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Lentiviral Gene Therapy for Hemophilia A Using CD34+ Hematopoietic Cells

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Abstract

Background—Severe hemophilia A (SHA) is managed with Factor VIII (FVIII) replacement products or mimetics to treat or prevent bleeding. We have developed gene therapy for SHA with hematopoietic stem cells (HSC) based expression of FVIII.

Methods—Autologous HSCs were transduced with a novel *F8* transgene *(ET3)* with a myeloid-directed CD68 promoter in lentiviral vector (LV)(LV-ET3-CD68) using two manufacturing protocols, without (G1) and with transduction enhancer (G2). Transduced HSCs were transplanted into recipients after myeloablative conditioning. Participants were assessed for safety (engraftment and regimen related toxicities) and efficacy (FVIII activity and bleeding).

Results—Five participants, aged 22–41 years, received LV-ET3-CD68 transduced autologous CD34+ HSC (5.0–6.1 × 10^6 cells/kg). The vector copy numbers (VCN) in the final drug product were 1.02 and 0.57 (G1) and 1.49, 0.62 and 2.24 (G2). The duration of severe neutropenia and thrombocytopenia were 7–11 and 1–7 days, respectively. The median one-stage FVIII activity (IU per deciliter) after day +28 until last follow-up is 5.2 (range, 3.0–8.7) and 1.7 (range, 1.0–4.0) with a peripheral blood VCN of 0.17 and 0.09 in G1 participants, respectively, and 37 (range, 18.3–73.6), 19.3 (range, 6.6–34.5) and 39.9 (range, 20.6–55.1) with a peripheral blood VCN of

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4.4, 3.2 and 4.8 in G2 participants, respectively. The annualized bleeding rate was zero over a cumulative follow-up of 81 months (median: 14; range: 9–27).

Conclusion—Gene therapy for hemophilia A using lentiviral vector transduced autologous HSCs resulted in stable factor VIII expression with FVIII activity correlating with VCN in the peripheral blood. (Funded by Ministry of Science and Technology, Government of India, and others. Clinicaltrials.gov number NCT05265767; CTRI/2022/03/041304)

INTRODUCTION

Severe hemophilia A is an X-linked disorder characterized by less than 1% measurable Factor VIII (FVIII) activity in plasma and is associated with frequent spontaneous bleeding into joints and muscles as well as sporadic life-threatening bleeding. (1). People with hemophilia (PWH) with FVIII activity >1 international unit (IU) per deciliter (dL) have a milder clinical profile while among those with FVIII activity >5 IU per dL, spontaneous bleeding is rare. (1) Prophylactic treatment with FVIII products or other hemostasis drugs are recommended for prevention of bleeding in severe hemophilia. (1)

Gene therapy for hemophilia has been attempted for more than two decades with different technologies.(2–4) An adeno-associated virus (AAV) vector-based hepatocyte directed gene therapy has been approved for the treatment of hemophilia A. (4) However, this technology has several limitations including i) variable initial expression; ii) gradual decline of expression after the first 6–12 months, often associated with transaminitis requiring immunosuppressive treatment, which has led to its own adverse events (4, 5); iii) age-related ineligibility until liver maturity (6); and iv) pre-existing anti-AAV antibodies from natural infections, precluding treatment. (7, 8)

We have shown it is possible to achieve FVIII expression in hemophilia A mice using mouse hematopoietic stem cells (HSC) and in immunodeficient mice using human CD34+ HSC with a CD68-ET3-LV self-inactivating lentiviral vector packaging a novel *F8* transgene (ET3). (9) These preclinical data led us to initiate a first-in-human clinical trial to evaluate the safety and feasibility of this technology for gene therapy of severe hemophilia A. This report describes the outcome of the completed phase 1 clinical trial involving five participants who have completed at least 6 months of follow-up.

METHODS

STUDY DESIGN AND OVERSIGHT

This is a single-center study involving five participants with severe hemophilia A without inhibitors who have undergone gene therapy by transplantation of autologous HSC transduced with the CD68-ET3-LV vector. The study was approved by the Institutional Review Board and the Ethical Committee of the Christian Medical College, Vellore, India as well as the Central Drugs Standard Control Organization (CDSCO) of the Ministry of Health and Family Welfare of Government of India and was conducted according to GCP guidelines. The investigators at the Christian Medical College (CMC), Vellore, India and the Centre for Stem Cell Research (CSCR), a unit of inStem, Bengaluru, India along with collaborators at the Emory University, Atlanta, GA, USA and Expression Therapeutics,

Tucker, GA, USA were responsible for design of this study and having the CD68-ET3-LV vector manufactured under suitable GMP conditions. The study was conducted at the CMC and CSCR, Vellore, India where site investigators and team members were responsible for collection and analysis of all data. The manuscript was prepared by the lead authors and reviewed by all authors who also had access to the complete data. The authors assure the completeness and accuracy of the data and compliance of the study with the protocol which is available at NEJM.org.

PATIENTS AND PROCEDURES

As a first of its kind study in India, a multistep process was followed for participant selection and informed consent. This started with cohort counselling for groups of people with hemophilia over an extended period to increase general awareness in the community about gene therapy technologies. After approval of the clinical trial protocol, specific information was shared with the same patient groups. Interested patients and their families were invited for further discussion to the study site following which detailed counselling was undertaken for the potential participant and his family. Formal consenting was video recorded according to regulatory requirements in India. Going beyond these regulatory requirements, an additional step of review by an independent external committee comprising of a physician, a family member of an unrelated person with hemophilia (PWH) and an HSC transplant survivor was included. This committee interviewed the potential participant and his family to evaluate their comprehension of this clinical trial and, if satisfied with their responses, certified the consenting process.

A total of eight participants were consented. Two participants could not be included in the study, one due to failed eligibility criteria and the second who refused participation due to concerns of possible infertility. The third participant was withdrawn when he developed a low titer FVIII inhibitor on the day his conditioning treatment was to start. His outcome will be reported separately. Data from the other five treated participants treated between June, 2022 and December, 2023 are included in this report. (Table S1)

Study participants, who were all on episodic treatment, received prophylactic extended half-life (EHL) recombinant FVIII (rFVIII) once or twice weekly after consenting to the study. CD34+ cells were mobilized with granulocyte colony-stimulating factor (G-CSF) at 5ug per kg given twice daily for 5 days along with plerixafor 0.24mg/kg given 12–14 hours before apheresis. Adequate numbers of autologous CD34+ peripheral blood stem cells (PBSC), defined as $>5\times10^6$ CD34+ cells/kg body weight, were obtained in a single apheresis procedure after positive selection using a CliniMACS® Plus system (Miltenyi Biotec, Bergisch Gladbach, Germany). An aliquot of PBSC with $>2\times10^6$/kg CD34+ cells per kg was cryo-preserved without manipulation.

The enriched CD34+ HSC were transduced with the clinical grade lentiviral vector, CD68-ET3-LV, (Figure S1) initially using a double exposure transduction protocol. (Suppl P1) After the outcome of the first two treated participants was reviewed, and as planned, the transduction protocol was modified to include an enhancer, (LentiBOOST, SIRION Biotech, Gräfelfing, Germany), and only a single exposure to the vector. Transduced HSCs were cryopreserved while the final drug product was qualified for release criteria.

Participants were conditioned with intravenous treosulfan at 14g/m^2 on days −6 to −4 along with fludarabine 30mg/m^2 on days −6 to −2 with no therapeutic drug monitoring. The day of infusion of the drug product was considered as day 0. Participants were nursed in HEPA-filtered isolation rooms during conditioning and ensuing cytopenia. Other supportive care was provided according to institutional protocols for autologous HSC transplantation (HSCT).

SAFETY AND EFFICACY ENDPOINTS

The endpoints related to safety included i) adverse events related to drug-product infusion and conditioning therapy, ii) engraftment by day +30, iii) survival at day +100 and iv) development of a FVIII inhibitor v) vector copy number and vector integration profile. The endpoints of efficacy were i) plasma FVIII activity (one stage assay – OSA and chromogenic substrate assay – CSA) and ii) annualized bleeding rate (ABR). (Table S2)

Participants were assessed regularly for adverse events according to the Common Terminology Criteria for Adverse Events (CTCAE) v5.0 (2017). The engraftment endpoint criteria were absolute neutrophil count of $>0.5\times10^9$ per liter and an unsupported platelet count of $>20\times10^9$ per liter. FVIII activity assay was performed daily during the conditioning and cytopenic phase to maintain trough levels >50 IU per dL after administration of EHL rFVIII. As engraftment ensued, the dose of EHL rFVIII was gradually reduced and stopped once the platelet count was $>100\times10^9$ per liter.

Vector copy number (VCN) in the drug product and in genomic DNA from peripheral blood post-gene therapy was assessed at regular intervals, as described previously. (9) (Suppl P2) In view of the underlying hemophilia, bone marrow assessment was performed on one occasion under additional FVIII cover at 4–22 months of follow-up for assessment of morphology, cytogenetics and VCN. Integration site analysis was performed by ligation mediated PCR (LM-PCR) on genomic DNA from peripheral blood and bone marrow (CD Genomics, New York, USA).

STUDY OVERSIGHT & DATA

The study was performed according to protocol approved by the regulator in India, the CDSCO. Serious adverse events were reported within 24 hours. An institutional data and safety monitoring board also reviewed serious adverse events. Additional scientific oversight was provided by an international scientific advisory board of experts.

The data reported are as of 30th September, 2024. Given the small number of participants in this study, all statements are qualitative based on inspection of data with no statistical analysis.

RESULTS

PARTICIPANTS AND TREATMENT

Five participants, aged 22–41 years at study recruitment, received the investigational gene therapy product candidate. The median follow-up is 14 months (range, 9–27) after gene modified HSC transplantation.

The target number of mobilized CD34+ HSC was collected in a single apheresis yielding a median of 7.5×10^6 cells per kg body weight (range, 7.0–9.2). (Table S3) This also allowed a minimum of 2×10^6/kg unmanipulated CD34+ HSCs to be cryopreserved as a back-up, if required, for delayed engraftment. The median drug product dose infused after transduction was 5.6×10^6 cells per kg (range, 5.0–6.1). (Table 1)

For the first two participants (G1) where the final gene therapy product, CD68-ET3-LV-CD34+, was manufactured with a double transduction protocol, the VCN was 0.57 and 1.02 in the drug product. For the next three participants, with the modified transduction protocol with a single transduction step with an enhancer, LentiBOOST®, the VCN in the drug product was 0.62, 1.49 and 2.24. (Table 1)

SAFETY OUTCOMES

All participants developed expected severe cytopenia with nadir absolute neutrophil counts $<0.1\times10^9$ per liter and platelet counts $<20\times10^9$ per liter. However, engraftment occurred in all participants with the median time to neutrophil engraftment of 11 days (range, 10–12) and platelet engraftment of 15 days (range, 12–15). (Table S4) All participants received G-CSF 5ug per kilogram per day from day +5 after drug product infusion. The median duration of severe neutropenia was 8 days (range, 7–11) and that of severe thrombocytopenia was 3 days (range, 1–7). Engraftment parameters of participants were not different in G1 or G2 groups. The blood counts have been in the normal range for all participants between 1–3 months after gene therapy and in P6 by the 14^{th} month. (Table S5). Exogenous EHL FVIII concentrate was administered for a median of 16 days (range: 11–20) during HSCT.

No other adverse events of grade >2 occurred in any participant. The most common adverse event was nausea with or without vomiting. All non-laboratory adverse events during the hospital stay and follow-up are listed in Table S6A,B. No participant developed inhibitors to FVIII after drug product infusion.

Integration site analysis performed at 4–22 months after drug-product infusion has shown no safety concerns. Integration is skewed toward intronic sequences which account for >70% of integrations (Table S7 A, B, C), with exonic sequences accounting for <3% integrations and the rest being predominantly in the intergenic non-coding regions. (Table S7D) The current data indicate that the transduction enhancer does not adversely affect the integration profile of this LV vector. Though integrations were observed near several oncogenic sequences (Table S7E), consistent with other LV vector therapies (10–12), no evidence of clonal dominance was noted in any participant.

Bone marrow examination performed at 4–22 months after gene therapy for all participants, showed normal cellularity and morphology. Karyotype analysis on the bone marrow was also normal. Analysis of VCN on the bone marrow sample and the peripheral blood taken on the same day was comparable. (Table 2)

EFFICACY OUTCOMES

Evidence of endogenous FVIII expression was observed as early as day +18 in P6. The FVIII activity (IU per dL) on day +60 was 7.9 and 4.0 in G1 participants and 28, 14.2 and

36.6 in G2 participants. Median FVIII activity (IU per dL) after day +28 until last follow-up are 5.2 (range, 3.0–8.7) and 1.7(range, 1.0–4.0) in the G1 participants and 37(range, 18.3–73.6), 19.3 (range, 6.6–34.5) and 39.9(range, 20.6–55.1-) in the G2 participants. All these FVIII levels are based on the OSA. The serial FVIII activities are shown in Figure 1 and Table S8. It is important to note that both the OSA and CSA FVIII activity showed similar results. (Table 2; Figure S2)

No spontaneous bleeding events occurred in any participant during HSCT or after gene therapy. Before gene therapy, all participants had reported an ABR of >20 events. (Table 1) The lack of bleeding in P3 with FVIII activity between 1–3 IU per dL is notable.

A positive correlation was noted between the VCN in peripheral blood 1 month after gene therapy and the corresponding FVIII activity. VCN analysis of peripheral blood and bone marrow also correlated well in both G1 and G2 participants. Both VCN and FVIII activity are much higher in G2 participants after HSCT (Table 2) even though the VCN in the gene therapy products were similar. This increase in FVIII activity also correlates with a more intense integration profile of the vector in the G2 participants. (Table S7B)

OTHER OUTCOMES

All participants reported higher physical activities without fear of bleeding and enhanced ability to engage in more intense leisure activities. Two participants from G2 had trauma. P6 (FVIII activity of about 40 IU per dL) had a minor motor vehicle accident (MVA) on day +302 with minor cuts that stopped bleeding spontaneously and transient loss of consciousness but full neurological recovery immediately thereafter. CT scan of the brain was normal. P7 (FVIII activity of about 20 IU per dL) had a superficial cat bite on day +102 for which anti-rabies vaccine was given intramuscularly and a minor MVA on day +179 with superficial abrasions. No additional FVIII replacement was required for any of these trauma related events.

Semen analysis carried out on all participants at least 6 months after HSCT showed normal sperm counts in four (one with low motility) and the fifth having a low sperm count at 8 months of follow-up.

DISCUSSION

The results from this clinical trial validate a novel approach to gene therapy of severe hemophilia A through transplantation after myeloablative conditioning of autologous HSCs transduced with a LV vector encoding a novel FVIII transgene driven by a CD68 promoter. No unexpected safety issues were encountered. All participants achieved clinically significant circulating FVIII activity sufficient to completely abrogate spontaneous bleeding and which remained stable after the first 3–6 months during follow-up ranging from 9–27 months.

The candidate CD68-ET3-LV $CD34^+$ gene therapy product in this study incorporates multiple design elements that distinguish it from existing options. First, the *ex vivo* gene transfer into adult stem cells utilizing an integrating and self-inactivating LV vector instead

of *in vivo* transfer to a hepatocyte through a non-integrating AAV vector. The correlative premise is lifelong durability of expression and the initial data out to more than two years of follow-up appear promising. Second, the human *CD68* gene directed promoter shifts gene expression away from HSCs to prevent FVIII related cellular toxicity. (13, 14).

Monocytes are abundant short lived circulating blood cells. (15) Tissue monocytes or macrophages also represent a second source of FVIII and can be long-lived (up to 1 yr) with ~ 200 billion macrophages present in an adult male. (16) Macrophages are also present at significant density in the synovium of joints and could contribute to local hemostasis even with low plasma FVIII activity. The third unique design element is the ET3 transgene, which results in 10 – 100-fold higher expression and secretion efficiency compared to B domain deleted human FVIII (17). The addition of a transduction enhancer in manufacturing produced a dramatic 5–10-fold increase in FVIII activity with the expected higher numbers of integration sites without affecting short term safety. The FVIII activity has been stable within the same clinically relevant range in all participants with some fluctuation during follow-up of >2 years in GI and >1 year in G2 participants. This could be related to analytical issues or reflect true variations in expression due to transient changes in the relevant blood cells during intercurrent events in the participants which were not clinically obvious.

Compared to AAV gene therapy, LV vector transduced HSC based gene therapy for hemophilia A overcomes nearly all the limitations related to highly variable and unpredictable expression, age and pre-existing anti-AAV antibody related ineligibilities. Interestingly, concordance between the OSA and CSA assays for FVIII activity is observed. However, the HSC approach also has limitations, including the elaborate and complex process of collection and manipulation of HSCs to prepare the patient-specific drug product. This approach has been used extensively for gene therapy of primary immune deficiency syndromes and also for the major hemoglobin disorders. (18, 19) Acute and long-term toxicities of myeloablative conditioning are also a potential concern (20), including severe mucositis and possible infertility. (21) Our results so far have been encouraging in both types of adverse events. Using treosulfan instead of busulfan, as in other myeloablative conditioning protocols for HSC based gene therapy, (18, 22) we show a much-reduced toxicity profile, as is well-known in allogeneic HSC transplant for high-risk cases. (23). What is most remarkable is the finding of normal sperm counts in four of the five participants in this study. Lower infertility rates have been reported with treosulfan conditioning compared to busulfan. (24) Finally, in severe genetic disorders, gene therapy should ideally be offered as soon as possible after diagnosis. This HSC-based approach moves the field closer to that goal. Treatment in early childhood well before puberty would also reduce infertility concerns (24, 25). Safety concerns related to random integration of the LV vector into the host genome will need close monitoring.

In conclusion, the early results of this clinical trial reveal a new opportunity for gene therapy of hemophilia A that can be offered to all patients, possibly at an early age, and which results in sustained expression of therapeutic levels of FVIII. Longer follow-up and additional clinical trials with more participants will clarify whether these expectations will hold true.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Srivastava A, Santagostino E, Dougall A, Kitchen S, Sutherland M, Pipe SW, et al. WFH Guidelines for the Management of Hemophilia, 3rd edition. Haemophilia : the official journal of the World Federation of Hemophilia. 2020 Aug;26 Suppl 6:1–158. [PubMed: 32744769]
2. Pierce GF, Fong S, Long BR, Kaczmarek R. Deciphering conundrums of adeno-associated virus liver-directed gene therapy: focus on hemophilia. Journal of thrombosis and haemostasis : JTH. 2024 May;22(5):1263–89. [PubMed: 38103734]
3. High KH, Nathwani A, Spencer T, Lillicrap D. Current status of haemophilia gene therapy. Haemophilia : the official journal of the World Federation of Hemophilia. 2014 May;20 Suppl 4:43–9. [PubMed: 24762274]
4. Ozelo MC, Mahlangu J, Pasi KJ, Giermasz A, Leavitt AD, Laffan M, et al. Valoctocogene Roxaparvovec Gene Therapy for Hemophilia A. The New England journal of medicine. 2022 Mar 17;386(11):1013–25. [PubMed: 35294811]
5. George LA, Monahan PE, Eyster ME, Sullivan SK, Ragni MV, Croteau SE, et al. Multiyear Factor VIII Expression after AAV Gene Transfer for Hemophilia A. The New England journal of medicine. 2021 Nov 18;385(21):1961–73. [PubMed: 34788507]
6. Krumb E, Lambert C, Hermans C. Patient selection for hemophilia gene therapy: Real-life data from a single center. Research and practice in thrombosis and haemostasis. 2021 Mar;5(3):390–4. [PubMed: 33870024]
7. Pabinger I, Ayash-Rashkovsky M, Escobar M, Konkle BA, Mingot-Castellano ME, Mullins ES, et al. Multicenter assessment and longitudinal study of the prevalence of antibodies and related adaptive immune responses to AAV in adult males with hemophilia. Gene therapy. 2024 May;31(5–6):273–84. [PubMed: 38355967]
8. Chhabra A, Bashirians G, Petropoulos CJ, Wrin T, Paliwal Y, Henstock PV, et al. Global seroprevalence of neutralizing antibodies against adeno-associated virus serotypes used for human gene therapies. Molecular therapy Methods & clinical development. 2024 Sep 12;32(3):101273. [PubMed: 39022744]
9. Doering CB, Denning G, Shields JE, Fine EJ, Parker ET, Srivastava A, et al. Preclinical Development of a Hematopoietic Stem and Progenitor Cell Bioengineered Factor VIII Lentiviral Vector Gene Therapy for Hemophilia A. Human gene therapy. 2018 Oct;29(10):1183–201. [PubMed: 30160169]
10. Cowan MJ, Yu J, Facchino J, Fraser-Browne C, Sanford U, Kawahara M, et al. Lentiviral Gene Therapy for Artemis-Deficient SCID. The New England journal of medicine. 2022 Dec 22;387(25):2344–55. [PubMed: 36546626]
11. Yan KK, Condori J, Ma Z, Metais JY, Ju B, Ding L, et al. Integrome signatures of lentiviral gene therapy for SCID-X1 patients. Science advances. 2023 Oct 6;9(40):eadg9959. [PubMed: 37801507]

12. Magnani A, Semeraro M, Adam F, Booth C, Dupre L, Morris EC, et al. Long-term safety and efficacy of lentiviral hematopoietic stem/progenitor cell gene therapy for Wiskott-Aldrich syndrome. Nature medicine. 2022 Jan;28(1):71–80.
13. Brown HC, Gangadharan B, Doering CB. Enhanced biosynthesis of coagulation factor VIII through diminished engagement of the unfolded protein response. The Journal of biological chemistry. 2011 Jul 8;286(27):24451–7. [PubMed: 21606503]
14. Chen Z, Herzog RW, Kaufman RJ. Cellular stress and coagulation factor production: when more is not necessarily better. Journal of thrombosis and haemostasis : JTH. 2023 Dec;21(12):3329–41. [PubMed: 37839613]
15. Sender R, Weiss Y, Navon Y, Milo I, Azulay N, Keren L, et al. The total mass, number, and distribution of immune cells in the human body. Proceedings of the National Academy of Sciences of the United States of America. 2023 Oct 31;120(44):e2308511120. [PubMed: 37871201]
16. Sender R, Milo R. The distribution of cellular turnover in the human body. Nature medicine. 2021 Jan;27(1):45–8.
17. Doering CB, Healey JF, Parker ET, Barrow RT, Lollar P. Identification of porcine coagulation factor VIII domains responsible for high level expression via enhanced secretion. The Journal of biological chemistry. 2004 Feb 20;279(8):6546–52. [PubMed: 14660593]
18. Hacein-Bey-Abina S, Pai SY, Gaspar HB, Armant M, Berry CC, Blanche S, et al. A modified gamma-retrovirus vector for X-linked severe combined immunodeficiency. The New England journal of medicine. 2014 Oct 9;371(15):1407–17. [PubMed: 25295500]
19. Locatelli F, Thompson AA, Kwiatkowski JL, Porter JB, Thrasher AJ, Hongeng S, et al. Betibeglogene Autotemcel Gene Therapy for Non-beta(0)/beta(0) Genotype beta-Thalassemia. The New England journal of medicine. 2022 Feb 3;386(5):415–27. [PubMed: 34891223]
20. Chaudhry HM, Bruce AJ, Wolf RC, Litzow MR, Hogan WJ, Patnaik MS, et al. The Incidence and Severity of Oral Mucositis among Allogeneic Hematopoietic Stem Cell Transplantation Patients: A Systematic Review. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation. 2016 Apr;22(4):605–16. [PubMed: 26409924]
21. Phelan R, Im A, Hunter RL, Inamoto Y, Lupo-Stanghellini MT, Rovo A, et al. Male-Specific Late Effects in Adult Hematopoietic Cell Transplantation Recipients: A Systematic Review from the Late Effects and Quality of Life Working Committee of the Center for International Blood and Marrow Transplant Research and Transplant Complications Working Party of the European Society of Blood and Marrow Transplantation. Transplantation and cellular therapy. 2022 Jun;28(6):335 e1–e17.
22. Locatelli F, Lang P, Wall D, Meisel R, Corbacioglu S, Li AM, et al. Exagamglogene Autotemcel for Transfusion-Dependent beta-Thalassemia. The New England journal of medicine. 2024 May 9;390(18):1663–76. [PubMed: 38657265]
23. Pai AA, Mohanan E, Panetta JC, Kulkarni UP, Illangeswaran RSS, Balakrishnan B, et al. Treosulfan Exposure Predicts Thalassemia-Free Survival in Patients with Beta Thalassemia Major Undergoing Allogeneic Hematopoietic Cell Transplantation. Clinical pharmacology and therapeutics. 2024 Jan;115(1):116–25. [PubMed: 37846495]
24. Saglio F, Pagliara D, Zecca M, Balduzzi A, Cattoni A, Prete A, et al. Long-Term Complications after Allogeneic Hematopoietic Stem Cell Transplantation with Treosulfan- or Busulfan-Based Conditioning in Pediatric Patients with Acute Leukemia or Myelodysplastic Syndrome: Results of an Associazione Italiana Ematologia Oncologia Pediatrica Retrospective Study. Transplantation and cellular therapy. 2024 Apr;30(4):433 e1–e10.
25. Tichelli A, Rovo A. Fertility issues following hematopoietic stem cell transplantation. Expert review of hematology. 2013 Aug;6(4):375–88. [PubMed: 23991924]

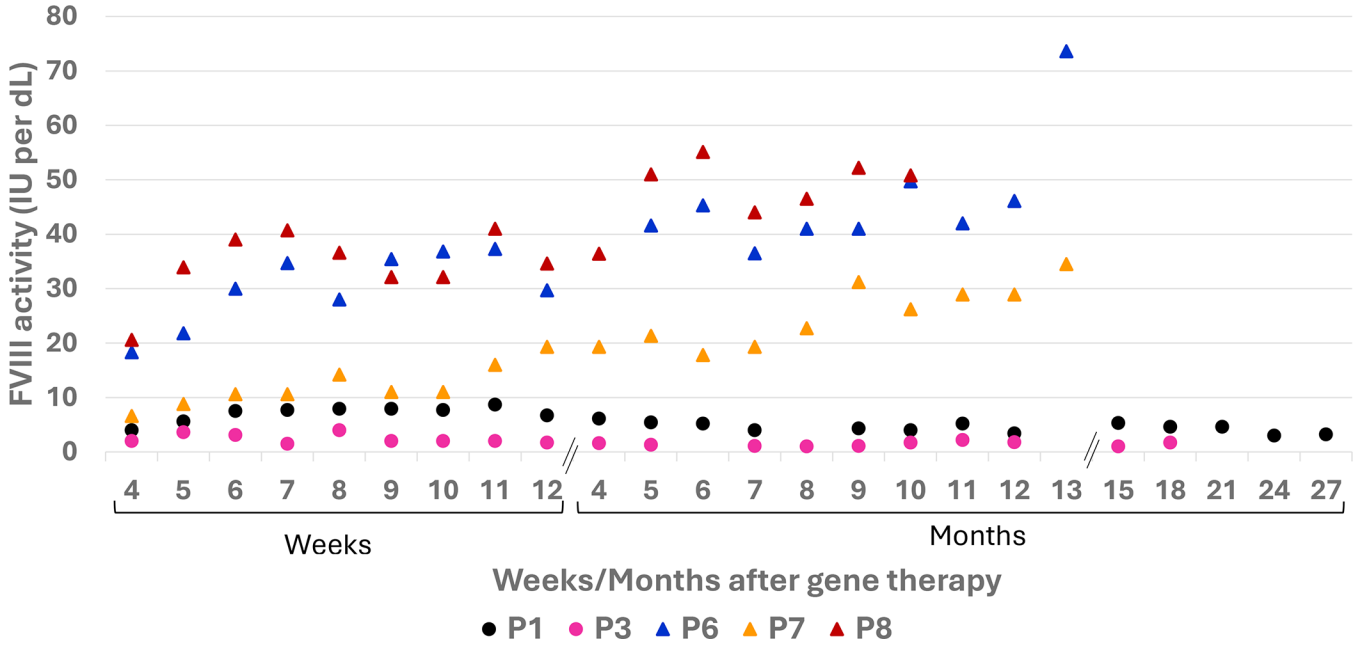

Figure 1A: Serial FVIII activity (one-stage assay) - Post gene therapy

FVIII activity measured by one stage assay weekly for post 12 weeks, per month for 12 months and then every 3 months. Data shown from 4th week onwards.

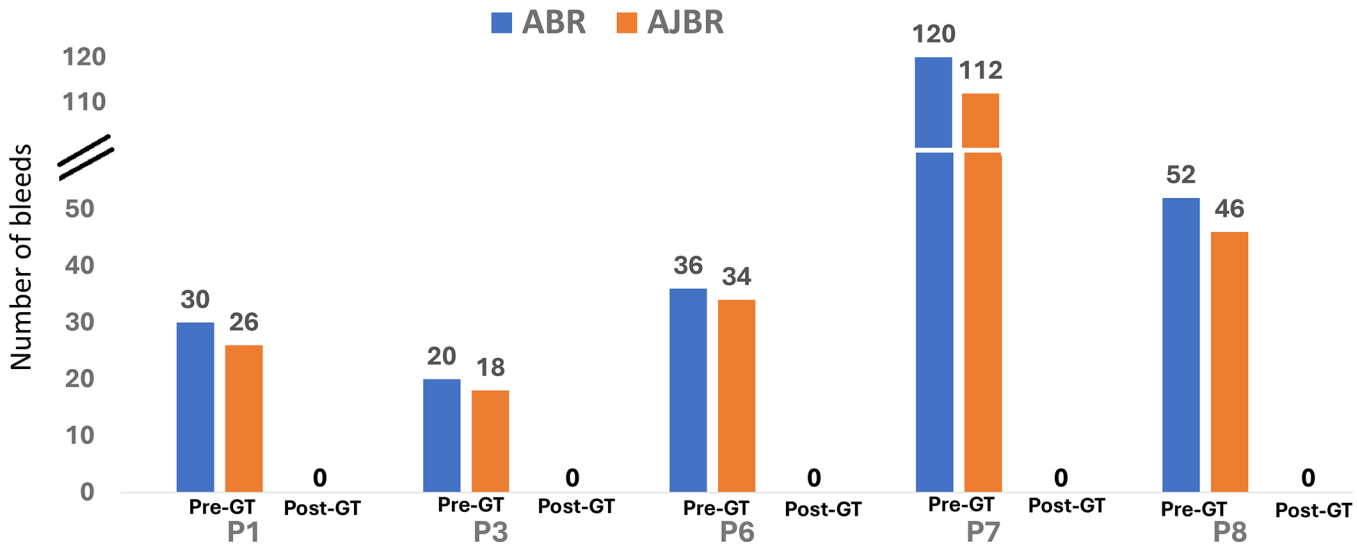

Figure 1B: Bleeding profile before and after gene therapy.

ABR-Annualized bleeding rate (all bleeds); AJBR-Annualized joint bleeding rate; GT – Gene therapy

Table - 1:

Baseline characteristics of treated participants and the final gene therapy drug product.

Participant Number	Age (Yrs)	Genotype	Bleeding symptoms (ABR)*	HJHS Total score - 124	FVIII replacement (Number of exposures)	Enriched CD34 Cell dose/kg of body weight ($\times 10^6$)	Cell dose infused / kg of body weight	VCN of Product	Follow-up (months)
P1	33	Duplication of Exon 7 to 13	30	46	Episodic (>100)	7.5	5.6	1.02	27
P3	31	Exon 13 (abnormal) TGG>TAG * Trp 637 Stop	20	33	Episodic (>100)	7.0	5.3	0.57	19
P6	34	Intron 22 inversion	36	26	Episodic (>100)	8.7	5.9	1.49	14
P7	22	Exon 13 c.1911T>G p.Asn637Lys	120	36	Episodic (>100)	9.2	6.1	0.62	12
P8	41	Exon 11 c.1733T>A p.Val578Glu	52	46	Episodic (>100)	7.2	5.0	2.24	9

* All ABR calculated as total bleeds for the previous 12 months. All participants had between 1–3 target joints.

Table - 2:

Correlation of vector copy number (peripheral blood or bone marrow) with FVIII activity

Source and Time Point	**P1**	**P3**	**P6**	**P7**	**P8**
Month post gene therapy	1	1	1	1	1
VCN (peripheral blood)	0.07	0.1	2.5	1.4	3.6
FVIII (one stage)	4	2	30	7	21
FVIII (chromogenic)	4	<1%	26	8	18
Months post gene therapy	22	14	8	6	4
VCN (peripheral blood)	0.17	0.09	4.4	3.2	4.8
VCN (bone marrow)	0.12	0.08	4.2	2.8	2.7
FVIII (one stage)	3.6	<1%	41	19.3	36.4

VCN - vector copy number