Author Manuscript

Gene Therapy for Hemophilia

Steven W. Pipe, MD

Correspondence: Steven W. Pipe, MD

Professor of Pediatrics and Pathology

University of Michigan

D4202 MPB

1500 E Medical Ctr Dr

Ann Arbor, MI, USA

48109-5718

Tel.: 734-647-2893

Fax: 734-615-0464

Email: ummdswp@med.umich.edu

Word count: Abstract 108, main text 3,957

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/pbc.26865.

This article is protected by copyright. All rights reserved.

Tables, Figures: 3

Running title: Gene therapy for hemophilia

Key words: hemophilia, gene therapy, adeno-associated virus, clinical trials

Abbreviations:

FVIII	Factor VIII
FIX	Factor IX
AAV	Adeno-associated virus
ITR	Inverted terminal repeats
ZFN	Zinc finger nucleases
TALEN	transcription activator-like effector nucleases
CRISPR	clustered regularly interspaced short palindromic repeat
NHEJ	Non-homologous end joining
HDR	Homology-directed repair
DSB	Double stranded break
iPSC	Induced pluripotent stem cell
HSC	Hematopoietic stem cell

Abstract

Individuals with the inherited bleeding disorder hemophilia, have achieved tremendous advances in clinical outcomes through widespread implementation of prophylactic replacement with safe and efficacious factor VIII and IX. However, despite this

This article is protected by copyright. All rights reserved.

Author Manuscript

therapeutic approach, bleeds still occur, some with serious consequence, joint disease has not been eradicated, and patients have not yet been liberated from the need for regular intravenous infusions. The shift from protein replacement to gene replacement is offering great hope to achieve durable levels of plasma factor activity levels high enough to remove the risk for recurrent joint bleeding. For the first time, clinical trial results are showing promise for "curative" correction of the bleeding phenotype.

Author Manuscript

This article is protected by copyright. All rights reserved.

Introduction

Hemophilia is an X-linked recessive bleeding disorder leading to spontaneous bleeding and bleeding following trauma and surgery. Though typically expressed in males, female genetic carriers may have clinical bleeding symptoms and even factor activity levels in the hemophilia range. It is characterized by a congenital deficiency of coagulation factor VIII (hemophilia A) and factor IX (hemophilia B) affecting approximately 20,000 individuals in the USA and over 400,000 individuals across the globe[1,2]. Hemophilia arises from mutations in the *F8* and *F9* genes with an incidence of about 1 in 5000 and 1 in 30,000 male births, respectively, with >30% of cases occurring due to spontaneous mutations, and affects all racial and ethnic groups[3]. Severity is defined by the residual plasma factor activity, with those with severe deficiency (<1% activity) accounting for about half of affected individuals. Without the availability of replacement therapy, individuals with severe disease are at risk for recurrent bleeding into joints (hemarthroses), muscles, soft tissues and other locations that can be life-threatening (eg. central nervous system). Long-term sequelae as a result of recurrent bleeding include chronic arthropathy, chronic pain, muscle atrophy, and loss of mobility with significant disability[4].

The mortality and morbidity of severe hemophilia has been significantly impacted by the development of factor VIII (FVIII) and factor IX (FIX) concentrates. The current standard of care is prophylactic factor replacement therapy[5,6]. The rationale for prophylaxis was the observation that individuals with moderate hemophilia (as little as 1-5% of residual activity) exhibited fewer hemarthroses and were much less likely to develop arthropathy[4,7]. The hypothesis was that regular infusions of factor concentrates with a goal of maintaining a plasma activity that did not fall below 1-2% would lead to a more moderate clinical phenotype with less joint bleeding and subsequent arthropathy. Primary prophylaxis initiated in infancy and early childhood in the absence of documented joint disease and continued

This article is protected by copyright. All rights reserved.

Author Manuscript

indefinitely has been proven to prevent overall bleeding, joint bleeding and arthropathy and has led to health-related quality of life measures that are indistinguishable from their unaffected peers.

Hemophilia in the recombinant DNA era

The recombinant era for hemophilia began in the 1980s with the cloning of the *F8* and *F9* genes and the subsequent expression of functional proteins for both FVIII and FIX within mammalian cell lines[8]. The rationale for recombinant clotting factors included: a) that they would be safer than their plasma-derived counterparts, especially as they were being developed on the backdrop of the catastrophic viral contamination of plasma-derived clotting factors, b) the development of consistent manufacturing and processing that was liberated from the uncertainties of securing source plasma, c) a potentially unlimited supply that could drive down costs of replacement therapy, d) that this would facilitate an increase in the utilization of prophylaxis and e) wider availability of replacement products for patients in developing countries. Over the past 20 years of clinical trial and worldwide experience, recombinant clotting factors have not had any infectious pathogen transmission, no safety signals on adverse event reporting and no evidence of increased rate of inhibitors in previously treated patients[9,10]. Further, recombinant DNA technology has facilitated efforts that are exploiting insights on the structure and function of FVIII and FIX to introduce targeted modifications that enhance their functional properties. This has best been exemplified in a recent wave of bioengineered molecules that have more efficient production and purification, optimized post-translational modifications and enhanced pharmacokinetics[11,12].

Author Manuscript

This article is protected by copyright. All rights reserved.

The efficient production of recombinant clotting factors in mammalian and eventually human cell culture systems required overcoming significant challenges due to the complex post-translational modifications that are integral to their procoagulant function[8]. In addition, preclinical animal models were utilized to conduct pharmacokinetic, efficacy and safety evaluations. These were particularly important to characterize and evaluate bioengineered molecules with enhanced properties. This laid the groundwork for moving from protein-based replacement therapy to gene-based replacement therapy (**Figure 1**).

What are the remaining unmet needs?

With widespread availability of safe and effective plasma-derived and recombinant clotting factors and effective implementation of primary prophylaxis, clinical outcomes in hemophilia have made outstanding advances. However, there are remaining barriers to the adoption and adherence to the demands of prophylaxis[12]. The protein replacement strategy requires venous access from a young age, significant demands in cost and time to patients and their caregivers as well as health system access challenges. Clinicians must adapt replacement therapy to a wide range of phenotypic and pharmacokinetic variability. Long-term follow up data with current approaches has been limited to 25-30 years but have shown that annualized bleeding rates are not zero and joint disease still appears in young adults[13,14]. Thus, what are the implications over a lifetime?

Individuals with hemophilia may also develop an immune response triggered by exposure to the FVIII or FIX protein in any of its forms (plasma-derived or recombinant) and these IgG antibodies (inhibitors) neutralize the coagulant effect of the infused factor. The incidence is highest in those with severe disease (20-30% of severe hemophilia A, 1-4% of severe hemophilia B)[15]. These typically develop early in life (median age 1.7-3.3 years) with

This article is protected by copyright. All rights reserved.

Author Manuscript

the greatest exposure within the first 50 exposure days to the infused product. Individuals with inhibitors must rely on alternative hemostatic agents, bypassing agents (activated prothrombin complex concentrates, recombinant factor VIIa), that have unpredictable efficacy. Eradication of the inhibitors can be achieved (in about 70%) through an immune tolerance therapy in which high doses of FVIII or FIX are given over a long period to time until the antibody has waned. However, the total cost of treating inhibitors is even more significant with increased factor utilization and an adverse impact on clinical outcomes with higher mortality and morbidity from joint disease and bleeding[16].

The relatively short half-life of FVIII and FIX leads to the most significant burden of treatment with a standard prophylaxis regimen for FVIII of three to four infusions per week (two to three infusions per week for FIX)[17]. This is what has typically been required to maintain trough factor levels above approximately 1%. Extended half-life versions of FVIII and FIX have received FDA approval. Although the extended half-life FVIII products offer very little change in the dosing frequency of infusions, the extended half-life FIX offer the possibility of weekly and even every 2 week infusion strategies. These products have also enabled the ability to achieve higher trough levels when clinically indicated with a reasonable infusion frequency, and potentially improve adherence to prophylaxis[12]. These incremental improvements in care may slow the transition away from protein-based replacement therapy to gene-based delivery. Nevertheless, these new therapies have not reduced the cost of care for patients and health systems, have not eliminated the burden of regular venous access, and it is not known if these will significantly impact the incidence of inhibitors.

Why gene therapy for hemophilia?

Author Manuscript

This article is protected by copyright. All rights reserved.

Moving from protein replacement to gene replacement overcomes many of the unmet challenges to hemophilia care. Gene therapy would rely on endogenous expression of the clotting factor leading to steady state levels and a sustained duration of action. This would liberate individuals from prophylaxis and the need for regular intravenous delivery. The efficacy of the therapy would not be tied to adherence. This would have the greatest impact on the overall burden of therapy. Endogenous expression of the factors could be less immunogenic as they would have altered interaction with the immune system and could potentially even be a more effective tolerizing therapy in those with established inhibitors[18]. With current therapy, more than 90% of the overall costs of care for hemophilia is the cost of the clotting factor concentrates[19,20]. These costs can be >$300,000 USD per year[21]. Gene therapy offers an opportunity for a "one and done" intervention and, if it allows for discontinuation of prophylaxis, would result in enormous cost savings over the course of a lifetime. In addition, due to the costs of care and health care access challenges in the developing world, >75% of individuals around the globe have limited or no access to any factor replacement therapy[22]. A gene therapy intervention that could convert those with severe disease to a mild phenotype would dramatically alter the outcomes for hemophilia around the world.

Why target the liver for gene expression?

The liver is a central organ for rare diseases with >400 described rare monogenic disorders associated with the liver, many of which can be cured through orthotopic liver transplantation[23]. There are a subset of these monogenic diseases associated with the liver that are well-suited to gene therapy. These include hemophilia A and B because of the following characteristics: a well-understood disease biology, restoration of protein levels to as little as 5-10% is clinically meaningful, the availability of preclinical models, well-described

This article is protected by copyright. All rights reserved.

Author Manuscript

biomarkers, readily identifiable patients, a short time to the primary outcome measure (plasma factor activity level) in order to shorten the time to proof of concept, and the opportunity for orphan drug designation to encourage research and development toward commercialization (**Figure 2**). In contrast, caution remains in targeting the liver as it is unknown how gene therapy would affect the risk for liver cancer, especially in a population with existing pathology from prior hepatitis infection. Malignancy was an issue with some early gene therapy trials targeting hematopoietic stem cells, however, the mechanism appeared to be related to the use of retrovirus vectors which integrated into target cell chromosomes leading to activation of cellular proto-oncogenes. Such risk could be minimized by adopting non-insertional (ie. non-integrative) approaches[24].

Which vectors efficiently target the liver?

The primary tools for gene transfer have included non-viral vectors, retroviral vectors, adenoviral vectors, lentiviral vectors and adeno-associated viral vectors. Each of these can target the liver and have their distinct advantages and disadvantages (see reviews[25-27]). Adeno-associated virus (AAV) is a non-enveloped parvovirus that was discovered as an accompanying virion to adenovirus infection, shows widespread infection in the human population and yet is not associated with any pathogenic disease[28]. Wild-type AAV contains overlapping genes which encode the replication (r*ep*) and capsid (*cap*) proteins between two inverted terminal repeats (ITRs). Significantly, the *rep* and *cap* genes can be provided in *trans*, thus the genome can be replaced with an expression cassette for a therapeutic protein between the ITRs. These recombinant AAV (rAAV) vectors have been engineered to remove their integrative capacity such that they persist intracellulary almost exclusively as episomal chromatin to provide a template leading to durable expression of the therapeutic protein. That integration events into the host genome are rare is a distinct safety advantage

This article is protected by copyright. All rights reserved.

Author Manuscript

for rAAV over retroviral and lentiviral vectors. Dozens of naturally occurring and genetically engineered AAV capsids (the protein shell of the virus) have been characterized. Small differences in the capsid sequence (characterized by serotyping) can significantly influence the tissue tropism of the vector and can therefore be exploited to improve the efficacy of the gene transfer. This has identified rAAV vectors with high tropism to the liver[29]. Other distinctive advantages for rAAV vectors have been their capacity to transduce post-mitotic cells, a low risk for germline transmission with systemic delivery and a reduced inflammatory response. Disadvantages include a cumbersome production system, a compact size which limits the capacity to accommodate larger therapeutic gene cassettes and significant pre-existing humoral immunity in the population. Overall, the relative efficacy and safety of rAAV vectors has made them the most highly suited for clinical gene therapy and the first to commercialization (AAV-based treatment for familial lipoprotein lipase deficiency[30]).

Observations from early clinical trials in hemophilia

The earliest gene therapy trial for hemophilia B was conducted in China using a retroviral vector that transduced autologous skin fibroblasts with a FIX construct *ex vivo* that was then subsequently injected into subjects[31]. This achieved transient expression of FIX up to 2% with partial correction of the bleeding phenotype. A subsequent trial utilized intramuscular injection but failed to achieve persistent elevations of FIX in the plasma[32] that was then followed by intravascular delivery of rAAV into the hepatic artery[33]. This led to expression of FIX between 10-12%. However, the expression was transient over several weeks with loss of expression following an asymptomatic and self-limited elevation in liver transaminases. This was not observed in preclinical animal models. Several hypotheses have been proposed for this observation[29]. First, capsid antigen presentation on the hepatocyte surface with accompanying memory T-cell activation may lead to clearance of

This article is protected by copyright. All rights reserved.

Author Manuscript

AAV-transduced cells. Secondly, expression of rep/cap from vector impurities or translation of alternative reading frames within the expression cassettes may trigger cytotoxic T cells directed against transduced cells. Lastly, the mechanism whereby AAV is taken up by antigen presenting cells may result in higher immunogenicity for specific serotypes. In an attempt to abrogate these mechanisms, recent trials have explored the use of alternative or engineered serotypes[34], strategies to reduce the AAV vector dose required to achieve therapeutic efficacy, and utilization of immunosuppression[35].

Academic proof of concept for hemophilia gene therapy

After more than a decade of preclinical and clinical trial exploration with AAV, academic proof of concept for hemophilia gene therapy was achieved in a clinical trial for hemophilia B conducted through a collaboration between the University College of London and St. Jude Children's Research Hospital[36]. This trial utilized a self-complementary AAV8 serotype which had shown strong liver tropism, rapid uncoating of the capsid, and instantaneous transgene expression upon nuclear localization of the virion. The expression cassette utilized a codon-optimized FIX gene construct driven by a short liver-specific promoter. These modifications lead to enhanced transduction efficiency in preclinical studies and the hypothesis was that a meaningful clinical effect could be achieved with lower vector dose delivered via peripheral infusion. Indeed, in the clinical trial, a dose-dependent rise in FIX to 1-6% of normal was achieved with an average drop of bleeding episodes from 15.5 to 1.5 per year, an average 92% decrease in replacement FIX use and durable effect that is now approaching 5 years[37]. However, four of the six patients in the highest dose cohort developed an asymptomatic rise in liver transaminases (primarily the ALT) with a concomitant decline of FIX activity. The investigators treated these subjects with a short course of oral prednisolone resulting in stabilization of the FIX levels and resolution of the

This article is protected by copyright. All rights reserved.

Author Manuscript

transaminase elevation. The success of this clinical trial has driven an explosive activity of hemophilia gene therapy programs across the world.

Author Manuscript

Ongoing hemophilia clinical trial programs

The ongoing gene therapy clinical trial programs are each building on the success reported by Nathwani et al. to further improve safety and efficacy as well as broadening the eligibility of subjects. Pre-existing neutralizing antibodies to AAV would reduce the efficacy of viral transduction and the prevalence in potential subjects can vary widely by age, geography and AAV serotype[38,39]. Thus, additional AAV serotypes have been explored including those with engineered capsids. Several programs are leveraging recombinant technologies in attempts to achieve improved transduction efficiency while minimizing the vector dosage. Strategies have included enhancements in codon optimization and utilization of bioengineered variants of the FVIII (B domain deletion[40]) and FIX constructs ("hyperactive" Padua FIX variant, R338L[41]) to facilitate improved viral packaging and higher specific activity. The Padua FIX variant was adopted as it was identified as a naturally occurring FIX variant in a family with thrombophilia, exhibits ~5- to 7-fold increased FIX specific activity, and allows for reduced vector dosing without sacrificing efficacy as determined by plasma FIX levels. Immunosuppression strategies have included early introduction of prednisolone at first evidence of transaminase elevation and even prophylactic steroids. The phase 1/2 clinical trials that have reported results[11,42-46] are summarized in **Figure 3**. These trials have demonstrated reassuring safety across a broad age range of subjects and evidence of dose-response, with the majority of subjects in the higher dose cohorts achieving FIX and FVIII levels that are in the mild hemophilia range (>5%) or higher. Notably, 6 of 8 patients in the highest dose cohort of the AAV-FVIII trial achieved curative FVIII expression (>50%)[46]. The expectation is that these programs and

This article is protected by copyright. All rights reserved.

others will move forward in clinical development with phase 3 pivotal trials toward commercialization.

Future directions for gene therapy

Genome editing

As hemophilia therapy has evolved from protein replacement to gene replacement, the next natural step would be gene correction. This has now become a reality through fundamental discoveries and engineering breakthroughs that have produced a toolkit of reagents for genome editing[47]. The four basic platforms are engineered meganucleases, zinc-finger nucleases (ZFN), transcription activator-like effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeat (CRISPR)/Cas9 nucleases. The basic premise of these technologies is to introduce a site-specific DNA double-stranded break (DSB) and then allow the cell's own endogenous repair machinery to repair the break. The 2 major repair pathways are non-homologous end joining (NHEJ) and homology-directed repair (HDR). NHEJ is error prone and often results in small insertions or deletions ("indels") at the cleavage site which can lead to functional disruptions in the targeted sequence. HDR requires a donor template to facilitate the repair. Whereas natural HDR relies on homologous sister chromatids to serve as the template, in genome editing, an extrachromosomal donor template may be used to integrate a DNA sequence of choice adjacent to the induced DSB. This then becomes a mechanism by which gene replacement or editing can be achieved. This technology has been applied in which an AAV vector is utilized to deliver ZFNs which mediate site-specific integration of a FIX transgene within the albumin gene locus[48]. This allows the transgene to come under control of the native expression machinery within the liver and is deemed a "safe harbor" for genetic

This article is protected by copyright. All rights reserved.

Author Manuscript

integration, avoiding random integration with potential deleterious effects. A distinct advantage of this strategy is that transgene expression would be anticipated to remain stable with cell division and turnover (versus current non-integrative strategies) and may be more amenable to gene therapy in younger subjects. This strategy has resulted in expression of therapeutic FIX levels (20-50%) in non-human primates and a Phase 1/2 clinical trial is ongoing[49]. Cas9 has been difficult to package in AAV due to its large size but truncated guide RNAs and computationally designed hepatocyte specific promoters can lead to liver-specific and targeted site-specific indels in murine models[50]. However, it's currently impractical in the context of a clinical gene therapy program to design reagents to correct each point mutation that can cause hemophilia. The "safe harbor" approach obviates the need to design reagents for each point mutation.

Other cellular targets for gene therapy for hemophilia

Patient-derived **induced pluripotent stem cells** (iPSCs) are a promising area of investigation for cell-based therapy for hemophilia. These can be derived from human dermal fibroblasts, although without modification, would retain the genetic defect causing the subject's hemophilia. However, the defective gene can be corrected *ex vivo* through genome editing. In one example, TALENs were used to invert a 140-kbp chromosomal segment of the *F8* gene in human iPSCs, thus recapitulating the commonest genetic cause of severe hemophilia A[51]. These model hemophilia A iPSC cell lines were then reverted back to the wild-type state through a similar strategy. The *ex vivo* approach also allows for characterization of the cell lines to ensure no off-target effects. This genomic rearrangement would likely be even more efficient with CRISPR/Cas9. However, this is evidence that engineered endonucleases could be used to rearrange large genomic sequences in iPSCs

This article is protected by copyright. All rights reserved.

Author Manuscript

and provides proof of concept that genomically modified iPSCs could be used to correct a genetic defect like hemophilia through autologous stem cell therapy.

Hematopoietic stem cell (HSC)-directed gene therapy is achieved through *ex vivo* transduction of autologous HSCs, typically utilizing integrating viral vectors such as retroviral or lentiviral vectors. The transduced HSCs are then transplanted into an HSC-depleted recipient after conditioning. Since HSCs undergo both self-renewal and differentiation, they then create a reservoir of transgene-expressing cells that persist in the bone marrow and are capable of amplifying within the recipient up to 10^6-fold. One strategy used lentivirus to transduce HSCs *ex vivo* with bioengineered high-expression FVIII transgenes that corrected the bleeding phenotype of hemophilia A mice[52].

Activated **platelets** mediate the primary response to vascular injury by adhering to the site of injury and secrete biologically active proteins. It was hypothesized that a FVIII transgene under control of a megakaryocyte-specific promoter would lead to a locally inducible mechanism to maintain hemostasis at sites of vascular injury in hemophilia A. Furthermore, since platelets would provide a confined site of synthesis and storage within platelets, FVIII would be protected from inhibition from anti-FVIII antibodies. This is an important feature as subjects with inhibitors to FVIII have been excluded from all clinical gene therapy trials to date. HSC-targeted lentiviral-mediated gene transfer of FVIII leads to trafficking of FVIII to the alpha-granule compartment of platelets and correction of the bleeding phenotype in murine and canine models of hemophilia. In mice, hemostasis was achieved even in the presence of high titer inhibitory antibodies[53]. FVIII expressed in this manner also did not elicit the formation of inhibitory antibodies in a line of dogs with hemophilia A previously known to readily form inhibitors to human FVIII[54]. A phase 1 clinical trial protocol has been proposed that would target subjects with hemophilia A and refractory high titer antibodies. A recent study demonstrated in a preclinical model that intra-osseus

This article is protected by copyright. All rights reserved.

Author Manuscript

injection of a lentiviral vector was capable of transducing bone marrow cells *in situ* with a FVIII transgene and target to platelets[55]. Notably, this approach would not require any pre-transplant conditioning which is still a significant drawback for HSC-targeted approaches.

Remaining questions

The cytotoxic cell-mediated immune response remains a stubborn challenge for all of the clinical trial programs. There is still debate as to the underlying mechanism(s), whether the decline in factor expression can be abrogated with steroids in all cases, and whether a reactive or prophylactic approach to instituting steroids is best. Is the ALT increase the best biomarker or could new biomarkers provide an earlier signal that could trigger an more consistent therapeutic response?

Given the enhanced pharmacokinetic characteristics of the most recently approved recombinant clotting factors, particularly the extended half-life FIX products that have been able to maintain trough factor IX levels as high as 20% with weekly dosing schedules, what level of durable expression does a gene therapy intervention have to achieve to be a viable option? Current outcomes show durable response over ~5 years, but considering that exposure to AAV universally leads to an immunological response that may preclude re-treatment, are the current gene therapy strategies likely to achieve durable expression over a lifetime?

What will be the potential application in children? It has been widely demonstrated that the earlier that joint bleeding can be abrogated, the better likelihood of joint preservation into adulthood. However, what long term outcome data will be necessary in order to give confidence to apply this intervention in younger children. Will hepatocellular turnover limit durable expression if gene therapy is applied early in life?

This article is protected by copyright. All rights reserved.

Author Manuscript

The economics of hemophilia therapy are an area of intense focus by commercial and public payer systems. Gene therapy certainly offers to dramatically reduce or even eliminate the need for regular factor replacement lifelong. How should this be valued and who should pay for what may be the definitive gene therapy intervention? Moreover, in nationalized health systems and in the developing world, should gene therapy be the preferable intervention to a lifetime of factor replacement therapy? Even considering a "one and done" approach and the anticipation of long-term savings, is it likely that nationalized health systems, particularly within economically disadvantaged countries, would be able to afford this technology?

These questions will need to be addressed through the phase 3 pivotal trial programs and considered by regulators, payers and consumer advocacy groups. Even with successfully phase 3 programs, there may be considerable challenges in the scalability of individual gene therapy programs to address the needs globally. However, these are likely to be surmountable hurdles as we enter this next "golden era" for treatment for hemophilia.

Author Manuscript

This article is protected by copyright. All rights reserved.

Disclosure: SWP has served as a consultant to Shire, Novo Nordisk, CSL Behring, Pfizer, Biogen, Bayer, Dimension Therapeutics, uniQure, Freeline, Biomarin, Freeline, Sangamo Biosciences and served as a Principal Investigator for Dimension Therapeutics.

Author Manuscript

This article is protected by copyright. All rights reserved.

References

1. Soucie JM, Evatt B, Jackson D. Occurrence of hemophilia in the United States. The Hemophilia Surveillance System Project Investigators. *Am J Hematol*. 1998;59(4):288-294.

2. Skinner MW. Haemophilia: provision of factors and novel therapies: World Federation of Hemophilia goals and achievements. *Br J Haematol*. 2011;154(6):704-714.

3. Mannucci PM, Tuddenham EG. The hemophilias--from royal genes to gene therapy. *N Engl J Med*. 2001;344(23):1773-1779.

4. Srivastava A, Brewer AK, Mauser-Bunschoten EP, et al. Guidelines for the management of hemophilia. *Haemophilia*. 2013;19(1):e1-47.

5. 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(6):535-544.

6. Oladapo AO, Epstein JD, Williams E, Ito D, Gringeri A, Valentino LA. Health-related quality of life assessment in haemophilia patients on prophylaxis therapy: a systematic review of results from prospective clinical trials. *Haemophilia*. 2015;21(5):e344-358.

7. Ljung R, Gretenkort Andersson N. The current status of prophylactic replacement therapy in children and adults with haemophilia. *Br J Haematol*. 2015;169(6):777-786.

8. Pipe SW. Recombinant clotting factors. *Thromb Haemost*. 2008;99(5):840-850.

9. Carcao M, Re W, Ewenstein B. The role of previously untreated patient studies in understanding the development of FVIII inhibitors. *Haemophilia*. 2016;22(1):22-31.

Author Manuscript

This article is protected by copyright. All rights reserved.

10. Fischer K, Lassila R, Peyvandi F, et al. Inhibitor development in haemophilia according to concentrate. Four-year results from the European HAemophilia Safety Surveillance (EUHASS) project. *Thromb Haemost*. 2015;113(5):968-975.

11. Hartmann J, Croteau SE. 2017 Clinical trials update: Innovations in hemophilia therapy. *Am J Hematol*. 2016;91(12):1252-1260.

12. Pipe SW. New therapies for hemophilia. *Hematology Am Soc Hematol Educ Program*. 2016;2016(1):650-656.

13. Mazepa MA, Monahan PE, Baker JR, Riske BK, Soucie JM. Men with severe hemophilia in the United States: birth cohort analysis of a large national database. *Blood*. 2016.

14. Oldenburg J. Optimal treatment strategies for hemophilia: achievements and limitations of current prophylactic regimens. *Blood*. 2015;125(13):2038-2044.

15. DiMichele DM, Hoots WK, Pipe SW, Rivard GE, Santagostino E. International workshop on immune tolerance induction: consensus recommendations. *Haemophilia*. 2007;13 Suppl 1:1-22.

16. Walsh CE, Jimenez-Yuste V, Auerswald G, Grancha S. The burden of inhibitors in haemophilia patients. *Thromb Haemost*. 2016;116 Suppl 1:S10-17.

17. Coppola A, Di Capua M, Di Minno MN, et al. Treatment of hemophilia: a review of current advances and ongoing issues. *J Blood Med*. 2010;1:183-195.

18. Sack BK, Herzog RW, Terhorst C, Markusic DM. Development of Gene Transfer for Induction of Antigen-specific Tolerance. *Mol Ther Methods Clin Dev*. 2014;1:14013.

19. Rocha P, Carvalho M, Lopes M, Araujo F. Costs and utilization of treatment in patients with hemophilia. *BMC Health Serv Res*. 2015;15:484.

This article is protected by copyright. All rights reserved.

Author Manuscript

20. Zhou ZY, Koerper MA, Johnson KA, et al. Burden of illness: direct and indirect costs among persons with hemophilia A in the United States. *J Med Econ*. 2015;18(6):457-465.

21. Manco-Johnson M. Comparing prophylaxis with episodic treatment in haemophilia A: implications for clinical practice. *Haemophilia*. 2007;13 Suppl 2:4-9.

22. Skinner MW. Gene therapy for hemophilia: addressing the coming challenges of affordability and accessibility. *Mol Ther*. 2013;21(1):1-2.

23. Fagiuoli S, Daina E, D'Antiga L, Colledan M, Remuzzi G. Monogenic diseases that can be cured by liver transplantation. *J Hepatol*. 2013;59(3):595-612.

24. Ferrua F, Aiuti A. Twenty-five years of gene therapy for ADA-SCID: from "bubble babies" to an approved drug. *Hum Gene Ther*. 2017; *in press*.

25. Chira S, Jackson CS, Oprea I, et al. Progresses towards safe and efficient gene therapy vectors. *Oncotarget*. 2015;6(31):30675-30703.

26. Rogers GL, Herzog RW. Gene therapy for hemophilia. *Front Biosci (Landmark Ed)*. 2015;20:556-603.

27. Kumar SR, Markusic DM, Biswas M, High KA, Herzog RW. Clinical development of gene therapy: results and lessons from recent successes. *Mol Ther Methods Clin Dev*. 2016;3:16034.

28. Brimble MA, Reiss UM, Nathwani AC, Davidoff AM. New and improved AAVenues: current status of hemophilia B gene therapy. *Expert Opin Biol Ther*. 2016;16(1):79-92.

29. Mingozzi F, High KA. Immune responses to AAV vectors: overcoming barriers to successful gene therapy. *Blood*. 2013;122(1):23-36.

This article is protected by copyright. All rights reserved.

Author Manuscript

30. Gaudet D, Methot J, Dery S, et al. Efficacy and long-term safety of alipogene tiparvovec (AAV1-LPLS447X) gene therapy for lipoprotein lipase deficiency: an open-label trial. *Gene Ther*. 2013;20(4):361-369.

31. Qiu X, Lu D, Zhou J, et al. Implantation of autologous skin fibroblast genetically modified to secrete clotting factor IX partially corrects the hemorrhagic tendencies in two hemophilia B patients. *Chin Med J (Engl)*. 1996;109(11):832-839.

32. Manno CS, Chew AJ, Hutchison S, et al. AAV-mediated factor IX gene transfer to skeletal muscle in patients with severe hemophilia B. *Blood*. 2003;101(8):2963-2972.

33. 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(3):342-347.

34. Martino AT, Basner-Tschakarjan E, Markusic DM, et al. Engineered AAV vector minimizes in vivo targeting of transduced hepatocytes by capsid-specific CD8+ T cells. *Blood*. 2013;121(12):2224-2233.

35. Arruda VR, Samelson-Jones BJ. Obstacles and future of gene therapy for hemophilia. *Expert Opin Orphan Drugs*. 2015;3(9):997-1010.

36. Nathwani AC, Tuddenham EG, Rangarajan S, et al. Adenovirus-associated virus vector-mediated gene transfer in hemophilia B. *The New England journal of medicine*. 2011;365(25):2357-2365.

37. 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(21):1994-2004.

Author Manuscript

This article is protected by copyright. All rights reserved.

Author Manuscript

38. 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(2):59-67.

39. Monahan PE. Gene therapy in an era of emerging treatment options for hemophilia B. *J Thromb Haemost*. 2015;13 Suppl 1:S151-160.

40. Gnatenko DV, Saenko EL, Jesty J, Cao LX, Hearing P, Bahou WF. Human factor VIII can be packaged and functionally expressed in an adeno-associated virus background: applicability to haemophilia A gene therapy. *Br J Haematol*. 1999;104(1):27-36.

41. Monahan PE, Sun J, Gui T, et al. Employing a gain-of-function factor IX variant R338L to advance the efficacy and safety of hemophilia B human gene therapy: preclinical evaluation supporting an ongoing adeno-associated virus clinical trial. *Hum Gene Ther*. 2015;26(2):69-81.

42. Monahan PE. Emerging genetic and pharmacologic therapies for controlling hemostasis: beyond recombinant clotting factors. *Hematology Am Soc Hematol Educ Program*. 2015;2015:33-40.

43. Leebeek FWG, Tangelder M, Meijer K, et al. Interim Results from a Dose Escalating Study of AMT-060 (AAV5-hFIX) Gene Transfer in Adult Patients with Severe Hemophilia B. *Blood*. 2016;128(22):2314-2314.

44. George LA, Sullivan SK, Giermasz A, et al. Spk-9001: Adeno-Associated Virus Mediated Gene Transfer for Hemophilia B Achieves Sustained Mean Factor IX Activity Levels of >30% without Immunosuppression. *Blood*. 2016;128(22):3-3.

45. Dimension Announces Interim Topline Results from Ongoing Phase 1/2 Clinical Program for DTX101, Dimension's Lead AAV Product Candidate in Development for Adult Patients with Moderate/Severe to Severe Hemophilia B. http://www.globenewswire.com/; 2017.

This article is protected by copyright. All rights reserved.

46. Pasi J, Wong WY, Rangarajan S, et al. Interim results of an open-label, phase 1/2 study of BMN 270, an AAV5-FVIII gene transfer in severe hemophilia A. *Haemophilia*. 2016;22(Suppl. 4):151.

47. Porteus MH. Towards a new era in medicine: therapeutic genome editing. *Genome Biol*. 2015;16:286.

48. Sharma R, Anguela XM, Doyon Y, et al. In vivo genome editing of the albumin locus as a platform for protein replacement therapy. *Blood*. 2015;126(15):1777-1784.

49. Wechsler T, Meyer KE, Spratt SK, et al. ZFN-Mediated Gene Targeting at the Albumin Locus in Liver Results in Therapeutic Levels of Human FIX in Mice and Non-Human Primates. *Blood*. 2015;126(23):200-200.

50. Senis E, Fatouros C, Grosse S, et al. CRISPR/Cas9-mediated genome engineering: an adeno-associated viral (AAV) vector toolbox. *Biotechnol J*. 2014;9(11):1402-1412.

51. Park CY, Kim J, Kweon J, et al. Targeted inversion and reversion of the blood coagulation factor 8 gene in human iPS cells using TALENs. *Proc Natl Acad Sci U S A*. 2014;111(25):9253-9258.

52. Johnston JM, Denning G, Doering CB, Spencer HT. Generation of an optimized lentiviral vector encoding a high-expression factor VIII transgene for gene therapy of hemophilia A. *Gene Ther*. 2013;20(6):607-615.

53. Kuether EL, Schroeder JA, Fahs SA, et al. Lentivirus-mediated platelet gene therapy of murine hemophilia A with pre-existing anti-factor VIII immunity. *J Thromb Haemost*. 2012;10(8):1570-1580.

54. Du LM, Nurden P, Nurden AT, et al. Platelet-targeted gene therapy with human factor VIII establishes haemostasis in dogs with haemophilia A. *Nat Commun*. 2013;4:2773.

This article is protected by copyright. All rights reserved.

Author Manuscript

55. Wang X, Shin SC, Chiang AF, et al. Intraosseous delivery of lentiviral vectors targeting factor VIII expression in platelets corrects murine hemophilia A. *Mol Ther*. 2015;23(4):617-626.

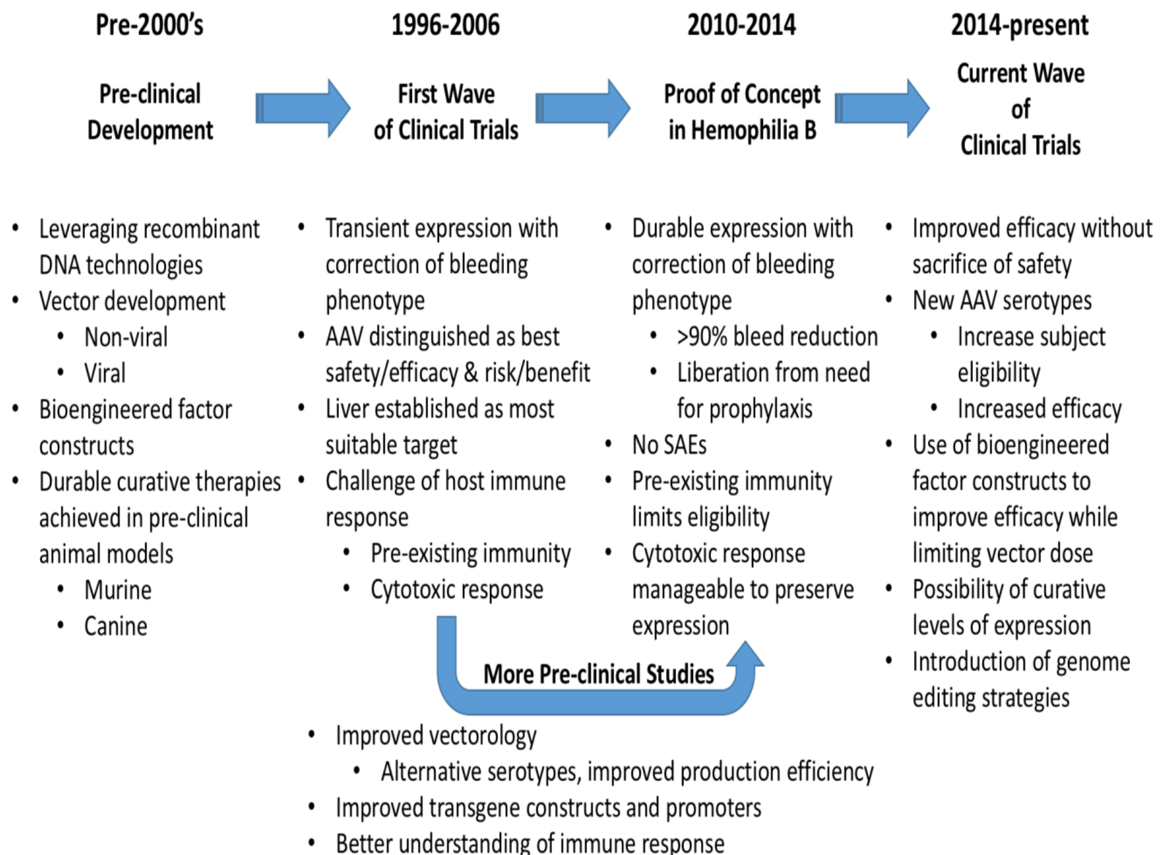
Author Manuscript

This article is protected by copyright. All rights reserved.

Figure Legends:

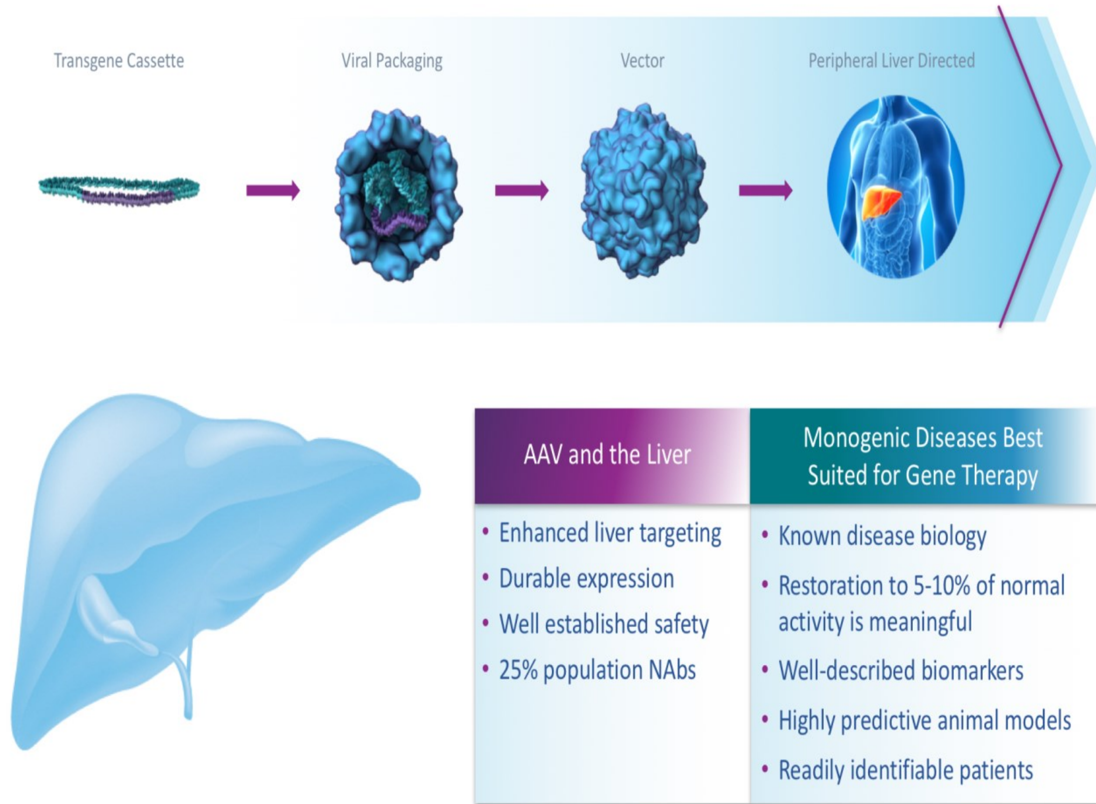
Figure 1. AAV and the Liver

NAbs, neutralizing antibodies



This article is protected by copyright. All rights reserved.

Figure 2. Evolution of Hemophilia Gene Therapy



Author M

This article is protected by copyright. All rights reserved.

Figure 3. Gene therapy trials for Hemophilia A and B with reported results.

sc, self-complementary; h, human; co, codon optimized; BDD, B domain deleted; ALT, alanine aminotransferase

Sponsor	Vector	Expression Construct	# of Subjects	Outcome	Management Of Cytotoxic Response	Trials Identifier
SJCRH/ UCL	scAAV2/8	hFIXco	10	Dose-dependent rise in FIX to 1-6%	4 of 6 subjects in highest dose cohort with transient ALT increase; treated with steroids	NCT00979238
Baxalta	scAAV8	hFIXR338Lco	8	Dose-dependent rise in FIX to 0.5->25%	5 of 8 subjects with transient ALT increase; treated with steroids	NCT01687608
uniQure	AAV5	hFIXco	10	Dose-dependent rise in FIX to >5%	3 of 10 subjects with transient ALT increase; treated with steroids	NCT02396342
Spark Therapeutics	Engineered AAV8	hFIXR338Lco	10	Rise in FIX to 14-81%	2 of 10 subjects with transient ALT increase; treated with steroids	NCT02484092
Dimension Therapeutics	rhAAV10	hFIXco	6	Dose-dependent rise in FIX to 5-20%	5 of 6 subjects with transient ALT increase; treated with steroids	NCT02618915
Biomarin	AAV5	BDD-hFVIII	15	Dose-dependent rise in FVIII to >50% in 6 of 7 subjects in highest dose cohort	6 of 7 subjects in highest dose cohort with transient ALT increase; steroid treatment included prophylactic dosing	NCT02576795

This article is protected by copyright. All rights reserved.