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Gene Therapy in Haemophilia: Updates from Clinical Trials and Insights to Future Technologies

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Gene Therapy in Haemophilia: Updates from Clinical Trials and Insights to Future Technologies

These presentations took place from 9th–13th July 2022 as part of the International Society on Thrombosis and Haemostasis (ISTH) Congress held in London, UK

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Meeting Summary

At the International Society on Thrombosis and Haemostasis (ISTH) 2022 Congress, held 9th–13th July, multiple oral and poster presentations were dedicated to gene therapy as a treatment for haemophilia A or B. These included updates from clinical trials of adeno-associated virus (AAV)-based gene therapy products and guidance on the real-world monitoring of patients with haemophilia who have received gene therapy, both in the short- and long-term. The unmet needs and challenges associated with gene therapy were also discussed, and several preclinical studies that aimed to refine AAV-based strategies were presented. Finally, there were a number of presentations providing an insight into the ongoing research into alternative gene therapy strategies, including the use of non-viral gene transfer, gene editing strategies, and nanobodies.

Introduction

Gene therapy is an attractive treatment option for patients with haemophilia A or B as it offers the potential to restore normal haemostatic function whilst relieving the burden of regular infusions with factor VIII (FVIII) or IX (FIX) concentrates.[1] Gene replacement strategies that use AAVs to deliver a FVIII or FIX transgene are at the forefront of gene therapy for haemophilia, and promising findings have been reported from multiple clinical trials.[2-7] This has culminated in the approval of the first gene therapy for haemophilia A (valoctocogene roxaparvovec) by the European Medicines Agency (EMA) in June 2022.[8] Although gene replacement therapy has improved outcomes for patients with haemophilia, there remain challenges associated with this approach, including the presence of pre-existing neutralising antibodies (NAb) to the AAV capsids (protein shell), and the variability and unpredictability of transgene expression.[1] Research is currently ongoing to refine the process of AAV-mediated gene transfer and also to develop alternative gene therapy strategies, such as the use of non-viral gene transfer methods, or exploitation of the CRISPR/Cas9 system to directly edit the mutated FVIII or FIX genes.[9]

Adeno-associated Virus Vector-Mediated Replacement Gene Therapy: Updates from Clinical Trials

Multiple gene therapies are currently in clinical development for haemophilia A and B, with many clinical trials ongoing or completed. Updates on several of these were provided at ISTH in 2022, and are summarised below.

Haemophilia A

Valoctocogene roxaparvovec is an AAV serotype 5 (AAV5)-based gene therapy that has been studied in patients with severe haemophilia A in a Phase I/II study,[5] and an open-label, multicentre, single-arm, Phase III trial (GENEr8-1).[7] Michael Laffan, Professor at the Centre for Haematology, Imperial College London, UK, presented 6-year follow-up data for 13 patients treated in the Phase I/II study, and reported that there were no new treatment-related safety signals in Years 5 or 6.[10] One serious adverse event (AE) of acinar cell carcinoma occurred, but was not associated with the treatment. Over the 6-year follow-up, a sustained reduction in the annualised bleeding rate (ABR) and an increase in the number of patients who were bleed-free compared with baseline was observed. Improvements in ABR and use of exogenous FVIII were noted even in patients with low FVIII transgene expression. Twelve out of 13 participants did not require FVIII prophylaxis at Year 6, and patients either improved or maintained their quality of life for up to 6 years following treatment.

The majority of patients enrolled in the GENEr8-1 study (112 out of 134) entered the trial from a previous non-interventional study of patients receiving FVIII prophylaxis. Johnny Mahlangu, Professor at the University of the Witwatersrand, Johannesburg, South Africa, and National Health Laboratory Service (NHLS), Johannesburg, South Africa, reported an

85% reduction in the treated ABR and a 98% reduction in annualised FVIII utilisation after treatment compared with baseline in this rollover population.[11] Furthermore, 30% of patients had zero bleeds at baseline, increasing to 58% and 67% in the first and second years after treatment. Of note, more than 80% of rollover patients had no treated bleeds each year, despite receiving no routine prophylaxis. The number of joint bleeds and matched median FVIII activity in GENEr8-1 aligned with estimates from an epidemiological study,[12] suggesting that transgene-derived FVIII provides similar protection as to native or exogenous FVIII. The safety profile of valoctocogene roxaparvovec in Year 2 was consistent with Year 1.[11]

In a post hoc analysis reported by Anthony Hatswell, Director at Delta Hat Limited, Nottingham, UK, propensity scoring was used to compare outcomes for patients enrolled in the GENEr8-1 rollover population (intervention cohort) with patients who were enrolled in the earlier non-interventional study but did not transfer into GENEr8-1 (control cohort).[13] Mean ABRs were significantly lower in the intervention versus control cohorts for both 'all bleeds' and 'treated bleeds'. In addition, the proportion of patients with zero bleeds (52.7% versus 28.5%; $p<0.05$) or zero treated bleeds (79.5% versus 32.9%; $p<0.001$) were significantly higher in the intervention versus control cohorts.

Suresh Agarwal, Director of Clinical Pharmacology at BioMarin Pharmaceutical Inc, San Rafael, California, USA, reported that in GENEr8-1, median peak vector DNA levels were observed 1–8 days after administration and were highest in blood, followed by saliva, semen, stool, and urine.[14] Encapsidated vector DNA levels then declined steadily with a maximum time to clearance of $\leq$12 weeks in both plasma and semen. The maximum time-to-clearance of all other residual vector DNA in semen was 36 weeks. Therefore, the risk of transmission to untreated individuals is considered to be very low; however, contraception is recommended for men for 6 months following treatment due to the time-to-clearance of residual vector DNA.

TAK754, an AAV serotype-8 (AAV8)-based gene therapy containing an FVIII transgene, is currently being investigated in a Phase I/II study in patients with severe haemophilia A.[15] John Chapin, Director at Takeda, Cambridge, Massachusetts, USA, reported that in the first three patients, no changes in serum cytokines were observed within 24 hours of infusion; however, an elevation in transaminases and a concomitant decline in FVIII expression occurred 4–9 weeks post-infusion despite glucocorticoid use. Using bulk mRNA transcriptomic analysis, no significant changes in natural killer cell, dendritic cell, NF-κB, IL-6, Toll-like receptors 1-8, or T cell pathway signals were observed, although transient increases in Toll-like receptor 9, TNFα, chemokine ligand 5, and interferon regulatory factor 7 occurred 8 hours after infusion.

Lastly, Gil Gonen-Yaacovi, Associate Director, Clinical and Regulatory Affairs at ASC Therapeutics, Milpitas, California, USA, described ASC618, a novel gene therapy treatment for haemophilia A composed of a bioengineered human FVIII transgene with liver-specific codon optimisation under the control of a hepatic combinatorial bundle promoter.[16] In preclinical studies, ASC618 was well tolerated at all doses evaluated. The safety, tolerability, and preliminary efficacy are currently being studied in a Phase I/II clinical trial. It is hypothesised that the human–porcine chimeric transgene will allow for lower AAV doses while still generating sufficient serum levels of FVIII and a more durable treatment effect.

Haemophilia B

Etranacogene dezaparvovec is a gene therapy product composed of an AAV5 vector and the highly active FIX Padua variant transgene that is currently being studied in the Phase III clinical trial, HOPE-B, in adults with severe or moderately-severe haemophilia. Steven Pipe, Professor at the University of Michigan, Ann Arbor, USA, reported statistically significant improvements in the EuroQoL 5 Dimension 5 Level (EQ-5D-5L) visual analogue scale (nominal p value: 0.0244) and index scores (nominal p value: 0.0132) after 2 years compared to the 6 months prior to baseline.[17] Statistically significant improvements were also observed in the haemophilia-specific quality of life (Haem-A-QoL) questionnaire (nominal p value: <0.0001), with the largest improvement observed for the reduction in treatment burden.

Verbrinacogene setparvovec (formerly FLT180a), which uses an AAVS3 synthetic capsid and the Padua FIX transgene, is also in clinical development for haemophilia B, with a commercial formulation currently being studied in the Phase I/II study B-LIEVE. Guy Young, Professor at the Cancer and Blood Diseases Institute, Children's Hospital Los Angeles, California, USA, and Keck School of Medicine, University of Southern California, Los Angeles, USA, reported that the first three patients treated in B-LIEVE achieved FIX levels in the normal range and had discontinued prophylaxis by follow-up at 33, 56, and 77 days, respectively.[18] Treatment was well tolerated, and all AEs were consistent with the known profile of immune management.

The Importance of Patient Selection and Ongoing Monitoring

In an introduction to a session on the importance of patient selection and monitoring, Mahlangu explained that although clinical experience with gene therapy is growing, there are multiple factors that still require standardisation across therapies and trials, including production of the vector, individualised vector selection, dosing, immunosuppression strategies, integration monitoring, choice of efficacy endpoints, and patient selection.[19]

Jerome Teitel, Professor of Medicine at the University of Toronto, Ontario, Canada, explained that the biggest barrier to eligibility for gene therapy remains the presence of pre-existing NAbs.[20] Other considerations for patient selection include psychological issues, patient lifestyle, liver health, thrombotic risk, other health problems, and history of treatment adherence. Patients themselves need to consider several factors when making treatment decisions. For instance, the advantage of gene therapy is that it is currently the only option for long-term control without the need for continuous therapy. Also, some patients are motivated by participating in clinical trials. Conversely, patients need to be aware that the degree of response is unpredictable, immunosuppressive therapies may need to be taken, they may need to limit alcohol intake and defer family planning, and they will require regular blood tests, and potentially liver biopsies. In addition, there is a low risk of vector integration, and treatment may prevent future eligibility for AAV-based gene therapy due to the development of NAbs.

Priyanka Raheja, Consultant Haematologist at Royal London Hospital, UK, described how the innate and adaptive immune response can drive inter-patient variability in responses to gene therapy.[21] This can be due to patient-dependent factors, such as pre-existing immunity, inflammation, age, human leukocyte antigen-type, and genetic background, as well as vector contaminants. To manage the immune response, rapid introduction of immunosuppressive therapy is imperative, with some trials using prophylactic corticosteroids. Alternatively, if mild inflammation occurs, it can be treated with a short course of steroids. Other proposed strategies to minimise the immune response include capsid engineering, reducing the CpG content, and codon optimisation.

Paul Batty, Associate Professor, University College London, UK, and Margareth Ozelo, Head of Hematology Division, University of Campinas, São Paulo, Brazil, both gave updates on current knowledge regarding the integration of AAV vectors into the host genome, and the potential risk of cancer.[22,23] Following AAV-mediated gene therapy, the majority of the transgene exist as episomes within the nuclei of hepatocytes; however, 0.1–1.0% will integrate within the genomic DNA.[24] In mice, recurrent integration events have been observed in the *Rian* locus, and this has been associated with the development of hepatocellular carcinoma (HCC).[25,26] However, this region is very specific to the mouse genome, and is not found in humans or other large animals. In canine studies of gene therapy for haemophilia, low-frequency integration events have been reported, although these occurred predominantly in non-coding regions of the genome.[26-30] Reassuringly, none of the dogs treated in these studies developed tumours or displayed evidence of altered liver function for up to 12 years of follow-up.[27,28]

In a human study, wild-type AAV2 genomes were found to be integrated in 5.7% of biopsies from patients with HCC, although the majority of these patients had established risk factors for HCC, or mutations associated with liver cancer.[31] In humans treated with rAAV2-hFIX, no

evidence of long-term liver toxicity or tumourigenesis was observed for up to 15 years post-hepatic artery infusion.[32] Finally, liver biopsies performed up to 4 years after treatment in five patients in the Phase I/II trial of valoctocogene roxaparvovec revealed no evidence of liver damage.[33] In these patients, one to six integration events per 1,000 cells were observed, but there was no enrichment in integration sites near protooncogenes, and no evidence of clonal expansion (data on file). To date, one case each of tonsillar carcinoma,[34] acinic cell carcinoma (data on file), and HCC[35] have been reported in patients with haemophilia post-gene therapy. In all cases, there was little to no evidence of AAV vector genome integration.

Donna Coffin, Director of Research and Public Policy at the World Federation of Hemophilia (WFH), Montreal, Quebec, Canada, described how gathering details of rare AEs in a small patient population over a large geographical area can be challenging. To address this, the WFH is currently collaborating with national registries to promote data integration into the international Gene Therapy Registry (GTR).[36] To date, a database partner has been selected, and collaborations with the American Thrombosis and Hemostasis Network (ATHN), Netherlands HemoNED, and the Canadian Bleeding Disorders Registry (CBDR) have been initiated. It is anticipated that the GTR will be available for data entry and linkage by mid-2022.

Barbara Konkle, Professor of Medicine in Hematology at the University of Washington, Seattle, USA, explained that as haemophilia is a rare disease, all gene therapy-treated patients need to be carefully monitored.[37] For safety assessments, the WFH-GTR[38] has defined multiple AEs of special interest, including FVIII/FIX inhibitors, liver disease, malignancy, thromboembolic events, hypersensitivity reactions, hepatitis B or C, and sensory paraesthesia. To determine efficacy and durability, FVIII/FIX activity along with the clinical bleeding phenotype should be assessed. Successful long-term monitoring of post-gene therapy will require clinical trial follow-up in accordance with regulatory requirements along with enrolment in the WFH-GTR. The primary objective of the GTR is to determine the long-term safety of FVIII and FIX gene therapy. Secondary objectives are to determine the long-term efficacy and durability of these therapies, and to assess long-term quality of life and burden of disease.

Adeno-associated Virus Vector-Mediated Gene Replacement Therapy: Preclinical Studies

In a canine model, Batty reported dose-dependent therapeutic expression of canine FVIII (cFVIII) following infusion of a codon-optimised AAV5-cFVIII product.[39] AAV5-cFVIII was detected in the liver of all animals at 3 months and a significant correlation was observed between liver vector genomes and FVIII mRNA copies ($r=0.88$; $p=0.002$). Capsid antibodies were detected within 7 days of infusion but no new FVIII inhibitors were detected. No blood biomarkers of innate immune activation were also observed.

Two liver-specific promoter elements have been used in haemophilia A clinical studies to date: human α-one antitrypsin/apolipoprotein E promoter/enhancer[4] and transthyretin.[3] A study in haemophilia A mice reported by Jonathan Lindgren, Research Tech II at the Children's Hospital of Philadelphia, Pennsylvania, USA, demonstrated that both of these promoter elements may drive transgene expression in both hepatocytes and liver sinusoidal endothelial cells.[40]

Anna Sternberg, Postdoctoral Fellow at the Children's Hospital of Philadelphia, Pennsylvania, USA, described how sustained AAV-mediated FVIII expression at levels necessary to eliminate bleeding has not yet been achieved in patients with haemophilia A.[41] However, a FVIII variant transgene with enhanced function could potentially achieve haemostatic efficacy at lower AAV vector doses. The FVIII-R336Q/R562Q (QQ) variant is resistant to activated protein C-mediated inactivation, and has demonstrated an approximate five-fold enhanced haemostatic function *in vivo*.[42] In this study, blood loss post-tail-clip was reduced by five- to 10-fold in mice treated with the QQ versus wild-type FVIII transgene. No significant survival

differences were observed between treatment groups at 3 months post-infusion, and preliminary safety data indicated that there was no enhanced prothrombotic or immunological risk with QQ versus wild-type FVIII.

In paediatric recipients of gene therapy for haemophilia B, growth of the liver and expanding blood volume in childhood may lead to a reduction in FIX levels. In a study presented by Debra Pittman, Senior Research Director of Rare Disease Research, Pfizer Inc., Cambridge, Massachusetts, USA, an AAV-FIX-R388L variant was infused into 12 juvenile dogs with haemophilia B: six 3-month-old dogs (to model 2–6-year-old children) and six 6-month-old dogs (to model 6–12-year-old children).[43] The treatment was well tolerated and no spontaneous bleeds occurred during the follow-up period of at least 12 months, despite liver growth and blood volume expansion. In all dogs, stable and durable reductions in haemostatic assay results were observed, indicating persistent FIX activity in these animals.

Across multiple clinical trials using AAV vectors, FVIII activity, as measured by one-stage clotting assays, was approximately 1.6-fold higher than that measured by chromogenic substrate assay,[3,5,44] whereas the opposite relationship has been observed for recombinant FVIII products.[45] In a study presented by Sternberg,[46] these observations were recapitulated in mice, and AAV-derived FVIII was shown to have enhanced FVIIIa function compared to the recombinant protein. Enhanced FVIIIa function did not appear to be due to improved stability of the FVIII A2 subunit, and assay discrepancies occurred independently of von Willebrand factor.

Gene Therapy for Haemophilia: A Look at Future Technologies

Ultrasound-mediated gene delivery with microbubbles is a potentially effective method of non-viral gene delivery. In a study described by Savannah Lawton, Research Scientist at Seattle Children's Research Institute, Washington, USA, mice were injected into the portal vein with plasmid DNA and microbubbles before a pulsed therapeutic ultrasound transducer was applied to the liver for 1 minute, either at low energy (LE) or high energy (HE), targeting predominantly endothelial cells and hepatocytes, respectively.[47] In both groups, FVIII activity levels stabilised at approximately 10% at 84 days post-treatment, and fluorescent microscopy revealed expression of human FVIII in liver sinusoidal endothelial cells. Transaminase levels indicated lower transient liver damage in the LE compared with the HE groups in the first week, with both groups returning to baseline levels by Week 2. FVIII inhibitors were detected in five out of 10 mice in the HE group, but none in the LE group.

Intraosseous gene therapy via delivery of lentiviral vectors into bone marrow to drive FVIII expression in platelets has been used to successfully treat mice with haemophilia A.[48] In a study presented by Cameron Rementer, Postdoctoral Researcher at Seattle Children's Research Institute, Washington, USA, this approach was extended to four dogs with haemophilia A.[49] After injection, cFVIII was detected in platelets and persisted for the duration of the study in all dogs. The whole blood clotting time was shortened at multiple time points after treatment, and the dogs experienced fewer bleeds compared with the period before treatment. The intraosseous gene therapy delivery was well tolerated, and no toxicity was observed.

Transduction of haematopoietic stem cells *ex vivo* with lentiviruses to generate genetically-modified platelets has the potential to control bleeding in patients with severe haemophilia A with inhibitors, and is supported by multiple pre-clinical studies.[50-54] David Wilcox, Associate Professor at the Medical College of Wisconsin, Milwaukee, USA, described the advantages of this approach,[55] including that it can be used in patients with anti-AAV antibodies or FVIII inhibitors and patients with liver damage. This approach also results in a more stable expression of the transgene compared with an AAV approach, and it can also be used in a diverse population, including paediatric patients. In addition, *ex vivo* haematopoietic stem cell manipulation should not transduce germ cells, and there is a low potential for insertional mutagenesis, clonal expansion, and cancer. This approach is currently being evaluated in a Phase I study in adults with severe haemophilia A and

a history of inhibitors to FVIII.[55] Results for the first treated patient have indicated excellent tolerance to the treatment, and sustained complete haematopoietic recovery. FVIII activity was detected in platelet lysates and no bleeding episodes were reported in the first month after treatment.[55]

Gene editing of DNA can potentially be used to correct missense and nonsense mutations in FVIII that cause haemophilia A. Elena Tonetto, PhD Researcher at the Department of Life Sciences and Biotechnology, University of Ferrara, Emilia-Romagna, Italy, described proof-of-principle efficacy for base editing in cellular models, with reversal of nonsense (p.R2166*) and missense (p.R2228Q) mutations detected at DNA level leading to rescue of secreted FVIII protein and activity levels up to 20% of wild-type FVIII.[56] Subsequently, Chun-Yu Chen, Research Scientist at Seattle Children's Research Institute, Washington, USA, described the use of lipid nanoparticle (LNP) technology to deliver a FVIII exon 1-targeting single guide RNA (sgRNA) with CRISPR/Cas9 expressing plasmid into immunodeficient mice with a frameshift deletion mutation in exon 1 of the *FVIII* gene.[57] The data suggested that LNPs can efficiently transfect both HepG2 and HUVEC cells *in vitro* and *in vivo*. Furthermore, LNPs carrying CRISPR/Cas9 mRNA and an mF8sgRNA were able to induce insertion-deletion mutations in the target site, leading to therapeutic levels of FVIII expression.

To overcome reductions in FVIII or FIX transgene expression over time, overexpression of FVIIa could bypass the need for FVIII or FIX and generate sufficient thrombin for clotting.[58] In this study presented by Pratiksha Sarangi, PhD researcher at the Indian Institute of Technology Kanpur, Uttar Pradesh, India, CRISPR/Cas9-based gene editing was used to insert a transgene-encoding murine FVIIa into the host genome of mice with haemophilia B.[59] Treated animals had an approximately four-fold higher expression of murine FVIIa and an up to 36% decline in prothrombin time for up to 15 weeks after vector administration compared with controls. Reduced blood loss in a tail-clip assay was also observed for the gene therapy group compared with controls.

Splice variant mutations often lead to exon skipping and can potentially be rescued by RNA therapeutics based on the small nuclear RNA component U1 spliceosomal RNA (U1snRNA). Laura Peretto, PhD Researcher at the Department of Life Sciences and Biotechnology, University of Ferrara, Emilia-Romagna, Italy, evaluated a FVIII c.1752+5g>c splice variant that resulted in skipping of exon 11 and the generation of low levels of spliced transcripts consistent with moderate haemophilia A.[60] Co-transfection of human hepatoma cells with this splice variant and an appropriately designed U1snRNA variant restored exon 11 inclusion in up to 92% of transcripts.

Elena Barbon, Postdoctoral Fellow at San Raffaele Telethon Institute for Gene Therapy, Milan, Lombardy, Italy, described how capsid engineering can be used to improve transduction efficiency, reduce immunogenicity, and increase production efficiency for AAV vectors.[61] Firstly, site-directed mutagenesis can be used to modify surface-exposed tyrosine residues and increase AAV2 transduction both *in vivo*[62] and in mice with haemophilia B.[63] Secondly, capsid shuffling has been used to generate an AAV chimaera composed of five parental AAV capsids that could efficiently transduce human primary hepatocytes in culture, and had higher resistance to neutralisation with pooled human IgG compared with AAV2.[64] In a human haemophilia A trial, use of this vector resulted in sustained FVIII expression in 16 out of 18 participants, permitting discontinuation of prophylaxis and a reduction in bleeding episodes.[3]

Nanobodies are functional, single-domain, antibody fragments that are much smaller than typical antibodies. Peter Lenting, Director of research of INSERM U1176, Université Paris-Saclay, Le Kremlin-Bicetre, France, explained the potential role of nanobodies in gene therapy for haemophilia,[65] including how they can be incorporated into AAV capsids and can increase cell-specific targeting with reduced off-target transduction.[66,67] Nanobodies can also be expressed at high levels via AAV vectors and are characterised by low immunogenicity. In one example, a nanobody that blocked the antithrombin pathway was packaged in an AAV8 vector, and was able to correct the bleeding phenotype in mice with haemophilia A.[68]

Conclusion

For many years, gene therapy has offered great promise as a treatment for monogenic disorders. In haemophilia, evidence to support AAV-based gene therapies is now beginning to accumulate, with one treatment now licensed for use in Europe. However, although the benefits are clear, current gene therapies are not curative and patient eligibility remains limited. Many challenges remain, but intensive research efforts are ongoing both to refine existing technologies and to develop novel treatment strategies.

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