similar to that of patients with mild haemophilia (as measured by thrombin generation assays).[23,70–72] Breakthrough bleeding events still occur, particularly with traumatic injuries, and the amount of protection from these drugs might not be adequate for some surgical interventions. Thus, such events require the use of additional haemostatic agents, as either FVIII and FIX concentrates or BPA, according to the patients' inhibitor status.[73–76]

The second aspect to consider is the risk of thrombotic complications. The need for intermittent treatment with additional procoagulants for breakthrough bleeds or surgery has been associated with a higher risk for thrombotic complications. Thromboembolic events and thrombotic microangiopathy were observed in the HAVEN 1 study in five patients treated with emicizumab who concomitantly received repeated infusions of activated prothrombin complex concentrate at more than 100 IU/kg per day for more than 1 day.[22] Subsequent to

these events, risk mitigation has included recommendations to use recombinant activated FVII instead of activated prothrombin complex concentrate during emicizumab prophylaxis if possible; and, if this concentrate is still required for effective haemostasis, to reduce daily dosing to less than 100 IU/kg per day when repeated doses are needed. Thrombotic complications have also been observed for the haemostatic rebalancing agents. A fatal case of cerebral thrombosis occurred in a young adult with severe haemophilia A without inhibitors who was on prophylaxis with fitusiran during the phase 2 trial and was given FVIII replacement at standard doses for a post-traumatic breakthrough bleed.[77] Since this event occurred, a risk mitigation plan was put in place with revised bleed management guidelines that recommend reduced doses of both FVIII and FIX replacement therapies and BPA.[78] The phase 2 trial with BAY1093884, a human anti-TFPI monoclonal antibody, has been prematurely terminated because of thrombotic adverse events that occurred with different drug doses and in the absence of a concomitant haemostatic treatment for breakthrough bleeds,[79] suggesting that there are relevant unknown factors concerning the mechanism of action and pharmacodynamic effect of such drugs. Moreover, phase 3 clinical trials with concizumab, another humanised anti-TFPI monoclonal antibody, have been put on hold because of the occurrence of non-fatal thrombotic adverse events in three patients.[80]

The third aspect is laboratory monitoring. One of the main advantages of replacement therapy is the availability of laboratory monitoring to guide the haemostatic coverage provided to patients in different clinical settings. The measurement of FVIII and FIX clotting activity in plasma is well-established in routine haemophilia care, and has mostly become standardised.[81] For non-replacement therapies, no specific routine laboratory monitoring is available; also, this method is not strictly recommended, because these drugs are administered according to a standard dosing regimen applied to all patients with either a fixed dose (ie, fitusiran) or with a fixed weight-based dosing schedule (ie, emicizumab or anti-TFPI antibodies). However, laboratory monitoring could be warranted in the event of reduced effectiveness or for guiding perioperative management: in these settings, specific assays should be made use of, owing to the potential interferences of these molecules on routine laboratory tests.[82,83]

Finally, arthropathy prevention should be taken into account. From available data, new non-replacement therapies have produced a high degree of efficacy based on Annualised Bleeding Rates that would suggest a positive effect on preserving long-term joint outcomes. However, a longer follow-up is needed to show this result. Moreover, there are unanswered but crucial questions pertaining to whether there might be biological roles for FVIII or FIX beyond their procoagulant function that might not be provided by new non-replacement therapies that could affect long-term bone and joint health.[84,85]

Future innovations for haemophilia prophylaxis

The current therapeutic armamentarium has provided a wide range of options suitable for different patient profiles, thus favouring treatment tailoring to meet specific clinical and personal needs.

More innovations are under investigation for haemophilia care, with the specific aim of providing a high degree of protection against bleeding with low burden treatment regimens that should continue to improve outcomes and quality of life.

Novel replacement therapies

BIVV001 is a novel EHL-FVIII fusion protein that consists of the recombinant FVIII Fc fusion protein molecule coupled with the FVIII binding D'D3 domain of VWF, as well as two XTEN® linkers, unstructured polypeptides that reduce clearance and degradation. This molecule was designed with the specific aim of overcoming the VWF-imposed half-life extension restrictions for FVIII. The safety, tolerability, and pharmacokinetic ability of BIVV001 have been evaluated in a first-in-human phase 1 and 2 clinical trial that showed an extended half-life of 37·6 h compared with the 12-h half-life of standard FVIII, and an average FVIII post-infusion activity of 5% at day 7.[86] A phase 3 trial to evaluate the safety and efficacy of BIVV001 in previously treated adults and adolescents with severe haemophilia A is ongoing (NCT04161495).

SubQ-8 is a novel strategy for subcutaneous delivery of the human cell line-derived recombinant FVIII (simoctocog alfa, Nuwiq) co-administered with a recombinant human VWF fragment dimer that contains the D'D3 domain of VWF and some additional VWF sequences that enhance bioavailability. The safety and efficacy of this molecule as regular prophylaxis in patients with severe haemophilia A is under investigation in a phase 1 and 2 study (NCT04046848). No results have been published yet.

Dalcinonacog alfa is a novel recombinant FIX with 22-times greater potency than native human FIX under investigation for subcutaneous delivery in a phase 2b study.[87] This molecule has been bioengineered with targeted amino acid substitutions to provide resistance to antithrombin inhibition, a higher affinity to FVIIIa, and increased catalytic activity. Interim data have reported an extended half-life ranging between 84 and 112 h, with post-injection FIX concentrations between 16 and 27% after 28 daily injections.[87]

Novel bypassing therapy

Marzeptacog alfa is a recombinant activated human factor VII variant with four site-specific amino acid changes: two within the catalytic domain to enhance procoagulant activity and two to increase the terminal half-life compared with that of wild-type recombinant

activated FVII. The safety, tolerability, pharmacokinetic and pharmacodynamic profile, and immunogenicity of this molecule have been investigated in a first-in-human study,[88] of which the positive results prompted further evaluation within a phase 1 study investigating the subcutaneous delivery of this drug as a prophylactic BPA in patients with haemophilia A or B with and without inhibitors (NCT04072237).

SerpinPC is a bioengineered serine protease inhibitor that rapidly inhibits activated protein C and has been shown to be effective in increasing thrombin generation in vitro and in mice with haemophilia.[30] This molecule is under investigation within a phase 1 and 2 trial, starting with healthy volunteers (NCT04073498) with a plan to eventually study it in patients with haemophilia A or B with and without inhibitors. This drug will be evaluated for safety and efficacy by intravenous and subcutaneous delivery, and has the potential for both the treatment and prevention of bleeding.

Gene therapy: endogenous prophylaxis or cure?

Both haemophilia A and B are ideal candidates for gene therapy because they are monogenic diseases that might be treated effectively by delivering a substitute copy of the FVIII (*F8*) and FIX (*F9*) genes. In fact, a successful gene therapy approach would result in a sustained endogenous production of FVIII and FIX proteins at concentrations that could provide effective prophylaxis without the need for exogenous factor replacement therapy. Ideally, sustained concentrations in the normal range in the long term would be a functional or phenotypic cure of the disease. Although simple in theory, for these results, advanced technologies are required to ensure the sufficient safety and efficacy to supplant highly efficacious treatment strategies.

The predominant strategy for gene transfer in patients with haemophilia is the liver-directed delivery of *F8* or *F9* with the use of recombinant non-integrating adeno-associated viral (AAV) vectors. These vectors can transfer therapeutic genes into post-mitotic tissues, such as the liver, through cellular targeting (tropism) driven by their protein coats (capsids). The different varieties of AAV capsid (serotypes) establishes the efficiency and specificity of the cellular targeting to hepatocytes. The therapeutic transgenes are predominantly in circularised episomal DNA within the transduced hepatocytes. Such non-integrating vectors are considered to reduce the risk of genotoxicity compared with that of vectors that are dependent on integrating into the genome (ie, lentiviral or retroviral vectors).

In AAV vectors, the viral genomic elements that facilitate viral replication are replaced with the therapeutic transgene, typically with a tissue (liver)-specific promoter or enhancer, or both, so that it will drive efficient expression within the target cell. Several AAV serotypes have been used for liver-directed gene therapy trials for haemophilia so far.[31–46] Table 4 shows the ongoing gene therapy clinical trials in haemophilia A and B.

The first successful gene therapy was reported for patients with haemophilia B in 2011.[31,32] *F9* is much smaller in size than *F8* and it is easier to package into the viral vectors used for this approach. However, this restriction has been overcome for haemophilia A by using *F8* transgenes that have been optimised for packaging through the deletion of genetic sequences that are not relevant for the clotting function of the mature protein (ie, the B domain).[33,38]

Data from multiple small phase 1 and 2 trials in haemophilia A and B, with a follow-up of up to 8–10 years, show that the vast majority of patients treated so far have measurable FVIII and FIX concentrations in plasma sufficient to be able to withdraw regular prophylaxis, at the same time as maintaining a good control of bleeding.[31–46] These initial studies have been assessed to show sufficient safety and efficacy to move forward with larger phase 3 pivotal trials in both haemophilia A and B with various AAV serotypes.

Gene therapy: current limitations and unknowns

Despite these positive results from gene therapy clinical trials to date, some limitations and potential safety concerns are likely to prevent the widespread use of such an approach.

Pre-existing antibodies against AAV vectors

Since AAV are common non-pathogenic viruses that infect humans beginning in childhood, pre-existing antibodies are frequently present in the general population and these antibodies can show the ability to neutralise the transduction efficiency of AAV vectors. Seroprevalence studies done in haemophilia cohorts have shown seropositivity rates up to 50%, with a co-prevalence of pre-existing immunity towards different AAV serotypes (ie, AAV2, AAV5, and AAV8) of approximately 40%.[89,90] Up to now, the presence of such antibodies is an exclusion criterion in many clinical trials, with few exceptions. Moreover, still there is no standardised universal assay to detect those antibodies and measure their titres across different investigational programmes, thus adding further uncertainties about comparisons of these programmes.

Liver toxicity

From the first trials of liver-directed AAV gene therapy, numerous patients showed an increase in aminotransferase concentrations that were associated with a subsequent decline in FVIII and FIX expression. Early evidence suggested that this decrease could be triggered by an immune response to the viral capsid with resultant cytotoxicity. This clinical feature represents the most common toxicity associated with liver-directed gene therapy, not typically associated with any effect on liver function; however, it potentially affects the clinical outcome for patients. In many, but not all, patients, the early introduction of a course of immunosuppression with corticosteroids can lead to resolution

	Transgene	Haemophilia type (number of patients)	FVIII and FIX concentrations	Follow-up	Trial status*	Sponsor	Reference
scAAV2/8	co-FIX-WT	Haemophilia B (20†)	1·3–8·0%	Up to 8 years	Active, not recruiting	St Jude Children's Research Hospital and University College London	Nathwani et al (2011),[31] (2014),[32] (2018)[39]
scAAV8	FIX Padua	Haemophilia B (16)	0·5–25·0%	Up to 2 years	Active, not recruiting	Baxalta and Shire	Monahan et al (2015)[40]
AAV-SPK100 (SPK9001)	FIX Padua	Haemophilia B (15)	22·9 ± 9·9%	Up to 1 year	Recruiting	Spark Therapeutics and Pfizer	George et al (2017),[34] (2019)[41]
rAAV5 (AMT-060)	co-FIX-wild type	Haemophilia B (10)	5·1–7·5%	Up to 4 years	Active, not recruiting	uniQure	Miesbach et al (2018),[36] (2019)[42]
rAAV5 (AMT-061)	FIX Padua	Haemophilia B (3)	30·0–54·0%	Up to 1 year	Recruiting	uniQure	Pipe et al (2019)[37]
rAAVS3	FIX Padua	Haemophilia B (2)	45·0 ± 5·0%	Up to 20 weeks	Recruiting	Freeline Therapeutics	Chowdary et al (2018)[43]
rAAV6 (zinc finger-mediated integration)	FIX-wild type	Haemophilia B (NR)	NR	NR	Active, not recruiting	Sangamo Therapeutics	Not published yet
rAAV5	co-BDD-FVIII	Haemophilia A (15)	13·0–20·0%	Up to 3 years	Active, not recruiting	BioMarin Pharmaceutical	Rangarajan et al (2017),[33] Pasi et al (2020)[38]
rAAV8	BDD-FVIII-V3	Haemophilia A (3)	6·0–76·0%	Up to 47 weeks	Recruiting	St Jude Children's Research Hospital and University College London	Nathwani et al (2018)[46]
AAV-LK03	BDD-FVIII	Haemophilia A (12)	13·0–30·0%	Up to 66 weeks	Recruiting	Spark Therapeutics	High et al (2018)[35]
rAAV6	BDD-FVIII	Haemophilia A (11)	7·0–169·0%	Up to 28 weeks	Recruiting	Sangamo Therapeutics	Konkle et al (2019)[44]
rAAVhu37	BDD-FVIII	Haemophilia A (2)	5·0–17·0%	Up to 24 weeks	Recruiting	Bayer	Pipe et al (2019)[45]
rAAV8	BDD-FVIII	Haemophilia A (NR)	NR	NR	Recruiting	Shire and Takeda	Not published yet

BDD=B-domain deleted. FVIII=factor VIII. FIX=factor IX. NR=not reported. *Trial status at the time of this publication. †10 plus 10 in two different trials.

Table 4: **Liver-directed gene therapy clinical trials with the use of different vectors for patients with haemophilia A and B**

of the aminotransferase elevation and protection from the loss of FVIII and FIX expression; however, the pathophysiological mechanism underlying this effect is still unclear and might not be the same in all patients. Different hypotheses have been posited to explain the aminotransferase increase: some experimental results suggest that the liver toxicity is the result of an immune-mediated destruction of transduced hepatocytes,[91] others suggest that the immune system is triggered by cryptic epitopes of the therapeutic protein,[91] and some have theorised that the viral transduction of hepatocytes might cause a protein overload of the endoplasmic reticulum that leads to cellular stress.[92] Clinical data show that this toxicity might be influenced by higher vector doses, required to ensure a high concentration of expression of the therapeutic protein, creating an interdependence between safety and effectiveness. For this reason, multiple strategies have been adopted to optimise the cellular transduction through alternative bioengineered AAV vectors and transgene modifications to enable the use of lower vector doses. Transgene modifications have included codon optimisation, more potent promoter and enhancer elements, and the use of gene variants with improved properties, such as more efficient secretion or enhanced functional activity. The most notable modification is the use of the FIX Padua variant, which contains a single missense mutation that yields an FIX protein with 6–8-times higher procoagulant activity.[34,37,40,41,43]

Risk for genotoxicity

By definition, the risk of insertional mutagenesis following AAV gene therapy is low, because the transgene is in the nucleus predominantly in an episomal form. However, studies have shown that chromosomal integration of the AAV vector genome, although rare, can occur in the liver.[93] This risk will be the focus of long-term follow up studies.

Durability of expression

Results from some haemophilia B trials have now shown the stable expression of FIX for up to 10 years. However, the first trials in haemophilia A with a follow-up of longer than 1 year have shown a decline in FVIII concentrations over time.[38] Longer follow-ups will be required to show if FVIII concentrations will stabilise at a lower steady state

	Strengths	Weaknesses
Standard half-life clotting factor concentrates	Effective for both bleeding control and prevention; well established safety and effectiveness profile for decades; measurable FVIII and FIX concentrations as surrogate marker of effectiveness; can result in normal concentrations of FVIII and FIX	Frequent intravenous injections; inhibitor development
Extended half-life clotting factor concentrates	Effective for both bleeding control and prevention; a reduced number of injections; higher trough concentrations; measurable FVIII and FIX concentrations as surrogate markers of effectiveness; can result in normal concentrations of FVIII and FIX	Intravenous route; inhibitor development
Non-replacement therapies (emicizumab, fitusiran, anti-tissue factor pathway inhibitor antibodies, SerpinPC)	Subcutaneous route; infrequent injections; standard doses for all patients	Need for adjunctive haemostatic treatment; steady state of coagulation activity not within the normal range; thrombotic risk
Gene therapy	Single intravenous injection; restoration of endogenous FVIII and FIX production	Pre-existing immunity against adeno-associated viral vectors; immune response against vectors and transfected cells; unknown durability of transduction; need for immunosuppressive therapy; unknown long-term safety

FVIII=factor VIII. FIX=factor IX.

Table 5: **Strengths and weaknesses of the old and new therapeutic options for haemophilia**

or if the decline will be progressive with the complete loss of expression.

Eligibility

Eligibility and exclusion criteria have restricted the patients who have been able to participate in gene therapy trials to date. Because of the liver-targeted approach, patients with active viral hepatitis, and those with other hepatic diseases or on hepatotoxic drugs (eg, some antiretroviral therapies for HIV), or both, are excluded from these trials. Children, who would represent the ideal candidates for gene therapy, are still excluded because of many practical restrictions and safety concerns. Notably, the episomal state of the transgene after transduction is not suitable to ensure stable transgene expression in a growing liver because of the dilution effect from cellular division. Furthermore, patients with inhibitors are now excluded from this therapeutic approach, even though an upcoming study will evaluate the potential for gene therapy (with tolerising effect) in this patient population (NCT03734588).

Affordability

It is reasonable to forecast a high cost for a gene therapy when it will become available. Such a therapy has the potential to produce huge savings for direct and indirect costs related to haemophilia care; however, the cost is still likely to restrict access to patients in many countries worldwide.

Future innovations for gene therapy

Beyond the gene addition strategies currently under investigation, there are other technologies that could be used to correct the gene defect of the host genome. One of these techniques, gene editing, relies on the use of clustered regularly interspaced short palindromic repeats (CRISPR) CRISPR-associated protein (Cas) 9. Those molecular scissors induce double-stranded breaks at the target genome site, thus favouring the insertion of template DNA with the wild-type gene.[94]

Concluding remarks

Haemophilia treatment has seen a rapid revolution in the last several decades. Table 5 summarises the main strengths and weaknesses of the aforementioned therapeutic options.

The availability of safer plasma-derived products and recombinant SHL concentrates favoured the adoption of prophylaxis, thus improving joint outcomes and quality of life. However, these advances came with several drawbacks, including difficult venous access, poor adherence, and scarce affordability on a global scale. The advent of EHL products have further improved prophylaxis effectiveness and feasibility, allowing the acceptance of prophylaxis as standard of care widely, and through the implementation of humanitarian programmes aiming at providing such therapies to low-income countries. Nevertheless, the intravenous route of administration, the risk of inhibitor development, and the need for treatment individualisation (to meet specific clinical and non-clinical needs) still represent a burden for both patients and clinicians. In light of these challenges, new non-replacement therapies display many advantages, including the subcutaneous route of administration, a similar effectiveness across a wide age range, the possibility to implement prophylaxis from young ages (from birth), and the availability of fixed-dose regimens for all, including patients with inhibitors. However, there are patients for whom the lifestyle or clinical conditions, or both, might benefit most from near-normal or normal FVIII and FIX concentrations, which cannot yet be reached with non-replacement therapies; indeed, the short follow up data accumulated until now are not informative enough with respect to long-term bleed control and joint outcomes, and few data are available on

the safety and efficacy of these therapies in newborn babies and infants younger than 1 year of age.

Looking forward, the scientific concept behind gene therapy embeds the promise of a functional haemophilia cure and, with a single intravenous infusion, offers the chance to have the stable expression of FVIII and FIX concentrations high enough not to require regular prophylaxis: a real game-changer, particularly effectual if it can be applied in the developing world. However, several issues around long-term safety and effectiveness are unsolved, and the current platform for delivery restricts the application of this approach to highly committed adult patients with haemophilia but without inhibitors and without relevant comorbidities or pre-existing immunity to AAV, or both. For this reason, there is continued interest in the development of novel replacement and non-replacement therapies, including novel BPA.

Finally, innovation can be revolutionary on the costs and availability of products. These factors can have an effect on product market shares and national tenders: the multiplicity of old and new therapies should imply competitive lower costs, which might have a relevant effect on the global availability of products to bring prophylaxis to an increasingly greater proportion of patients worldwide.

Contributors

MEM conceived the idea and wrote the first draft of the paper. All authors contributed to the literature review, interpretation, and discussion of the findings.

Declaration of interests

MEM served on clinical trial steering committees for Bayer HealthCare and Roche; is member of the scientific advisory committee for Bayer HealthCare, Biomarin Pharmaceutical, Catalyst Biosciences, CSL Behring, Grifols, Kedrion, Novo Nordisk, Octapharma, Roche, Sobi, and Takeda; and is a member of the speaker bureau of Bayer HealthCare, Biomarin Pharmaceutical, Biotest, Catalyst Biosciences, CSL Behring, Grifols, Kedrion, Novo Nordisk, Octapharma, Pfizer, Roche, Sobi, and Takeda. JNM has received research grants from Bayer, Biogen, BioMarin Pharmaceutical, CSL Behring, Novo Nordisk, Sobi, Roche, and Uniqure; he is member of the scientific advisory committee of Amgen, Bayer, Baxalta, Biogen, Biotest, CSL Behring, Catalyst Biosciences, Novo Nordisk, Roche, and Spark Therapeutics; and is a member of the speaker bureau of Alnylam, Bayer, Biogen, Biotest, Novo Nordisk, Pfizer, Roche, Sobi, and Shire/Takeda. SWP has received research funding from Shire/Takeda and Pfizer; serves on the clinical trial steering committees for BioMarin Pharmaceutical, uniQure, and Bayer HealthCare; and is a consultant for Shire/Takeda, Pfizer, BioMarin Pharmaceutical, uniQure, Bayer HealthCare, Roche/Genentech, CSL Behring, Alnylam Pharmaceuticals, Novo Nordisk, HEMA Biologics, Bioverativ, Catalyst Biosciences, DNARx, and Spark Therapeutics.

References

1 Mannucci PM, Tuddenham EG. The hemophilias--from royal genes to gene therapy. *N Engl J Med* 2001; **344:** 1773–79.

2 Astermark J, Petrini P, Tengborn L, Schulman S, Ljung R, Berntorp E. Primary prophylaxis in severe haemophilia should be started at an early age but can be individualized. *Br J Haematol* 1999; **105:** 1109–13.

3 Manco-Johnson MJ, Abshire TC, Shapiro AD, et al. Prophylaxis versus episodic treatment to prevent joint disease in boys with severe hemophilia. *N Engl J Med* 2007; **357:** 535–44.

4 Andersson NG, Auerswald G, Barnes C, et al. Intracranial haemorrhage in children and adolescents with severe haemophilia A or B - the impact of prophylactic treatment. *Br J Haematol* 2017; **179:** 298–307.

5 Srivastava A, Santagostino E, Dougall A, et al. WFH guidelines for the management of hemophilia, 3rd edition. *Haemophilia* 2020; **26:** 1–158.

6 Oldenburg J. Optimal treatment strategies for hemophilia: achievements and limitations of current prophylactic regimens. *Blood* 2015; **125:** 2038–44.

7 Skinner MW. WFH: closing the global gap--achieving optimal care. *Haemophilia* 2012; **18** (suppl 4): 1–12.

8 Iorio A, Iserman E, Blanchette V, et al. Target plasma factor levels for personalized treatment in haemophilia: a Delphi consensus statement. *Haemophilia* 2017; **23:** e170–79.

9 Nugent D, Kalnins W, Querol F, et al. Haemophilia experiences, results and opportunities (HERO) study: treatment-related characteristics of the population. *Haemophilia* 2015; **21:** e26–38.

10 Mahdi AJ, Obaji SG, Collins PW. Role of enhanced half-life factor VIII and IX in the treatment of haemophilia. *Br J Haematol* 2015; **169:** 768–76.

11 Powell JS, Pasi KJ, Ragni MV, et al. Phase 3 study of recombinant factor IX Fc fusion protein in hemophilia B. *N Engl J Med* 2013; **369:** 2313–23.

12 Mahlangu J, Powell JS, Ragni MV, et al. Phase 3 study of recombinant factor VIII Fc fusion protein in severe hemophilia A. *Blood* 2014; **123:** 317–25.

13 Collins PW, Young G, Knobe K, et al. Recombinant long-acting glycoPEGylated factor IX in hemophilia B: a multinational randomized phase 3 trial. *Blood* 2014; **124:** 3880–86.

14 Konkle BA, Stasyshyn O, Chowdary P, et al. Pegylated, full-length, recombinant factor VIII for prophylactic and on-demand treatment of severe hemophilia A. *Blood* 2015; **126:** 1078–85.

15 Santagostino E, Martinowitz U, Lissitchkov T, et al. Long-acting recombinant coagulation factor IX albumin fusion protein (rIX-FP) in hemophilia B: results of a phase 3 trial. *Blood* 2016; **127:** 1761–69.

16 Giangrande P, Andreeva T, Chowdary P, et al. Clinical evaluation of glycoPEGylated recombinant FVIII: efficacy and safety in severe haemophilia A. *Thromb Haemost* 2017; **117:** 252–61.

17 Reding MT, Ng HJ, Poulsen LH, et al. Safety and efficacy of BAY 94-9027, a prolonged-half-life factor VIII. *J Thromb Haemost* 2017; **15:** 411–19.

18 Pipe SW, Montgomery RR, Pratt KP, Lenting PJ, Lillicrap D. Life in the shadow of a dominant partner: the FVIII-VWF association and its clinical implications for hemophilia A. *Blood* 2016; **128:** 2007–16.

19 Königs C, Liesner R, Ozelo MC, et al. Incidence of inhibitors in previously untreated patients with severe haemophilia A treated with rFVIIIFc: the PUPs A-LONG study. *Haemophilia* 2019; **25:** 32.

20 Chan AK, Alamelu J, Barnes C, et al. Nonacog beta pegol (N9-GP) in hemophilia B: first report on safety and efficacy in previously untreated and minimally treated patients. *Res Pract Thromb Haemost* 2020; **4:** 1101–13.

21 Teitel JM. Treatment and prevention of bleeding in congenital hemophilia A patients with inhibitors. *Transfus Apheresis Sci* 2018; **57:** 466–71.

22 Oldenburg J, Mahlangu JN, Kim B, et al. Emicizumab prophylaxis in hemophilia A with inhibitors. *N Engl J Med* 2017; **377:** 809–18.

23 Pasi KJ, Rangarajan S, Georgiev P, et al. Targeting of antithrombin in hemophilia A or B with RNAi therapy. *N Engl J Med* 2017; **377:** 819–28.

24 Shapiro AD, Angchaisuksiri P, Astermark J, et al. Subcutaneous concizumab prophylaxis in hemophilia A and hemophilia A/B with inhibitors: phase 2 trial results. *Blood* 2019; **134:** 1973–82.

25 Cardinal M, Kantaridis C, Zhu T, et al. A first-in-human study of the safety, tolerability, pharmacokinetics and pharmacodynamics of PF-06741086, an anti-tissue factor pathway inhibitor mAb, in healthy volunteers. *J Thromb Haemost* 2018; **16:** 1722–31.

26 Gu JM, Zhao XY, Schwarz T, et al. Mechanistic modeling of the pharmacodynamic and pharmacokinetic relationship of tissue factor pathway inhibitor-neutralizing antibody (BAY 1093884) in cynomolgus monkeys. *AAPS J* 2017; **19:** 1186–95.

27 Kitazawa T, Igawa T, Sampei Z, et al. A bispecific antibody to factors IXa and X restores factor VIII hemostatic activity in a hemophilia A model. *Nat Med* 2012; **18:** 1570–74.

28 Sehgal A, Barros S, Ivanciu L, et al. An RNAi therapeutic targeting antithrombin to rebalance the coagulation system and promote hemostasis in hemophilia. *Nat Med* 2015; **21:** 492–97.

29 Hilden I, Lauritzen B, Sørensen BB, et al. Hemostatic effect of a monoclonal antibody mAb 2021 blocking the interaction between FXa and TFPI in a rabbit hemophilia model. *Blood* 2012; **119:** 5871–78.

30 Polderdijk SGI, Adams TE, Ivanciu L, Camire RM, Baglin TP, Huntington JA. Design and characterization of an APC-specific serpin for the treatment of hemophilia. *Blood* 2017; **129:** 105–13.

31 Nathwani AC, Tuddenham EG, Rangarajan S, et al. Adenovirus-associated virus vector-mediated gene transfer in hemophilia B. *N Engl J Med* 2011; **365:** 2357–65.

32 Nathwani AC, Reiss UM, Tuddenham EG, et al. Long-term safety and efficacy of factor IX gene therapy in hemophilia B. *N Engl J Med* 2014; **371:** 1994–2004.

33 Rangarajan S, Walsh L, Lester W, et al. AAV5-factor VIII gene transfer in severe hemophilia A. *N Engl J Med* 2017; **377:** 2519–30.

34 George LA, Sullivan SK, Giermasz A, et al. Hemophilia B gene therapy with a high-specific-activity factor IX variant. *N Engl J Med* 2017; **377:** 2215–27.

35 High KA, George LA, Eyster E, et al. A phase 1/2 trial of investigational SPK-8011 in hemophilia A demonstrates durable expression and prevention of bleeds. *Blood* 2018; **132** (suppl 1)**:** 487.

36 Miesbach W, Meijer K, Coppens M, et al. Gene therapy with adeno-associated virus vector 5-human factor IX in adults with hemophilia B. *Blood* 2018; **131:** 1022–31.

37 Pipe S, Giermasz A, Castaman G, et al. One-year data from a phase 2b trial of AMT-061 (AAV5-Padua hFIX variant), an enhanced vector for gene transfer in adults with severe or moderate-severe hemophilia B. *Blood* 2019; **134** (suppl 1)**:** 3348.

38 Pasi KJ, Rangarajan S, Mitchell N, et al. Multiyear follow-up of AAV5-hFVIII-SQ gene therapy for hemophilia A. *N Engl J Med* 2020; **382:** 29–40.

39 Nathwani AC, Reiss U, Tuddenham E, et al. Adeno-associated mediated gene transfer for hemophilia B: 8-year follow-up and impact of removing "empty viral particles" on safety and efficacy of gene transfer. *Blood* 2018; **132** (suppl 1)**:** 491.

40 Monahan PE, Walsh CE, Powell JS, et al. Update on phase 1/2 open-label trial of BAX335, an adeno-associated virus 8 (AAV8) vector-based gene therapy program for hemophilia B. *J Thromb Haemost* 2015; **13:** 87.

41 George LA, Sullivan SK, Rasko JEJ, et al. Efficacy and safety in 15 hemophilia B patients treated with the AAV gene therapy vector fidanacogene elaparvovec and followed for at least 1 year. *Blood* 2019; **134** (suppl 1)**:** 3347.

42 Miesbach W, Meijer K, Coppens M, et al. Stable FIX expression and durable reductions in bleeding and factor IX consumption for up to 4 years following AMT-060 gene therapy in adults with severe or moderate-severe hemophilia B. *Blood* 2019; **134** (suppl 1)**:** 2059.

43 Chowdary P, Shapiro S, Davidoff AM, et al. A single intravenous infusion of FLT180a results in factor IX activity levels of more than 40% and has the potential to provide a functional cure for patients with haemophilia B. *Blood* 2018; **132** (suppl 1)**:** 631.

44 Konkle B, Stine K, Visweshwar N, et al. Updated follow-up of the Alta Study, a phase 1/2, open label, adaptive, dose-ranging study to assess the safety and tolerability of SB-525 gene therapy in adult patients with severe hemophilia A. *Blood* 2019; **134** (suppl 1)**:** 2060.

45 Pipe S, Becka M, Detering E, Vanevski K, Lissitchkov T. First-in-human gene therapy study of AAVhu37 capsid vector technology in severe hemophilia A. *Blood* 2019; **134** (suppl 1)**:** 4630.

46 Nathwani AC, Tuddenham E, Chowdary P, et al. GO-8: preliminary results of a Phase I/II dose escalation trial of gene therapy for haemophilia A using a novel human factor VIII variant. *Blood* 2018; **132** (suppl 1)**:** 489.

47 Den Uijl IE, Mauser Bunschoten EP, Roosendaal G, et al. Clinical severity of haemophilia A: does the classification of the 1950s still stand? *Haemophilia* 2011; **17:** 849–53.

48 Ahlberg A. Haemophilia in Sweden. VII. Incidence, treatment and prophylaxis of arthropathy and other musculo-skeletal manifestations of haemophilia A and B. *Acta Orthop Scand Suppl* 1965; **36** (suppl 77)**:** 3–132.

49 Collins PW, Blanchette VS, Fischer K, et al. Break-through bleeding in relation to predicted factor VIII levels in patients receiving prophylactic treatment for severe hemophilia A. *J Thromb Haemost* 2009; **7:** 413–20.

50 Wu R, Luke KH, Poon MC, et al. Low dose secondary prophylaxis reduces joint bleeding in severe and moderate haemophilic children: a pilot study in China. *Haemophilia* 2011; **17:** 70–74.

51 Verma SP, Dutta TK, Mahadevan S, et al. A randomized study of very low-dose factor VIII prophylaxis in severe haemophilia - a success story from a resource limited country. *Haemophilia* 2016; **22:** 342–48.

52 Fischer K, Steen Carlsson K, Petrini P, et al. Intermediate-dose versus high-dose prophylaxis for severe hemophilia: comparing outcome and costs since the 1970s. *Blood* 2013; **122:** 1129–36.

53 Feldman BM, Rivard GE, Babyn P, et al. Tailored frequency-escalated primary prophylaxis for severe haemophilia A: results of the 16-year Canadian Hemophilia Prophylaxis Study longitudinal cohort. *Lancet Haematol* 2018; **5:** e252–60.

54 Nilsson IM, Berntorp E, Löfqvist T, Pettersson H. Twenty-five years' experience of prophylactic treatment in severe haemophilia A and B. *J Intern Med* 1992; **232:** 25–32.

55 Saxena K, Lalezari S, Oldenburg J, et al. Efficacy and safety of BAY 81-8973, a full-length recombinant factor VIII: results from the LEOPOLD I trial. *Haemophilia* 2016; **22:** 706–12.

56 Mahlangu J, Kuliczkowski K, Karim FA, et al. Efficacy and safety of rVIII-SingleChain: results of a phase 1/3 multicenter clinical trial in severe hemophilia A. *Blood* 2016; **128:** 630–37.

57 Lissitchkov T, Rusen L, Georgiev P, et al. PK-guided personalized prophylaxis with Nuwiq® (human-cl rhFVIII) in adults with severe haemophilia A. *Haemophilia* 2017; **23:** 697–704.

58 Lentz SR, Janic D, Kavakli K, et al. Long-term safety and efficacy of turoctocog alfa in prophylaxis and treatment of bleeding episodes in severe haemophilia A: final results from the guardian 2 extension trial. *Haemophilia* 2018; **24:** e391–94.

59 Mahlangu J, Young G, Hermans C, Blanchette V, Berntorp E, Santagostino E. Defining extended half-life rFVIII-A critical review of the evidence. *Haemophilia* 2018; **24:** 348–58.

60 Wiley RE, Khoury CP, Snihur AWK, et al. From the voices of people with haemophilia A and their caregivers: challenges with current treatment, their impact on quality of life and desired improvements in future therapies. *Haemophilia* 2019; **25:** 433–40.

61 Valentino LA, Ewenstein B, Navickis RJ, Wilkes MM. Central venous access devices in haemophilia. *Haemophilia* 2004; **10:** 134–46.

62 Konkle BA, Ebbesen LS, Erhardtsen E, et al. Randomized, prospective clinical trial of recombinant factor VIIa for secondary prophylaxis in hemophilia patients with inhibitors. *J Thromb Haemost* 2007; **5:** 1904–13.

63 Leissinger C, Gringeri A, Antmen B, et al. Anti-inhibitor coagulant complex prophylaxis in hemophilia with inhibitors. *N Engl J Med* 2011; **365:** 1684–92.

64 Morfini M, Haya S, Tagariello G, et al. European study on orthopaedic status of haemophilia patients with inhibitors. *Haemophilia* 2007; **13:** 606–12.

65 Mahlangu J, Oldenburg J, Callaghan MU, et al. Health-related quality of life and health status in persons with haemophilia A with inhibitors: a prospective, multicentre, non-interventional study (NIS). *Haemophilia* 2019; **25:** 382–91.

66 Young G, Liesner R, Chang T, et al. A multicenter, open-label phase 3 study of emicizumab prophylaxis in children with hemophilia A with inhibitors. *Blood* 2019; **134:** 2127–38.

67 Mahlangu J, Oldenburg J, Paz-Priel I, et al. Emicizumab prophylaxis in patients who have hemophilia A without inhibitors. *N Engl J Med* 2018; **379:** 811–22.

68 Pipe SW, Shima M, Lehle M, et al. Efficacy, safety, and pharmacokinetics of emicizumab prophylaxis given every 4 weeks in people with haemophilia A (HAVEN 4): a multicentre, open-label, non-randomised phase 3 study. *Lancet Haematol* 2019; **6:** e295–305.

69 Shima M, Nogami K, Nagami S, et al. A multicentre, open-label study of emicizumab given every 2 or 4 weeks in children with severe haemophilia A without inhibitors. *Haemophilia* 2019; **25:** 979–87.

70 Pasi KJ, Lissitchkov T, Georgiev P, et al. Fitusiran, an RNAi therapeutic targeting antithrombin to restore hemostatic balance in hemophilia: interim analysis from the open-label extension study. *Res Pract Thromb Haemost* 2019; **3** (suppl 1)**:** 86.

71 Shima M, Hanabusa H, Taki M, et al. Factor VIII-mimetic function of humanized bispecific antibody in hemophilia A. *N Engl J Med* 2016; **374:** 2044–53.

72 Eichler H, Angchaisuksiri P, Kavakli K, et al. Concizumab restores thrombin generation potential in patients with haemophilia: pharmacokinetic/pharmacodynamic modelling results of concizumab phase 1/1b data. *Haemophilia* 2019; **25:** 60–66.

73 Barg AA, Avishai E, Budnik I, et al. Emicizumab prophylaxis among infants and toddlers with severe hemophilia A and inhibitors-a single-center cohort. *Pediatr Blood Cancer* 2019; **66:** e27886.

74 Zimowski KL, Batsuli GM, Bryant P, et al. Severe bleeding events in hemophilia A patients receiving emicizumab prophylaxis. *Blood* 2019; **134** (suppl 1)**:** 1126.

75 Ebbert PT, Xavier F, Seaman CD, Ragni MV. Emicizumab prophylaxis in patients with haemophilia A with and without inhibitors. *Haemophilia* 2020; **26:** 41–46.

76 Santagostino E, Mancuso ME, Novembrino C, Solimeno LP, Tripodi A, Peyvandi F. Rescue factor VIII replacement to secure hemostasis in a patient with hemophilia A and inhibitors on emicizumab prophylaxis undergoing hip replacement. *Haematologica* 2019; **104:** e380–82.

77 World Federation of Hemophilia. Alnylam suspends fitusiran dosing due to thrombotic event in phase 2 open-label extension study. Sept 7, 2017. https://news.wfh.org/alnylam-suspends-fitusiran-dosing-due-thrombotic-event-phase-2-open-label-extension-study/ (accessed Dec 1, 2020).

78 World Federation of Hemophilia. Update: FDA lifts suspension of fitusiran trial. Dec 19, 2017. https://news.wfh.org/update-fda-lifts-suspension-fitusiran-trial/ (accessed Dec 1, 2020).

79 Ferrante F, Ingham S, Kunze M, Michaels LA. Anti-TFPI antibody BAY1093884: early termination of phase II dose escalation study due to thrombosis. *Haemophilia* 2020; **26:** 77–78.

80 Figueiredo M. Novo Nordisk pauses 3 clinical trials of concizumab amid safety concerns. March 18, 2020. https://hemophilianewstoday.com/2020/03/18/novo-nordisk-pauses-three-clinical-trials-of-concizumab-due-to-safety-concerns/ (accessed Dec 1, 2020).

81 Adcock DM, Strandberg K, Shima M, Marlar RA. Advantages, disadvantages and optimization of one-stage and chromogenic factor activity assays in haemophilia A and B. *Int J Lab Hematol* 2018; **40:** 621–29.

82 Adamkewicz JI, Chen DC, Paz-Priel I. Effects and interferences of emicizumab, a humanized bispecific antibody mimicking activated factor VIII cofactor function, on coagulation assays. *Thromb Haemost* 2019; **119:** 1084–93.

83 Leksa NC, Aleman MM, Goodman AG, Rabinovich D, Peters R, Salas J. Intrinsic differences between FVIIIa mimetic bispecific antibodies and FVIII prevent assignment of FVIII-equivalence. *J Thromb Haemost* 2019; **17:** 1044–52.

84 Lenting PJ, Denis CV, Christophe OD. Emicizumab, a bispecific antibody recognizing coagulation factors IX and X: how does it actually compare to factor VIII? *Blood* 2017; **130:** 2463–68.

85 Samuelson Bannow B, Recht M, Négrier C, et al. Factor VIII: long-established role in haemophilia A and emerging evidence beyond haemostasis. *Blood Rev* 2019; **35:** 43–50.

86 Konkle BA, Shapiro A, Quon D, et al. BIVV001 fusion protein as factor VIII replacement therapy for hemophilia A. *N Engl J Med* 2020; **383:** 1018–27.

87 Mahlangu J, Levy H, Negrier C, et al. Phase 2b trial to evaluate the safety and factor IX levels resulting from a daily subcutaneous prophylaxis treatment regimen of dalcinonacog alfa (DalcA) in haemophilia B. *Haemophilia* 2020; **26:** 23.

88 Gruppo RA, Malan D, Kapocsi J, et al. Phase 1, single-dose escalating study of marzeptacog alfa (activated), a recombinant factor VIIa variant, in patients with severe hemophilia. *J Thromb Haemost* 2018; **16:** 1984–93.

89 Stanford S, Pink R, Creagh D, et al. Adenovirus-associated antibodies in UK cohort of hemophilia patients: a seroprevalence study of the presence of adenovirus-associated virus vector-serotypes AAV5 and AAV8 neutralizing activity and antibodies in patients with hemophilia A. *Res Pract Thromb Haemost* 2019; **3:** 261–67.

90 Rajavel K, Ayash-Rashkovsky M, Tang Y, Gangadharan B, de la Rosa M, Ewenstein B. Co-prevalence of pre-existing immunity to different serotypes of adeno-associated virus (AAV) in adults with hemophilia. *Blood* 2019; **134** (suppl 1)**:** 3349.

91 Ertl HCJ, High KA. Impact of AAV capsid-specific T-cell responses on design and outcome of clinical gene transfer trials with recombinant adeno-associated viral vectors: an evolving controversy. *Hum Gene Ther* 2017; **28:** 328–37.

92 Zolotukhin I, Markusic DM, Palaschak B, Hoffman BE, Srikanthan MA, Herzog RW. Potential for cellular stress response to hepatic factor VIII expression from AAV vector. *Mol Ther Methods Clin Dev* 2016; **3:** 16063.

93 Nowrouzi A, Penaud-Budloo M, Kaeppel C, et al. Integration frequency and intermolecular recombination of rAAV vectors in non-human primate skeletal muscle and liver. *Mol Ther* 2012; **20:** 1177–86.

94 Cong L, Zhang F. Genome engineering using CRISPR-Cas9 system. *Methods Mol Biol* 2015; **1239:** 197–217.