The evolving landscape of gene therapy for congenital severe hemophilia: a 2024 state of the art

Giovanni Di Minno,[1] Gaia Spadarella,[2] Ilenia Lorenza Calcaterra,[1] Giancarlo Castaman,[3] Paolo Simioni,[4] Raimondo De Cristofaro,[5] Cristina Santoro,[6] Flora Peyvandi,[7] Matteo Di Minno[1]

[1]Department of Clinical Medicine and Surgery, Hub Center for Congenital Hemorrhagic Diseases and Thrombophilia, Federico II University, Naples; [2]Department of Translational Medicine, Federico II University, Naples; [3]Center for Bleeding Disorders and Coagulation, Careggi University Hospital, Florence; [4]Medical Clinic 1, Department of Medicine (DIMED), Padua University Hospital; [5]Section of Hemorrhagic and Thrombotic Diseases, Department of Medicine and Translational Surgery, Sacro Cuore Catholic University, Rome; [6]Hematology Unit, Umberto I University Hospital, Rome; [7]Fondazione IRCCS Ca' Granda Hospital, Milan; Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Department of Pathophysiology and Transplantation, University of Milan, Italy

Correspondence: Giovanni Di Minno, Clinical Medicine, Federico II University, School of Medicine, Naples, Italy; Governance Headquarters, Campania Network for Inherited Bleeding Disorders, Via S. Pansini 5, 80131 Napoli, Italy.
E-mail: diminno@unina.it

Key words: adeno-associated virus gene therapy; person-reported outcomes; laboratory monitoring; emicizumab; extended half-life products.

Contributions: Gaia Spadarella and Ilenia Lorenza Calcaterra equally contributed to the present paper.

Conflict of interest: GDM speaker or a member of a speaker bureau for: BioMarin, Bayer, CSL Behring, Roche, Takeda, Viatris Pharmaceuticals; consultant or ad-hoc speaker/consultant for: BioMarin, Bayer, Pfizer, Takeda, Viatris Pharmaceuticals. PS: Grant/Research support from: CSL Behring. GC: Grant/Research support from: CSL Behring, Pfizer, Sobi, Consultant for: blynx, Alexion, Bayer, Takeda, CSL Behring, Novo Nordisk, Pfizer, Roche, Sanofi, SOBI, uniQure (Membership on an entity's Board of Directors or advisory committees), Speaker Bureau of: Bayer, Roche, Sobi, Grifols, Novo Nordick, Werfen, Kedrion. RDC: Speaker Bureau of Bayer, Sobi, CSL Behring, and Takeda, CS: speaker or a member of a speaker bureau for: Bayer, CSL Behring, Novo Nordisk, Takeda; consultant for: Bayer, Novo Nordisk, CSL Behring, Sobi, Roche, Biomarin, Takeda, Kedrion; FP: speaker or a member of a speaker bureau for Grifols and Roche, on advisory boards for Sanofi, SOBI, Takeda, Roche, and BioMarin; MDM: grants and honoraria from Sobi, Pfizer, Bayer, Novo-Nordisk, Takeda, Roche, and Daiichi Sankyo. GS, and ILC, declare no interests which might be perceived as posing a conflict or bias.

Acknowledgements: the authors wish to thank Maria Tommasi and Daniele Vecchio for helpful discussions.

Availability of data and material: data and materials are available by the authors.

Informed consent: the manuscript does not contain any individual person's data in any form.

Received: 21 June 2024.
Accepted: 27 June 2024.

Publisher's note: all claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article or claim that may be made by its manufacturer is not guaranteed or endorsed by the publisher.

Bleeding, Thrombosis and Vascular Biology 2024; 3:144
doi:10.4081/btvb.2024.144

ABSTRACT

Despite major advances in prophylaxis, no repeated dosing regimen with currently employed extended-half-life or non-factor products replaces the advantages of a long-term cure in persons with severe congenital hemophilia A and B (HA, HB). They indeed live with the risk of breakthrough bleedings, and treatment is still invasive, both physically and psychologically. Early studies showed that adeno-associated virus-based *in vivo* gene therapy (AAV-based *in vivo* GT), could convert haemophilia persons from a severe to mild a phenotype for years. However, the proportion of the hemophilia population likely to benefit from this transformative strategy was uncertain. Current evidence is expanding the eligibility criteria, and helps to predict risks, complications and unexpected side effects of this advanced treatment. Thus, among future options, AAV-based *in vivo* GT is likely to become the treatment of choice in HA and HB, if real-life data confirm its negligible short-term adverse events. However, while the global use of AAV-based *in vivo* GT is endorsed as a key objective of future studies in hemophilia, the liberating capability of a potentially one-off treatment on individuals with chronic diseases for whom lifelong cure has been inaccessible so far remains to be thoroughly recognized by government bodies. This is critical for reimbursement agencies to absorb the cost of *the cure* and calls for a partnership between health care systems and the pharmaceutical industry. However, bridging the gap between the costs of the advanced treatments approved for commercialization and their readiness to persons with HA and HB is still a challenging task.

Introduction

Thirty-two advanced therapeutic medicinal products (ATMPs), based on cell or gene therapy (GT) sometimes in combination, are presently approved for commercialization in Europe and the United States of America. Eleven out of them provide new

historical options for severe single gene hematological (*e.g.*, hemophilia) and non-hematological chronic diseases for which life-long cure had been inaccessible so far.[1] This milestone in modern science is the result of major events in the past half-century: a) the understanding of basic virology; b) the imperative for translational impact of NIH-funded research; c) public investments in molecular genetics; d) developments in genomic technologies, and e) developments of economic models for commercialization of rare disease medicines.[2,3] Due to the long-term transgene expression, the ease-of-use, and the preclinical safety and efficacy data, recombinant second-generation adeno-associated virus (rAAV) vectors emerged as the most safe and efficient mode to correct inherited single-gene defects in nondividing cells.[4] The extensive knowledge of a virus in long-term symbiosis with its human host has further boosted the possibility of using rAAV for long-term gene transfer in humans.[5,6]

Over the last decades evidence has been gathered that, with AAV-based *in vivo* GT, severe congenital hemophilia A and B (HA and HB) could substantially progress from a severe to a mild phenotype for years.[7] This lends credence to the possibility that, among future options, AAV-based *in vivo* GT will become the treatment of choice in hemophilia. However, how wide was the spectrum of persons with HA and HB who could benefit from this treatment modality remained largely unclear.[8] Newer data in the last 3-4 years addressed issues concerning the eligibility criteria to expand the indications of the rAAV-based *in vivo* GT, the predictability of the response, the possibility to re-administer rAAV vectors, and have documented short-term side effects and potential long-term complications, including the risk of vector integration.

Unmet needs in the current treatment of hemophilia A and B

Traditionally, persons with HA and HB have been managed with on-demand replacement of the deficient protein (or with bypassing agents) to prevent or treat acute bleeding episodes. In the '90s of the last century (approximately when *in vivo* GT for hemophilia was conceived), life-long *intravenous (IV) prophylaxis with plasma-derived or recombinant standard half-life (SHL) FVIII/IX products* initiated after the first recognized joint/muscle bleed – *e.g.*, at 1-2 years of age – to maintain circulating plasma FVIII/IX levels ≥1% of normal[9] was the gold standard for bleeding prevention in high-income countries.[10] A marked reduction in bleeding frequency, and in joint bleed,[11] and an improved quality of life (QoL) in persons with severe (and some with mild/moderate) hemophilia was observed in Western Countries,[12,13] and life expectancy for persons with HA and HB became comparable to that of males without hemophilia.[14-16] However, prophylaxis has a variety of shortcomings,[17-19] and its burden is high. Physical, logistical,[20] psychological,[21-24] and economic shortcomings are critical for some persons with hemophilia (PWHs), and painful repeated infusions significantly impact treatment adherence in children.[25,26] Although early joint damage has been seen also in highly compliant HA and HB persons on prophylaxis since childhood,[27] *hemophilic arthropathy* is usually correlated with poor adherence to prophylaxis, low trough levels and breakthrough bleeds.[28-32] Bleeds causing joint damage, pain, disability, and reduced QoL may also occur with sports.[18,33-37] Joint bleeding is the leading cause of morbidity in HA.[38,39] Uncontrolled repeated and untreated (micro)bleeds can progress to arthropathy. Even a single episode of joint bleeding can cause irreversible damage and foster new local bleedings leading to severe arthropathy.[40] Joint changes are marked by pain, functional impairment, and progressive loss of function (ankyloses) with poor mobility.[41] Deformity, and functional impairment lead to isolation, anxiety, and depression,[23,24] and negatively impact social relations, participation,[21,22,24,42-44] and sex life.[42] *Pain* in PWH is a pervasive often under-recognized problem,[41,45] that hampers the possibility to be socially active.[25] Reports argue for: a) more than 85% of PWH having suffered from pain over a period of the last six months; b) up to 89% of PWH stating that pain interfered with activities in daily life function;[46] c) acute pain being primarily driven by frequency of bleeding in target joints and chronic pain by joints with a low range of motion,[47] and d) ≈50% of PWH sustaining chronic pain.[48] By promoting depression and anxiety,[49] chronic pain adversely impacts medication adherence and QoL.[45] Because of pain and motion limitations,[50] both physical and mental health-related QoL is compromised in PWH,[21,22] major drawbacks being related to discomfort (≈70%), poor mobility (≈60%), restrictions in usual activities (≈50%), anxiety/depression (≈50%), and self-care (≈ 20%).[22] On the other hand, because of visits, hospitalizations, and drug treatments, direct costs of day life are higher in PWH with than in those without arthropathy.[22] Indirect costs (loss of school days and parental/caregiver engagement, low work productivity, high absenteeism, early retirement) should be also considered.[51] Thus, PWH may face more financial concerns than the general population.[49,52] Accordingly, person-relevant (*e.g.* annual infusion rate, pain, mental health status) and person-reported outcomes (*e.g.* well-being, physical health, physical activity, sport participation) show a poor QoL in most PWH.[23,53] *Inhibitor formation* – the most common and severe complication in PWH on prophylaxis – is due to the development of neutralizing antibodies to therapeutically administered FVIII/FIX.[54-56] Compared to inhibitor-free PWH, those with high-titer inhibitors have a worse joint status and QoL, and higher mortality and morbidity related to major limitations (*e.g.*, effectiveness) of replacement options.[38,57] Finally, in high-income countries, due to the very *high cost of prophylaxis* in hemophilia care, objective measures of health were needed both in children and adults to evaluate whether this strategy was worth its cost.[58] Being highly expensive even in countries with national contract negotiations, prophylaxis with SHL factor concentrates to reduce mortality and improve QoL was beyond the means of many PWH across the world.[59,60] In these settings, treatment is still the same it was one century ago.[13] Drawbacks in persons with inhibitors,[61] and hurdles of access in low-income countries pushed the search for alternatives to SHL FVIII/IX products in PWH.

FVIII/IX products with extended half-lives (EHL) allow for less frequent venipunctures to achieve zero bleeding (the current gold standard of prophylaxis).[18] Half-lives of EHL concentrates currently employed for the treatment of persons with HB range 82-111 hrs. Thus, a once-weekly administration of an EHL concentrate converts persons with HB to a mild phenotype. On the other hand, by targeting 1.5-1.7-fold higher trough levels than SHL products, twice weekly intravenous (IV) injections of *EHL FVIII products* convert persons with HA to a moderate phenotype.[62,63] All in all, by reducing the number of infusions, *EHL FIX/FVIII products* enable more flexible treatment regimens, and improve individualization, efficacy and treatment adherence

to handle the risk of bleeding. However, prophylaxis with EHL products still requires frequent IV administration. This leads to peaks-and-troughs in factor levels. Moreover, clotting factors concentrates administration requires considerable time commitment and is tough when using a fistula. The need to replenish the drug supply implies the regular pick up of the medication and/or to be home to receive it. Ensuring suitable medical supplies, attending fixed clinical visits to update treatment, and moving with large amounts of products are key issues during travel or holidays, and significant logistical limitations.[20] When multiple injections are needed, establishing and maintaining a routine treatment may be awkward in PWHs.[26] The impact of extensive venous damage should be also considered.[24] Finally, PWH are aware that there is a wide variability in the minimal factor levels needed to achieve an annualized bleeding rate (ABR) of zero, the cogent objective of personalized prophylaxis.[53] They also know that bleeding protection is not *physiologic* when employing prophylaxis: indeed, despite repeated infusions to maintain safe circulating factor levels, troughs (and, in turn, bleeds) are expected when using SHL as well as EHL products.[28-30] Accordingly, both in clinical trials for drug registration and in real-life reports, not all persons receiving prophylaxis with EHL FIX/FVIII products achieve zero bleeding.[18] While no real-life data on adverse drug reactions (ADR) are available as to prophylaxis with EHL FIX products for HB, the EudraVigilance 2021 retrieved 275 hemorrhagic (including intracranial, hemarthroses, hematomas, gastrointestinal, and post-procedure) during a year of observation of the post-marketing assessment of EHL FVIII products.[64] The report also enlists 9 thrombotic events associated with the use of EHL FVIII products in HA persons (4 arterial and 3 venous thromboses for efmoroctocog alfa; 1 arterial thrombosis for turoctocog alfa pegol, and 1 consumption coagulopathy for damoctocog alfa pegol). In some of these events, eptacog alfa (activated), octocog alfa,or emicizumab had been used along with the EHL product.[64]

The subcutaneous (SC) administration – every week, every two weeks or once every 4 weeks – of the FVIII-mimetic agent emicizumab reduces spontaneous and target joint bleedings and converts PWHs with HA from severe to a mild phenotype.[65,66] Weekly SC injections of emicizumab are both FDA and EMA approved in adults and children to prevent/reduce the frequency of bleeds in HA persons regardless of the presence of inhibitors.[67] However, zero bleeding is not achieved in all persons using emicizumab.[65,66] During a year of observation in the post marketing assessment of ADRs, the EudraVigilance 2021 report retrieved 232 hemorrhagic ADRs (104 being severe bleeds), 14 arterial and 8 venous thromboses, 1 thrombotic microangiopathy, 1 consumption coagulopathy.[64] Among the 24 thrombotic ADRs, 6 occurred when eptacog alfa (activated) and 2 when FVIII concentrate was used along with emicizumab.[64] At emicizumab approval for clinical use, a black box alert on thrombotic microangiopathy and thromboembolism had been issued by the FDA.[62]

Adeno-associated virus-based gene therapy in hemophilia

Efficacy and safety data

A survey covering over 140 clinical trials of AAV-based gene therapies and involving more than 3000 persons treated for more than 20 years documents – like in long-term follow-ups in animals[68] – the effectiveness and the limited toxicity of treatment with systemically infused rAAV vectors.[69] AAV genomes replicate extra chromosomally (*i.e.,* they persist primarily as episomes), and cause negligible short-term adverse events (self-limited fever, myalgias, and/or hypotension) within the first month after AAV administration.[70,71] In 21% of the trials, low-grade adverse events were detected. Increase in alanine aminotransferase (ALT) levels, occurring in high dose [$>10^{13}$ vector genomes per kg (vg/kg) of body weight] systemically (mostly IV) administered cohorts, was first detected in persons with HB undergoing GT.[72] No preclinical model helped to predict this hepatotoxic event – attributed to vector and transgene-specific T-cell responses against AAV-transduced hepatocytes – that had a positive response to corticosteroid administration.[72-75] No sign of liver toxicity of a systemic rAAV *in vivo* administration emerges from long-term (up to 15 years) information in 4 adults with severe HB who participated in the first in-human trial of systemic IV AAV delivery.[76] Like in the first AAV-based *in vivo* GT study in HB with the rAAV2 vector,[72] studies with rAAV8 (a vector with a stronger hepatic tropism than rAAV2), and with rAAV5 [the serotype with the lowest prevalence of neutralizing antibodies (Nabs) to AAV vectors in the general population][77,78] document dose-dependent cytotoxic T-cell response against transduced hepatocytes in some persons.[79,80] This led to lysis of transduced cells (and asymptomatic ALT increase), and loss of transgene expression.[81]

In a trial in persons with HA who received a single IV dose of valoctocogene roxaparvovec, an increase in ALT levels was detected in 115/134 participants. Of them, 106 (79.1%) received glucocorticoids (230 days, median treatment duration, range: 22 to 551). Glucocorticoid-related adverse events were reported in 71.8% of participants.[82] At 2-year data cut, no new safety signals emerged and no treatment- or drug-related serious adverse events occurred.[83] In addition to identifying potential factors contributing to interindividual variability of transgene expression, liver biopsies taken up 4 years after GT established successful transduction, and showed no evidence of drug-induced liver injury.[84] A phase 3 evaluation of the prophylactic use of steroids with valoctocogene roxaparvovec is under way (GENEr8-3; ClinicalTrials.gov number, NCT04323098). Less common increase in ALT levels was reported in the HOPE-B trial (NCT03569891) in HB males who received etranacogene dezaparvovec.[85] Together with mild, asymptomatic increases in ALT levels matching with a detectable corticosteroid- controlled anti-AAV capsid T-cell response,[86-88] ALT levels 1.5-to 2-fold higher than the upper normal limit might not be linked with hepatocyte loss,[89,90] *i.e.,* capsid response and ALT level increase may be independent, parallel events.[87,90-92]

Supraphysiologic FVIII: C levels (odds ratios: 8.8-21.3) are independent risk-factors of venous thrombosis, mainly in the elderly.[93] Because of transgene-derived circulating FVIII activity levels >150% (upper limit of normal) in some PWH who had undergone AAV-based *in vivo* GT with giroctocogene fitelparvovec (rAAV2/6 SB-525 vector), the study has been paused and the protocol amended. During the pause, a thrombotic event occurred in an infused PWH with upper normal FVIII activity levels and a marked decrease in physical activity.[62] High, stable (up of 1 year) expression levels of FIX (24 to 168 U/dL at 3 weeks) were also detected after GT in the phase 1 B-AMAZE study using

FLT180a, a AAVS3 capsid carrying a *F9* variant with the Padua gain-of-function mutation.[94] A participant with a high FIX expression (>200 U/dL) had his arteriovenous fistula occluded.[95,96]

A major theoretical safety concerns of high-dose AAV vector infusion is the risk of genotoxicity leading to cancer.[97] In the HOPE-B trial (NCT03569891), 54 adult males with HB were enrolled –regardless of a history of hepatitis B or hepatitis C virus infection – to receive a single IV dose of etranacogene dezaparvovec (2×10^{13} gc/kg), comprising a AAV-based rAAV5 vector containing a codon-optimized Padua-variant human FIX transgene, and a liver-selective promoter.[85] Molecular and vector integration analyses of a case of HCC 1 year after GT, in a participant with a longstanding history of HCV infection, established no relationship with rAAV administration and provided. Using a similar approach, no relationship has been found for the tonsil cancer in a participant in the BAX-335 trial.[89] Finally, in two persons who had been infused with valoctocogene roxaparvovec 3 and 5 years before for HA and developed a salivary gland carcinoma and a B-cell acute lymphoblastic leukemia, respectively, whole genome sequencing analysis led the trials Data Monitoring Committee to argue against such malignancies as related to GT.[98]

The safety of AAV vectors was first questioned by a study reporting HCC in mice after systemic delivery of AAV vector to treat mucopolysaccharidosis type VII.[99] In a subset of tumors in the treated mice, AAV integrations were tightly clustered in the RNA imprinted and accumulated in nucleus (*Rian*) locus on chromosome 12.[99] This region encodes several regulatory RNAs, including microRNAs.[100] Integration of AAV2 at sites of active transcription had already been documented in mice.[101] HCC have been documented in mice with different inborn errors of metabolism several months after neonatal AAV injections and associated with vector integration and overexpression of microRNA-341 proximal to the RNA imprinted and accumulated in nucleus (*Rian*) locus.[102] Systemic administration of AAV vector to newborn mice with growing livers resulted in very high penetrance HCC. Accordingly, the aberrant expression of proximal small noncoding regulatory RNAs induced by AAV vector integration was interpreted as a mechanism for carcinogenesis.[99] However, no insertional mutagenesis and cancer have been observed in mice receiving *recombinant AAV* (*rAAV vectors, i.e.,* those employed in current GT studies).[103,104]

In dogs and in non-human primates genome microRNA-341 is not present.[8,105] Low-frequency AAV integration in sites of active transcription has been documented in dogs, together with AAV integration and clonal expansion of cells with insertions near genes associated with growth control.[106] However, no nodule formation or transformation (or abnormal liver function related to AAV administration) has been documented in the 10 years follow-up after transgene delivery to dogs.[107] Single nuclear domains of vector DNA were documented in >10% of hepatocytes that persisted despite the loss of transgene expression. Genomic integration of vector sequences was detected in 1/100 cells at broadly distributed loci that were not in proximity to genes associated with HCC.

Overall, the risk of genotoxicity and cancer remains a concern of high-dose AAV vector infusion. Conclusive information is expected from infants with spinal muscular atrophy receiving high systemic AAV vector doses with a ubiquitous promoter (*e.g.* NCT03306277, Zolgensma®).[97] Because of the rapid growth of the liver and the high rates of cellular division, such clinical setting resembles the risk of genotoxicity active in mice following AAV administration.[102]

Expanding the indications of early studies

Given the potential risk of long-term hepatic toxicity of AAV vectors, phase 1/2 GT studies and current phase 3 trials with AAV vectors -alluded to in the previous section- have limited inclusion to HA persons with stable hepatic function and no history of inhibitor formation to therapeutically administered FVIII/FIX.[102] Expanding the indications from the *ideal* persons to be enrolled [PWH under 18 years of age; with less than 30-50 exposure days to therapeutically administered coagulation products; without active hepatitis B virus or hepatitis C virus (HCV) infections or underlying liver disease;[108,109] without a history of inhibitors to FVIII,[54-56] and without pre-existing Nabs to AAV vectors higher than a cut-off value], to sub-populations usually found in clinical practice is crucial to establish whether AAV-based *in vivo* GT may provide a potentially uniform progress in hemophilia management.

The prevalence of hepatitis C virus (HCV) infections and of hepatocellular carcinoma (HCC) – that were both elevated among ageing PWH with hemophilia that had received plasma-derived FVIII/FIX products – is expected to decrease in the >90% of people that achieved clearance of the virus from the circulation in response to anti-HCV drugs.[110-112] In parallel, the burden of fatty liver syndrome – that includes both non-alcoholic and alcoholic fatty liver disease – is progressively increasing in PWH and emerging as a common cause of chronic liver disease.[113] The steadily increasing prevalence of metabolic syndrome and/or of diabetes mellitus in persons with HA and HB,[114] is critical for future indications of GT in PWH. The likely progression of fatty liver disease to hepatic fibrosis and liver-related complications is usually assessed via a percutaneous liver biopsy. However, due to the inherent risk of complications of this invasive procedure, and the poor availability of trans-jugular liver biopsy, noninvasive procedures are employed to stratify the stage of the fatty liver syndrome in PWH (Table 1 and Supplementary Material), to predict long-term outcomes, and to monitor responses to diet/drugs.[115-117]

None of the persons that have undergone GT has developed inhibitors to transgene-derived proteins.[2] However, in GT studies,[62] exclusion criteria only allowed for enrollment of PWH with the lowest tendency to develop neutralizing inhibitors to FVIII or FIX (less than 30-50 exposure days to therapeutically administered coagulation products). In keeping with data in humans, no post-GT inhibitor formation has been shown in dog studies (whose hemophilia strongly resembles that of human beings).[118-122] Moreover, canine data support tolerance to transgene products after long-term FVIII expression by AAV-based *in vivo* GT.[123] Amelioration of the phenotype because of inhibitor eradication has also been achieved when an immune response to FVIII was already present.[73] These considerations argue for the efficacy of AAV-based *in vivo* GT for tolerance induction in persons with inhibitors.[124] Emicizumab is the current standard of care to prevent bleeds in persons with inhibitors to FVIII.[61] Its easy use compares well with the treatment burden and costs of Immune tolerance induction (ITI), the former standard of care to eradicate high-titer inhibitors in HA persons.[125,126] However, the need to use FVIII for

emergency bleeds and surgery and the lack of data on long-term outcomes of emicizumab, argue for restoring FVIII use in young PWHs.[127] A dose-finding trial in persons with HA and with FVIII inhibitors (NCT03734588) has been completed (February 2024). FVIII doses used to achieve inhibitor eradication in primary ITI courses in persons with HA and high responding (HR) inhibitors,[128,129] should have provided useful information as to circulating FVIII levels by transgene expression needed for inhibitor eradication in AAV-based GT studies.

Due to natural AAV infections, pre-formed NAbs to AAV vectors commonly used in clinical trials, are present in ~30-40% of the general population.[130] NAbs prevent treatment with the same rAAV. Animal data also imply that,[131] NAbs against one serotype may cross-react with others making ineffective the serotype switching.[132] Thus, when high-levels of NAbs are present, the benefit of a second administration of the same AAV serotype or switching to a different rAAV vector might be precluded.[132] Efforts are currently made to mitigate this limitation leading to a *one chance only* therapeutic opportunity.

Pre-existing Nab*s* to AAV5, up to a titer of 678, were not an exclusion criterion for effective transgene expression in the phase III study AMT-061, NCT03569891 in which 2×10 genome copies (gc)/kg of AAV5 expressing the *FIX* Padua transgene (etranacogene dezaparvovec) were administered to 54 persons with severe and moderate HB.[133] After achieving a stable FIX production, 52/53 participants (98%) discontinued prophylaxis, with an overall 97% reduction in mean annualized factor consumption. Up to a titer of 678, persons with (n=31) and those without (n=23) pre-existing AAV5 NAbs behaved similarly as to FIX activity. Among the serotypes used as GT vectors, AAV5 has a least-conserved capsid sequence.[134,135] Recognition and cross-neutralization by non-AAV5 Nabs is less likely to occur.[136] Whether, due to low binding affinity or high *off* rates, NAbs inhibiting *in vitro* AAV vector transduction, might not impede the *in vivo* effect should be explored.[137] The neutralizing potential of anti-AAV NAbs deserves better understanding.[136]

Both in mouse and nonhuman primates, imlifidase improves vector delivery in the context of pre-existing anti-AAV NAbs.[138] Immunosuppression;[139,140] plasmapheresis;[141] serotype switch;[142] inclusion of empty capsids (to serve as decoys);[143] novel vectors,[6,130] and co-administration of rapamycin nanoparticles with initial vector infusion to modulate the anti-AAV immune response,[144] need to be validated in non-human primates. The risk of increasing presentation of capsid proteins on hepatocytes causing loss of transduction inheres the infusion of empty capsids;[145] plasmapheresis does not decrease NAbs titer below the threshold for infusion in persons with high-titer anti-rAAV antibodies,[141] and only a transient effect is achieved in non-human primates by combining methylprednisolone, rituximab, mycophenolate mofetil, tacrolimus, and anti-thymocyte globulin.[140]

NAbs titers are low during the first year of age,[130,146] and the immune system of toddlers makes GT administration in pediatrics theoretically advantageous.[147] Very young persons are a large proportion of the hemophilia population with inhibitors to FVIII.[133] However, the adult liver is 16 times heavier than the neonatal one.[148] Due to liver growth, a drawback in GT performed at early age would be the loss of efficacy occurring because of dilution of transduced hepatocytes. Moreover, the obvious difference in the blood volume between a five-year-old and a twenty-year-old hemophilia person argues for an increase in the amount of FVIII/FIX needed to maintain the plasma concentration of the factor. Due to the normal growth of both the liver and intravascular space, it is unlikely that a neonatal (or early in life) injection will suffice to provide sustained correction of the hemophilia phenotype. Therefore, vector re-administration should be envisaged for young PWHs during the phase of rapid liver growth (and/or PWHs with loss of expression over their lifetime). A gene-editing program that uses a CRISPR/Cas9-based *in vivo* genome-editing to enable chromosomal integration of a modified human B-domain–deleted FVIII at the albumin locus (to prevent the loss of AAV vector due to hepatocyte proliferation) in liver cells is currently pursued (ASC Therapeutics) to move towards durable treatment options in young persons with HA.[149,150]

New standards, goals, open issues

Both in HA and HB, current data argue for AAV-based *in vivo* GT being successful to attain zero bleeds for years. Moreover, the estimated and observed risk of joint bleeding in HA persons that had undergone AAV-based *in vivo* GT reflects that of persons with mild-to-moderate HA.[151] Reduction in bleeds re-

Table 1. Non-invasive tests to assess hepatic fibrosis in fatty liver disease.

Variables evaluated*	Rating the risk: algorithms		
	Low risk	Intermediate risk°	High risk
Non-invasive tests			
FIB4 index	<1.3	1.3-2.67	>2.67
AAR	<0.8	0.8-1	>1
NFS	<-1.455	-1.455-0.676 m	>0.676 m
TE	<8kPa	8-13kPa	>13kPa
MRE	(<2.5 kPa)	2.5-4 kPa	(>4 kPa)
Key clinical information	BMI<30, no MS	BMI≥30, MS	BMI>30, MS

FIB4, fibrosis-4; AAR, aldosterone-renin ratio; NFS, NAFLD fibrosis score; TE, transient elastography; MRE, magnetic resonance elastography; BMI, body mass index.
*The following variables have been evaluated. *Person demographics*: age, sex, BMI, history of diabetes mellitus or of metabolic syndrome [≥3 of the following criteria: waist circumference >101.6 cm (men) or 88.9 cm (women), blood pressure >130/85 mmHg, fasting triglyceride level >150 mg/dL, fasting high-density lipoprotein cholesterol level <40 mg/dL (men) or 50 mg/dL (women), and fasting blood sugar >100 mg/dL]. *Routine blood tests*: alanine aminotransferase, aspartate aminotransferase, platelet counts, fasting plasma glucose, serum albumin. *Elastography data*: transient elastography, magnetic resonance elastography, and ultrasound-based shear wave elastography. The data on shear wave elastography are limited and not robust, especially in persons with alcoholic fatty liver disease.
°MRE mandatory, liver biopsy needed.
Additional information in *Supplementary Material*.

duces hospitalizations, outpatient visits, and drug treatment.[152] Thus, a persistent functionally efficacious transgene expression after a single GT treatment embodies a shift in the tendency to bleed and improves the QoL of HA persons. The pivotal phase 3 HOPE-B trial with etranacogene dezaparvovec documented sustained FIX activity and bleed protection in HB participants with baseline FIX levels ≤2%. ABR was reduced by 64% (p=0.0002) compared with infusion-based prophylaxis, and 96% of participants (p<0.0001) withdrew prophylaxis.[153] Compared to the results obtained 6 months pre-GT, health-related QoL was significantly improved as early as one- and two-years post-infusion, using a variety of generic and disease-specific pre-defined person reported outcomes. Significant differences were found in trial participants for the Hem-A-QoL total score (-6.0; p<0.0001); for the treatment (-13.94; p<0.0001), feelings (-9.01; p<0.0001), future (-6.45; p=0.0004) and work/school (-5.21; p=0.0098) domains. PWH with ≥15-point improvement ranged from 45.83% (95% CI: 31.37%, 60.83%) for treatment to 13.89% (95% CI: 4.67%, 29.50%) for family planning.[154] While it is presently unclear whether and to what extent GT can prevent new joint damage from occurring,[151] person reported questionnaires in AAV-based *in vivo* GT with valoctocogene roxaparvovec (Table 2), confirm and extend, in HA persons, the concepts summarized above for HB, and argue for such progress in HA largely depending on improvements in role functioning, and physical functioning.[155,156] Newer patient-relevant and patient-reported outcomes should be used to establish efficacy in persons that have undergone AAV-based *in vivo* GT.

Ongoing observations also argue for the long-term efficacy (*i.e.,* durability) of valoctocogene roxaparvovec for AAV-based treatment of HA being shorter than that of etranacogene dezaparvovec-drlb in AAV-based *in vivo* GT for HB.[82,133,151,157] Inherent to the decline in transgene expression is the fear of a progressive loss of treatment effect (*i.e.*, a relapse in the tendency to bleed). The World Federation of Hemophilia guidelines advise prophylaxis for moderate hemophilia persons (FVIII activity levels ≤5%) with a severe bleeding phenotype (*i.e.*,≥2 treated bleeds in 6 months).[158] Accordingly, a bleeding tendency is expected when a person with HA who has undergone AAV-based *in vivo* GT exhibits circulating FVIII activity levels <5% (*i.e.*, enters the moderate hemophilia range) and exhibits a severe bleeding phenotype (*i.e.*, ≥2 treated bleeds in 6 months).[159] The combination of clinical endpoints (two or more treated bleeds within 6 months), and an objectively measurable biomarker of

Table 2. Person reported outcomes questionnaires in gene therapy clinical trials for hemophilia A.

Study [Numbers of PWH studied]	Major finding(s)	Comments
Study 270-201: mean total HaemoQol-score over time [6E13 vg/kg cohort (n=7)] [4E13 vg/kg cohort (n=6)]	Sustained improvement in QoL over 6 years, as measured by Haemo-QoL-A: • Consequences of bleeding • Emotional impact • Physical functioning • Role functioning • Treatment concern • Worry	Other person reported outcomes questionnaires employed in gene therapy clinical trials for HA are: *EQ-5D-5L*: general questionnaire designed to measure health status. *HAL* (Hemophilia Activities List): questionnaire that measures the impact of HA on functional abilities in adults. *WPAI+CIQ: HS*: (Hemophilia Specific Work Productivity and Activity Impairment plus Classroom Impairment Questions): questionnaire designed to measure effect of symptom severity on work productivity and activity/classroom impairment.
Study 270-301 (GENEr8-1): quality of life [134 enrolled participants]	104-week study with planned subsequent 4-year long-term follow-up By week 156, persons experience clinically meaningful and statistically significant improvement in Haemo-QoL-A total score and the following domains: • Physical functioning • Role functioning • Consequences of bleeding	Improvements in scores reflect meaningful improvements in anxiety, pain and discomfort, ability to perform activities of daily living, social and family relationships.
Study 270-301 (GENEr8-1)q: predicting treated joint bleeds from FVIIIa activity [134 enrolled participants]	Count of treated joint bleeds and evaluation of FVIII activity (CSA and OSA, median). Follow-up divided into 4- or 6-week intervals for all 134 participants. Results compared to the estimate of treated joint bleeding rates and self-reported FVIII activity in 433 individuals. The relationship between transgene-derived FVIII activity the risk of joint bleeding reflect that reported with the use of epidemiologic data for persons with mild-to-moderate HA.	Relationship between count of joint bleeds and matched median FVIII activity was modeled using negative binomial regression.

PWH, persons with hemophilia; Haemo-QoL-A, Hemophilia-specific, health-related quality of life questionnaire for adults; HA, hemophilia A; FVIII, factor VIII; CSA, chromogenic assay; OSA, one-stage assay.

treatment effect (FVIII levels ≤5 U/dL) is indeed needed to establish the return to prophylaxis due to a *total loss of response* of GT. If only bleeds were considered, they could reflect lifestyle choices (*e.g.,* traumatic bleeds due to practicing sports). If only FVIII levels are considered, all persons that have ≤5% would lose response: however, data from the GENEr8-1 study (270-301) show that some PWH still have good hemostatic control when their endogenous FVIII levels are <5.[82]

Both for HA and HB, after AAV-based *in vivo* GT, circulating FIX/FVIII activity levels rise rapidly, and rapidly decline before levelling off and entering the slower decline phase. Data from the 2-3 year follow-up of 55 HB participants from phase 2 b (NCT03489291; n=3) and 3 (NCT03569891; n=52) studies that received a single administration of etranacogene dezaparvovec for AAV-based *in vivo* GT, show that, regardless of pre-treatment AAV5 NAb status, they would likely be free from prophylaxis for 25.5 years (Frequentist and Bayesian linear mixed models).[160] As to HA, the maximum follow-up of the 270-301 (GENEr8-1) study is 266 weeks (entire population, n=134 cases). For the BMN 270-201 study (where 7 HA persons received the licensed dose of 6x1013 vg/kg of valoctocogene roxaparvovec, *i.e.*, the same dose used in the GENEr8-1 study) a 374-week follow-up is available. Combining the data of the two studies reduces uncertainty as to the time to loss of response to valoctocogene roxaparvovec. In an individual-level modelling, the response to BMN 270 is lost 12.8 years (mean, 9.8 years median) after dosing (Table 3).

Information on the risk of a relapse in the tendency to bleed is mandatory for payers to absorb the cost of the *cure* and define new financial models.[161] However, durability predictions are based on statistical methods and *in vivo* results may dramatically differ. Long-term data are thus expected to address the issue. On the other hand, additional information to compute the costs and uncertainties of this *cure* are poorly available: 1) *vis-à-vis* the request of upfront reimbursements for a single high-cost GT treatment, current reimbursement models of prophylaxis for PWH fit the yearly assessment of coverage, *i.e.,* both benefits and treatment costs are spread out over time, and 2) price of EHL FIX products,[162] and of emicizumab and of EHL FVIII products,[163] vary from country to country in Europe (Tables 4 and 5). Overall, both for HA and HB, the durability issue (≥5% transgene FVIII/FIX expression) should be critical to define GT costs, and the latter should not exceed what each country is paying for FIX, FVIII, or for emicizumab.

Like in the nervous system,[164] nascent proteins are folded and secreted in the endoplasmic reticulum (ER). The unfolded protein response (UPR) pathway detects the conformity of protein folding in the ER lumen,[165] and transfers information to the nucleus to adjust the protein folding capacity or to induce cell apoptosis.[166] Function overload induced by a greater demand for

Table 3. Estimated duration of the effect of a dose of BMN 270.

Time elapsed after vector dose, years, (mean)	Loss of response (% of treated persons)
5	8.1
8	37.1
10	55.5
12.8 (9.4)	100 (total loss of response)

Additional information in *Supplementary Material*.

Table 4. Cost of prophylaxis with extended half-lives factor IX products for persons with hemophilia in Europe.

EU country (currency)	Product, cost/unit (list price)		
	Eftrenonacog alfa Alprolix®	Albutrepenonacog alfa Idelvion®	Nonacog beta pegol Refixia®
UK (£)	0.60	2.09	2.83
France (€)	0.96	1.71	NA
Italy (€)	1.38	2.20	2.20
Spain (€)	1.24	2.30	–
Germany (€)	1.47	1.41	1.70

NA, not available.
Calculations based on Burke *et al.*[167]

Table 5. Five-year costs/person in Europe using emicizumab and efmoroctocog alfa (Fc-fusion FVIII).*

EU country	Emicizumab 1.5 mg/kg once weekly		rFVIII Fc 85.4 U/kg/weekly	
	Emicizumab list price (mg)	5-yr cost/person	rFVIIIFc list price (U)	5-yr cost/person
UK	80.51 £	2.532.404	0.89 £	1.500.408
France	77.13 €	2.420.730	0.65 €	1.095.435
Italy	55.07 €	1.734.174	0.65 €	1.095.435
Spain	76.70 €	2.404.307	0.59 €	994.318
Germany	64.66 €	2.048.088	0.53 €	893.201

Calculations (in persons with hemophilia ≥12 years of age) based on Mancuso *et al.*[168]
*In addition to efmoroctocog alfa (Fc-fusion FVIII), a dramatic variability in costs is also documented for PEGylated EHL FVIII turoctocog alfa pegol and damoctocog alfa pegol.

protein folding, is involved in nervous system toxicities in non-human primates.[164,167,168] Transgene expression in AAV-based *in vivo* GT in hemophilia is limited to a subset of liver cells. At variance with FIX (whose native site of production is the hepatocyte), the native site for FVIII production is liver sinusoidal endothelial cells, which differs from the AAV vector target cell. The role of stress-induced apoptosis in the loss of FVIII expression overtime is unclear so far.

Perspectives

No repeated dosing regimen of EHL or non-factor products currently employed suffices to allow persons to feel free from the fears and the obligations of HA and HB. Breakthrough bleeding may still occur regardless of prophylaxis, and treatment is still invasive, negatively affecting the QoL in PWH. The achieving of sustained transgene factor IX levels that exceed those at which bleeds occur in GT trials – both in skeletal muscle[147,169] and in the circulation of HB persons undergoing AAV-based in vivo GT[74,164] – suggested the promise of a long-lasting cure for the bleeding tendency in hemophilia.[164] A progressive rise in the proportion of PWH persons likely to benefit from AAV-based *in vivo* GT emerges from data collected over the last 3-4 years, and helps to establish whether, among newer options, AAV-based *in vivo* GT is likely to become the treatment of choice in HA and HB. Recent information also provides data on the durability of transgene expression, and new laboratory methods for early detection and long-term monitoring of transgene related FVIII and FIX levels. This is a key to predict side effects, and to manage/monitor unanticipated risks and complications in PWH receiving rAAV-based *in vivo* GT.[62] Overall, a paradigm shift is underway that may change the treatment landscape and the structure of hemophilia Centers. *Curative levels* (*i.e.,* ≥15-20 U/mL) does not equal to *normal levels* (≥50 U/mL of FVIII/FIX).[170] This poses unique challenges to the "*former severe hemophilia person* and his care team. In this context, the sense of *a normal life* for PWH (and the setting of a new PWH-physician alliance) should stem from the awareness of the distinctive circumstances of the person (resources, culture, personality, behavior) and of his environment (family, friends, community, religion).[171] For stable solutions of unsolved issues of AAV-based *in vivo* GT, gene editing,[154] and antisense approaches,[172] are expected to complement gene augmentation. Together with a program in young persons with HA,[154,155] proof of concept of F9 gene editing has been tested in NHP models to allow GT treatments to provide a lifetime cure to PWH.[173] Although FDA requires all persons receiving any lentiviral vector for gene-editing to be followed for 15 years, this prospect is supported by the circumstance that none of those treated with newer lentivirus vectors for transfusion-dependent β-thalassemia (included children) has developed any leukemia or myelodysplastic syndrome following GT.[174]

Despite that progress in the area, the liberating potential of this transformative strategy on individuals for whom long-lasting cure has been inaccessible so far, still is to be fully recognized by government bodies. This is a pre-requisite for reimbursement agencies (to compute uncertainties and to absorb the cost of a *cure* for persons with chronic congenital diseases *e.g.,* hemophilia). To what extent the expense for AAV-based *in vivo* GT in PWH justifies a reduction in life-time costs for prophylaxis and hospitalizations implies a new sense for the *therapeutic success* model. A concerted action of health care professionals, PWH and their associations, pharmaceutical companies, researchers, and other stakeholders is needed for *ad hoc* educational programs to improve the perception of the advantages of a potentially one-off cure.[173] At least in the early stages, the affordability and sustainability of GT should also consider risk mitigation and coverage of specific treatment-associated issues. Because of the unusual delivery and monitoring, GT treatment is limited to centers with multidisciplinary, well-trained teams that hold the equipment and the ability for the intervention and the access to baseline and follow-up visits (centers of excellence). In most countries, ability for AAV-based *in vivo* GT is limited to very few centers. Persons with chronic diseases, (*e.g*., hemophilia) develop high confidence in their referring centers and may not wish to move to a new center. Ensuring active interactions between referring and dosing centers is critical for health equity.

Hemophilia – the most common inherited chronic disorder of blood coagulation (prevalence: 17.1 cases/100,000 males) – is estimated to affect 500,000 people globally.[13] The hope that GT will allow persons to achieve stable clotting factor expression and protect from spontaneous bleeding for years is critical for PWH living in low-income countries (350,000 persons, *i.e.,* ∼>3/4 of the whole PWH population). Mortality due to severe bleeding (*e.g.,* intracerebral bleeding) or to bleeding becoming severe (*e.g*., bleeding linked with circumcision in the absence of appropriate measures), is thought to explain the discrepancy between the expected (according to the population) *vs*. the observed number of PWH in low-income countries.[175] While the goal of a uniform progress in hemophilia handling is recommended as a key objective of future studies of AAV-based *in vivo* GT,[171] the technical complexity of the treatment argues against protocols for the advanced treatment of hemophilia being applicable where the greatest demand for AAV-based *in vivo* GT for this monogenic disease lies (*i.e*., low-medium income countries).[176] Improving diagnosis and medical reviews by promoting centers for patient management and quality programs for treaters (to regiment GT treatment) in low-medium income countries should be an obvious direction for pharmaceutical companies to strengthen their investments for ATMPs.

In high-income countries, aligning the interests of health systems and pharmaceutical companies appears as a key issue with respect to the therapeutic readiness of ATMPs. Due to the high costs to the person (€ 1.11 million for an average adult); the rarity of the disease (congenital lipoprotein-lipase deficiency, prevalence: 1 per million livebirths), and the expense to maintain therapeutic readiness by the company, Glybera™ (the first approved GT in Europe) has been withdrawn from the market in 2018. At that time, only 31 people in the world had been treated with this ATMP. Strimvelis™ (γ-retroviral transduction of hemopoietic stem cells) is the first GT product for treatment of adenosine deaminase deficiency causing severe combined immuno-deficiency (ADA-SCID).[177] Since, according to the company, the current cost of the product (€ 594,0000 for an average adult) does not guarantee a return of investment, the EMA has authorized the non-profit association TELETHON to produce and commercialize Strimvelis™ to prevent troubles for patients due to the risk of arrest of Strimvelis™ commercialization in Europe. Same threat also emerged for Zynteglo™ for GT treat-

Table 6. Durability and costs (for an average adult) of some approved advanced therapy medicinal products for rare diseases.

Product	Durability	Treatment costs	Comparator	Disease/comments	References*
Strimvelis™°	5 years (mean 2.4-15.4)	€ 594,0000 or $ 650,000	The price tag cost does not guarantee a return of investment (company policy)	Adenosine deaminase deficiency causing severe combined immuno-deficiency	179
Zynteglo™ *betibeglogene autotemcelor beti-cel etibe*	Lifelong efficacy	€ 1,650,000 or $ 1,900,000	Savings in supportive care for 50 years of thalassemia-free survival	Transfusion-dependent severe β-thalassemia.[#] Marketing authorisation withdrawn at the request of the marketing-authorisation holder	178
Glybera™ *Alipogene tiparvovec*	6 years	€ 1.11 million	Affordable when compared to yearly costs of the alternatives: small molecule drugs, monoclonal antibodies, enzyme replacement therapy	Congenital lipoprotein-lipase deficiency. Withdrawn from the market due to economic concerns (2018)[§]	180
Luxturna™ *Voretigene Neparvovec*	4-7.5 years[^]	$ 850,000	Difficult to put a price on regaining vision	Leber's congenital amaurosis. Criticisms for the high price tag ($ 425,000/eye)	181, 182
Zolgensma™ *Onasemnogene abeparvovec*	7.5 years	$ 2.1 million for children weighing 13.5-21 kg	Lower cost compared to Spinraza: $ 750,000 first year $ 375,000 annually for life[@]	Spinal muscular atrophy. The world's most expensive drug on the market: $ 425,000/yr for 5 years	183, 184

*Rather than to a total loss of response of GT, durability refers here to what has been observed and published to date. Please refer also to Muhuri *et al.*[185]
°Autologous CD34+ enriched cell fraction containing CD34+ cells transduced with retroviral vector that encodes for the human adenosine deaminase (ADA) cDNA sequence from human haematopoietic stem/progenitor (CD34+) cells.
[#]The incidence of transfusion-dependent severe β-thalassemia rates 15.9 cases/100,000 newborns in the Northern Mediterranean coast (*i.e.*, in Europe, where significant health infrastructures are available); 31.4/100,000 newborns in the Eastern coast; and 36.8 per 100,000 newborns in the Southern coast.
[§]The rarity of the inherited deficiency (prevalence: 1 per million livebirths), the cost to the person, and the expense to maintain therapeutic readiness by the Company made it very difficult to continue gene delivery commercially.
[^]>75% of the functional protein expressed.
[@]The only alternative treatment approved for spinal muscular atrophy.

ment of persons with transfusion-dependent severe β-thalassemia (TDT).[178] Based on the savings in supportive care that 50 years of hypothetical thalassemia-free survival would represent, the manufacturer asked for a price tag of €1,650,000 or $1,900,000, which makes the cost of Zynteglo™ 3 times higher than the current standard of care of TDT (allogeneic hematopoietic stem cell transplantation),[178] and makes its use too expensive for payers even in many high-income countries. Criticism for the high price tag and the rationale behind it (difficult to put a price on regaining vision) has been raised for the cost of Luxturna™ for the treatment of Leber's congenital amaurosis disease. As to Zolgensma™ for spinal muscular atrophy (SMA), its costs have been claimed to be lower compared to the price of Spinraza™ – the alternative cure approved for this neuromuscular disease (Table 6).[178-185] Price per patient of etranacogene dezaparvovec for AAV-based *in vivo* GT treatment for HB is €1,500,000,[186] that of valoctocogene roxaparvovec for GT treatment of HA is $900,000.[187] Tailored approaches to product pricing (*e.g.*, costs similar/cheaper than the global costs of current lifetime treatments; varied pricing in different countries) and economic models other than value-based pricing (*e.g.,* payment systems based upon costs of goods) are assumed to enable the commercialization of ATMPs and the advanced treatment of rare diseases in low-medium income countries. A partnership to align the interests of pharmaceutical companies and health systems, under the auspices of key scientific societies, is expected to be vital to this end. In parallel, guidelines that encompass the World Health Organization may simultaneously pledge both governments and the communities they serve to match regulatory frameworks and provide to supervisors a path to control the efficacy and safety, and to advance the suitability of ATMPs therapies.[176] Yet, bridging the gap between the promise of approved ATMPs and their readiness to those who may maximally benefit of them is a challenging task that calls for more intensive efforts to be made.[188,189]

References

1. High KA. Turning genes into medicines - what have we learned from gene therapy drug development in the past decade? Nat Commun 2020;11:5821.
2. Mendell JR, Al-Zaidy SA, Rodino-Klapac LR, al. Current clinical applications of in vivo gene therapy with AAVs. Mol Ther 2021;29:464-88.
3. Flotte TR, Afione SA, Zeitlin PL. Adeno-associated virus vector gene expression occurs in nondividing cells in the absence of vector DNA integration. Am J Respir Cell Mol Biol 1994;11:517-21.
4. Crenshaw BJ, Jones LB, Bell CR, et al. Perspective on Adenoviruses: epidemiology, pathogenicity, and gene therapy. Biomedicines. 2019;7.
5. Keeler AM, Flotte TR. Recombinant adeno-associated virus gene therapy in light of luxturna (and zolgensma and glybera): where are we, and how did we get here? Annu Rev Virol 2019;6:601-21.

6. Di Minno G, Miesbach W, Castaman G, Peyvandi F. Next-generation strategies to improve safety and efficacy of adeno-associated virus-based gene therapy for hemophilia: lessons from clinical trials in other gene therapies. Haematologica. 2024 Mar 7. [Online ahead of print]
7. Peyvandi F, Garagiola I. Clinical advances in gene therapy updates on clinical trials of gene therapy in hemophilia. Hemophilia 2019;25:738-46.
8. Spadarella G, Di Minno A, Brunetti-Pierri N, et al. The evolving landscape of gene therapy for congenital hemophilia: An unprecedented, problematic but promising opportunity for worldwide clinical studies. Blood Rev 2021;46:100737.
9. Nijdam A, Altisent C, Carcao MD, et al. Bleeding before prophylaxis in severe hemophilia: paradigm shift over two decades. Haematologica 2015;100:e84-6.
10. Oldenburg J, Dolan G, Lemm G. Hemophilia care then, now and in the future. Hemophilia 2009;15:2-7.
11. Mannucci PM. Treatment of hemophilia: building on strength in the third millennium. Hemophilia 2011;17:1-24.
12. Nijdam A, Kurnik K, Liesner R, et al. How to achieve full prophylaxis in young boys with severe hemophilia A: different regimens and their effect on early bleeding and venous access. Hemophilia 2015;21:444-50.
13. Mannucci PM. Hemophilia therapy: the future has begun. Haematologica 2020;105:545-53.
14. Chorba TL, Holman RC, Clarke MJ, Evatt BL. Effects of HIV infection on age and cause of death for persons with hemophilia A in the United States. Am J Hematol 2001;66:229-40.
15. Plug I, Van Der Bom JG, Peters M, et al. Mortality and causes of death in patients with hemophilia, 1992-2001: a prospective cohort study. J Thromb Haemost 2006;4:510-6.
16. Tagliaferri A, Rivolta GF, Iorio A, et al. Mortality and causes of death in Italian persons with hemophilia, 1990-2007. Hemophilia 2010;16:437-46.
17. Khair K, Ranta S, Thomas A, Lindvall K. The impact of clinical practice on the outcome of central venous access devices in children with hemophilia. Hemophilia 2017;23:e276-e81.
18. Di Minno A, Spadarella G, Nardone A, et al. Attempting to remedy sub-optimal medication adherence in hemophilia: the rationale for repeated ultrasound visualisations of the patient's joint status. Blood Rev 2019;33:106-16.
19. Iorio A, Marchesini E, Marcucci M, et al. Clotting factor concentrates given to prevent bleeding and bleeding-related complications in people with hemophilia A or B. Cochrane Database Syst Rev 2011:CD003429.
20. Balkaransingh P, Young G. Novel therapies and current clinical progress in hemophilia A. Ther Adv Hematol 2018;9:49-61.
21. Carvalhosa AM, Henrard S, Lambert C, Hermans C. Physical and mental quality of life in adult patients with hemophilia in Belgium: the impact of financial issues. Hemophilia 2014;20:479-85.
22. Chen SL. Economic costs of hemophilia and the impact of prophylactic treatment on patient management. Am J Manag Care 2016;22:s126-33.
23. Miesbach W, O'Mahony B, Key NS, Makris M. How to discuss gene therapy for hemophilia? A patient and physician perspective. Hemophilia 2019;25:545-57.
24. Wiley RE, Khoury CP, Snihur AWK, et al. From the voices of people with hemophilia A and their caregivers: Challenges with current treatment, their impact on quality of life and desired improvements in future therapies. Hemophilia 2019;25:433-40.
25. Lee Mortensen G, Strand AM, Almen L. Adherence to prophylactic haemophilic treatment in young patients transitioning to adult care: a qualitative review. Hemophilia 2018;24:862-72.
26. Thornburg CD, Duncan NA. Treatment adherence in hemophilia. Patient Prefer Adherence 2017;11:1677-86.
27. Olivieri M, Kurnik K, Pfluger T, Bidlingmaier C. Identification and long-term observation of early joint damage by magnetic resonance imaging in clinically asymptomatic joints in patients with hemophilia A or B despite prophylaxis. Hemophilia 2012;18:369-74.
28. Arruda VR, Doshi BS, Samelson-Jones BJ. Novel approaches to hemophilia therapy: successes and challenges. Blood 2017;130:2251-6.
29. Weyand AC, Pipe SW. New therapies for hemophilia. Blood 2019;133:389-98.
30. Jiménez-Yuste V, Auerswald G, Benson G, et al. Achieving and maintaining an optimal trough level for prophylaxis in hemophilia: the past, the present and the future. Blood Transf 2014;12:314-9.
31. Dodd C, Watts RG. A comparison of traditional vs. Canadian tailored prophylaxis dosing of prophylactic factor infusions in children with hemophilia A and B in a single hemophilia treatment center. Hemophilia 2012;18:561-7.
32. Krishnan S, Vietri J, Furlan R, Duncan N. Adherence to prophylaxis is associated with better outcomes in moderate and severe hemophilia: results of a patient survey. Hemophilia 2015;21:64-70.
33. Kraft J, Blanchette V, Babyn P, et al. Magnetic resonance imaging and joint outcomes in boys with severe hemophilia A treated with tailored primary prophylaxis in Canada. J Thromb Haemost 2012;10:2494-502.
34. Seuser A, Bohm P, Wermes C. Early orthopaedic challenges in hemophilia patients and therapeutic approach. Thromb Res 2014;134:S61-7.
35. 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.
36. Mazepa MA, Monahan PE, Baker JR, et al. Men with severe hemophilia in the United States: birth cohort analysis of a large national database. Blood 2016;127:3073-81.
37. Oladapo AO, Epstein JD, Williams E, et al. Health-related quality of life assessment in hemophilia patients on prophylaxis therapy: a systematic review of results from prospective clinical trials. Hemophilia 2015;21:e344-58.
38. Di Minno MN, Ambrosino P, Quintavalle G, et al. Assessment of hemophilic arthropathy by ultrasound: where do we stand? Semin Thromb Hemost 2016;42:541-9.
39. Lak M, Peyvandi F, Mannucci PM. Clinical manifestations and complications of childbirth and replacement therapy in 385 Iranian patients with type 3 von Willebrand disease. Br J Haematol 2000;111:1236-9.
40. Mannucci PM, Cortesi PA, Di Minno MND, et al. Comparative analysis of the pivotal studies of extended half-life re-

combinant FVIII products for treatment of hemophilia A. Hemophilia 2021;27:e422-e33.
41. Santoro C, Di Minno MND, Corcione A, et al. Improving assessment and management of pain in hemophilia: an Italian Delphi consensus statement. Blood Rev 2022;51:100885.
42. Blamey G, Buranahirun C, Buzzi A, et al. Hemophilia and sexual health: results from the HERO and B-HERO-S studies. Patient Relat Outcome Meas 2019;10:243-55.
43. Cassis FR, Buzzi A, Forsyth A, et al. Hemophilia Experiences, Results and Opportunities (HERO) study: influence of hemophilia on interpersonal relationships as reported by adults with hemophilia and parents of children with hemophilia. Hemophilia 2014;20:e287-95.
44. Cassis FR, Querol F, Forsyth A, Iorio A, Board HIA. Psychosocial aspects of hemophilia: a systematic review of methodologies and findings. Hemophilia 2012;18:e101-14.
45. Tagliaferri A, Franchini M, Rivolta GF, et al. Pain assessment and management in hemophilia: a survey among Italian patients and specialist physicians. Hemophilia 2018;24: 766-73.
46. Witkop M, Neff A, Buckner TW, al. Self-reported prevalence, description and management of pain in adults with hemophilia: methods, demographics and results from the Pain, Functional Impairment, and Quality of life (P-FiQ) study. Hemophilia 2017;23:556-65.
47. Auerswald G, Dolan G, Duffy A, et al. Pain and pain management in hemophilia. Blood Coagul Fibrinolysis 2016;27: 845-54.
48. Salzman R, Cook F, Hunt T, et al. Addressing the value of gene therapy and enhancing patient access to transformative treatments. Mol Ther 2018;26:2717-26.
49. Witkop ML, Lambing A, Nichols CD, et al. Interrelationship between depression, anxiety, pain, and treatment adherence in hemophilia: results from a US cross-sectional survey. Patient Prefer Adherence 2019;13:1577-87.
50. Davari M, Gharibnaseri Z, Ravanbod R, Sadeghi A. Health status and quality of life in patients with severe hemophilia A: a cross-sectional survey. Hematol Rep 2019;11:7894.
51. Mahlangu J, Oldenburg J, Callaghan MU, et al. Health-related quality of life and health status in persons with hemophilia A with inhibitors: a prospective, multicentre, non-interventional study (NIS). Hemophilia 2019;25:382-91.
52. O'Hara J, Walsh S, Camp C, et al. The impact of severe hemophilia and the presence of target joints on health-related quality-of-life. Health Qual Life Outcomes 2018;16:84.
53. Machin N, Ragni MV. Measuring success in hemophilia gene therapy using a factor level and outcomes yardstick. Expert Rev Hematol 2018;11:83-6.
54. Coppola A, Di Minno MN, Santagostino E. Optimizing management of immune tolerance induction in patients with severe hemophilia A and inhibitors: towards evidence-based approaches. Br J Haematol 2010;150:515-28.
55. Kempton CL, White GC, 2nd. How we treat a hemophilia A patient with a factor VIII inhibitor. Blood 2009;113:11-7.
56. Lusher JM, Scharrer I. Evolution of recombinant factor VIII safety: KOGENATE and Kogenate FS/Bayer. Int J Hematol 2009;90:446-54.
57. Oladapo AO, Lu M, Walsh S, et al. Inhibitor clinical burden of disease: a comparative analysis of the CHESS data. Orphanet J Rare Dis 2018;13:198.
58. Aledort L, Bullinger M, von Mackensen S, et al. Why should we care about quality of life in persons with hemophilia? Hemophilia 2012;18:e154-7.
59. O'Mahony B, Noone D, Prihodova L. Survey of coagulation factor concentrates tender and procurement procedures in 38 European Countries. Hemophilia 2015;21:436-43.
60. Srivastava A, Brewer AK, Mauser-Bunschoten EP, et al. Guidelines for the management of hemophilia. Hemophilia 2013;19:e1-47.
61. Ljung R, Auerswald G, Benson G, et al. Inhibitors in hemophilia A and B: management of bleeds, inhibitor eradication and strategies for difficult-to-treat patients. Eur J Haematol 2019;102:111-22.
62. Di Minno G, Castaman G, De Cristofaro R, et al. Progress, and prospects in the therapeutic armamentarium of persons with congenital hemophilia. Defining the place for liver-directed gene therapy. Blood Rev 2023;58:101011.
63. Mannucci PM, Kessler CM, Germini F, et al. Bleeding events in people with congenital hemophilia A without factor VIII inhibitors receiving prophylactic factor VIII treatment: a systematic literature review. Hemophilia 2023;29: 954-62.
64. Maria A, Alessandro C, Declan N, Peyvandi F. Hemorrhagic and thrombotic adverse events associated with emicizumab and extended half-life factor VIII replacement drugs: EudraVigilance data of 2021. J Thromb Haemost 2023;21:546-52.
65. Kitazawa T, Esaki K, Tachibana T, et al. Factor VIIIa-mimetic cofactor activity of a bispecific antibody to factors IX/IXa and X/Xa, emicizumab, depends on its ability to bridge the antigens. Thrombosis Haemostasis 2017;117: 1348-57.
66. 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;18:1570-4.
67. Mahlangu J. Emicizumab for the prevention of bleeds in hemophilia A. Exp Opin Biol Ther 2019;19:753-61.
68. Ferla R, Alliegro M, Dell'Anno M, et al. Low incidence of hepatocellular carcinoma in mice and cats treated with systemic adeno-associated viral vectors. Mol Ther Methods Clin Dev 2021;20:247-57.
69. Kuzmin DA, Shutova MV, Johnston NR, et al. The clinical landscape for AAV gene therapies. Nat Rev Drug Discov 2021;20:173-4.
70. 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.
71. Nathwani AC. Gene therapy for hemophilia. Hematology Am Soc Hematol Educ Program 2022:569-78.
72. Manno CS, Pierce GF, Arruda VR, et al. Successful transduction of liver in hemophilia by AAV-Factor IX and limitations imposed by the host immune response. Nat Med 2006;12:342-7.
73. Doshi BS, Arruda VR. Gene therapy for hemophilia: what does the future hold? Ther Adv Hematol 2018;9:273-93.
74. 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.
75. van der Loo JC, Wright JF. Progress and challenges in viral vector manufacturing. Hum Mol Genet 2016;25: R42-52.
76. George LA, Ragni MV, Rasko JEJ, et al. Long-term follow-

up of the first in human intravascular delivery of AAV for gene transfer: AAV2-hFIX16 for severe hemophilia B. Mol Ther 2020;28:2073-82.
77. Boutin S, Monteilhet V, Veron P, et al. Prevalence of serum IgG and neutralizing factors against adeno-associated virus (AAV) types 1, 2, 5, 6, 8, and 9 in the healthy population: implications for gene therapy using AAV vectors. Hum Gene Ther 2010;21:704-12.
78. Klamroth R, Hayes G, Andreeva T, et al. Global seroprevalence of pre-existing immunity against AAV5 and other AAV serotypes in people with hemophilia A. Hum Gene Ther 2022;33:432-41.
79. Pipe S, Leebeek FWG, Ferreira V, et al. Clinical considerations for capsid choice in the development of liver-targeted AAV-based gene transfer. Mol Ther Methods Clin Dev 2019;15:170-8.
80. Patton KS, Harrison MT, Long BR, et al. Monitoring cell-mediated immune responses in AAV gene therapy clinical trials using a validated IFN-gamma ELISpot method. Mol Ther Methods Clin Dev 2021;22:183-95.
81. Kotterman MA, Schaffer DV. Engineering adeno-associated viruses for clinical gene therapy. Nat Rev Genet 2014;15: 445-51.
82. Ozelo MC, Mahlangu J, Pasi KJ, et al. Valoctocogene roxaparvovec gene therapy for hemophilia A. N Engl J Med 2022;386:1013-25.
83. Ozelo MC, Mahlangu J, Pasi J, et al. Efficacy and safety of valoctocogene roxaparvovec adeno-associated virus gene transfer for severe hemophilia A: results from the phase 3 GENEr8-1 trial. Oral presentation at ISTH 2021 congress. Res Pract Thromb Haemost 2021;5:e12591.
84. Fong S, Yates B, Sihn CR, et al. Interindividual variability in transgene mRNA and protein production following adeno-associated virus gene therapy for hemophilia A. Nat Med 2022;28:789-97.
85. Schmidt M, Foster GR, Coppens M, et al. Molecular evaluation and vector integration analysis of HCC complicating AAV gene therapy for hemophilia B. Blood Adv 2023;7:4966-9.
86. 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.
87. 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.
88. 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.
89. Konkle BA, Walsh CE, Escobar MA, et al. BAX 335 hemophilia B gene therapy clinical trial results: potential impact of CpG sequences on gene expression. Blood 2021;137:763-74.
90. Pierce GF. Uncertainty in an era of transformative therapy for hemophilia: addressing the unknowns. Hemophilia 2021;27:103-13.
91. Nathwani AC, Rosales C, McIntosh J, et al. Long-term safety and efficacy following systemic administration of a self-complementary AAV vector encoding human FIX pseudotyped with serotype 5 and 8 capsid proteins. Mol Ther 2011;19: 876-85.
92. 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.
93. Rietveld IM, Lijfering WM, le Cessie S, et al. High levels of coagulation factors and venous thrombosis risk: strongest association for factor VIII and von Willebrand factor. J Thromb Haemost 2019;17:99-109.
94. Simioni P, Tormene D, Tognin G, et al. X-linked thrombophilia with a mutant factor IX (factor IX Padua). N Engl J Med 2009;361:1671-5.
95. Pierce GF, Iorio A. Past, present and future of hemophilia gene therapy: From vectors and transgenes to known and unknown outcomes. Hemophilia 2018;24:60-7.
96. Naso MF, Tomkowicz B, Perry WL, 3rd, Strohl WR. Adeno-associated virus (AAV) as a vector for gene therapy. BioDrugs 2017;31:317-34.
97. Samelson-Jones BJ, George LA. Adeno-associated virus gene therapy for hemophilia. Annu Rev Med 2023;74: 231-47.
98. Consortium EH. BioMarin announced an additional serious adverse event in its gene therapy clinical trial for hemophilia A. 2022 Available from: https://www.ehc.eu/biomarin-announced-an-additional-serious-adverse-event-in-its-gene-therapy-clinical-trial-for-hemophilia-a/
99. Donsante A, Miller DG, Li Y, et al. AAV vector integration sites in mouse hepatocellular carcinoma. Science 2007;317:477.
100. Hatada I, Morita S, Obata Y, et al. Identification of a new imprinted gene, Rian, on mouse chromosome 12 by fluorescent differential display screening. J Biochem 2001;130:187-90.
101. Nakai H, Montini E, Fuess S, et al. AAV serotype 2 vectors preferentially integrate into active genes in mice. Nat Genet 2003;34:297-302.
102. Chandler RJ, LaFave MC, Varshney GK, et al. Vector design influences hepatic genotoxicity after adeno-associated virus gene therapy. J Clin Invest 2015;125:870-80.
103. La Bella T, Imbeaud S, Peneau C, et al. Adeno-associated virus in the liver: natural history and consequences in tumour development. Gut 2020;69:737-47.
104. Nault JC, Datta S, Imbeaud S, et al. Recurrent AAV2-related insertional mutagenesis in human hepatocellular carcinomas. Nat Genet 2015;47:1187-93.
105. Chandler RJ, LaFave MC, Varshney GK, et al. Genotoxicity in mice following AAV gene delivery: a safety concern for human gene therapy? Mol Ther 2016;24:198-201.
106. Nguyen GN, Everett JK, Kafle S, et al. A long-term study of AAV gene therapy in dogs with hemophilia A identifies clonal expansions of transduced liver cells. Nat Biotechnol 2021;39:47-55.
107. Greig JA, Martins KM, Breton C, et al. Integrated vector genomes may contribute to long-term expression in primate liver after AAV administration. Nat Biotechnol 2023 Nov 6. [Online ahead of print].
108. Goedert JJ, Chen BE, Preiss L, et al. Reconstruction of the hepatitis C virus epidemic in the US hemophilia population, 1940-1990. Am J Epidemiol 2007;165:1443-53.
109. Svicher V, Cento V, Bernassola M, et al. Novel HBsAg markers tightly correlate with occult HBV infection and strongly affect HBsAg detection. Antivir Res 2012;93:86-93.

110. Conti F, Buonfiglioli F, Scuteri A, et al. Early occurrence and recurrence of hepatocellular carcinoma in HCV-related cirrhosis treated with direct-acting antivirals. J Hepatol 2016;65:727-33.
111. Qvigstad C, Tait RC, Rauchensteiner S, et al. The elevated prevalence of risk factors for chronic liver disease among ageing people with hemophilia and implications for treatment. Medicine (Baltimore) 2018;97:e12551.
112. Llovet JM, Villanueva A. Liver cancer: effect of HCV clearance with direct-acting antiviral agents on HCC. Nat Rev Gastroenterol Hepatol 2016;13:561-2.
113. Han MAT. Noninvasive tests (NITs) for hepatic fibrosis in fatty liver syndrome. Life (Basel) 2020;10.
114. Grundy SM, Cleeman JI, Daniels SR, et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circulation 2005;112:2735-52.
115. Venkatesh SK, Yin M, Ehman RL. Magnetic resonance elastography of liver: technique, analysis, and clinical applications. J Magn Reson Imaging 2013;37:544-55.
116. Shah AG, Lydecker A, Murray K, et al. Comparison of noninvasive markers of fibrosis in patients with nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol 2009;7:1104-12.
117. Angulo P, Hui JM, Marchesini G, et al. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. Hepatology 2007;45:846-54.
118. LoDuca PA, Hoffman BE, Herzog RW. Hepatic gene transfer as a means of tolerance induction to transgene products. Curr Gene Ther 2009;9:104-14.
119. Crudele JM, Finn JD, Siner JI, et al. AAV liver expression of FIX-Padua prevents and eradicates FIX inhibitor without increasing thrombogenicity in hemophilia B dogs and mice. Blood 2015;125:1553-61.
120. Finn JD, Nichols TC, Svoronos N, et al. The efficacy and the risk of immunogenicity of FIX Padua (R338L) in hemophilia B dogs treated by AAV muscle gene therapy. Blood 2012;120:4521-3.
121. Finn JD, Ozelo MC, Sabatino DE, et al. Eradication of neutralizing antibodies to factor VIII in canine hemophilia A after liver gene therapy. Blood 2010;116:5842-8.
122. Mingozzi F, Hasbrouck NC, Basner-Tschakarjan E, et al. Modulation of tolerance to the transgene product in a nonhuman primate model of AAV-mediated gene transfer to liver. Blood 2007;110:2334-41.
123. Arruda VR, Samelson-Jones BJ. Gene therapy for immune tolerance induction in hemophilia with inhibitors. J Thromb Haemost 2016;14:1121-34.
124. Ragni MV, George LA, Members of working group tNSotSWofViGanbffr. The national blueprint for future factor VIII inhibitor clinical trials: NHLBI State of the Science (SOS) Workshop on factor VIII inhibitors. Hemophilia 2019;25:581-9.
125. Gringeri A, Mantovani LG, Scalone L, et al. Cost of care and quality of life for patients with hemophilia complicated by inhibitors: the COCIS study group. Blood 2003;102: 2358-63.
126. Carcao M, Escuriola-Ettingshausen C, Santagostino E, et al. The changing face of immune tolerance induction in hemophilia A with the advent of emicizumab. Hemophilia 2019;25:676-84.
127. Gollomp KL, Doshi BS, Arruda VR. Gene therapy for hemophilia: progress to date and challenges moving forward. Transfus Apher Sci 2019;58:602-12.
128. Di Minno G, Coppola A, Margaglione M, et al. Predictors of inhibitor eradication by primary immune tolerance induction in severe hemophilia A with high responding inhibitors. Hemophilia 2022;28:55-64.
129. Hay CR, DiMichele DM, International Immune Tolerance S. The principal results of the International Immune Tolerance study: a randomized dose comparison. Blood 2012;119:1335-44.
130. Mimuro J, Mizukami H, Shima M, Matsushita T, Taki M, Muto S, et al. The prevalence of neutralizing antibodies against adeno-associated virus capsids is reduced in young Japanese individuals. J Med Virol 2014;86:1990-7.
131. Monahan PE, Negrier C, Tarantino M, et al. Emerging immunogenicity and genotoxicity considerations of adeno-associated virus vector gene therapy for hemophilia. J Clin Med 2021;10.
132. 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.
133. Pipe SW, Leebeek FWG, Recht M, et al. Gene therapy with etranacogene dezaparvovec for hemophilia B. N Engl J Med 2023;388:706-18.
134. Chiorini JA, Afione S, Kotin RM. Adeno-associated virus (AAV) type 5 Rep protein cleaves a unique terminal resolution site compared with other AAV serotypes. J Virol 1999;73:4293-8.
135. Gao G, Vandenberghe LH, Wilson JM. New recombinant serotypes of AAV vectors. Curr Gene Ther 2005;5:285-97.
136. Halbert CL, Miller AD, McNamara S, et al. Prevalence of neutralizing antibodies against adeno-associated virus (AAV) types 2, 5, and 6 in cystic fibrosis and normal populations: implications for gene therapy using AAV vectors. Hum Gene Ther 2006;17:440-7.
137. Majowicz A, Nijmeijer B, Lampen MH, et al. Therapeutic hFIX activity achieved after single AAV5-hFIX treatment in hemophilia B patients and NHPs with pre-existing anti-AAV5 NABs. Mol Ther Methods Clin Dev 2019;14:27-36.
138. Leborgne C, Barbon E, Alexander JM, et al. IgG-cleaving endopeptidase enables in vivo gene therapy in the presence of anti-AAV neutralizing antibodies. Nat Med 2020;26: 1096-101.
139. Corti M, Elder M, Falk D, et al. B-cell depletion is protective against anti-AAV capsid immune response: a human subject case study. Mol Ther Methods Clin Dev 2014;1.
140. Unzu C, Hervas-Stubbs S, Sampedro A, et al. Transient and intensive pharmacological immunosuppression fails to improve AAV-based liver gene transfer in non-human primates. J Translat Med 2012;10:122.
141. Monteilhet V, Saheb S, Boutin S, et al. A 10 patient case report on the impact of plasmapheresis upon neutralizing factors against adeno-associated virus (AAV) types 1, 2, 6, and 8. Mol Ther 2011;19:2084-91.
142. Buchlis G, Podsakoff GM, Radu A, et al. Factor IX expression in skeletal muscle of a severe hemophilia B patient 10 years after AAV-mediated gene transfer. Blood 2012;119: 3038-41.

143. Mingozzi F, High KA. Immune responses to AAV vectors: overcoming barriers to successful gene therapy. Blood 2013;122:23-36.
144. Meliani A, Boisgerault F, Hardet R, et al. Antigen-selective modulation of AAV immunogenicity with tolerogenic rapamycin nanoparticles enables successful vector re-administration. Nat Commun 2018;9:4098.
145. 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; 132:491.
146. Louis Jeune V, Joergensen JA, Hajjar RJ, Weber T. Pre-existing anti-adeno-associated virus antibodies as a challenge in AAV gene therapy. Hum Gene Ther Methods 2013; 24:59-67.
147. McKay TR, Rahim AA, Buckley SM, et al. Perinatal gene transfer to the liver. Curr Pharm Design 2011;17:2528-41.
148. Coppoletta JM, Wolbach SB. Body length and organ weights of infants and children: a study of the body length and normal weights of the more important vital organs of the body between birth and twelve years of age. Am J Pathol 1933;9:55-70.
149. Chen H, Shi M, Gilam A, et al. Hemophilia A ameliorated in mice by CRISPR-based in vivo genome editing of human Factor VIII. Sci Rep 2019;9:16838.
150. Johnson V. First patient with hemophilia A dosed in new gene therapy trial. 2023 [cited 2023 December 29th]; Available from: https://www.cgtlive.com/view/first-patient-hemophilia-a-dosed-new-gene-therapy-trial
151. Mahlangu J, Kaczmarek R, von Drygalski A, et al. Two-year outcomes of valoctocogene roxaparvovec therapy for hemophilia A. N Engl J Med 2023;388:694-705.
152. Cook K, Forbes SP, Adamski K, et al. Assessing the potential cost-effectiveness of a gene therapy for the treatment of hemophilia A. J Med Econ 2020;23:501-12.
153. Pipe S, Leebeek WG, Recht M, et al. Gene therapy with etranacogene dezaparvovec for hemophilia B. N Engl J Med 2023;388:706-18.
154. Itzler R, Buckner TW, Leebeek FWG, et al. Effect of etranacogene dezaparvovec on quality of life for severe and moderately severe hemophilia B participants: results from the phase III HOPE-B trial 2 years after gene therapy. Hemophilia 2024;30:709-19.
155. Quinn J, Delaney KA, Wong WY, et al. Psychometric validation of the Haemo-QOL-A in participants with hemophilia A treated with gene therapy. Patient Relat Outcome Meas 2022;13:169-80.
156. Pasi KJ, Laffan M, Rangarajan S, et al. Persistence of haemostatic response following gene therapy with valoctocogene roxaparvovec in severe hemophilia A. Hemophilia 2021;27:947-56.
157. von Drygalski A, Gomez E, Giermasz A, et al. Stable and durable factor IX levels in patients with hemophilia B over 3 years after etranacogene dezaparvovec gene therapy. Blood Adv 2023;7:5671-9.
158. Srivastava A, Santagostino E, Dougall A, et al. WFH Guidelines for the management of hemophilia, 3rd ed. Hemophilia 2020;26:1-158.
159. Nathwani AC, Davidoff AM, Tuddenham EGD. Advances in gene therapy for hemophilia. Hum Gene Ther 2017;28: 1004-12.
160. Shah J, Kim H, Sivamurthy K, et al. Comprehensive analysis and prediction of long-term durability of factor IX activity following etranacogene dezaparvovec gene therapy in the treatment of hemophilia B. Curr Med Res Opin 2023;39:227-37.
161. Vokinger KN, Avorn J, Kesselheim AS. Sources of innovation in gene therapies - approaches to achieving affordable prices. N Engl J Med 2023;388:292-5.
162. Burke T, Asghar S, O'Hara J, et al. Clinical, humanistic, and economic burden of severe haemophilia B in adults receiving factor IX prophylaxis: findings from the CHESS II real-world burden of illness study in Europe. Orphanet J Rare Dis 2021;16:521.
163. Mancuso ME, Castaman G, Pochopien M, et al. Cost-minimization analysis of recombinant factor VIII Fc versus emicizumab for treating patients with hemophilia A without inhibitors in Europe. J Med Econ 2022;25:1068-75.
164. Hordeaux J, Buza EL, Jeffrey B, et al. MicroRNA-mediated inhibition of transgene expression reduces dorsal root ganglion toxicity by AAV vectors in primates. Sci Transl Med 2020;12.
165. Hetz C, Zhang K, Kaufman RJ. Mechanisms, regulation and functions of the unfolded protein response. Nat Rev Mol Cell Biol 2020;21:421-38.
166. Pipe SW, Gonen-Yaacovi G, Segurado OG. Hemophilia A gene therapy: current and next-generation approaches. Exp Opin Biol Ther 2022;22:1099-115.
167. Ghemrawi R, Khair M. Endoplasmic reticulum stress and unfolded protein response in neurodegenerative diseases. Int J Mol Sci 2020;21.
168. Prasad V, Greber UF. The endoplasmic reticulum unfolded protein response - homeostasis, cell death and evolution in virus infections. FEMS Microbiol Rev 2021;45.
169. Jiang H, Pierce GF, Ozelo MC, et al. Evidence of multiyear factor IX expression by AAV-mediated gene transfer to skeletal muscle in an individual with severe hemophilia B. Mol Ther 2006;14:452-5.
170. Spadarella G, Di Minno A, Milan G, et al. Paradigm shift for the treatment of hereditary hemophilia: towards precision medicine. Blood Rev 2020;39:100618.
171. Di Minno G, Tremoli E. Tailoring of medical treatment: hemostasis and thrombosis towards precision medicine. Haematologica 2017;102:411-8.
172. Ponomarev AS, Chulpanova DS, Yanygina LM, et al. Emerging gene therapy approaches in the management of spinal muscular atrophy (SMA): an overview of clinical trials and patent landscape. Int J Mol Sci 2023;24.
173. Targeted Gene Insertion of Factor 9 as a Potential Durable Treatment for Hemophilia B. CRISPR/Cas9-mediated F9 gene insertion therapy / Intellia Therap, Regeneron - LARVOL DELTA 2023 [cited; Available from: https://delta.larvol.com/Products/?ProductId=234344ff-0ff4-493f-8c30-4f719fd027ae
174. Soni S. Gene therapies for transfusion dependent beta-thalassemia: current status and critical criteria for success. Am J Hematol 2020;95:1099-112.
175. Lambert C, Meite N, Sanogo I, et al. Hemophilia in Cote d'Ivoire (the Ivory Coast) in 2017: Extensive data collection

as part of the World Federation of Hemophilia's twinning programme. Hemophilia 2019;25:236-43.
176. Adair JE, Androski L, Bayigga L, et al. Towards access for all: 1st Working Group Report for the Global Gene Therapy Initiative (GGTI). Gene Ther 2023;30:216-21.
177. Aiuti A, Roncarolo MG, Naldini L. Gene therapy for ADA-SCID, the first marketing approval of an ex vivo gene therapy in Europe: paving the road for the next generation of advanced therapy medicinal products. EMBO Mol Med 2017;9:737-40.
178. Thuret I, Ruggeri A, Angelucci E, Chabannon C. Hurdles to the adoption of gene therapy as a curative option for transfusion-dependent thalassemia. Stem Cells Translat Med 2022;11:407-14.
179. Migliavacca M, Barzaghi F, Fossati C, et al. Long-term and real-world safety and efficacy of retroviral gene therapy for adenosine deaminase deficiency. Nat Med 2024; 30:488-97.
180. Gaudet D, Stroes ES, Méthot J, et al. Long-term retrospective analysis of gene therapy with alipogene tiparvovec and its effect on lipoprotein lipase deficiency-induced pancreatitis. Hum Gene Ther 2016;27:916-25.
181. Maguire AM, High KA, Auricchio A, et al. Age-dependent effects of RPE65 gene therapy for Leber's congenital amaurosis: a phase 1 dose-escalation trial. Lancet 2009;374: 1597-605.
182. Maguire AM, Russell S, Chung DC, et al. Durability of Voretigene Neparvovec for Biallelic RPE65-mediated inherited retinal disease: phase 3 results at 3 and 4 years. Ophthalmology 2021;128:1460-8.
183. Mendell JR, Al-Zaidy SA, Lehman KJ, et al. Five-Year Extension Results of the Phase 1 START trial of onasemnogene abeparvovec in spinal muscular atrophy. JAMA Neurol 2021;78:834-41.
184. Retson L, Tiwari N, Vaughn J, et al. Epithelioid neoplasm of the spinal cord in a child with spinal muscular atrophy treated with onasemnogene abeparvovec. Mol Ther 2023;31:2991-8.
185. Muhuri M, Levy DI, Schulz M, et al. Durability of transgene expression after rAAV gene therapy. Mol Ther 2022;30: 1364-80.
186. Meier N, Fuchs H, Galactionova K, et al. Cost-effectiveness analysis of etranacogene dezaparvovec versus extended half-life prophylaxis for moderate-to-severe haemophilia B in Germany. Pharmacoecon Open 2024;8:373-87.
187. Chapman M. German health insurance fund to reimburse Roctavian for hem A. 2023 Available from: https://hemophilianewstoday.com/news/german-health-insurance-fund-will-reimburse-rocatvian-hem-a/
188. Mannucci PM. Gene transfer in hemophilia A: not cogent yet. Bleed Thromb Vascul Biol 2022;1:32.
189. Castaman G. Gene transfer in hemophilia B: a big step forward. Bleed Thromb Vascul Biol 2023;2:70.

Online supplementary material:
Additional information to Tables.