THERAPEUTIC ADVANCES in
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Review

Gene therapy in haemophilia: literature review and regional perspectives for Turkey

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***Abstract*:** Haemophilia is an X-linked lifelong congenital bleeding disorder that is caused by insufficient levels of factor VIII (FVIII; haemophilia A) or factor IX (FIX; haemophilia B) and characterized by spontaneous and trauma-related bleeding episodes. The cornerstone of the treatment, factor replacement, constitutes several difficulties, including frequent injections due to the short half-life of recombinant factors, intravenous administration and the risk of inhibitor development. While extended half-life factors and subcutaneous novel molecules enhanced the quality of life, initial successes with gene therapy offer a significant hope for cure. Although adeno-associated viral (AAV)-based gene therapy is one of the most emerging approaches for treatment of haemophilia, there are still challenges in vector immunogenicity, potency and efficacy, genotoxicity and persistence. As the approval for the first gene therapy product is coming closer, eligibility criteria for patient selection, multidisciplinary approach for optimal delivery and follow-up and development of new pricing policies and reimbursement models should be concerned. Therefore, this review addresses the unmet needs of current haemophilia treatment and explains the rationale and principles of gene therapy. Limitations and challenges are discussed from a global and national perspective and recommendations are provided to adopt the gene therapies faster and more sufficient for the haemophilia patients in developing countries like Turkey.

Keywords: Hemophilia-A, Hemophilia-B, gene therapy, AAV vectors

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Introduction

Haemophilia A and B are rare, X-linked inherited bleeding disorders caused by mutations in the *F8* and *F9* genes, resulting in missing or reduced production/function of clotting factor VIII (FVIII; haemophilia A) and clotting factor IX (FIX; haemophilia B), respectively.[1] The current global incidence of haemophilia A is estimated at 1:5000 and haemophilia B is 1:30,000 male live births.[2] Therefore, the expected worldwide number of patients with haemophilia is 1,125,000, of whom 418,000 should have severe haemophilia.[3]

Patients with severe haemophilia have a plasma FVIII or FIX activity less than 1%, resulting in spontaneous or posttraumatic bleeding, or both, into joints and other tissues, which cause morbidity and mortality.[1,4] The cornerstone of the treatment is the replacement therapy with intravenous injections of FVIII and FIX concentrates, either episodically to treat acute bleedings or prophylactically to prevent them.[5] Existing therapies for haemophilia have been summarized in Figure 1. Long-term prophylaxis has been shown to be very effective and is now accepted as the standard of care.[6,7] Due to the terminal half-life of traditional factor replacement, frequent injections are needed. This may be burdensome and costly for patients and healthcare systems, which consequently causes poor compliance and globally limited access to therapy for patients.[8]

Bioengineered extended half-life clotting factors produced by fusion techniques and covalent binding to polyethylene glycol (PEG) can reduce the burden of treatment.[9,10] However, replacement therapy is still associated with a risk for inhibitor development that reduces the effect of

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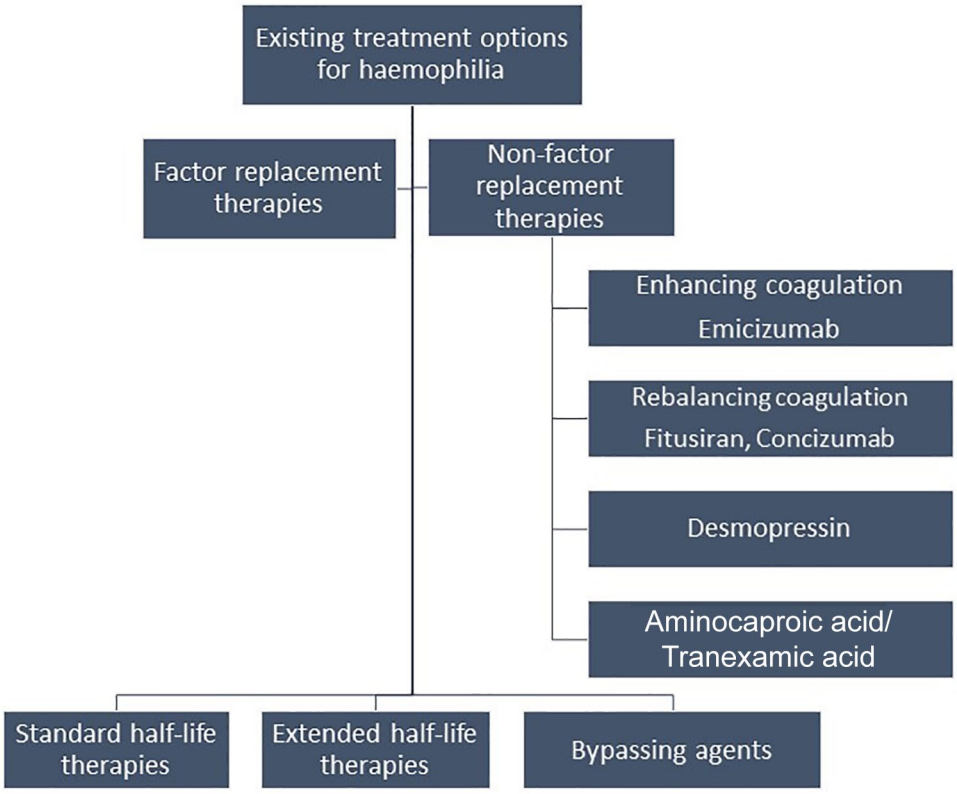

Figure 1. Existing treatment options for haemophilia.

bleeding prevention.[11] Therefore, subcutaneously delivered novel molecules provide effective prophylaxis in the presence or absence of inhibitors, either substituting for the procoagulant function of clotting factors or targeting the natural inhibitors of coagulation.[11–13] Both approaches have shown efficacy in reducing the rate of bleeding, but their usage may be limited by the risk of thrombogenicity, and both still require lifelong injections without restoring normal haemostasis.[12] Hence, there is a need for phenotypical cure that may be achievable with gene therapy, which is currently in progress with many in-human clinical trials; however, there is still a significant heterogeneity in the levels of clotting factor expression.[14]

Although gene therapies will be available in the near future, accurate and in-depth knowledge on this emerging treatment among the healthcare teams and scientists is still lacking. There are currently 409 known haemophilia centres in Europe. Comprehensive care for haemophilia is a multidisciplinary approach to the treatment of haemophilia. Patients can access comprehensive care services in one place: either a European Haemophilia Treatment Centre (HTC) or a European Comprehensive Care Centre (CCC).[15] The criteria for being certified as either of these types of haemophilia centres can be found in the European guidelines for the certification of haemophilia centres in Europe. Emerging novel therapies, particularly gene therapy, will require adjustments in these treatment centres as well as establishment of 'hub and spoke model' with long-term safety and efficacy surveillance systems.[16] Moreover, gene therapy was likely to be cost-effective compared with on-demand treatment and prophylaxis for patients with severe haemophilia, whereas the annual costs exceed $100,000 per patient.[17,18]

In this review article, we aimed to

- Summarize the current therapies for haemophilia addressing unmet needs and explaining the rationale of gene therapy and general principles of gene therapy.

- Review preclinical studies and clinical trials for gene therapy for haemophilia and provide a historical journey with new improvements and advancements.
- Assess the clinical implications of gene therapy, necessity and benefits of data collection, infrastructure, opportunities and challenges for gene therapy launching.
- Provide specific recommendations for future treatment landscape in Turkey and developing countries.

Rationale of gene therapy for haemophilia

Haemophilia treatment has a long journey of efficient translation of protein biochemistry and application of molecular biology to patient care.[8] As gene therapy provides a functional copy of the disease-causing gene that is either absent or expressed as a nonfunctional protein, haemophilia is an optimal target for gene therapy due to the monogenic nature of inheritance. Cloning of the *F8* and *F9* genes led to the production of recombinant clotting factors as well as initiated the gene therapy efforts to potentially cure the disease.[19–21] During its biosynthesis, mature FVIII and FIX are secreted into the circulation and studies have demonstrated that small amount of transgenic factors in a fraction of hepatocytes could substantially decrease bleeding.[22] Moreover, gene therapy for haemophilia allows for a wide therapeutic range of FVIII and FIX, and clinical experience and natural history studies revealed that even modest increases in clotting factor activity can dramatically improve the clinical outcome.[21,23] The presence of well-characterized murine and canine haemophilia models has also empowered broad preclinical research for gene therapy.[8,24] Finally, challenges of intravenous factor administration in young patients and lack of access to factor concentrates resulting from inadequate factor supply in developing countries highlight the possible benefit of providing a continuous source of clotting factor by a single gene therapy administration.[21,22]

Evolution of AAV vector gene therapy for haemophilia

Gene therapy is the re-establishment of cellular function by transferring or editing genetic material that aims to cure a disease. Depending on the chosen delivery strategy, gene therapy can be performed *in vivo* or *ex vivo* several different vectors such as lentiviral or non-AAV vectors.[25]

The leading approach for gene transfer in patients with haemophilia is the liver-directed delivery of *F8* or *F9* genes by recombinant AAV vectors.[26,27] These vectors can transfer therapeutic genes into postmitotic tissues, such as the liver, through cellular tropism determined by their protein capsids. To avoid the genotoxicity of the more integrating vectors, AAVs are considered in the most recent clinical trials. AAV gene therapy clinical trials for both haemophilia A and B are listed in Table 1.

Table 1. AAV gene therapy clinical trials for haemophilia A and B.

Disease type	NCT	Investigational medical product	Dose (vg/kg)	Phase	Number of patients	Trial status	Up-to-date outcome	Reference
Haemophilia B	NCT00979238	scAAV2/8-LP1-hFIXco	2×10^{11} 6×10^{11} 2×10^{12}	1	14	Active, not recruiting	Mean FIX activity 2–11% at 6–16 months postinfusion Mean FIX activity 1–6% up to 3.2 years postinfusion Mean FIX activity levels 1.9 ± 0.6, 2.3 ± 0.3 and 5.1 ± 1.4 IU/l in three different dose cohorts up to 8 years	Yen *et al.*,[24] Buchlis *et al.*,[28] Manno *et al.*[29]
	NCT01687608	AAV8sc-TTR-FIXco-Padua (AskBio009)	2×10^{11} 1×10^{12} 3×10^{12}	1,2	30	Active, not recruiting	Only one participant achieved sustained FIX activity of ~20% at 4 years postinfusion	Von Drygalski *et al.*,[30] Miesbach *et al.*[31]

(Continued)

Table 1. (Continued)

Disease type	NCT	Investigational medical product	Dose (vg/kg)	Phase	Number of patients	Trial status	Up-to-date outcome	Reference
	NCT02396342	AAV5-hFIXco (AMT-060)	5×10^{12} 2×10^{13}	1,2	10	Completed	Mean FIX activity was increased to 4.4 IU/dl and 6.9 IU/dl at 1-year postinfusion	Nathwani *et al.*[32]
	NCT03489291	AAV5-FIXco-Padua (AMT-061)	2×10^{13}	2	3	Active, not recruiting	Mean FIX activity 31% at week 6, 47% at week 26 postinfusion	Monahan *et al.*[33]
	NCT03569891	AAV5-FIXco-Padua (AMT-061)	2×10^{13}	3	56	Active, not recruiting	Mean FIX activity 36.9% and 39% at 18 and 6 months postinfusion	Crudele *et al.*[34]
	NCT02484092	AAV-SPARK100-FIXco-Padua (SPK-9001)	5×10^{11}	2	15	Completed	Mean steady-state FIX activity 35.5 $\pm$ 18.7% at week 14	Konkle *et al.*[35]
	NCT03307980	AAV-SPARK100-FIXco-Padua (PF-06838435, formerly SPK-9001)	SPK-9001 extension study	2	20	Recruiting	Mean steady-state FIX activity 22.9 $\pm$ 9.9% at 1-year postinfusion	Weber *et al.*[36]
	NCT03861273	AAV-SPARK100-FIXco-Padua (Fidanacogene elaparvovec, PF-06838435, formerly SPK-9001)	NA	3	55	Recruiting	NA	NA
	NCT03369444	AAVS3-FIXco-Padua (FLT180a)	6×10^{11} 2×10^{12}	1	18	Recruiting	Mean FIX activity $>$ 40% at week 12 postinfusion	George *et al.*[37]
	NCT03641703	AAVS3-FIXco-Padua (FLT180a)	FLT180a extension study	2,3	50	Active, not recruiting	NA	NA
Haemophilia A	NCT02576795	AAV5-FVIII-BDD (Valoctocogene roxaparvovec, BMN-270)	6×10^{12} 2×10^{13} 6×10^{13}	1,2	15	Active, not recruiting	Mean FVIII activity 77 IU/dl at week 52 postinfusion (high-dose cohort) Mean FVIII activity 20 IU/dl 3 years postinfusion (high-dose cohort) Median FVIII levels $>$ 5 IU/dl 5 years postinfusion (high-dose cohort)	ClinicalTrials.gov.,[38] Park *et al.*,[39] Rangarajan *et al.*[40]
	NCT03392974	AAV5-FVIII-BDD (Valoctocogene roxaparvovec, BMN-270)	4×10^{13}	3	1	Active, not recruiting	NA	NA
	NCT03370913	AAV5-FVIII-BDD (Valoctocogene roxaparvovec, BMN-270)	6×10^{13}	3	134	Active, not recruiting	Mean FVIII activity had increased by 41.9 IU/dl 49 through 52 weeks postinfusion	Pasi *et al.*[41]
	NCT03520712	AAV5-FVIII-BDD (Valoctocogene roxaparvovec, BMN-270)	6×10^{13}	1,2	10	Enrolling by invitation	NA	NA

(Continued)

Table 1. (Continued)

Disease type	NCT	Investigational medical product	Dose (vg/kg)	Phase	Number of patients	Trial status	Up-to-date outcome	Reference
	NCT04323098	AAV5-FVIII-BDD (Valoctocogene roxaparvovec, BMN-270)	6×10^{13}	3	20	Recruiting	NA	NA
	NCT03003533	AAV-SPARK200-FVIII-BDD (SPK-8011)	5×10^{11} 1×10^{12} 2×10^{12}	1,2	50	Recruiting	Mean FVIII activity 12.9 ± 6.9% of the normal value at 26–52 weeks when the participants were not receiving glucocorticoids *versus* 12.0 ± 7.1% of the normal value at >52 weeks postinfusion	Yilmaz *et al.*[25]
	NCT03432520	AAV-SPARK200-FVIII-BDD (SPK-8011)	SPK-8011 extension study	1,2	40	Enrolling by invitation		
	NCT03734588	SPK-8016	Dose-finding pre-FVIII inhibitor study	1,2	30	Active	FVIII activity levels increased to 5.9–21.8% and remain stable for more than a year after a single 5×10^{11} vg/kg dose of SPK-8016	Pasi *et al.*[42]
	NCT03001830	AAV2/8-HLP-FVIII-V3	6×10^{11} 2×10^{12} 6×10^{12}	1	18	Recruiting	Factor VIII activity has remained stable at 7 ± 1 IU/dl in patient 1 over a period of 47 weeks (6×10^{11} vg/kg dose cohort). The second participant has steady state at 20 weeks postinfusion with FVIII activity of 6 ± 2 IU/dl. In the third subject, the steady-state FVIII activity was 69 ± 7 IU/dl (both in 2×10^{12} vg/kg dose cohort).	Ozelo *et al.*[43]
	NCT03061201	AAV2/6-FVIII-BDD (SB-525, PF-07055480)	9×10^{11} 2×10^{12} 1×10^{13} 3×10^{13}	1,2	11	Active	FVIII levels within the normal range, with no bleeding events reported up to 24 weeks postinjection (in 3×10^{13} vg/kg dose cohort). Mean FVIII activity maintained in the mild to normal range through 104 weeks postinfusion	Sullivan *et al.*,[44] Nathwani *et al.*[45]
	NCT04370054	AAV2/6-FVIII-BDD (SB-525, PF-07055480)		3	63	Recruiting	NA	NA
	NCT03370172	AAV8-FVIII-BDD (BAX888)	2×10^{12} 6×10^{12} 1.2×10^{13}	1,2	12	Active	NA	NA
	NCT03588299	BAY2599023 (DTX201)	N/A	1,2	30	Recruiting	FVIII expression ~5% and ~17% in two patients at the starting dose of 0.5×10^{13} vg/kg	Konkle *et al.*[46]

AAV, adeno-associated viral; FIX, factor IX; FVIII, factor VIII.

Haemophilia B

Due to the limited packaging capacity of the AAV (~4.7 kb), initial studies were planned for haemophilia B as the *F9* cDNA is 1.6 kb in size.[20] The first in-human AAV study utilized an AAV2-FIX vector [2×10^{11}–1.8×10^{12} vector genome copies (vg)/kg], administered by intramuscular injection with transient low-level FIX expression (<2%).[47] Although this injection did not lead to a permanent transgene expression in the plasma, there was a FIX-AAV persistence (AAV-FIX DNA or FIX expression) in the muscles in two patients.[28] Thereafter, this vector administered by hepatic artery injection (8×10^{10}–2×10^{12} vg/kg) in seven patients and one patient treated with the high dose of the vector (2×10^{12} vg/kg) had a transient FIX expression (peak FIX:C 11%).[29] Loss of FIX expression was coincidentally seen in this patient with a transient asymptomatic transaminitis 4 weeks after the injection.

The first successful AAVFIX-based gene therapy trial (NCT00979238) administered recombinant AAV8–containing codon-optimized (co) FIX (scAAV2/8-LP1-hFIXco) to six severe haemophilia B patients in three different doses.[26] FIX expression was at 2–11% of normal levels in all participants that was sufficient to improve the bleeding phenotype. Two participants in the high dose cohort had a transient, asymptomatic elevation of serum aminotransferase levels, which was associated with capsid-mediated cellular immunity in one of them. Transaminase levels were normalized with a short course of corticosteroid therapy and FIX levels were maintained in the range of 3–11% of normal values.[26] In the long-term study, 10 patients in the high-dose cohort had a constant increase in FIX levels that led to more than 90% decrease in both frequency of the bleeding episodes and usage of prophylactic factor concentrates. Transaminitis occurred in four patients between week 7 and 10, but became normal after prednisolone treatment.[32] Dose-dependent expression without any late toxicity has been observed up to 8-year follow-up.[48]

A multinational, open-label study administered a single dose of AAV5 vector with human *FIX* gene, AAV5-co-FIX (AMT-060, NCT02396342) in two different dose cohorts to 10 adults with moderate/severe haemophilia B (FIX ⩽ 2% of normal). Annual FIX usage was reduced by 81% and 73%, whereas mean annual bleeding rate (ABR) had a 53% and 70% decrease in the low and high dose cohorts, respectively. Asymptomatic transient transaminitis, in one patient in the low-dose and two patients in the high-dose cohort, was successfully treated with prednisolone.[49] To increase the FIX expression six- to eightfold, a naturally occurring gain-of-function single nucleotide variation (R338 L, FIX-Padua), Padua transgene was used in the following preclinical studies.[33,34] Thereafter, to obtain higher expression, the AMT-060 FIX transgene was changed with Padua transgene as AAV5-hFIXco-Padua (AMT-061, etranacogene dezaparvovec). A single dose of 2×10^{13} vg/kg AMT-061 led to complete bleed cessation without a need for FIX replacement up to 26 weeks (NCT03489291).[30] According to that, an expanded evaluation of the AMT-061 in the multinational HOPE-B (Health Outcomes With Padua Gene; Evaluation in Haemophilia-B) phase III trial (NCT03569891) is ongoing. Results revealed durable, sustained increases in FIX activity at 18 months postinfusion.[31] These encouraging results illustrate the potential for a regulatory approval.

A phase I/II, open-label dose-escalation study investigated BAX 335 (AskBio009, AAV8.sc-TTR-FIXR338Lopt), an AAV8-based FIX Padua gene therapy, in patients with haemophilia B (NCT01687608). Eight adult male participants were involved in three different dose cohorts. One participant achieved sustained therapeutic FIX activity of ~20%, without bleeding or replacement therapy for 4 years; in others, FIX activity was not sustained beyond 5–11 weeks. In contrast to some previous studies, corticosteroid treatment did not stabilize FIX activity loss.[35,36]

On the contrary, 10 moderate/severe haemophilia B (FIX ⩽ 2%) patients were injected a single-stranded AAV vector with a bioengineered capsid (AAV-Spark100) with a FIX Padua transgene (SPK-9001) at a dose of 5×10^{11} vg/kg (NCT02484092). Continuous expression of FIX activity was achieved and allowed to stop baseline prophylaxis and led to a significant decrease in bleedings and factor usage.[37] Long-term follow-up (⩾5 years) is ongoing (NCT03307980). PF-06838435 (Fidanacogene elaparvovec, former SPK-9001) was well tolerated in 15 patients with no serious adverse events. All patients showed a significant reduction in bleeding frequency and exogenous FIX use at 52 weeks after

injection.[50] A phase III, open-label study with PF-06838435 (BENEGENE-2) is recruiting (NCT03861273).

Early data have recently been presented from a phase I/II study for FLT-180a (NCT03369444), using AAV-co-FIX-Padua for two participants at a dose of 4.5×10^{11} vg/kg. This single infusion sustained FIX levels over 40% with a reduced risk of spontaneous or traumatic bleeds.[51] A long-term follow-up study is also ongoing (NCT03641703). Moreover, ECLIPSE study that has a screening/observational protocol will be recruiting in four Turkish centres (NCT04272554). This study aims to collect prospective data to characterize bleeding events and FIX concentrate consumption in haemophilia B patients as a baseline and to screen participants for antibodies against a novel AAV vector to assess eligibility for a further Freeline gene therapy study.[38]

Genome editing tools, including zinc-finger nucleases (ZFNs), homing endonucleases (meganucleases), transcription activator–like effector-based nucleases (TALENs), and the clustered regularly interspaced short palindromic repeats (CRISPR)-Cas systems, were developed to induce a double-strand break at specific genomic loci that is subsequently resolved by cellular DNA repair pathways.[25] It was first explored in haemophilia B patients in whom AAV6 was used to deliver ZFNs and a promoterless FIX transgene, targeting the albumin locus. However, the phase I study was terminated after enrolling the first patient, for an unknown reason (NCT02695160).[38]

Haemophilia A

Clinical studies in haemophilia A have been slower because of the larger size of *F8* cDNA (7 kb), although there is a higher global prevalence. As the DNA packaging size of recombinant AAVs is limited to $\leqslant$ 5 kb, truncation of the *F8* cDNA, removing the sequence encoding nonfunctional domain (B-domain deletion, BDD), has allowed incorporation into AAV vectors.[39] The first successful application of this approach was reported in 2017, using a single intravenous dose of an AAV5 vector encoding a BDD *F8* (AAV5-hFVIII-SQ) in nine patients in three dose cohorts (BMN270, Valoctocogene Roxaparvovec, NCT02576795).[40] Within the high dose cohort, normalization of FVIII activity was sustained over 1 year with stabilization of haemostasis and a profound reduction in prophylactic FVIII usage.[40] Up to 5 years follow-up, there was a constant clinical improvement in higher dose cohorts.[41,42] Phase III open-label study in patients with residual FVIII levels $\leqslant$1 IU/dl and under prophylactic FVIII infusions is still ongoing (NCT03392974). BMN270 treatment provided endogenous factor VIII production and significantly reduced bleeding and need for factor VIII concentrate usage in severe haemophilia A patients (NCT03370913).[43] In the meanwhile, a phase I/II safety and efficacy study in patients with preexisting antibodies against AAV5 is recruiting by invitation (NCT03520712). An ongoing phase III clinical study will evaluate the safety and effectiveness of BMN270 in combination with prophylactic corticosteroids (GENEr8-3; NCT04323098). The patent owner company has done a licence application for valoctocogene roxaparvovec gene therapy for severe haemophilia A on August 2020; however, FDA recommended 2-year observation for ABR as a primary endpoint for ongoing phase III study. Approval may be obtained in late 2022.

A phase I/II study of SPK-8011, a bioengineered AAV capsid expressing BDD-FVIII in 12 men with severe ($n = 11$) or moderately severe ($n = 1$) haemophilia used three different dose cohorts (NCT03003533). There was a 97% reduction both in annual bleeding and infusion rates at 12 weeks after injection.[27] Further data are expected from an extension study (NCT03432520). Preliminary findings from a phase I/II trial of a single of 5×10^{11} vg/kg dose of SPK-8016 revealed stable and durable FVIII activity with a safety profile supporting further evaluation at a very low vector dose (NCT03734588).[44]

Early results of an ongoing open-label phase I/II dose escalation study of AAV8-HLP-hFVIII-V3 have been reported in three adult men (FVIII activity levels $\leqslant$ 1% of normal) (NCT03001830, GO-8). Transgenic FVIII levels were more than 5 IU/dl in all subjects with normalization in one patient.[45]

ALTA study is an ongoing, phase I/II, dose-ranging study to assess the safety and tolerability of SB-525 (PF-07055480), a liver-tropic rAAV6 vector carrying a BDD *F8* gene in four different dose groups (NCT03061201). Preliminary report revealed dose-dependent and sustained increases in FVIII levels, with a substantial decrease in FVIII usage, and no bleeding episodes recorded

in the highest dose cohort.[46] Updated follow-up revealed that four patients in the highest dose cohort had maintained their mean FVIII activity levels in the mild to normal range through week 104.[52] A long-term follow-up study has been recently placed on clinical hold by Federal Drug Administration (FDA) until the review of a proposed protocol amendment of the ongoing phase III study (NCT04370054, AFFINE). This study has been recruiting in four Turkish centres.

BAY 2599023 (AAVhu37FVIII), a nonreplicating AAV vector, is based on the AAV serotype hu37 and encoding BDD *F8*. Preliminary report of the phase I/II open-label, first time in human dose-finding study (NCT03588299) showed that two patients had measurable expression of endogenous FVIII and an early read-out of haemostatic efficacy at the starting dose of 0.5×10^{13} vg/kg.[53]

An open-label, multicenter, phase I/II study of the safety and dose escalation of BAX 888, an AAV8 vector expressing BDD-FVIII in severe haemophilia A patients (NCT03370172), is ongoing.

In terms of genome editing, *in vivo* genome targeting of the human transgene into the albumin locus by CRISPR/Cas9 led to human FVIII production in the liver and ameliorated severe haemophilia A phenotype in mice.[54] Most recently, lipid nanoparticle (LNP)-mediated delivery of Cas9 mRNA along with single guide RNA targeting antithrombin (AT) resulted in the inhibition of AT and improvement in thrombin generation in the mouse liver. Bleeding-associated phenotypes were recovered in both haemophilia A and B mice.[55]

A multinational phase I/II study of SHP648, an AAV vector for gene transfer in haemophilia B subjects involving Turkey, has been suspended due to the re-evaluation of the development strategy (NCT04394286).

Challenges for haemophilia gene therapy

Global perspectives

Although there is a recent progress in the field of gene therapy for haemophilia, there are still some obstacles to overcome to obtain better efficacy without any toxicities.

Pre-existing immunity for the AAV vectors can be a deterrent for the gene therapy eligibility. Although there is not any symptomatic clinical infection, immunological confirmation of past exposure to AAV can be seen in 30–80% of subjects depending upon the AAV serotype, age, sex and geographical location.[56,57] Global seroprevalence was 58.5% for AAV2, 34.8% for AAV5, 48.7% for AAV6, 45.6% for AAV8, and 46.0% for AAVrh10 in haemophilia A patients $\geqslant$ 12 years of age.[58] A study from the UK reported the prevalence of neutralizing antibodies as 23%, 35% and 18% for AAV-LK03, AAV3B and AAV8, respectively, with the lowest seroprevalence between 3 and 17 years of age for all serotypes.[59] Most of the studies showed that the presence of anti-AAV antibodies, even at low titers, can significantly diminish AAV vector delivery.[60] However, AAV5-mediated FIX gene transfer has shown successful transgene expression, while participants had pre-existing anti-AAV5 antibodies.[61]

Due to the robust and long-lasting immune response against the capsid, AAV vector re-administration is currently not possible. A novel strategy for overcoming this obstacle is the use of the IgG endopeptidase, Imlifidase (IdeS). In the preclinical studies, IdeS remarkably increased vector delivery to the liver even in the presence of neutralizing antibodies.[62] This might be an opportunity for overcoming the immune responses and readministration.

Early transient liver toxicity may occur in approximately 60% of patients between 4 and 12 weeks after vector delivery.[26,40] In most cases, this is a combination of mild/moderate increase in serum transaminase levels due to the death of transduced hepatocytes and a fall or loss of expression in the plasma level of the transgenic protein expression and cytotoxic T-cell response against AAV capsid.[60] Although short course of corticosteroids is often successful to save transgene expression and bring the enzymes back to normal levels, it is not obvious that what proportion of patients will require high-dose steroids as well as the duration of steroid administration is individually variable. Therefore, increased risk of steroid-associated side effects should also be cautiously considered.[26,32,37,40] Moreover, further studies are needed to clarify whether short-term immunosuppression with steroids will be effective at higher vector doses.

Duration and level of transgene expression are another concern for the outcome of the

gene therapy products. Human FIX gene therapy studies showed long-term liver-mediated expression up to 10 years with a minimal decline in plasma FIX levels.[48] Haemophilia A trials have still been gathering data, while the results of the longest trial using an AAV5 vector has shown a decline in FVIII levels over the first 4 years, levels of FVIII are still preventing bleeding.[41] Rather than durability, there is a significant variability in the levels of individual plasma level of transgenic protein. In human trials to date, there is a remarkable heterogeneity in these factor levels even with the same vector dose given.[41] It is obvious that even levels of native FVIII and FIX have a normal population variability of fourfold and that the balance and complexity of the production, secretion and clearance of these proteins are not completely understood.[60] Recent studies have shown that FVIII is synthesized in endothelial cells, whereas hepatocytes produce no detectable FVIII-likely attribute to its molecular chaperon von Willebrand factor (vWF), which is synthesized exclusively in endothelial cells and megakaryocytes.[63] However, hepatocyte is the only cell type that biochemically produces functional FIX *in vivo*.[64] This may also have a role in more stable FIX expression compared with FVIII after a liver-directed gene therapy approach. However, further studies are still needed to clarify.

On the contrary, as high coagulation factor levels may comprise a risk factor for thrombosis, supraphysiological levels of factor activity achieved by the gene therapy may be a trigger too.[65] FIX-Padua was first identified as the cause of a rare X-linked thrombophilia due to a missense mutation in the gene for FIX that led to increased specific FIX activity.[66] Thus, gene therapy studies started to use the high specific activity of FIX-Padua with the rationale of lowering the vector dose to mitigate the toxicity. While there is a significant efficacy with FIX-Padua transgene, there might also be a potential for thrombogenicity. Although this appears to be minimal as thrombosis occurred only in FIX-Padua patients with activity levels >700% of normal and there is no evidence of thrombosis in a total of six dogs in an 11-year period expressing FIX-Padua following AAV delivery, thrombogenicity should be concerned in human haemophilia gene therapy trials.[34,66,67]

As a low proportion of AAV vector sequences integrates into the host genome, there is a lower risk for long-term insertional oncogenicity. Despite more than 170 AAV-based human trials approved, ongoing or completed, no tumorigenic events have been reported so far.[68–70] The association between AAV gene transfer and risk for oncogenicity is still unknown. Although detailed autopsy and histopathological examination of haemophilic dogs lived over 10 years following AAV delivery has not reported any malignant tumours in the liver, in another study analysis of integration sites in liver samples from six AAV-treated haemophilia A dogs identified 1741 unique AAV integration events in genomic DNA and expanded cell clones in five dogs.[71,72] Moreover, one patient was diagnosed with hepatocellular carcinoma (HCC) in the Hope B gene therapy trial. However, the patient had multiple risk factors associated with HCC, including a 25-year history of hepatitis C (HCV) and history of hepatitis B (HBV). Analysis performed to the resected tumour and adjacent liver tissue showed that AAV vector integration in the patient's tissue sample was extremely rare and whole genome sequencing of the tumour confirmed typical genetic mutations for HCC are independent of vector integration.[73] However, genotoxicity still remains as a potential risk for gene therapy trials that requires long-term monitoring.

The risk of germ line transmission of vector sequences in humans is also an important safety concern, as the enrolment of subjects of reproductive age in gene therapy clinical trials continues to increase. So far adult patients enrolled in AAV trials with systemic delivery have been required to use contraception. Although vector sequences have been detected transiently in semen of treated patients in AAV2 or AAV8 trials with the latest clearance of the vector observed at 12 weeks postinjection, no vector was detected in semen samples in an AAV5 trial.[29,32,74] While vector was observed in seminal fluid, no transduction was seen in mature sperm and spermatogonia.[75,76]

Up to date, adult patients have been enrolled in haemophilia gene therapy trials and it is still unknown that the recent successes could be maintained in a paediatric population. The major challenge for using AAV vector delivery in children is the nonintegrating nature of these vectors, a big part of the vector could be lost from dividing cells during the substantial liver enlargement in the childhood. However, in the dog models, 10 years after delivery, many integrated vector copies and persistent episomal copies have been detected.[71]

Challenges and recommendations for Turkey and other developing countries

- Reports about seroprevalence of AAV in patients with haemophilia are currently lacking in Turkey and in other developing countries. However, pre-existing antibody seroprevalence was found highest in Turkey [67% in Turkey, followed by patients from the Dutch (27%) and Italian (14%) referral centres].[77] This higher prevalence may reduce the eligible candidate numbers in Turkey for upcoming gene therapies.
- In a meta-analysis of 129 prevalence studies in Turkey, the estimated number of HBV carriers in Turkey was found to be 3.3 million with an overall HBV prevalence of 4.57%; moreover, it is the major cause of acute viral hepatitis.[78,79] An average of 60% of the hospitalized acute viral hepatitis cases in adults (20–40 years of age) were due to HBV, and among children, this was only 22.4%.[79] As active viral hepatitis B or C infections (HBV/HCV) are an exclusion criterion for the gene therapy trials, this may also be a significant challenge for Turkey and for other countries where HBV/HCV infection rate is high.
- There are less gene therapy trial sites in developing countries compared with Europe and the United States, which may delay the initiation of these therapies once they are approved.[38] Clinical trials are known to increase awareness, experience and trust within the acquisition of new treatments. From eligibility criteria to side effects management, existing trial sites will surely adopt gene therapies faster and more sufficient than those that didn't participate.

Clinical implications of gene therapy, transfer from bench to bedside

Global perspectives

In the last 35 years, since the cloning of the *F8* and *F9* genes, there have been significant advances in the haemophilia care; hence, the first haemophilia gene therapy product will be approved for clinical use within the next few years according to the results of the ongoing phase III studies.

The success of the gene therapy in the clinical practice will require a wide-range, comprehensive multistakeholder arrangements, including scientists, manufacturers, governmental regulators, health technology assessors, patients and families, national advocacy groups and multidisciplinary healthcare teams.[80] To adequately assess the safety and efficacy of gene therapy, global data collection across products and countries is extremely necessary. American Thrombosis and Hemostasis Network (ATHN) established a longitudinal, observational cohort study as ATHN14 in participants with haemophilia A or B who are not principally tied to a particular manufacturer. While the primary outcome is to determine the safety of AAV or lentiviral vector–mediated *F8* and *F9* gene therapies, the secondary outcome is describing dose regimens, use of steroids and other immunomodulatory medications, determining the effectiveness of gene therapy by evaluating endogenous factor activity levels, bleeding rates and exogenous factor concentrate usage.[81]

The World Federation of Haemophilia (WFH) Gene Therapy Registry (GTR) is a prospective, observational and longitudinal registry that involves healthcare professionals, patient advocates, industry representatives and regulatory agency liaisons. All patients who receive gene therapy, *via* clinical trials or postregulatory approval, will be encouraged to participate, with an aim to enrol all of the eligible patients globally. Safety and efficacy data with quality of life parameters will be covered.[81]

While data collection is an important issue for long-term improvement, there is also a lack of knowledge about the emerging gene therapy among the healthcare teams and scientists. International Society on Thrombosis and Hemostasis conducted a survey about the understanding and awareness of gene therapy; 66% of the responders were physicians and 59% of them were directly involved in the care of haemophilia patients. Almost one-third of them had a difficulty in explaining the basic scientific principles of AAV gene therapy and 40% declared that they are not competent enough to answer patient questions about gene therapy.[82] Another survey given by the WFH to 103 national member organizations and 109 physicians from 76 countries showed that 68% of the patients have a primary conception of gene therapy and 44% of the medical professionals have only basic or intermediate knowledge.[83] Therefore, there is an

urgent need for the clear and reliable sources to advance the knowledge of the healthcare professionals about the gene therapy before the clinical administration. Thus, the WFH, European Haemophilia Consortium and the National Haemophilia Foundation have also partnered with Medscape to deliver CME content intended to enhance the knowledge about the basic principles and clinical application of gene therapy for haemophilia.[80]

Gene therapy offers a life-changing opportunity for patients to decrease bleeding risk as well as a reduction or cessation of the exogenous factor administration. However, switching to a new and investigational therapy is a real challenge for patients. A survey including 12 haemophilia patients and two mothers in Netherlands reported that the ease of use of the medications is important for them and they are aware of the promising new treatments. However, they had doubts about the safety and clarity of the effects of gene therapy. However, they want to be informed when they are eligible for a new treatment.[84] Patients should be educated to discuss and judge the benefits and drawbacks of the treatment with their physicians in an individual base.[85]

If the phase III clinical trials confirm the safety and efficacy of the AAV gene therapy products, the next step will be the regulatory approval and boosting the manufacturing capacity.

Both the European Medicines Agency (EMA) and the FDA Center for Biologics Evaluation and Research have provided draft guidance for industry on the development and long-term follow-up for gene therapy, with the FDA indicating specific guidance on haemophilic gene therapy.[86] Manufacturers will try to improve their capacity to ascend production efficiently with the support of developing technologies.[80]

To overcome the barriers at the individual and healthcare system levels, the coreHEM project was established as a multistakeholder action to determine the outcome measures required to evaluate efficacy, safety, comparative effectiveness and value of gene therapy for haemophilia with the goal of streamlining regulatory approval, health technology assessment and market access decisions. Forty-nine participants (five patients, five clinicians, five researchers, four regulators, three research agencies, six health technology assessors, nine payers and 12 drug developers) were involved in the study. Active dialogues among participants may enable extensive utilization of the determined outcomes in future clinical trials.[87]

Moreover, successful clinical delivery of gene therapy is another important issue that needs instant consideration.[88] Many hospitals do not have aseptic facilities for the reconstitution of gene therapy medicines or the appropriate freezers for storage as well as the lack of the experienced staff. Therefore, special regional/national treatment centres such as excellence or research centres should be established by implementing these requirements. In the long term, other hospitals will also be prepared for the clinical delivery.[88] To ensure the safe introduction, usage and monitoring, the European Association for Haemophilia and Allied Disorders (EAHAD) and the European Haemophilia Consortium (EHC) jointly recommend to use 'a hub-and-spoke model' for all first-generation gene therapies. The 'Hub' is an HTC and experienced in both comprehensive care and gene therapy, whereas the 'Spoke' is another centre with no or minimal gene therapy experience, which will be the home centre for the patient. Eligibility should be evaluated individually for each patient, considering the patient selection criteria of current gene therapy. Gene therapy should be exclusively prescribed and administered by the hubs and longitudinally monitored by spokes in close communication with the primary expert hub.[16] HTCs should be established to ensure the access to clinical specialties, emergency departments and appropriate laboratory facilities with a strong multidisciplinary team comprised of pharmacists, nurses, hepatologists, psychologists, physiotherapists, biomedical scientists and haematologists.[89] It is often recommended that patients should have a monitoring plan with a brief clinical history and sample testing at the following time points: pretreatment; 3, 6 and 12 months after treatment; and then yearly thereafter for a minimum of 5 years. Figure 2 summarizes the algorithm for optimal gene therapy delivery and follow-up.[86]

The opportunity provided to haemophilia patients by gene therapies may be hindered by economic concerns that can delay access. Therefore, gene therapy pricing policies and reimbursement models should be developed with multistakeholder engagement.

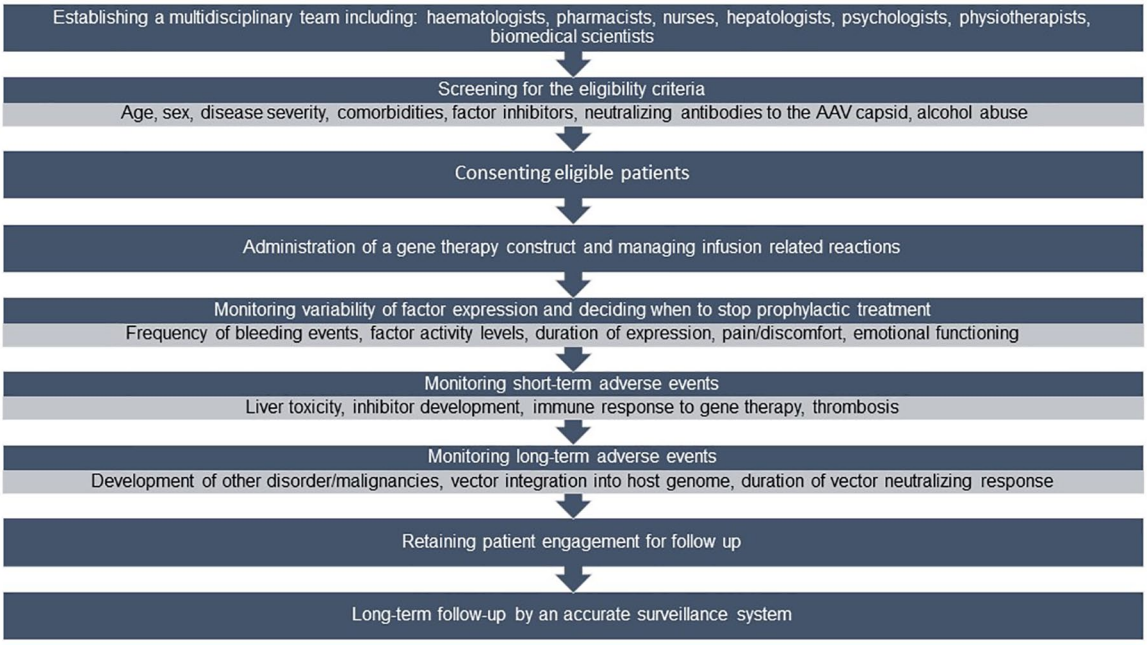

Figure 2. The algorithm for an optimal gene therapy delivery and follow-up.

Obstacles and opportunities for Turkey and other developing countries

- The SARS-CoV-2 pandemic has caused remarkable breakdown to the research, manufacturing, clinical development, and market launch of gene therapy products for non-SARS-CoV-2-related diseases in all around the world and surely developing countries like Turkey will face a foreseeable economic impairment due to the pandemic. Turkey and similar developing countries will need to develop new strategies and beneficial solutions have access to gene therapies in the upcoming years.
- Lack of centralized haemophilia patient registry in Turkey is the biggest barrier to identify suitable patients who may benefit from these curative but expensive therapies the most. Establishment of centralized haemophilia patient registries by Ministry of Health will surely be the most impactful factor on the consumption of the limited resources for the right reasons.
- Lack of knowledge about the gene therapy among the healthcare teams, scientists and patients in Turkey is also another important barrier to adapt these therapies for the right patients. There is an urgent need for the clear and reliable sources in Turkish language to advance the knowledge of the healthcare professionals about the gene therapy. Patient associations in developing countries, including Turkey, are not as sufficient as in the Western countries; therefore, healthcare professionals' knowledge will also contribute to optimize the patients' expectations from gene therapies before they become available in these countries.
- Another barrier to initiate gene therapies successfully in developing countries is the lack of qualified and experienced gene therapy application centres. Turkey has a great potential in the acquisition of these novel treatments with nine European Haemophilia Comprehensive Care Centre (EHCCC)-certified centres (Turkey is the fourth most certified country after Italy in Europe) and with four active gene therapy trial sites. Therefore, Turkey's experience can provide guidance to the neighbouring countries in the region.[15]

In conclusion, gene therapy is a potential life-changing opportunity and a possible milestone in haemophilia management. With the huge effort for overcoming the current obstacles, it can be expected that patients will significantly benefit from the gene therapy with a remarkable increase in the quality of life. To make this promise a reality in developing countries, strong collaborations

among the scientific community with regulators and patients' organizations for data collection and nation-wide registries are needed. All the stakeholders of the haemophilic community, including patients, should be educated properly to make a shared decision of switching to gene therapy in an individual base.

Declarations

Ethics approval and consent to participate

N/A since this is a narrative review article with expert opinions, this study did not require ethical approval.

Consent for publication

Not applicable.

Author contribution(s)

Kaan Kavaklı: Conceptualization; Investigation; Methodology; Supervision; Validation; Writing – original draft; Writing – review & editing.

Bülent Antmen: Conceptualization; Investigation; Validation; Writing – review & editing.

Vahap Okan: Conceptualization; Investigation; Validation; Writing – review & editing.

Fahri Şahin: Conceptualization; Investigation; Validation; Writing – review & editing.

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Can Balkan: Conceptualization; Investigation; Validation; Writing – review & editing.

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Alphan Küpesiz: Conceptualization; Investigation; Validation; Writing – review & editing.

Bülent Zülfikar: Conceptualization; Investigation; Supervision; Validation; Writing – review & editing.

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Not applicable.

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