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क्षेत्रीय आयुर्विज्ञान संस्थान, इंफाल: मणिपुर
REGIONAL INSTITUTE OF MEDICAL SCIENCES, IMPHAL, MANIPUR
(स्वास्थ्य और परिवार कल्याण मंत्रालय,भारत सरकार के अंतर्गत एक स्वायत्त संस्थान)
(An Autonomous Institute under the Ministry of Health & Family Welfare, Govt. of India

CIRCULAR

Imphal, the 15th July, 2026

No. 137/RIMS-MRU/2026: It is to notify that, the **14th Research Masterclasses 2026**, of the Department of Health Research, Ministry of Health and Family Welfare, Government of India, will be conducted virtually, **on 31st July, 2026 (Friday)**.

2. All the faculties (RIMS, Dental College, and College of Nursing), members of EC, LRAC of MRU, Principal Investigators undertaking MRU funding projects (including under process projects) and residents are invited to attend the session at **Banting Hall, RIMS, Imphal.**

Date: 31.07.2026 (Friday)
Time: 3:00 PM onwards
Venue: Banting Hall, RIMS, Imphal **(Designated Site for Participation for RIMS)**
Event Name: Research Masterclass under DHR-ICMR Research Grand Rounds
Speaker Name: Dr. Alok Srivastava, Professor & Head, Haematology Research Unit, St. John's Research Institute, Bengaluru, Karnataka.

3. The **research paper** to be discussed during the Masterclass will be uploaded on the **RIMS website** and circulated to the concerned Departments/Colleges through official email.

4. As per the directives issued by the DHR, **maximum participation** from our institute is highly encouraged. MRU is submitting the attendance sheet to the DHR after the session concludes.

15.7.26

(Prof. T. Jeetenkumar Singh)
Nodal Officer
Multi-Disciplinary Research Unit,
RIMS, Imphal

Copy to:

1. The P.S. to Director, RIMS, Imphal for kind information of Director
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10. The Principal Investigator, MRU, RIMS, Imphal .....
11. The IT Cell, RIMS, Imphal – with a request for uploading the notice in the website & technical support on **31.07.26**
12. Asst. Engineer (Elect. /Civil), RIMS, Imphal - with a request for ensuring uninterrupted power supply & optimum AC functioning.
13. The Care Taker, Banting Hall, RIMS, Imphal - for proper upkeep of the venue & accompanying facilities.
14. Guard file.

ORIGINAL ARTICLE

Lentiviral Gene Therapy with CD34+ Hematopoietic Cells for Hemophilia A

A. Srivastava, A. Abraham, F. Aboobacker, G. Singh, T. Geevar, U. Kulkarni, S. Selvarajan, A. Korula, R.G. Dave, M. Shankar, A.S. Singh, A. Jeba, N. Kumaar, C. Benjamin, K.M. Lakshmi, V.M. Srivastava, R.V. Shaji, S.C. Nair, H.C. Brown, G. Denning, P. Lollar, C.B. Doering, and T. Spencer

ABSTRACT

BACKGROUND

Severe hemophilia A is managed with factor VIII replacement or hemostatic products that stop or prevent bleeding. Data on gene therapy with hematopoietic stem-cell (HSC)–based expression of factor VIII for the treatment of severe hemophilia A are lacking.

METHODS

We conducted a single-center study involving five participants 22 to 41 years of age with severe hemophilia A without factor VIII inhibitors. Autologous HSCs were transduced with CD68-ET3-LV — a lentiviral vector including a new *F8* transgene (*ET3*) with a myeloid-directed CD68 promoter — either without transduction enhancer (group 1) or with transduction enhancer (group 2). Transduced HSCs were transplanted into recipients after myeloablative conditioning. The treatment was assessed for safety (engraftment and regimen-related toxic effects) and efficacy (factor VIII activity and annualized bleeding rate).

RESULTS

Participants received CD68-ET3-LV–transduced autologous CD34+ HSCs at doses of 5.0×10^6 to 6.1×10^6 per kilogram of body weight. The vector copy numbers in the final drug product were 1.0 and 0.6 copies per cell for the two participants in group 1 and 1.5, 0.6, and 2.2 copies per cell for the three participants in group 2. The duration of severe neutropenia was 7 to 11 days and of severe thrombocytopenia was 1 to 7 days. The median factor VIII activity level, measured with the use of a one-stage assay, after day 28 until the last follow-up visit was 5.2 IU per deciliter (range, 3.0 to 8.7) and 1.7 IU per deciliter (range, 1.0 to 4.0) with a peripheral-blood vector copy number of 0.2 and 0.1 copies per cell, respectively, in the two group 1 participants, and 37.1 IU per deciliter (range, 18.3 to 73.6), 19.3 IU per deciliter (range, 6.6 to 34.5), and 39.9 IU per deciliter (range, 20.6 to 55.1) with a peripheral-blood vector copy number of 4.4, 3.2, and 4.8 copies per cell, respectively, in the three group 2 participants. The annualized bleeding rate was zero for all five participants over a cumulative follow-up of 81 months (median follow-up, 14 months; range, 9 to 27).

CONCLUSIONS

Gene therapy for hemophilia A with the use of lentiviral vector–transduced autologous HSCs resulted in stable factor VIII expression, with factor VIII activity correlating to vector copy number in the peripheral blood. (Funded by the Ministry of Science and Technology, Government of India, and others; ClinicalTrials.gov number, NCT05265767; Clinical Trials Registry–India number, CTRI/2022/03/041304.)

The authors' full names, academic degrees, and affiliations are listed in the Appendix. Dr. Srivastava can be contacted at asriv@haematology.in or at St. John's Research Institute, 100 Feet Rd., Bengaluru 560034, India.

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Severe hemophilia A is an X-linked disorder characterized by less than 1% measurable factor VIII activity in plasma and is associated with frequent spontaneous bleeding into joints and muscles as well as sporadic life-threatening bleeding.[1] Persons with hemophilia with a factor VIII activity level of more than 1 IU per deciliter have a milder clinical profile than those with lower levels, and among persons with hemophilia with a factor VIII activity level of more than 5 IU per deciliter, spontaneous bleeding is rare.[1] Prophylactic treatment with factor VIII products or other hemostatic drugs is recommended for the prevention of bleeding in persons with severe hemophilia.[1]

Gene therapy for hemophilia has been attempted over a period of more than two decades with the use of 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 variable initial expression of factor VIII; gradual decline of expression after the first 6 to 12 months, a condition that is often associated with transaminitis requiring immunosuppressive treatment, which has led to its own adverse events[4,5]; age-related ineligibility of patients to receive therapy until liver maturity[6]; and preexisting anti-AAV antibodies from natural infections, which preclude treatment.[7,8]

We have previously shown that achieving factor VIII expression is possible in mice with hemophilia A with the use of mouse hematopoietic stem cells (HSCs) and in immunodeficient mice with the use of human CD34+ HSCs transduced with a CD68-ET3-LV self-inactivating lentiviral vector encoding *ET3*, a novel *F8* transgene.[9] These preclinical data led us to initiate a first-in-human clinical study to evaluate the safety and feasibility of this technology as gene therapy for severe hemophilia A. We describe the outcome of the completed phase 1 clinical study involving five participants who have completed at least 6 months of follow-up.

METHODS

STUDY DESIGN AND OVERSIGHT

We conducted a single-center study involving five participants with severe hemophilia A without factor VIII inhibitors (alloantibodies to factor VIII) who underwent gene therapy through transplantation of autologous HSCs transduced with the CD68-ET3-LV vector. The study was approved by the institutional review board and the ethics committee of the Christian Medical College (CMC) in Vellore, India, as well as the Central Drugs Standard Control Organization (CDSCO) of the Ministry of Health and Family Welfare of the Government of India and was conducted according to Good Clinical Practice guidelines. The authors were responsible for the design of this study and for having the CD68-ET3-LV vector manufactured under suitable conditions for Good Manufacturing Practice. The study was conducted at CMC and the Center for Stem Cell Research, a unit of inStem in Bengaluru, India, where site investigators and team members were responsible for the collection and analysis of all data. The manuscript was prepared by the first and last authors and reviewed by all authors, who also had access to the complete data. The authors vouch for the accuracy and completeness of the data and for the fidelity of the study to the protocol (available with the full text of this article at NEJM.org).

The study was performed according to a protocol approved by the CDSCO, the regulatory agency in India. Serious adverse events were reported within 24 hours after being recognized. 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.

Data collected up to September 30, 2024, are reported. Given the small number of participants in this study, all statements are qualitative and are based on inspection of data with no statistical analysis.

PARTICIPANTS AND PROCEDURES

A multistep process was followed for participant selection and informed consent. This process started with cohort counseling for groups of patients with hemophilia over an extended period to increase general awareness in the community about gene-therapy technologies. After the clinical study protocol was approved, specific information about the study was shared with these patient groups. Interested patients and their families were invited to the study site for further discussion, after which potential participants and their families underwent detailed counseling. Formal consent from the participants was video-recorded in accordance with regulatory requirements in India. Beyond these regulatory requirements,

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an additional step was included that involved review of the study protocol by an independent external committee comprising a physician, a family member of a person with hemophilia who was not involved in the study, and a person who had previously undergone HSC transplantation (HSCT). This committee interviewed potential participants and their families to evaluate their comprehension of this clinical study, and if their responses were satisfactory, the committee then certified the consenting process.

A total of eight participants provided consent. Two participants could not be included in the study; one did not meet the eligibility criteria, and the other declined to participate because of concerns regarding possible infertility. A third participant withdrew when a low titer of factor VIII inhibitors developed on the day his conditioning treatment was to start. His outcome is not reported here. Data from the other five participants, who were treated from June 2022 through December 2023, are included in this article (Table S1 in the Supplementary Appendix, available at NEJM.org).

Study participants, all of whom were receiving episodic treatment, received prophylactic extended half-life recombinant factor VIII once or twice weekly after providing consent. CD34+ cells were mobilized with granulocyte colony-stimulating factor (G-CSF), administered at a dose of 5 μg per kilogram of body weight twice daily for 5 days, along with plerixafor, administered at a dose of 0.24 mg per kilogram 12 to 14 hours before apheresis. An adequate number of autologous CD34+ peripheral-blood stem cells, which was defined as more than 5×10^6 CD34+ cells per kilogram of body weight, was obtained during a single apheresis procedure after positive selection of these cells with the use of a CliniMACS Plus system (Miltenyi Biotec). An aliquot of the peripheral-blood stem cells (>2×10^6 CD34+ cells per kilogram) was cryopreserved without manipulation.

Initially, the enriched CD34+ HSCs were transduced with the clinical-grade lentiviral vector CD68-ET3-LV (Fig. S1) according to a double-exposure transduction protocol (Protocol P1 in the Supplementary Appendix). After the outcomes in the first two participants treated with this protocol (Participants 1 and 3 [group 1]) were reviewed as planned, the transduction protocol was modified for the three remaining participants (Participants 6, 7, and 8 [group 2]) to include a transduction enhancer (LentiBOOST, SIRION Biotech) and only a single exposure to the vector. Transduced HSCs were cryopreserved while the final drug product awaited qualification for clinical use.

Participants underwent myeloablative conditioning with intravenous treosulfan at a dose of 14 g per square meter of body-surface area on days −6 to −4 (where day 0 is considered the day of infusion of the drug product), along with fludarabine at a dose of 30 mg per square meter of body-surface area on days −6 to −2, with no therapeutic drug monitoring. Participants received care in high-efficiency particulate air–filtered isolation rooms during conditioning and the ensuing cytopenia. Other supportive care was provided according to institutional protocols for autologous HSCT.

SAFETY AND EFFICACY END POINTS

The safety end points were adverse events related to the drug-product infusion and conditioning therapy, engraftment by 30 days after the infusion, survival at 100 days after the infusion, and development of a factor VIII inhibitor vector copy number and vector-integration profile. The efficacy end points were plasma factor VIII activity (assessed with the use of a one-stage assay and a chromogenic substrate assay) and annualized bleeding rate (Table S2).

Participants were assessed regularly for adverse events according to the Common Terminology Criteria for Adverse Events, version 5.0. The engraftment end-point criteria were absolute neutrophil count of more than 0.5×10^9 per liter and an unsupported platelet count of more than 20×10^9 per liter. The factor VIII activity assay was performed daily during the conditioning and cytopenia phase to maintain trough levels of more than 50 IU per deciliter after administration of extended half-life recombinant factor VIII. As engraftment ensued, the dose of extended half-life recombinant factor VIII was gradually reduced and then stopped once the platelet count was more than 100×10^9 per liter.

The vector copy number in the genomic DNA of the drug product and of the peripheral blood of the patients after gene therapy was assessed at regular intervals, as described previously[9] (Protocol P2). Because of the underlying hemophilia, bone marrow was assessed one time after additional factor VIII replacement at 4 to 22 months of follow-up for assessment of morphology, cytogenetics, and vector copy number. Genomic DNA

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from peripheral blood and bone marrow underwent integration-site analysis by ligation-mediated polymerase chain reaction (CD Genomics).

RESULTS

PARTICIPANTS AND TREATMENT

Five participants, 22 to 41 years of age at study recruitment, received the investigational gene-therapy product. At a cumulative follow-up of 81 months, the median follow-up after gene-modified HSCT was 14 months (range, 9 to 27).

The target number of mobilized CD34+ HSCs was collected during a single apheresis procedure in each participant, which yielded a median of 7.5×10^6 cells per kilogram of body weight (range, 7.0 to 9.2) (Table S3). This quantity also allowed an adequate amount of unmanipulated CD34+ HSCs (a minimum of 2×10^6cells per kilogram) to be cryopreserved as a backup for delayed engraftment. The median drug-product dose infused after transduction was 5.6×10^6 cells per kilogram (range, 5.0 to 6.1) (Table 1).

For the first two participants (group 1), who received the final gene-therapy product (CD34+ HSCs transduced with CD68-ET3-LV [CD68-ET3-LV-CD34+]) that had been manufactured under a double-exposure transduction protocol, the vector copy number in the drug product was 1.0 (Participant 1) and 0.6 (Participant 3) copies per cell. For the next three participants (group 2), who received the product that had been manufactured with the modified transduction protocol involving a single transduction with an enhancer (LentiBOOST), the vector copy number in the drug product was 1.5 (Participant 6), 0.6 (Participant 7), and 2.2 (Participant 8) copies per cell (Table 1).

SAFETY END POINTS

Severe cytopenia developed in all participants, as expected, with nadir absolute neutrophil counts of less than 0.1×10^9 per liter and platelet counts of less than 20×10^9 per liter. However, engraftment occurred in all participants, with a median time to neutrophil engraftment of 11 days (range, 10 to 12) and a median time to platelet engraftment of 15 days (range, 12 to 15) (Table S4). All participants received G-CSF at a dose of 5 μg per kilogram per day beginning on day 5 after drug-product infusion. The median duration of severe neutropenia was 8 days (range, 7 to 11) and that of severe thrombocytopenia was 3 days (range, 1 to 7). Engraftment characteristics of participants were not different between group 1 and group 2. Four of the five participants had blood counts in the normal range by 1 to 3 months after gene therapy; Participant 6 had normal blood counts by 14 months after therapy (Table S5). Exogenous extended half-life recombinant factor VIII concentrate was administered for a median of 16 days (range, 11 to 20) after HSCT.

Except for neutropenia and thrombocytopenia, no other adverse events greater than grade 2 occurred in any participant. The most common adverse event was nausea with or without vomiting. All other adverse events that occurred during the hospital stay or follow-up are listed in Table S6. Inhibitors to factor VIII did not develop in any participant after drug-product infusion.

Integration-site analysis performed at 4 to 22 months after drug-product infusion showed no safety concerns. Integration is skewed toward intronic sequences, which account for more than 70% of integrations, with exonic sequences accounting for less than 3% of integrations and the rest being predominantly in the intergenic noncoding regions (Table S7). The current data indicate that the transduction enhancer does not adversely affect the integration profile of this lentiviral vector. Although integrations were observed near several oncogenic sequences, which is consistent with other lentiviral vector therapies,[10-12] no evidence of clonal dominance was noted in any participant.

Bone marrow examination performed at 4 to 22 months after gene therapy in all participants showed normal cellularity and morphology. Karyotype analysis of the bone marrow was also normal. Results of analyses of the vector copy number in samples of bone marrow and peripheral blood obtained on the same day were similar (Table 2).

EFFICACY END POINTS

Evidence of endogenous factor VIII expression was observed as early as day 18 after infusion of the drug product in Participant 6. In group 1, the factor VIII activity level on day 60 was 7.9 (Participant 1) and 4.0 (Participant 3) IU per deciliter; in group 2, the activity level was 28.0 (Participant 6), 14.2 (Participant 7), and 36.6 (Participant 8) IU per deciliter. In group 1, the median factor VIII activity level after day 28 until the last follow-up visit was 5.2 IU per deciliter (range, 3.0 to 8.7) (Participant 1) and 1.7 IU per deciliter (range, 1.0 to 4.0) (Participant 3); in

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Table 1. Baseline Characteristics of Treated Participants and the Final Gene-Therapy Drug Product.*

Characteristic	Participant 1	Participant 3	Participant 6	Participant 7	Participant 8
Age — yr	33	31	34	22	41
Genotype	Duplication of exon 7 to 13	Exon 13, c.1967G→A, p.Trp656Stop	Intron 22 inversion	Exon 13, c.1911T→G, p.Asn637Lys	Exon 11, c.1733T→A, p.Val578Glu
Annualized bleeding rate†	30	20	36	120	52
HJHS total score‡	46	33	26	36	46
Factor VIII replacement (no. of exposures)	Episodic (>100)	Episodic (>100)	Episodic (>100)	Episodic (>100)	Episodic (>100)
Enriched CD34 cell dose — cells/kg of body weight	7.5×10^6	7.0×10^6	8.7×10^6	9.2×10^6	7.2×10^6
Drug-product dose — cells/kg of body weight	5.6×10^6	5.3×10^6	5.9×10^6	6.1×10^6	5.0×10^6
VCN in drug product — copies/cell	1.0	0.6	1.5	0.6	2.2
Duration of follow-up — mo	27	19	14	12	9

* VCN denotes vector copy number.
† Annualized bleeding rate was calculated as the total number of bleeding events during the previous 12 months. All participants had between one and three target joints (defined as joints with more than three bleeding events in the previous 6 months).
‡ Scores for the Hemophilia Joint Health Score (HJHS) range from 0 to 124, with higher scores indicating worse joint health.

group 2, the median activity level was 37.1 IU per deciliter (range, 18.3 to 73.6) (Participant 6), 19.3 IU per deciliter (range, 6.6 to 34.5) (Participant 7), and 39.9 IU per deciliter (range, 20.6 to 55.1) (Participant 8). All these factor VIII activity levels were measured with the one-stage assay. The serial factor VIII activity levels are shown in Figure 1 and Table S8. The one-stage assay and the chromogenic substrate assay showed similar results with respect to factor VIII activity (Table 2 and Fig. S2).

No spontaneous bleeding events occurred in any participant during HSCT or after gene therapy. Before gene therapy, all participants had reported an annualized bleeding rate of at least 20 events (Table 1). The lack of bleeding after gene therapy in Participant 3, who continued to have a factor VIII activity level of 1 to 3 IU per deciliter, is notable.

A positive correlation was noted between the vector copy number in peripheral blood 1 month after gene therapy and the corresponding factor VIII activity level. The vector copy number in peripheral blood also correlated well with that in bone marrow among both group 1 and group 2 participants. Both the vector copy number and the factor VIII activity level after HSCT were much higher in group 2 participants than in group 1 participants (Table 2) even though the vector copy numbers in the gene-therapy products administered in each group were similar. This increase in factor VIII activity also correlated with a more intense integration profile of the vector in the group 2 participants (Table S7B).

OTHER OUTCOMES

All participants reported increased duration and intensity of physical activities without fear of bleeding and enhanced ability to engage in more-intense leisure activities. Two participants from group 2 had trauma. Participant 6 had a minor motor vehicle accident on day 302 (factor VIII activity level of approximately 40 IU per deciliter), with minor cuts that stopped bleeding spontaneously and transient loss of consciousness but full neurologic recovery immediately thereafter. A computed tomographic scan of the brain was normal. Participant 7 had a superficial cat bite on day 102, for which an antirabies vaccine was given intramuscularly, and a minor motor vehicle accident on day 179, with superficial abrasions (factor VIII activity level of approximately 20 IU per

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Table 2. Correlation of Vector Copy Number (Peripheral Blood or Bone Marrow) with Factor VIII Activity.

Time Point and Source	Participant 1	Participant 3	Participant 6	Participant 7	Participant 8
1 Month after gene therapy					
Peripheral-blood VCN — copies/cell	0.1	0.1	2.5	1.4	3.6
Factor VIII, one-stage assay — IU/dl	4.0	2.0	30.0	7.0	20.6
Factor VIII, chromogenic assay — IU/dl	4.0	<1.0	26.0	8.0	18.0
More than 1 month after gene therapy					
No. of months	22	14	8	6	4
Peripheral-blood VCN — copies/cell	0.2	0.1	4.4	3.2	4.8
Bone marrow VCN — copies/cell	0.1	0.1	4.2	2.8	2.7
Factor VIII, one-stage assay — IU/dl	3.6	<1.0	41.0	19.3	36.4

deciliter at both time points). The participants did not receive additional factor VIII replacement for any of these trauma-related events. Semen analysis performed at least 6 months after HSCT showed normal sperm counts in four participants (one with low motility) and a low sperm count at 8 months of follow-up in one participant.

DISCUSSION

The results from this clinical study validate a new approach to gene therapy for severe hemophilia A through transplantation, after myeloablative conditioning, of autologous HSCs transduced with a lentiviral vector encoding a novel factor VIII transgene driven by a CD68 promoter. No unexpected safety issues were encountered. After receiving gene therapy, all participants had clinically significant circulating factor VIII activity levels that were sufficient to completely abrogate spontaneous bleeding and that remained stable after the first 3 to 6 months during a follow-up period ranging from 9 to 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 design includes ex vivo gene transfer into adult stem cells with the use of an integrating and self-inactivating lentiviral vector instead of in vivo transfer to a hepatocyte through a nonintegrating AAV vector. The correlative premise is lifelong durability of expression, and the initial data from more than 2 years of follow-up appear promising.

Second, the human *CD68* gene–directed promoter shifts gene expression away from HSCs to prevent factor VIII–related cellular toxicity.[13,14] Monocytes are abundant, short-lived circulating blood cells.[15] Tissue monocytes or macrophages also represent a second source of factor VIII and can be long-lived (up to 1 year), with approximately 200 billion macrophages present in an adult man.[16] Macrophages are also present at substantial density in the synovium of joints and could contribute to local hemostasis even if plasma factor VIII activity is low.

The third unique design element is the *ET3* transgene, which results in expression and secretion efficiency that is higher by a factor of 10 to 100 than that with B-domain–deleted human factor VIII.[17] The addition of a transduction enhancer during manufacturing produced a dramatic increase by a factor of 5 to 10 in factor VIII activity with the expected higher numbers of integration sites, without affecting short-term safety. The factor VIII activity has remained stable within the same clinically relevant range in all participants, with some fluctuation during a follow-up of more than 2 years in group 1 participants and of more than 1 year in group 2 participants. This fluctuation could be related to analytical issues or may reflect true variations in expression owing to transient changes in the relevant blood cells during intercurrent events in the participants that were not clinically obvious.

Gene therapy with lentiviral vector–transduced HSCs, as compared with AAV gene therapy, for hemophilia A overcomes nearly all the limitations related to highly variable and unpredictable expression and the ineligibility of patients on the basis of age and preexisting anti-AAV antibody–related conditions. Interestingly, concordance between the one-stage assay and the chromogenic

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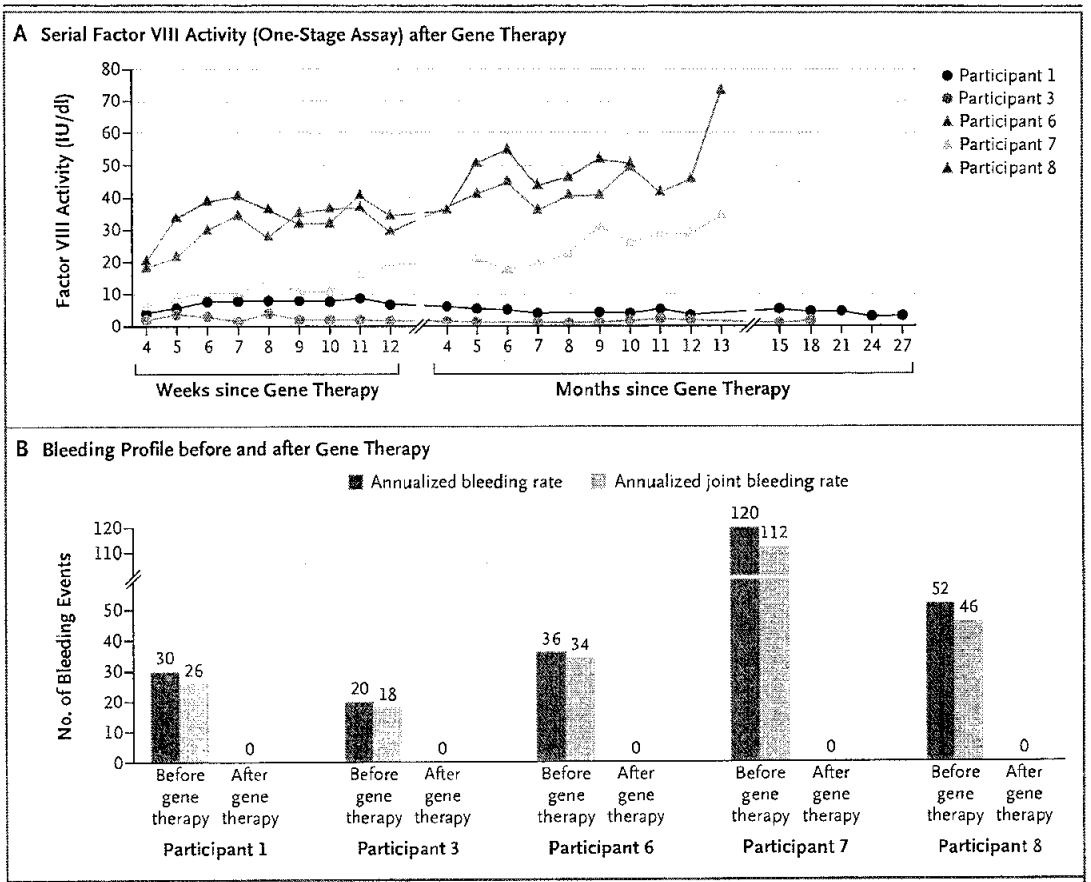

Figure 1. Serial Factor VIII Activity and Bleeding Profile.

Panel A shows the factor VIII activity in each of five participants after they received gene therapy with CD68-ET3-LV–transduced autologous CD34+ hematopoietic stem cells. Factor VIII activity was measured with the use of a one-stage assay once weekly for the first 12 weeks after gene therapy, then once per month until 12 months after therapy, and then every 3 months. Panel B shows the number of bleeding events for each participant before and after gene therapy.

substrate assay with respect to factor VIII activity was observed. However, the HSC approach also has limitations, including the elaborate and complex process of collecting and manipulating HSCs to prepare the patient-specific drug product. This approach has been used extensively in gene therapy for primary immune deficiency syndromes and for the major hemoglobin disorders.[18,19] Acute and long-term toxic effects of myeloablative conditioning are also a potential concern,[20] including severe mucositis and possible infertility.[21] Our results so far have been encouraging with respect to both types of adverse events. By using treosulfan instead of busulfan, which has been used in other myeloablative conditioning protocols for HSC-based gene therapy,[18,22] we showed a much-reduced toxic-effects profile, as is well-known with allogeneic HSCT for high-risk cases of major hemoglobin disorders.[23] Most notable 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 than with busulfan.[24] Finally, for patients with 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 lentiviral vector into the host genome will need close monitoring.

The early results of this clinical study reveal a new opportunity for gene therapy for hemophilia A that can be offered to all patients, possibly at an early age, and that results in sustained expression of therapeutic levels of factor VIII.

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Longer follow-up and additional clinical trials with more participants can clarify whether these expectations will hold true.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

A data sharing statement provided by the authors is available with the full text of this article at NEJM.org.

We thank the external scientific advisory committee members (Drs. Marina Cavazzana, Mark Walters, Thierry Vandendriessche, and Mammen Chandy) for their advice during this clinical trial.

APPENDIX

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