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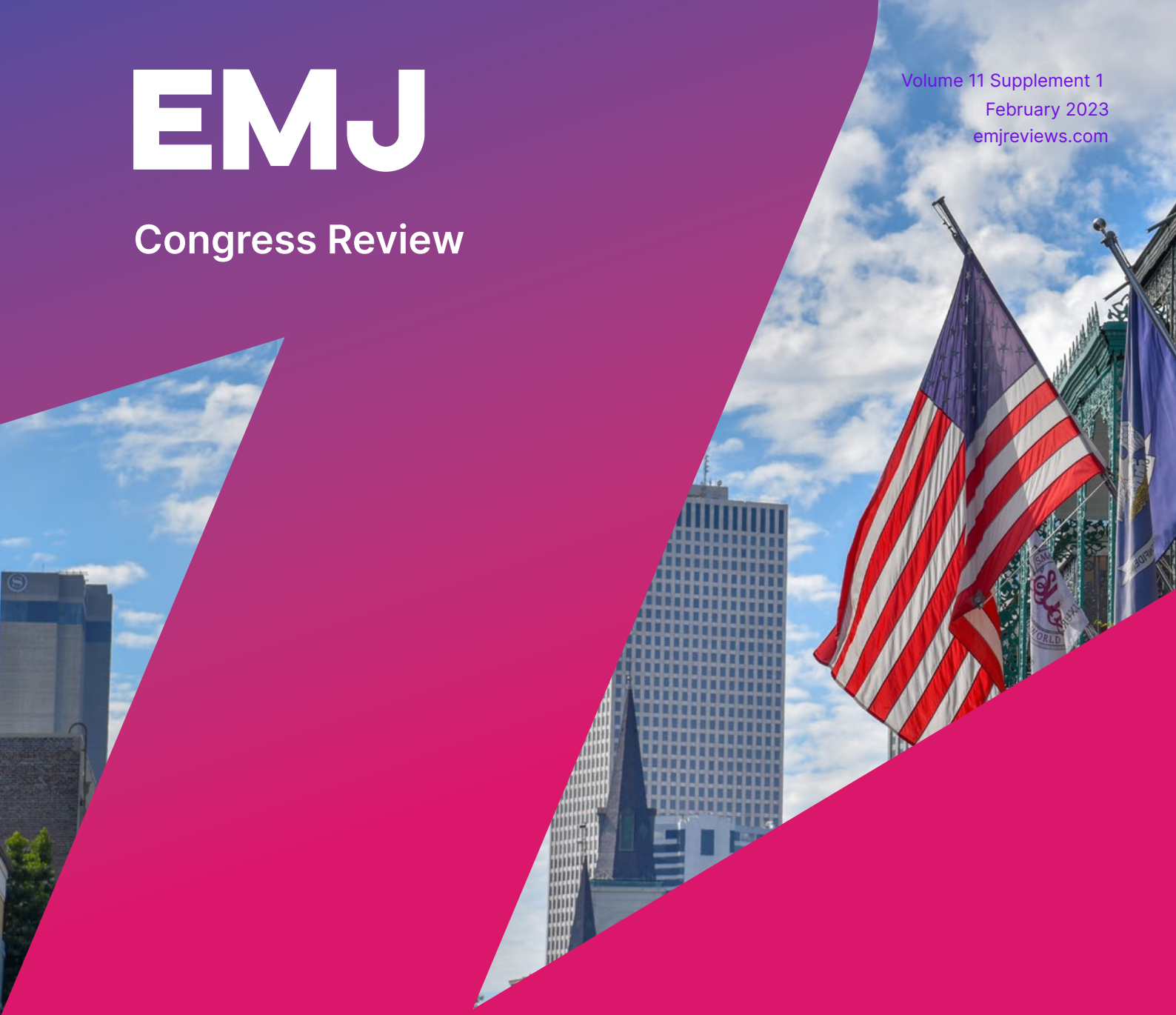

Realising the Promise of Gene Therapy for Haemophilia: Current Status and Future Innovations

Hematology

Realising the Promise of Gene Therapy for Haemophilia: Current Status and Future Innovations

These presentations took place from 10th–13th December 2022 as part of the 64th American Society of Hematology (ASH) Annual Meeting held in New Orleans, Louisiana, USA

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Meeting Summary

Gene therapy for haemophilia A and B was discussed during several sessions at the 64th American Society of Hematology (ASH) Annual Meeting, held 10th–13th December 2022. These presentations included updates from clinical trials of recombinant adeno-associated virus (rAAV)-based gene therapy products, along with recent preclinical data and insights into non-viral gene therapy strategies. Following the recent regulatory approval of two gene therapies, logistical aspects of transitioning gene therapy from a research-based treatment to routine clinical practice were also a major focus of some of the presentations.

INTRODUCTION

Gene therapy has long held promise as a treatment for haemophilia, and in 2022, that potential was finally realised with the regulatory approval of two rAAV vector-mediated replacement gene therapies: valoctocogene roxaparvovec[1] and etranacogene dezaparvovec.[2] Other gene therapy approaches that utilise an rAAV vector are currently at an advanced stage of clinical development, and there is ongoing research into other innovative therapy strategies, such as methods of non-viral gene transfer.

GENE THERAPY FOR HAEMOPHILIA: CURRENT STATE OF PLAY

At an educational session, Amit Nathwani, Director of the Katharine Dormandy Haemophilia Centre and Thrombosis Unit at the Royal Free Hospital, London, UK, highlighted that haemophilia is an excellent model disorder for the development of gene therapy (unpublished data, presented at ASH 2022). He described the promising results obtained from early gene therapy studies in haemophilia B, before highlighting the improvements achieved using the highly active Factor IX (FIX) Padua variant

transgene. More recently, an rAAV-mediated gene therapy for haemophilia B (verbrinacogene setparvovec) has been developed that uses a novel synthetic capsid designed to transduce hepatocytes more efficiently, with promising results reported from a Phase I/II trial.[3]

Developing transgenes for the treatment of haemophilia A has historically been more challenging, as the FVIII gene is above the carrying capacity of rAAV vectors. To circumvent this, the transgene used in valoctocogene roxaparvovec has the large B domain deleted, except for the portion containing six critical glycosylation sites. Positive results were achieved from a Phase I study and from the GENEr8-1 Phase III study, leading to the recent approval by the European Medicines Agency (EMA), and submission of a Biologic License Application to the US Food and Drug Administration (FDA).[1,4-6] Several other rAAV-mediated gene therapies for haemophilia A that include a B-domain deleted transgene are currently in an advanced stage of clinical development. Nathwani concluded with a summary of current challenges facing gene therapy in haemophilia, including improving the durability of transgene expression, achieving long-term transgene expression in children to avoid chronic joint damage, controlling vector-induced increases in transaminase liver enzymes, and improving access to gene therapy.

In a satellite symposium, Christine Kempton, Associate Professor at the Emory School of Medicine, Atlanta, USA; Annette Von Drygalski, Director of the Hemophilia and Thrombosis Treatment Center, University of California, San Diego, USA; Andrew Leavitt, Co-Director of the University of California, San Francisco Hemophilia Treatment Center, USA; and Steven Pipe, Professor at the University of Michigan, Ann Arbor, USA, discussed the current standard of care and unmet needs of patients with haemophilia B, and emphasised that patients continue to experience pain and restrictions to their daily life despite treatment (unpublished data, presented at ASH 2022). They noted that even a low annual bleed rate combined with subclinical microbleeds might have a cumulative impact on the development of arthropathy. The speakers emphasised that there are currently no interventions that can be implemented in paediatric patients that can sustain healthy joints throughout adult life. They also highlighted the promising results achieved so far with gene therapy and discussed how these could be recreated in a clinical setting. In terms of patient selection, the importance of ongoing education was strongly emphasised, with Pipe recommending that discussions are initiated in the paediatric setting so that patients have a good understanding of their options and treatment goals by the time they become eligible for gene therapy.

When determining eligibility, a full psychosocial evaluation should be performed to identify potential challenges. Patients must also understand the importance of lifestyle choices to maintain healthy liver function following gene therapy. The speakers emphasised that an unbiased approach to treatment decision-making should be taken, with the decision to receive gene therapy considered in the context of the novel non-factor therapies that are now becoming available. The logistics of implementing gene therapy in clinical practice were also discussed, including establishing treatment centres, the need to ensure sufficient staffing to support intensive follow-up, and the potential benefits of remote patient engagement supported by domiciliary nursing care. A 'hub and spoke' model has been proposed, with 'hubs' developing the infrastructure for treatment dosing and short-term follow-up, and 'spoke' centres being involved in patient identification and longer-term follow-up.

The implementation of gene therapy in clinical practice was also discussed by Michael Recht, Professor of Clinical Pediatrics at Yale Medical School, Connecticut, USA; Guy Young, Director of the Hemostasis and Thrombosis Center, Children's Hospital Los Angeles, USA, and Professor of Pediatrics at the University of Southern California, USA; Jennifer Maahs, Nurse Practitioner at Indiana Hemophilia and Thrombosis Center, Indiana, USA; and Luke Pembroke, Creative Director at Haemnet, London, UK, during a satellite symposium (unpublished data, presented at ASH 2022). Pembroke underwent gene therapy for haemophilia B in 2020 and provided a patient's perspective on the process. In particular, he emphasised that the side effects associated with immunosuppression should not be underestimated, and that the follow-up can be

intensive in the early months. Also, gene therapy does not treat pre-existing arthropathies. However, overall the treatment was enormously positive in terms of providing mental freedom from the disease, and the chance to live life more spontaneously. Implementing gene therapy in the clinic was acknowledged by all speakers to be challenging, and it was felt that there will be a steep learning curve following the commercialisation of the products.

The importance of implementing multidisciplinary care was discussed, with Pembroke acknowledging the vital contribution of nursing staff in the first year after his treatment. Peer support from other patients was also noted to be useful, as patients considering gene therapy will be keen to learn from others' experiences. For long-term monitoring of treatment, Recht described the World Federation of Hemophilia (WFH) Gene Therapy Registry, which will capture patient demographics, infusion details, safety and effectiveness data, quality of life parameters, and disease burden. In the USA, the American Thrombosis and Hemostasis Network (ATHN) Transcends study will be the primary source of data for the WFH Gene Therapy Registry. This is a real-world study that will follow patients with haemophilia for at least 15 years after gene therapy with any product.

AAV-MEDIATED GENE THERAPY: UPDATES FROM CLINICAL TRIALS

Multiple gene therapy strategies for haemophilia are currently being evaluated, with those based on rAAVs currently at the most advanced stage of clinical development.[3-5,7-9] At ASH 2022, several presentations provided an update on the most recent data on rAAV-mediated gene therapy for haemophilia A and B.

Haemophilia A

SPK-8011 comprises an rAAV vector containing a codon-optimised B-domain deleted FVIII transgene. A Phase I/II trial has previously demonstrated sustained FVIII expression and reduction in bleeding episodes with no major safety concerns in patients with moderate or severe haemophilia A.[7] At ASH 2022, further results from this study were reported.[10-12] Stacey Croteau, Attending Physician at Boston Children's Hospital, Massachusetts, USA, reported that 21 out of 23 participants had sustained expression of FVIII, and two lost expression following a presumed capsid immune response.[10] In 16 participants with ≥1-year follow-up, no decrease in FVIII activity was observed over time, with most participants maintaining FVIII activity in the mild haemophilia A range. Clinically meaningful reductions in annualised bleeding rates (ABR) and annualised FVIII infusion rates were observed for up to 5 years of follow-up, and no major safety signals were reported. Huyen Tran, a Professor at Monash University, Melbourne, Australia, reported that following a single infusion of SPK-8011, vector genome concentrations were below the limit of quantitation for all participants with available data (n=21) in saliva, semen, serum, and urine within 3 weeks, and in peripheral blood mononuclear cells within 12 weeks.[11] Knowing the rate of clearance allows limits to be determined for the duration of sample collection and safety precautions such as barrier contraception, thereby reducing the treatment burden for patients.

Matthew Evans, Associate Director at the Hemophilia Treatment Center at Penn State Health, Pennsylvania, USA, reported that of the 23 participants in the study, 17 received either reactive or prophylactic corticosteroids.[12] Two of the 12 participants who received reactive corticosteroids lost FVIII expression. All five participants who received prophylactic corticosteroids maintained FVIII expression, although, for four participants, the length of exposure to corticosteroids was prolonged at between 31 and 49 weeks. In all five study participants receiving prophylactic corticosteroids, and in four participants receiving reactive corticosteroids, attempts to taper the corticosteroid dose resulted in laboratory evidence of a capsid immune response, which could not be resolved by increased corticosteroid use. Adverse events associated with corticosteroid use such as weight gain and insomnia, were observed in four participants, and were associated with increased duration of corticosteroid use.

Giroctocogene fitelparvovec is an rAAV6 vector that encodes a modified B-domain-deleted FVIII transgene. Adam Giermasz, Associate Professor of Medicine at the University of California Davis, USA, reported updated results from the ongoing

ALTA Phase I/II dose-ranging study.[13] Overall, 11 patients with severe haemophilia A received a single infusion of giroctocogene fitelparvovec, and were followed up for 95–195 weeks. Six patients (55%) experienced a treatment-related adverse event, including five patients with an increase in alanine aminotransferase, three patients with an increase in aspartate aminotransferase, and one patient with Grade 3 hypotension. An increase in FVIII levels into the moderate to normal range was observed for participants in the highest dose cohort and minimal bleeding was observed for these patients.

GS001, an rAAV8 vector expressing B-domain-deleted FVIII, was described by Wei Liu, from the Chinese Academy of Medical Sciences, Tianjin, China.[14] In a pilot clinical trial, six patients with haemophilia A received a single infusion of GS001 after 1 week of prophylaxis with either prednisone (patients 1–3) or prednisone combined with tacrolimus (patients 4–6). Overall, 55 adverse events were reported, of which only seven (12.7%) were possibly related to GS001. No severe adverse events were reported and no participants developed FVIII inhibitors. FVIII activity levels reached a peak at 7 weeks post-infusion for patients 1–3 and within the first week post-infusion for patients 4–6. Vector-derived FVIII activity was sufficient to prevent bleeding events and minimise the need for replacement therapy. Liu concluded that adding tacrolimus to prednisone might provide an effective immunosuppressive regimen against the vector capsid.

Haemophilia B

Etranacogene dezaparvovec consists of a rAAV5 vector and the highly active FIX-Padua variant transgene. In the HOPE B Phase III clinical trial, etranacogene dezaparvovec was evaluated in adults with severe or moderately-severe haemophilia B irrespective of baseline AAV5 neutralising antibody status. Efficacy and safety data for the overall study population enrolled in HOPE-B during a 24-month follow-up period were reported by Steven Pipe.[15] Of the 54 participants, 52 (96.3%) remained free from continuous FIX prophylaxis up to Month 24. The mean ABR for all bleeds was reduced by 64% and the mean FIX consumption was reduced by 96% compared with the period before treatment. The mean ABR for other bleed types was also substantially reduced, and the mean FIX activity was sustained through Month 24. The safety profile was consistent with previously reported data.

Treatment-related adverse events were reported for 70% of participants, with only one occurring during Months 18–24. In a separate presentation, Pipe also reported a subgroup analysis from HOPE-B in patients either with pre-existing AAV5 neutralising antibodies up to a titre of 1:700 (NAb+; n=20) or without pre-existing neutralising antibodies (NAb-; n=33).[16] During 24 months of follow-up, a 79% and 62% reduction in ABR was observed for NAb- and NAb+ HOPE-B participants, respectively. The safety profile was similar between subgroups, and participants experienced freedom from continuous FIX prophylaxis, irrespective of baseline neutralising antibody status. FIX activity was stable during 24 months of follow-up, and there was no association between baseline neutralising antibody status and long-term durability of FIX expression.

Wolfgang Miesbach, Professor of Medicine at Frankfurt University Hospital, Germany, also described the durability of the response to etranacogene dezaparvovec and its precursor AMT-060, using data from three clinical trials.[17] In a Phase I/II study of AMT-060, FIX activity levels were stable for 5 years after dosing. Greater FIX activity levels were achieved with etranacogene dezaparvovec, with levels remaining in the near-normal range after 3 years of follow-up in a Phase IIb study and 2 years of follow-up in the Phase III HOPE-B study. All three studies reported low bleeding rates during the follow-up, and no new safety signals were identified.

BAX 335, an rAAV8-based gene therapy encoding the FIX-Padua variant, has also been investigated in clinical trials. An interim analysis of a Phase I/II trial reported no serious adverse events related to BAX 335 and no clinical thrombosis, inhibitors, or other FIX-directed immunity.[18] In a presentation at ASH 2022, John Chapin, Director at Takeda, Cambridge, Massachusetts, USA, reported long-term results from this trial.[19] In this study, eight patients with haemophilia B received BAX 335; of these, five were included in the long-term follow-up analysis. The majority of adverse events reported (72%) occurred within the first 2 years after infusion. Six serious adverse events

occurred, of which four were reported within the first 12 months. All serious adverse events resolved, and none were considered related to BAX 335. No FIX inhibitors were observed during the study. One study participant achieved persistent FIX transgene activity for 7.2 years and remained free from bleeding and the need for FIX replacement therapy. Consistent with other studies, at Year 5, AAV-neutralising antibodies persisted at titres that would limit re-dosing.

AAV Neutralising Antibodies

Although rAAV vectors have shown promising results in gene therapy for haemophilia, a proportion of the population has pre-existing neutralising antibodies against natural AAVs, which can limit their eligibility for rAAV vector-based gene therapy. In a presentation at ASH 2022, John Rasko, Head of the Cell and Molecular Therapies Department at Royal Prince Alfred Hospital, and Head of the Gene and Stem Cell Therapy Program at the Centenary Institute of Cancer Medicine & Cell Biology, University of Sydney, Australia, reported the results from an observational, retrospective study to assess the prevalence and titre levels of neutralising antibodies.[20] Overall, samples from 502 adults and 50 children from 10 countries were analysed. In adults and at a serum dilution of 1:1, the most prevalent neutralising antibodies were against AAV1 (74.9%), AAV6 (70.1%), and AAV5 (63.9%). At a serum dilution of 1:2 and all subsequent dilutions, AAV1 antibodies had the highest and AAV5 antibodies had the lowest seroprevalence. Children in this study had lower seroprevalence compared with adults, possibly reflecting reduced exposure to natural infections. In adults, the highest seroprevalence was observed in South Korea, whereas samples from Japan, Australia, and the USA had the lowest seroprevalence.

AAV-MEDIATED GENE THERAPY: PRECLINICAL STUDIES

Privately-owned dogs are a useful preclinical model for gene therapy in haemophilia as they allow long-term real-world evaluation of the safety and effectiveness of gene therapy beyond that observed under laboratory conditions. Bhavya Doshi, Assistant Professor of Pediatrics at the Children's Hospital of Philadelphia, Pennsylvania, USA, gave an overview of the real-world effectiveness of liver-directed AAV gene therapy in privately-owned dogs with severe haemophilia A (n=11) or severe haemophilia B (n=1).[21] AAV-based gene therapy led to a sustained increase in factor activity, and both the ABR and annual infusion rate decreased dramatically post-treatment. Gene therapy also eradicated a pre-existing high-titre canine FVIII inhibitor in one animal, and no new inhibitors were detected. Three animals died during follow-up, including one from an unrelated cause, one from a bleeding episode 8.9 years after gene therapy, and one from a multicentric lymphoma.

Lucas Van Gorder, a research student at the University of Pennsylvania, USA, went on to provide more details of the case of multicentric lymphoma, which occurred in a dog with severe haemophilia A after liver-directed rAAV8-based gene therapy.[22] Treatment resulted in a 100% reduction in ABR, but the dog developed a multicentric B-cell lymphoma requiring euthanasia 3.5 years after vector administration. Vector copy number (VCN) analysis indicated no detectable rAAV vector genomes in the lymph nodes, suggesting that vector integration could not have contributed to lymphoma development. Furthermore, canine FVIII expression remained stable before and after the diagnosis of lymphoma, further supporting the lack of vector integration into malignant cells.

Due to the immune responses that can occur against the viral vector, there is interest in optimising gene therapy constructs to reduce the dose required. Anna Sternberg, Postdoctoral Fellow at the Children's Hospital of Philadelphia, Pennsylvania, USA, described the FVIII-R336Q/R562Q (QQ) variant transgene, which is resistant to activated protein C cleavage and could potentially achieve haemostatic efficacy at lower rAAV vector doses.[23] In a study of mice treated with the QQ versus wild-type transgene, no significant differences in survival were observed after 7–11 months of follow-up, and there were no differences in D-dimer levels after 8 months. Mice with the QQ variant generated in the endogenous FVIII gene by CRISPR/Cas9 were also viable, and D-dimer levels did not differ significantly from wild-type mice. In a mouse model of haemophilia A, a five-fold to 10-fold lower vector dose was required to normalise blood loss following tail-clip for the QQ versus wild-type transgene.

Denise Sabatino, Professor of Pediatrics at the University of Pennsylvania, USA, also described a novel FVIII variant (Δ3-SP/DE), which is secreted significantly more efficiently than wild-type FVIII.[24] Using an rAAV8 vector, the Δ3-SP/DE variant demonstrated increased expression compared with wild-type FVIII in a haemophilia A mouse model (two-fold to three-fold), a haemophilia A canine model (two-fold to four-fold), and in non-human primates (two-fold to five-fold). In non-human primates, levels of Δ3-SP/DE FVIII were shown to be dose-dependent.

Other preclinical studies have attempted to further optimise the process of AAV-mediated gene therapy. Tirthadipa Pradhan-Sundd, Associate Professor of Medicine at the University of Pittsburgh, Pennsylvania, USA, reported data from a study that aimed to evaluate the mechanism of liver-directed gene transfer in an FVIII knockout mouse model using rAAV8-GFP.[25] The results showed that liver cells from FVIII knockout mice were less likely to express the transgene and were more likely to undergo apoptosis following infusion of rAAV8-GFP compared with control mice. FVIII knockout mice also had increased liver sinusoidal endothelial cell capillarisation compared with control mice, and this was associated with the upregulation of components of the Notch pathway and changes in nitric oxide/soluble guanylyl cyclase signalling. Further studies are required to understand how modulation of Notch and nitric oxide/soluble guanylyl cyclase can be manipulated to improve the efficiency of liver-directed gene transfer.

Patients cannot typically be retreated with AAV-based gene therapy due to the development of neutralising antibodies against the viral capsid following the first treatment. In an oral presentation, Jyoti Rana, a Postdoctoral Research Associate at Indiana University–Purdue University Indianapolis, USA, described the use and timing of different immunosuppressive regimes to control neutralising antibody formation in a mouse model infused with an rAAV8 vector.[26] When immunosuppressive therapies were administered concurrently with gene therapy, up to 75% of mice lost transgene expression in the liver and circulation. Furthermore, all mice developed neutralising antibodies to the rAAV8 vector, which precluded re-administration of rAAV8-based gene therapy. In contrast, when immunosuppressive therapies were initiated 3 weeks before gene therapy, rAAV8 could be re-administered to 70%, 75%, and 80% of animals treated with anti-CD20 antibodies, anti-CD20 antibodies plus rapamycin, or anti-CD20 antibodies plus anti-B-cell activating factor (BAFF) antibodies, respectively. Extending treatment with anti-BAFF for an additional 4 weeks allowed successful re-dosing in 100% of mice. Rana and colleagues concluded that treatment with anti-CD20 combined with extended treatment with anti-BAFF before initial gene therapy could be used to enable re-dosing with clinically relevant doses of AAV vectors.

NOVEL TECHNOLOGIES

Brian Truong, Research Scientist at Poseida Therapeutics, introduced the piggyBac® DNA delivery platform (Hera BioLabs, Lexington, Kentucky, USA), which facilitates the stable insertion of a therapeutic transgene into the host genome.[27] The use of liver-tropic nanoparticles to deliver the transgene offers multiple advantages compared with rAAV-based approaches, including increased transgene cargo capacity and potential for re-dosing. Proof of concept studies in wild-type mice resulted in sustained human FVIII antigen levels in the anticipated therapeutic range. In a mouse model of haemophilia A, a single treatment resulted in 30–150% of human FVIII activity in a dose-responsive manner, which was generally sustained throughout the 6-month study.

Platelet-targeted FVIII expression under the control of a platelet-specific promoter (2bF8) is a novel gene therapy approach for haemophilia A. Since anti-platelet drugs are commonly used in clinical practice, Hongyin Yu, a graduate student at the Children's Research Institute, Milwaukee, USA, evaluated whether the clinical efficacy of 2bF8-mediated gene therapy would be affected by treatment with anti-platelet agents.[28] In this study, in mice with haemophilia A treated with 2bF8, treatment with a platelet αIIbβ3 receptor antagonist impaired all parameters from rotational thromboelastometry analysis, indicating that αIIbβ3-mediated platelet aggregation might be critical for the haemostatic function of platelet-derived FVIII in a haemophilia A model.

LV-F8-299 is a novel gene therapy for haemophilia A that consists of a lentiviral vector expressing the FVIII transgene with the B-domain deleted except for the N-glycosylation sites.[29] In a study presented by Jie Gong, from the University of Electronic Science and Technology of China, the expression and safety of LV-F8-299 with either a universal (EF1a) or vascular endothelial-specific promoter were investigated.[30] In FVIII knock-out mice, therapeutic FVIII activities occurred within 60 days after infusion, with EF1a-299 and VEC-299 achieving 90% and 40% of the normal range, respectively. VEC-299 infusion resulted in a steady increase in FVIII activity, whereas after infusion of EF-299, FVIII activity dropped to 3% after 120 days. Kinetic analyses demonstrated that VEC-299-treated mice exhibited the lowest FVIII inhibitory immune response over time. In a macaque monkey treated with LV-VEC-299, the VCN in the blood remained stable but decreased over time in faeces, saliva, and urine. The VCN in all body fluids was nearly undetectable after 14 weeks. After a second injection of VEC-299 using a modified non-myeloablative conditioning regimen, the VCN in the blood reached 20% from Day 1 to Day 7, then reduced to 5% and remained stable until Week 6. Blood biochemical analyses remained in the normal range before and after treatment.

CONCLUSION

Multiple gene therapies for haemophilia A and B are undergoing preclinical and clinical development, with two rAAV vector-mediated replacement gene therapies, valoctocogene roxaparvovec and etranacogene dezaparvovec, receiving regulatory approval in 2022. Although not curative, these treatments have shown impressive and durable results and have the potential to be life-changing for people with haemophilia. Along with ongoing research to refine the process and broaden patient eligibility, successfully establishing these treatments in routine clinical practice will be the next stage in realising the promise of gene therapy for haemophilia.

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