

# Factor IX Padua AAV gene therapy in adolescents with hemophilia B: a phase 1 trial

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Adeno-associated virus (AAV)-mediated factor IX Padua gene therapy has demonstrated good safety and efficacy in adult patients with hemophilia B, but its safety and efficacy in adolescent patients with hemophilia B remain unknown. Here we report a multicenter, single-arm, phase 1 study involving 11 adolescent participants (aged 12–18 years) with severe or moderately severe hemophilia B (factor IX coagulant activity (FIX:C)  $\leq 2$  IU dl<sup>-1</sup>) in China. All of them received the AAV gene therapy BBM-H901 at a dose of  $5 \times 10^{12}$  vector genomes per kilogram of body weight and were then enrolled in a 52-week follow-up. The primary endpoint was safety. No dose-limiting toxicity was observed throughout the study. The most common adverse events included elevation of white blood cell count, elevation of neutrophil count and rash associated with corticosteroid use. One serious adverse event and two grade 3 adverse events occurred. Abnormal liver function was observed in one participant, with elevated alanine aminotransferase (197.0 U l<sup>-1</sup>) and aspartate aminotransferase (75.0 U l<sup>-1</sup>) levels, which returned to normal 4 weeks after immunosuppression therapy. The key secondary endpoints included the mean (s.d.) FIX:C at week 52 following infusion, which was 41.8 (30.1) IU dl<sup>-1</sup> (one-stage SynthASil method), and the mean annualized bleeding rate, which decreased from 13.9 to 0.5. In this study, we show that the gene therapy drug BBM-H901 for hemophilia B is safe in adolescent patients, providing initial insights into its efficacy. ClinicalTrials.gov identifier: [NCT05709288](https://clinicaltrials.gov/ct2/show/study/NCT05709288).

Hemophilia B is an X-linked inherited bleeding disorder caused by variants in the *F9* gene, leading to deficient or absent factor IX (FIX) coagulant activity (FIX:C). Individuals with severe or moderately severe hemophilia B (FIX:C  $\leq 2$  IU dl<sup>-1</sup>) experience recurrent spontaneous bleeding, particularly into joints and muscles, leading to progressive joint damage (arthropathy) and an increased risk of life-threatening hemorrhage<sup>1</sup>. Although prophylaxis with FIX concentrates or non-factor agents<sup>2</sup> has considerably improved clinical outcomes, lifelong invasive injections and the associated financial burden compromise quality of life<sup>3,4</sup>.

Furthermore, patients with arthropathy often experience irreversible joint deterioration even with regular treatment, highlighting the necessity of early intervention<sup>5,6</sup>.

Three gene therapy products for hemophilia have been approved by the US Food and Drug Administration and the European Medicines Agency. As of now, etranacogene dezaparvovec for hemophilia B is available in the USA and Europe<sup>7–9</sup>. Liver-targeted adeno-associated virus (AAV)-mediated (serotype: AAV5) gene therapies with the hyperactive FIX Padua (R338L) variant have recently achieved long-lasting bleeding

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control in patients with hemophilia B after a single administration<sup>10–12</sup>. Long-term follow-up data showed that, 4–6 years after treatment, FIX:C levels remained satisfactory in most patients<sup>13,14</sup>. BBM-H901 (generic name: dalnacogene ponparvovec), a gene therapy product for hemophilia B developed in China, was approved by the National Medical Products Administration (NMPA) of China in April 2025 and became the first product for hemophilia B in Asia and the third globally<sup>15</sup>. The results of the phase 3 trial of BBM-H901 demonstrated good safety, with a mean (s.d.) FIX:C at 52 weeks of 55.1 (35.9) IU dl<sup>-1</sup> (one-stage (OS) SynthASil method). In the phase 1 long-term follow-up, 90% of participants achieved stable FIX:C levels without new bleeding episodes. Of them, 70% maintained FIX:C levels above 48 IU dl<sup>-1</sup> for up to 210 weeks, with one participant successfully undergoing knee replacement surgery without requiring supplementation with FIX concentrates<sup>16</sup>.

To date, all three licensed hemophilia B gene therapies have been approved only for adults, with no established safety or efficacy data in adolescents. Given that early and sustained FIX:C elevation is critical for preventing bleeds and hemophilic arthropathy<sup>16,17</sup>, we conducted this study to evaluate the safety, tolerability and efficacy of BBM-H901 in adolescent patients aged 12–18 years with hemophilia B.

## Results

### Patient disposition

Between April 20, 2023, and April 16, 2024, 12 male adolescent participants were screened, and 11 of them were enrolled in the study. One participant was excluded due to a fracture requiring treatment during screening (Fig. 1). Participants were sequentially enrolled by age group as follows: group 1, 16 ≤ age < 18 years, *n* = 3; group 2, 14 ≤ age < 16 years, *n* = 4; and group 3, 12 ≤ age < 14 years, *n* = 4. Due to the small number of participants in each group, all subsequent descriptions and analyses were conducted on the entire cohort. Before enrollment, all participants were on prophylaxis and had a mean annualized bleeding rate (ABR) of 13.9 (95% confidence interval (CI) 0.6–27.2). Baseline characteristics of all participants are presented in Table 1. Changes in body mass index (BMI) for each participant are summarized in Table 2.

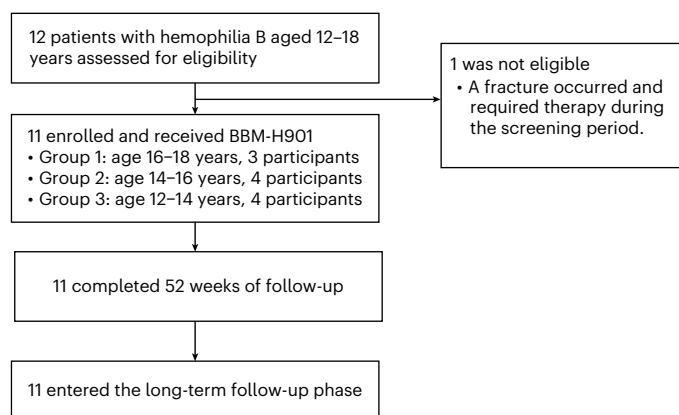
The sentinel participant in group 1 received a single intravenous infusion of BBM-H901 at 5 × 10<sup>12</sup> vector genomes per kilogram of body weight (vg kg<sup>-1</sup>), in accordance with the protocol. After a 2-week follow-up confirming good tolerability, two additional participants were enrolled in the group. With no dose-limiting toxicities (DLTs) observed after the last participant completed the 10-week follow-up, the safety review committee approved enrolling the next age group. Finally, 11 participants across three age groups were enrolled, and no DLTs occurred throughout the study (Extended Data Fig. 1).

All participants received prophylactic oral prednisone at the recommended dose, initiated 1–3 days before vector administration (Supplementary Table 1). The mean (s.d.) and median (range) duration of prophylactic prednisone use (number of days from the first prednisone dose to the first discontinuation or dose escalation) were 76.5 (24.9) and 77 (61–110) days, respectively. The mean (s.d.) and median (range) total prednisone exposures (cumulative days throughout the observation period) were 156.6 (124.1) and 98 (61–444) days, respectively.

### Primary outcome (safety)

The primary endpoint was met, as no DLTs (predefined in the protocol) were observed in any of the 11 participants throughout the study. A total of 92 adverse events (AEs) were reported among the 11 participants during the 52-week follow-up (Supplementary Table 3).

Participant 6 experienced one serious AE (SAE): acute exacerbation of chronic apical periodontitis, managed with tooth extraction at week 38. FIX:C remained stable during this period (40.1 IU dl<sup>-1</sup> at week 32; 37.3 IU dl<sup>-1</sup> at week 42) (Extended Data Fig. 2c). As a precaution, recombinant FIX was administered seven times perioperatively at a dose of 2,000 IU (33 IU kg<sup>-1</sup>) each time. Two grade 3 AEs, as defined by



**Fig. 1 | Participant disposition in the BBM-H901 adolescent study.** The flowchart shows how the participants were screened and divided into three age groups.

the Common Terminology Criteria for Adverse Events, were reported: one episode of finger infection (participant 5) and one episode of acute bronchitis (participant 6). All three events were confirmed to be unrelated to BBM-H901. All other AEs were grade 1 or 2. The most common AEs were leukocytosis (7 of 11, 63.6%), neutrophilia (5 of 11, 45.5%) and rash (3 of 11, 27.3%). All of them were related to corticosteroid use.

Participant 5 experienced transaminitis at week 14 following BBM-H901 infusion, with elevation of alanine aminotransferase (ALT) (197.0 U l<sup>-1</sup>, reference range: 7–43 U l<sup>-1</sup>) and aspartate aminotransferase (AST) (75.0 U l<sup>-1</sup>, reference range: 12–37 U l<sup>-1</sup>) levels, accompanied by a substantial decrease in FIX:C. Intervention included high-dose methylprednisolone (500 mg daily for 3 days), followed by prednisone (40 mg per day) combined with tacrolimus (1.5 mg per day). Despite normalization of liver enzymes by week 18, this participant exhibited only a transient increase in FIX:C, which decreased to 6.2 IU dl<sup>-1</sup> at week 52 (Extended Data Fig. 3c). No FIX inhibitors or thrombotic events developed through the data cutoff date.

### Secondary outcomes

Baseline FIX:C was ≤2 IU dl<sup>-1</sup> in all participants (Table 1). After BBM-H901 infusion, the mean FIX:C (s.d.) (OS SynthASil method) increased to 51.2 (14.0) IU dl<sup>-1</sup> by day 3, peaked at 92.8 (27.5) IU dl<sup>-1</sup> on day 6, reached 63.0 (27.9) IU dl<sup>-1</sup> by week 12 and was 41.8 (30.1) IU dl<sup>-1</sup> at week 52. When the OS Actin FSL method was used, the corresponding values were 41.6 (15.5) IU dl<sup>-1</sup> at week 12 and 26.4 (19.3 IU dl<sup>-1</sup>) at week 52 (Fig. 2a,b). The ratio of FIX:C measured with SynthASil and Actin FSL reagents was 1.49 (s.d. 0.36). At week 52, the FIX:C level was >40 IU dl<sup>-1</sup> in 6 participants (54.5%) and >5 IU dl<sup>-1</sup> in 11 participants (100%), as measured using the OS SynthASil method; with the OS Actin FSL method, the FIX:C level was >40 IU dl<sup>-1</sup> in 3 participants (27.3%) and >5 IU dl<sup>-1</sup> in 10 participants (90.9%) (Fig. 3a,b).

Vector DNA in all participants was rapidly cleared from saliva, urine, plasma, feces and peripheral blood mononuclear cells (PBMCs) (Supplementary Fig. 1). The maximum times to complete clearance were day 28 for saliva, day 224 for plasma, day 84 for feces and day 49 for urine. At week 52, the vector DNA concentration in PBMCs ranged from 0.07 to 6.72 copies per µl, although follow-up had not been completed by the cutoff date.

The baseline median (interquartile range (IQR)) number of target joints was 0 (0–1), while the mean (s.d.) value was 0.9 (1.7). After gene therapy, the number decreased to zero. The proportion of patients with zero target joints increased from 63.6% (7 of 11) at baseline to 100% (11 of 11) at week 52.

ABR decreased from a mean (95% CI) of 13.9 (0.6–27.2) before treatment to 0.5 (0.1–2.9) after gene therapy. During the 52-week follow-up

**Table 1 | Demographic and clinical characteristics of the participants**

| Category                                        | Group           |          |          |              |                 |          |                                     |          |                 |          |          |
|-------------------------------------------------|-----------------|----------|----------|--------------|-----------------|----------|-------------------------------------|----------|-----------------|----------|----------|
|                                                 | 16≤Age<18 years |          |          |              | 14≤Age<16 years |          |                                     |          | 12≤Age<14 years |          |          |
| Participant no.                                 | 1               | 2        | 3        | 4            | 5               | 6        | 7                                   | 8        | 9               | 10       | 11       |
| Age (years)                                     | 17              | 16       | 17       | 15           | 15              | 15       | 14                                  | 13       | 13              | 12       | 13       |
| Height (cm)                                     | 168.7           | 181.0    | 173.0    | 179.0        | 173.0           | 178      | 161.0                               | 161.0    | 160.0           | 153      | 163.0    |
| Day −1 weight (kg)                              | 48.6            | 59.7     | 65.5     | 88.0         | 82.7            | 59.0     | 43.6                                | 45.7     | 42.6            | 38.5     | 54.0     |
| BMI (kg m <sup>−2</sup> )                       | 17.3            | 18.7     | 21.9     | 27.5         | 27.3            | 18.4     | 16.9                                | 18.2     | 17.1            | 16.7     | 20.6     |
| HBV/HCV antibody                                | Negative        | Negative | Negative | Negative     | Negative        | Negative | Negative                            | Negative | Negative        | Negative | Negative |
| FIX:C on day −1 (IU dl <sup>−1</sup> )          | 0.40            | 0.70     | 0.80     | 1.80         | 1.10            | 1.40     | 0.50                                | 0.40     | 0.30            | 0.50     | 0.90     |
| Number of target joints                         | 0               | 2        | 0        | 0            | 0               | 0        | 0                                   | 1        | 6               | 0        | 1        |
| Titer of binding antibody to AAV843 at baseline | <1:50           | 1:50     | <1:50    | <1:50        | <1:50           | <1:50    | <1:50                               | <1:50    | 1:50            | <1:50    | <1:50    |
| Titer of nAb to AAV843 at baseline              | <1:1            | <1:1     | <1:1     | <1:1         | <1:1            | <1:1     | <1:1                                | <1:1     | <1:1            | <1:1     | <1:1     |
| ABR (number of bleeding episodes per year)      | 1               | 13       | 2        | 2            | 81              | 27       | 0                                   | 13       | 3               | 4        | 7        |
| Number of FIX injections per year               | 108             | 54       | 49       | 113          | 154             | 71       | 72                                  | 74       | 52              | 54       | 54       |
| Replacement therapy                             | PCC+FIX+FFP     | PCC      | PCC      | PCC+FIX+rFIX | rFIX            | PCC+rFIX | PCC+FIX                             | PCC      | rFIX            | rFIX     | FIX      |
| Inhibitor history and treatment                 | None            | None     | None     | None         | None            | None     | Yes; eradicated by ITI <sup>a</sup> | None     | None            | None     | None     |

FFP, fresh frozen plasma; FIX, factor IX concentrate; rFIX, recombinant factor IX; HBV, hepatitis B virus; HCV, hepatitis C virus. <sup>a</sup>This participant developed a FIX inhibitor after 20 exposure days to PCC in January 2015. The peak inhibitor titer was 56 BU ml<sup>−1</sup>. In November 2015, ITI (PCC with low-dose rituximab) was initiated, and the inhibitor was successfully eradicated within 17 months. Gene therapy was initiated 80 months after inhibitor eradication.

after gene therapy, 81.8% of patients (9 of 11) had zero bleeds compared to 9.1% (1 of 11) at baseline.

The median (IQR) and mean (s.d.) number of annual FIX concentrate infusions decreased from 71 (54–91) and 77.7 (32.0) before gene therapy to 0 (0–1.5) and 1.45 (2.46) after gene therapy, respectively (Fig. 2c). Participant 6 received seven prophylactic infusions due to apical periodontitis-related tooth extraction. Participant 9 received two prophylactic infusions after trauma to prevent bleeding. Participant 7 received three on-demand infusions for trauma and two on-demand infusions for spontaneous bleeding. No participant resumed prophylaxis through the data cutoff date.

The median (IQR) and mean (s.d.) Canadian Hemophilia Outcomes–Kids' Life Assessment Tool (CHO-KLAT) scores on day −1, week 26 and week 52 were 73.6 (66.9–78.9) and 72.6 (8.4), 79.2 (73.0–84.0) and 78.3 (9.2), and 76.4 (73.7–89.6) and 78.5 (14.0), respectively. The median (IQR) and mean (s.d.) Hemophilia Joint Health Score (HJHS) scores on day −1, week 26 and week 52 were 6 (4.8–11) and 8 (5.8), 4 (1.5–5.3) and 4.4 (4.1), and 4 (2–8.5) and 5.5 (4.2), respectively. The median (IQR) and mean (s.d.) Hemophilic Early Arthropathy Detection with UltraSound in China (HEAD-US-C) scores on day −1, week 26 and week 52 were 5 (3–11.6) and 7.6 (6.1), 5 (4.3–9.3) and 7.6 (5.9), and 8 (3.8–10) and 7.6 (5.2), respectively (Fig. 3c).

At baseline screening, all 11 participants exhibited anti-AAV843 neutralizing antibody (nAb) titers <1:1 and binding antibody titers

≤1:50. After gene therapy, all participants exhibited high titers of nAbs and binding antibodies, which persisted throughout the follow-up period (Extended Data Fig. 4 and Supplementary Table 5).

The participants underwent physical examinations, electrocardiography, and monitoring of α-fetoprotein levels and other parameters before and after treatment. No changes or abnormalities related to BBM-H901 were observed. For bone mineral density, although an overall analysis could not be conducted due to differences in measurement instruments among participants, comparisons within individuals before and 1 year after gene therapy did not reveal any notable downward trend (Supplementary Table 4).

### Single-cell RNA sequencing analysis

We conducted single-cell RNA sequencing (scRNA-seq) analysis on PBMCs from three participants at five time points. Accordingly, in the clinical trial of AAV gene therapy for pediatric hemophilia, we designed five critical time points to systematically monitor dynamic immune changes during glucocorticoid (GC) tapering: 1 day before vector infusion (day −1); 3 days after vector infusion (day 3); 4 weeks after vector infusion (week 4, during which GC was tapered to approximately 50% of the peak dose); when GC tapering reached 10 mg (10 mg GC); and at complete GC withdrawal (Stop GC). Integrating the data and using UMAP (Uniform Manifold Approximation and Projection) for dimensionality reduction and clustering, we identified 20 immune

**Table 2 | Factor IX activity and BMI velocity**

| Participant no. | Week 1 FIX:C (IU dl <sup>-1</sup> ) | Maximal FIX:C (IU dl <sup>-1</sup> ) | Week 52 FIX:C (IU dl <sup>-1</sup> ) | BMI velocity (kg m <sup>-2</sup> per year) |
|-----------------|-------------------------------------|--------------------------------------|--------------------------------------|--------------------------------------------|
| 1               | 117.8                               | 117.8                                | 17.6                                 | 0.07                                       |
| 2               | 100.5                               | 113.2                                | 61.7                                 | 0.27                                       |
| 3               | 109.2                               | 109.2                                | 83.4                                 | -0.13                                      |
| 4               | 102.9                               | 122.0                                | 48.5                                 | 2.40                                       |
| 5               | 154.6                               | 154.6                                | 6.2                                  | 0.60                                       |
| 6               | 77.7                                | 98.9                                 | 40.5                                 | 2.20                                       |
| 7               | 77.7                                | 91.3                                 | 15.4                                 | -0.20                                      |
| 8               | 61.7                                | 72.5                                 | 56.2                                 | 0.13                                       |
| 9               | 92.2                                | 92.2                                 | 10.5                                 | 1.07                                       |
| 10              | 65.5                                | 65.5                                 | 23.8                                 | 0.47                                       |
| 11              | 93.8                                | 117.1                                | 96.0                                 | 1.80                                       |

cell subsets (Fig. 4a). Following tapering of the prednisone dose to 10 mg and subsequent treatment cessation, the proportions of plasmablasts, CD8<sup>+</sup> effector T (T<sub>eff</sub>) cells, proliferating T cells and CD16<sup>+</sup> monocytes continued to increase persistently (Fig. 4b). Additionally, four activated immune cell subsets—activated B cells, plasmablasts, CD8<sup>+</sup> T<sub>eff</sub> cells and CD16<sup>+</sup> monocytes—exhibited consistently high expression of the *FOS* gene (Supplementary Fig. 3b). Compared to other time points, the enrichment scores of the interferon- $\alpha$  (IFN $\alpha$ ) signature (Human Gene Set M5911) and the IFN $\gamma$  signature (Human Gene Set M5913) at week 4 were substantially increased. Upon the 10 mg GC time point, the nuclear factor- $\kappa$ B (NF- $\kappa$ B) signature (Human Gene Set M5890) was substantially elevated in nearly all activated immune cell subsets, with the most pronounced increase detected in CD8<sup>+</sup> T<sub>eff</sub> cells. Upon the Stop GC time point, we observed a trend of coordinated increases in the interferon and NF- $\kappa$ B scores of activated B cells, plasmablasts and CD8<sup>+</sup> T<sub>eff</sub> cell subsets (Fig. 4c). Subsequently, we systematically examined immune alterations on a per-patient basis to reveal individual immune response patterns. For participants 4 and 7, FIX:C showed a continuous decline following the reduction of GC to 10 mg and its complete withdrawal. The percentages of plasmablasts, CD8<sup>+</sup> T<sub>eff</sub> cells, proliferating T cells and CD16<sup>+</sup> monocytes were markedly elevated relative to the interval from day -1 to week 4. In participant 2, the aforementioned immune cell subsets showed a transient increase at the 10 mg GC time point and a decrease at Stop GC (Fig. 4d). Figure 4e shows a cell-cell communication plot, where the right y-axis represents the signal senders and the left y-axis represents the signal receivers. The analysis results demonstrated that the overall strength of immune crosstalk was notably enhanced at the 10 mg GC tapering stage. Alongside the *TNF-TNFRSF1B* and *TNF-TNFRSF1A* pairs, we detected pronounced activation of adhesion-related ligand-receptor pairs (*ICAM1-ITGAX-ITGB2*, *ITGB2-CD226*) as well as interleukin ligand-receptor pairs, including *IL18-IL18R1-IL18RAP*, *IL7-IL7R-IL2RG* and *IL1B-IL1R2* (Fig. 4e).

**Long-term follow-up of the phase 1 study**

All 11 participants completed 78 weeks of follow-up (data cutoff on January 21, 2026). The median (range) and mean (s.d.) FIX:C were 45.6 (5.5–118.2) IU dl<sup>-1</sup> (Fig. 2a) and 43.0 (34.5) IU dl<sup>-1</sup> at week 78 after gene therapy (OS SynthASil method), respectively. Extended Data Figs. 2 and 3 show the individual FIX:C levels (OS SynthASil method) up to 130 weeks of follow-up. Participant 1 experienced one spontaneous bleeding event (gastrointestinal hemorrhage) at week 103 (FIX:C 12.8 IU dl<sup>-1</sup> at week 104). Participant 7 had oral mucosal bleeding due to trauma at week 61, with FIX:C levels of 25.5 IU dl<sup>-1</sup> at week 56 and 12.9 IU dl<sup>-1</sup> at week 78 (Extended Data Table 1).

**Post-hoc analysis**

For participants 2, 8 and 11, we conducted ELISpot assays, which showed marked increases in immunospots at weeks 6–12, 6–16 and 3–6, respectively. Participant 11 experienced a transient decrease in FIX:C at weeks 2–4, whereas no obvious fluctuations in FIX:C associated with ELISpot responses were observed in the other two participants (Supplementary Fig. 2).

No significant correlations were found between peak FIX:C and baseline BMI, between FIX:C decline (peak to week 52) and change in BMI, or between total vector dose and peak ALT and AST levels.

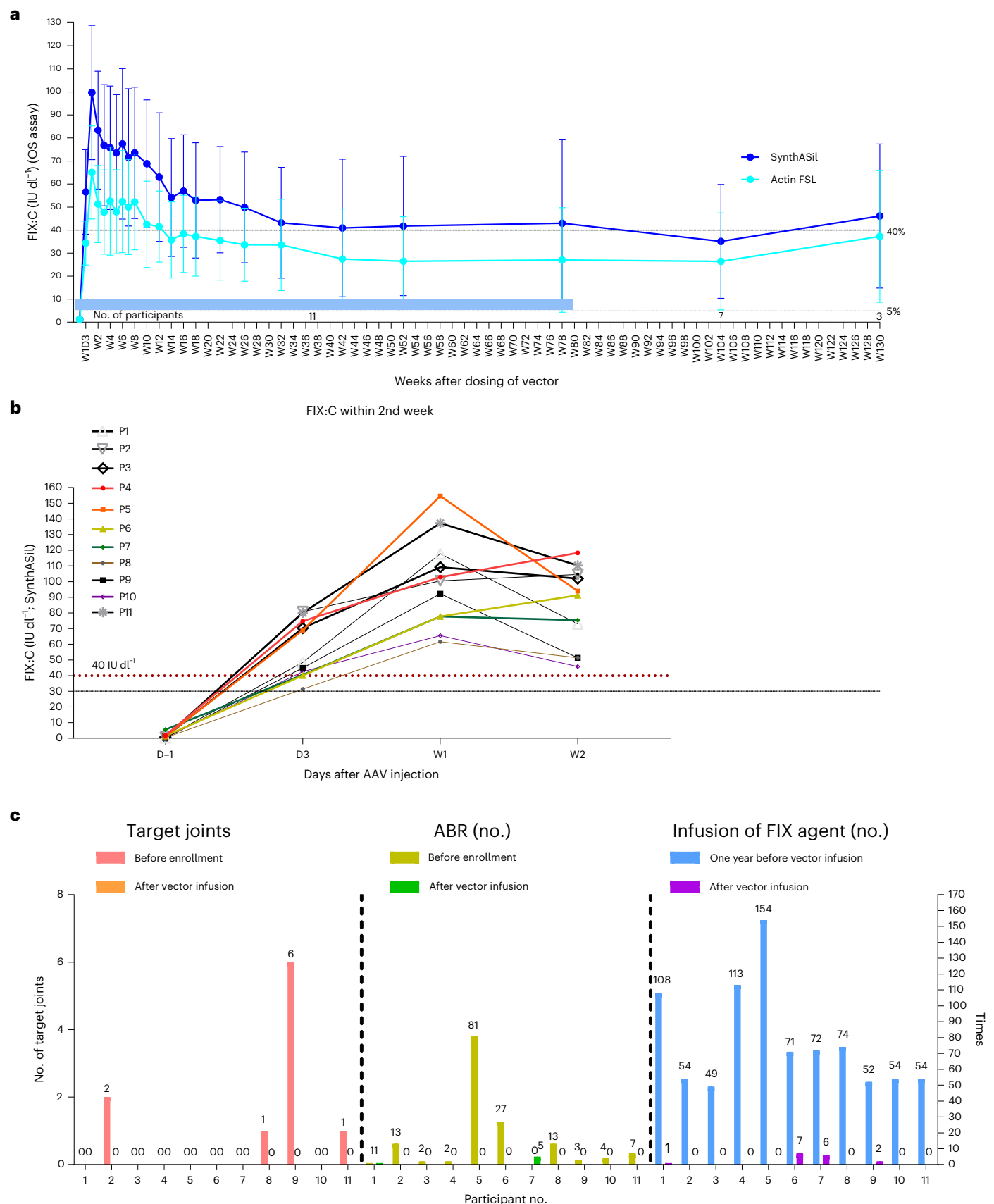
**Individual participant details**

**Participants without additional immunosuppressive therapy.** At the individual level, for participants 2, 3, 6, 8, 9, 10 and 11, FIX:C levels at week 52 were 61.7, 83.4, 40.5, 56.2, 10.5, 23.8 and 96.0 IU dl<sup>-1</sup>, respectively (Extended Data Fig. 2a–g).

**Participant with high-titer FIX inhibitor history.** Participant 7 had a history of FIX inhibitor development (peak 56 BU ml<sup>-1</sup> by the Bethesda method, January 2015, after 20 exposure days to prothrombin complex concentrate (PCC)), which was successfully eradicated by immune tolerance induction (ITI) using PCC combined with low-dose rituximab over 17 months, without hypersensitivity or nephrotic syndrome. Thereafter, the participant continued prophylaxis for more than 150 exposure days and remained inhibitor-negative. He received BBM-H901 treatment 80 months later. After treatment, he achieved a maximal FIX:C level of 91.3 IU dl<sup>-1</sup> at week 6 but demonstrated a continuous decline in levels starting at week 14. The ELISpot results also indicated a substantial increase during weeks 8–12, followed by a marked decrease after week 14. Prednisone was increased to 40 mg per day, gradually tapered and discontinued by week 32. However, FIX:C decreased again, reaching a nadir of 4 IU dl<sup>-1</sup> at week 50. Prednisone was reintroduced at 30 mg per day, tapered and discontinued by week 65. FIX:C was 12.9 IU dl<sup>-1</sup> at the last visit (week 78). This participant was the only one to experience bleeding events during the 52-week follow-up, with a total of two traumatic bleeds (left back and left inguinal region) and two spontaneous bleeds (left buttock and left thumb). At week 61, he experienced another traumatic bleed in the oral mucosa and received an infusion of 1,500 IU PCC. A total of six infusions of FIX were administered (Extended Data Fig. 3d). Throughout the study, this participant had an inhibitor titer of <0.6 BU ml<sup>-1</sup>, as measured by the Bethesda method.

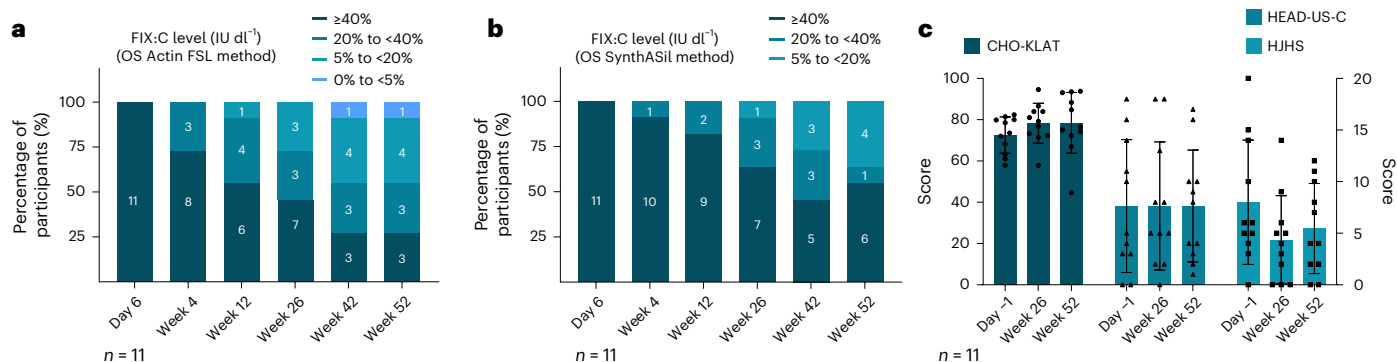
**Participants with additional immunosuppressive therapy.** Participant 1 achieved peak FIX:C (117.8 IU dl<sup>-1</sup>) at week 1, followed by a rapid decline to 55.6 IU dl<sup>-1</sup> by week 3. This prompted the initiation of methylprednisolone (500 mg daily for 3 days) and then tacrolimus (1.5 mg per day) from week 7. Prednisone and tacrolimus were discontinued at week 18 (FIX:C 54.2 IU dl<sup>-1</sup>) and week 26 (FIX:C 40.1 IU dl<sup>-1</sup>), respectively. FIX:C declined further to 10.7 IU dl<sup>-1</sup> at week 42, leading to a second course of methylprednisolone (500 mg daily for 3 days), tacrolimus (1.5 mg per day) and prednisone (40 mg per day, gradually tapered and discontinued by week 54). His FIX:C was 17.6 IU dl<sup>-1</sup> at week 52. At week 103, he experienced spontaneous gastrointestinal hemorrhage and received a single dose of 3,000 IU plasma-derived FIX. His FIX:C was 12.8 IU dl<sup>-1</sup> at 104 weeks after gene therapy (Extended Data Fig. 3a).

Participant 4 initially received prednisone treatment at 80 mg per day, which was tapered and discontinued by week 11. However, FIX:C exhibited a sustained decline starting at week 10. The ELISpot results showed a substantial increase during weeks 12–16 (Supplementary Fig. 2). Prednisone was reinitiated at 40 mg per day at week 14, then tapered and discontinued by week 32. FIX:C was 45.1 IU dl<sup>-1</sup> at week 104 (Extended Data Fig. 3b).



**Fig. 2 | FIX:C and clinical outcomes after gene therapy. a**, Mean FIX:C after gene therapy. FIX:C was measured using an OS method with both Actin FSL and SynthASil aPTT (activated partial thromboplastin time) reagents. Data are presented as mean  $\pm$  s.d. All 11 participants ( $n = 11$ ) have data for the first 78 weeks;

7 participants ( $n = 7$ ) have data for week 104; and 3 participants ( $n = 3$ ) have data for week 130. **b**, FIX:C in the first 2 weeks after gene therapy, measured using an OS method with SynthASil aPTT reagents. **c**, Comparison of target joints, ABR and infusion of FIX agents before and after gene therapy for each participant.



**Fig. 3** FIX:C at week 52 and joint and quality-of-life assessments. **a**, Distribution of participants within the variant FIX:C range (OS Actin FSL method). **b**, Distribution of participants within the variant FIX:C range (OS SynthASil method). **c**, CHO-KLAT,

HEAD-US-C and HJHS scores assessed on day -1, week 26 and week 52 for the 11 participants. Data are presented as mean ± s.d. and each data point represents the score value of each participant.

Participant 5 experienced a marked decline in FIX:C levels from 48.8 to 10.4 IU dl<sup>-1</sup> between weeks 12 and 14, accompanied by transaminitis. Treatment included methylprednisolone (500 mg daily for 3 days) starting at week 15, tacrolimus (1.5 mg per day) and prednisone (40 mg per day, tapered and discontinued by week 30). Liver enzymes normalized by week 18, but FIX:C showed limited recovery. The last measured FIX:C level was 5.3 IU dl<sup>-1</sup> (week 104) (Extended Data Fig. 3c). No bleeding events occurred through the data cutoff date.

## Discussion

BBM-H901 has completed a phase 3 clinical trial in adults with favorable outcomes and has been approved for marketing by the NMPA in China<sup>15</sup>. Given the demonstrated safety and efficacy in adults, we initiated a multicenter phase 1 clinical trial to evaluate the safety, tolerability and efficacy of BBM-H901 in adolescents aged 12–18 years with hemophilia B. Therefore, this study provides initial insights into gene therapy for hemophilia B in the pediatric population.

The dosing regimen of BBM-H901 in adults was established through exploration in phase 1 pilot and phase 1/2 studies, and was finally determined to be  $5 \times 10^{12}$  vg kg<sup>-1</sup> in the phase 3 study<sup>17</sup>. However, the clinical outcomes of gene therapy in pediatric patients with hemophilia B remain unknown. Considering the dynamic growth trajectories of pediatric patients—including age-dependent changes in body composition, hepatic volume and metabolic maturation—we selected adolescents (aged 12–18 years) as the initial pediatric population for BBM-H901 gene therapy. Accordingly, this approach balances scientific rigor, safety pragmatism and regulatory alignment—particularly with the International Council for Harmonisation's E11(R1) principles supporting the targeted extrapolation of adult data to adolescents when justified by physiological and disease-related maturity. The dose used was identical to that in adults; to further ensure safety, participants were enrolled sequentially by age group from oldest to youngest.

The primary endpoint (safety) was achieved, with no DLTs observed throughout the trial. During the 52-week follow-up period after infusion, only one AE related to BBM-H901 occurred, which was resolved following intensified immunosuppressive treatment. No inhibitor development or thrombotic events were reported, confirming the favorable safety and tolerability profile of BBM-H901 in adolescents. At 52 weeks after gene therapy, prespecified secondary outcomes included a mean (s.d.) FIX:C increase to 41.9 (28.7) IU dl<sup>-1</sup> (OS SynthASil method), zero target joints in 100% of participants and 81.8% achieving bleeding-free status.

All participants developed high titers of binding antibodies and nAbs to the AAV capsid following treatment, as expected. Currently, gene therapy remains a one-time treatment unless new AAV serotypes are used to circumvent cross-reactivity. Although several in vitro

studies on repeated gene therapy are underway<sup>18–22</sup>, clinical application remains distant.

In the phase 3 BBM-H901 trial for adults with hemophilia B, the ABR at week 52 was 0.60 (95% CI 0.18–1.99), markedly lower than the prespecified mean ABR of 5.0 among Chinese patients receiving prophylaxis. Mean (s.d.) FIX:C at week 52 was 41.9 (28.7) IU dl<sup>-1</sup> (OS Actin FSL method). In the phase 1 long-term follow-up cohort (median 210 weeks; range 159–270 weeks), 70% of participants sustained FIX:C levels >35 IU dl<sup>-1</sup>, with 40% exceeding 55 IU dl<sup>-1</sup>, indicating durable expression over more than 4 years of observation<sup>23</sup>. The phase 3 BENEGENE-2 trial treated 45 patients with hemophilia B with fidanacogene elaparvovec ( $5 \times 10^{11}$  vg kg<sup>-1</sup>), achieving a mean FIX:C of 26.9 IU dl<sup>-1</sup> (median 22.9 IU dl<sup>-1</sup>) at 15 months ( $n = 38$ , OS SynthASil method) and a 78% reduction in ABR versus prophylaxis<sup>24</sup>. The multiyear follow-up of the phase 1/2a fidanacogene elaparvovec study showed mean FIX:C levels ranging from 7.4 to 44.2 IU dl<sup>-1</sup> between years 4 and 6, sustained in the mild hemophilia range<sup>13</sup>. In the HOPE-B study, a single  $2 \times 10^{13}$  vg kg<sup>-1</sup> dose of etranacogene dezaparvovec in 54 patients with hemophilia B led to a mean (s.d.) FIX:C of 36.7 (19) IU dl<sup>-1</sup> at 24 months (OS SynthASil method), with ABR decreasing from 4.18 to 1.51 (ref. 25). Regarding long-term expression, participants maintained a mean (s.d.) FIX:C of 36.1 (15.7) IU dl<sup>-1</sup> at year 5 (ref. 14). Moreover, one study has reported gene therapy data in juvenile dogs with hemophilia B. Seventeen puppies, including the youngest at 2 days of age, were followed for 1.5–5 years after gene therapy, during which no relevant AEs were observed and the ABR was markedly reduced<sup>26</sup>. These findings support the safety of AAV-mediated gene therapy in neonatal, juvenile and growing dogs and provide indirect evidence supporting the potential safety of gene therapy in adolescent patients.

BBM-H901 treatment led to a rapid increase in FIX:C after infusion, which was first observed in the phase 1 pilot trial and consistently noted in the phase 1/2 and 3 studies in adults<sup>17</sup>. In the current adolescent study, FIX:C reached 51.2 (14.0) IU dl<sup>-1</sup> on day 3 and 92.8 (27.5) IU dl<sup>-1</sup> at week 1, with 8 of 11 participants achieving their peak levels at week 1, confirming that BBM-H901 can rapidly increase FIX:C in adolescent patients as well.

The CHO-KLAT scores showed an overall upward trend after gene therapy, indicating an improved quality of life compared to prior replacement therapy. HJHS scores generally decreased, suggesting improved joint status with stable factor levels, while HEAD-US-C scores showed no notable change, implying that structural joint improvement may require a longer follow-up.

In the adult phase 1/2 and 3 BBM-H901 trials, asymptomatic elevations of liver transaminases accompanied by declines in FIX:C were observed<sup>17</sup>. Currently, cytotoxic T lymphocytes are believed to be the primary cause of gene therapy-related hepatotoxicity and reduced efficacy<sup>27</sup>. Immunosuppression with corticosteroids is commonly used to control transaminase elevations and preserve FIX expression,

although the response varies<sup>28,29</sup>, and no consensus exists regarding the optimal regimen. In the BBM-H901 adult trials, prophylactic corticosteroids were started on day -7 or day -1 relative to infusion<sup>17</sup>. Although valoctocogene roxaparvec GENEr8-3 did not show benefit from prophylactic corticosteroids in maintaining FVIII levels<sup>30</sup>, in the BBM-H901 phase 3 trial, the only participant who did not receive prophylactic corticosteroids experienced a substantial decline in FIX:C that was unresponsive to rescue immunosuppression. Therefore, in the adolescent trial, prophylactic corticosteroids were initiated between days -3 and -1.

Only one adolescent participant developed a substantial liver function abnormality, while four participants exhibited marked declines in FIX:C. Although transaminase levels did not exceed the upper limit of normal (ULN), transient elevations relative to baseline correlated temporally with declines in activity. High-dose methylprednisolone combined with tacrolimus immunosuppression was used as an intervention. While responses varied, timely immunosuppressive treatment appeared to help mitigate the loss of activity.

Compared to 26 participants in the adult phase 3 data (Extended Data Table 2), adolescents reached a higher peak FIX:C level (106.4 (s.d. 25.1) IU dI<sup>-1</sup> versus 97.7 (s.d. 57.0) IU dI<sup>-1</sup>); however, their levels declined more rapidly by week 52 (41.8 (s.d. 30.1) IU dI<sup>-1</sup> versus 55.1 (s.d. 35.9) IU dI<sup>-1</sup>)<sup>23</sup>. The mechanism behind the faster decline in adolescents requires further investigation. Given that the participants' BMI did not change notably (Table 2), one hypothetical explanation is increased hepatocyte proliferation in adolescents. Because recombinant AAV vectors predominantly persist as episomes instead of integrating into the host genome, dilution through hepatocyte division might reduce long-term expression<sup>18,31</sup>. However, this hypothesis requires further validation. Another finding was that, although adolescents exhibited a rapid post-infusion peak in FIX:C—similar to adults but with higher peak levels—72.7% experienced a marked decline at weeks 2–4, followed by a further decline at weeks 8–14. In contrast, most adult participants in the phase 3 trial showed only a single pronounced decline between weeks 8 and 14. No clear differences in vector shedding were observed among adolescents. Whether the early decline reflects immune-mediated effects remains unclear. Given that multiple adolescent participants showed early declines, a slower tapering of corticosteroids before week 14 may be beneficial in future studies.

In this study, four participants exhibited a sustained decline in FIX:C. scRNA-seq analysis was performed on samples from two of these participants and from one participant with relatively stable FIX:C levels. In our center's clinical trial of hemophilia A, a sustained decline in therapeutic efficacy that may be associated with prednisone tapering was observed<sup>32</sup>. From another clinical trial of hemophilia A conducted at our center, scRNA-seq evidence revealed that pretreatment

with prednisone combined with tacrolimus markedly suppressed the immune response of CD8<sup>+</sup> T cells. Nevertheless, marked activation of the interferon pathway in CD8<sup>+</sup> T cells was still detectable at week 4 after vector infusion, even during combined drug administration—a pattern highly consistent with the immune mobilization profiles observed in the present study. This suggests that initial GC tapering and AAV-induced responses jointly drive immune activation at this time point. This study further elucidated the stepwise immune evolutionary characteristics during GC tapering. When prednisone was tapered to 10 mg, participants exhibited high expression of the *FOS* gene, and the immune system mainly remained in a state characterized by antigen presentation, metabolic mobilization and NF- $\kappa$ B initiation, with relatively mild T cell activation—a phenotype particularly prominent in CD8<sup>+</sup> T<sub>eff</sub> cells. Concurrently, ligand–receptor crosstalk within the cell adhesion molecule and interleukin families was notably enhanced. Such drastic molecular fluctuations underscore the necessity of implementing combined immunosuppressive regimens during GC tapering. Complete GC withdrawal triggers robust synergistic activation of both the interferon and NF- $\kappa$ B pathways. Such marked withdrawal-induced T cell activation was observed in both of the aforementioned participants with sustained efficacy decline. In summary, addressing the challenge of reduced coagulation factor activity following AAV gene therapy in pediatric patients with hemophilia, this study suggests that the development of new combined immunosuppressive regimens targeting relevant molecular signatures—including the *FOS* gene, the NF- $\kappa$ B pathway, adhesion molecules and interleukin pathways—should be considered. In future clinical practice, based on close monitoring of drug-related adverse reactions, precise optimization of the timing of immune intervention will provide a more scientific and reliable reference for improving the long-term efficacy of AAV gene therapy.

Although differences exist compared to the adult trial results, the adolescent trial demonstrated an overall favorable safety profile and acceptable efficacy, with most participants benefiting from the treatment. These differences require validation using longer follow-up periods and larger cohorts, and it remains important to determine whether similar patterns are observed in other pediatric gene therapy studies.

This study has limitations, including a small sample size and a short follow-up duration. Only adolescents aged  $\geq 12$  years were enrolled; therefore, safety and efficacy in younger children remain unknown. In addition, a subset of participants was selected from seronegative individuals enrolled in a previous AAV antibody study. Together with the small sample size, this resulted in a relatively homogeneous population. Consequently, certain findings, such as the incidence of nAbs, may not fully reflect what is observed in real-world settings. Another limitation is that the mechanisms underlying the differences in efficacy between adolescents and adults remain unclear. Therefore, future studies with

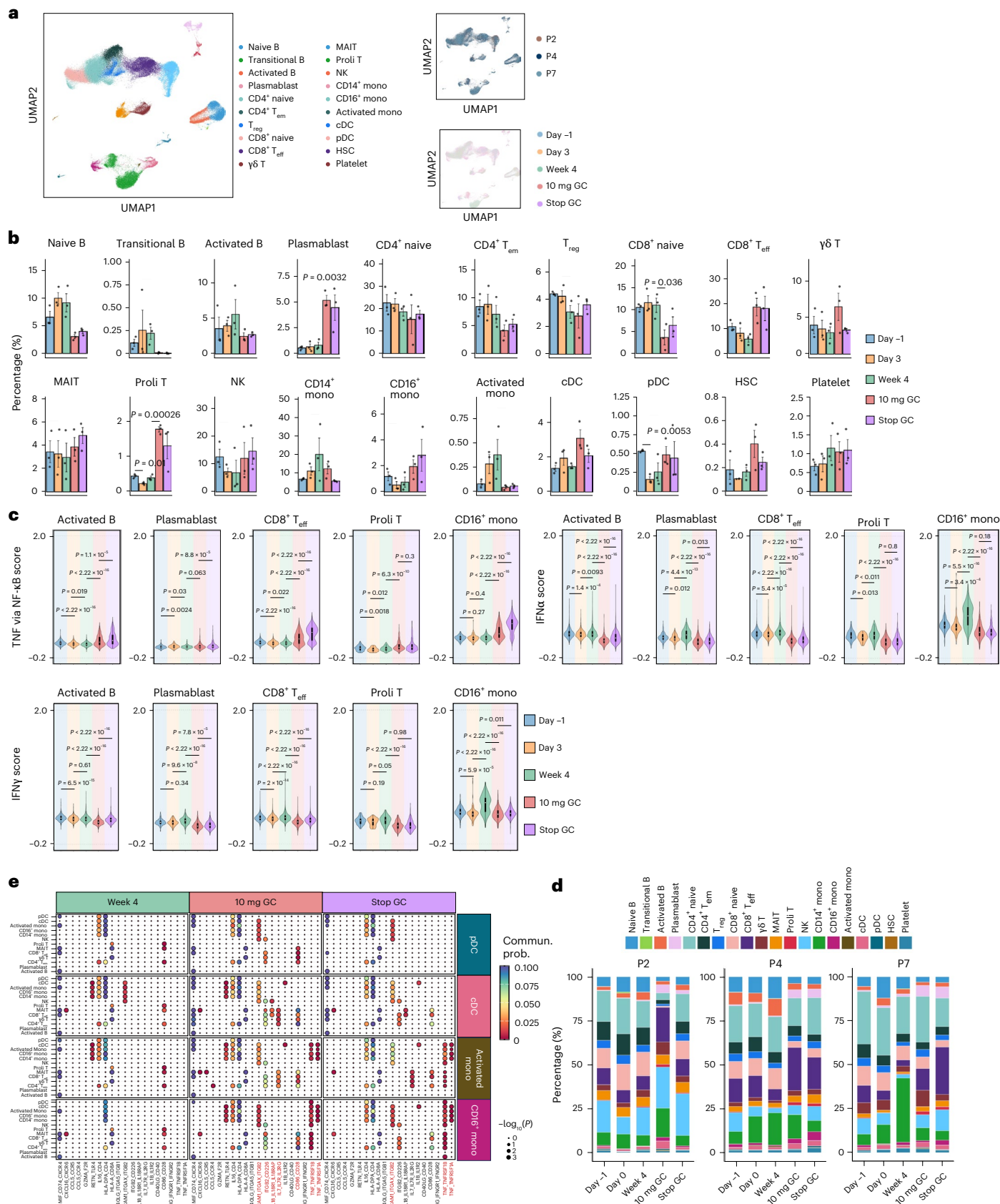
**Fig. 4 | Single-cell transcriptomic analysis of peripheral blood immune cells before and after gene therapy.** **a**, Single-cell transcriptome sequencing and subsequent batch integration were performed on PBMCs obtained from three patients at five distinct time points. **b**, Bar plots illustrating the percentage distribution of distinct immune cell subsets within PBMCs at five key time points during AAV gene therapy. The height of the bars represents the mean value, with error bars indicating  $\pm$ s.e.m. Each dot represents an individual participant ( $n = 3$  per group). Pairwise comparisons were performed using a two-sided unpaired Welch's *t* test without adjustment for multiple comparisons, with exact *P* values indicated above the brackets. The exact *P* values, *t* statistics, degrees of freedom and 95% CIs are provided as source data. **c**, Transcriptional levels of IFN $\alpha$ , IFN $\gamma$  and NF- $\kappa$ B in five cell subsets. Hallmark scores were assessed at distinct time points of infusion (right legends). Within each violin plot, the embedded box plot elements are defined as follows: the center line represents the median (50th percentile); the lower and upper bounds of the box represent the 25th (lower quartile) and 75th (upper quartile) percentiles, respectively; and the whiskers extend to the minimum and maximum data points within 1.5 $\times$  the IQR (calculated as the difference between the 75th and 25th percentiles) from the box bounds. The y axis indicates batch-corrected expression levels and enrichment

scores derived from single-cell sequencing data across multiple participants and time points. Each compared group contains more than 48 individual cells. Pairwise comparisons were performed using a two-sided Wilcoxon rank-sum test, with exact *P* values indicated above the brackets. **d**, Stacked bar plots depicting the relative proportions of immune cell subsets from three adolescent participants with hemophilia B across five key time points. **e**, Cell–cell communication networks in 13 cell subsets at three key time points: week 4, 10 mg GC and Stop GC. The plots are grouped by major signal-sending cell types: plasmacytoid dendritic cells, conventional dendritic cells, activated monocytes and CD16<sup>+</sup> monocytes (right y axis). Signal-receiving cell types are annotated to indicate the target of each ligand–receptor interaction (left y axis). Ligand–receptor pairs are presented separately for each of the three time points (top x axis), and the activated ligand–receptor pairs are marked in red (bottom x axis). Bubble size represents the  $-\log_{10}(P)$  of communication significance, while bubble color indicates communication probability (commun. prob.), with a color gradient from red (low) to blue (high). T<sub>eff</sub>, effector memory T cell; MAIT, mucosal-associated invariant T cell; NK, natural killer cell; cDC, conventional dendritic cell; pDC, plasmacytoid dendritic cell; HSC, hematopoietic stem cell; mono, monocyte; proli, proliferating; T<sub>reg</sub>, regulatory T cell.

larger sample sizes and longer follow-up periods are warranted to address these limitations.

In summary, the BBM-H901 adolescent study confirms the safety and tolerability of the therapy in children aged 12–18 years with hemophilia B who had at least 1 year of follow-up, expanding the age range for

hemophilia gene therapy. Most participants showed a marked increase in FIX:C, which substantially reduced their risk of bleeding. However, some exhibited later declines in FIX:C. Therefore, longer follow-up periods and larger sample sizes are needed to further confirm the long-term efficacy and safety.



## Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41591-026-04636-8>.

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## Methods

This multicenter, single-arm, open-label, single-dose, phase 1 study aimed to evaluate the safety, tolerability, pharmacokinetic profile and efficacy of a single intravenous infusion of BBM-H901 in adolescent patients with hemophilia B (aged  $\geq 12$  years but  $< 18$  years) with FIX:C levels  $\leq 2$  IU  $\text{dl}^{-1}$  at two Chinese clinical centers. This study was preregistered on ClinicalTrials.gov on January 19, 2023, with the identifier [NCT05709288](#). The study was reviewed and approved by the institutional review board of the Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College. A long-term follow-up study is included as part of this research. This study complies with all relevant ethical regulations and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants and their guardians before screening. Participants would not receive any compensation for participating in the study; however, the sponsor would cover the costs of transportation and nutrition related to biosample collection. The study design is shown in Extended Data Fig. 1.

## Study design and participants

We planned to enroll nine evaluable participants (subject to adjustment based on the actual situation) and divide them into three age groups (group 1, group 2, group 3) in descending order of age. Ultimately, 11 evaluable participants were enrolled and divided into group 1 (3 participants aged  $\geq 16$  years but  $< 18$  years), group 2 (4 participants aged  $\geq 14$  years but  $< 16$  years) and group 3 (4 participants aged  $\geq 12$  years but  $< 14$  years). Group 1 received the BBM-H901 infusion first, followed by groups 2 and 3. A sentinel participant in each group was enrolled and observed for at least 2 weeks after dosing. The enrollment of the remaining two participants in each group was assessed by the investigator. After the last participant completed the 10-week follow-up, the safety review committee determined whether to proceed to the next group. After the last participant in group 3 completed the 10-week follow-up, the safety review committee comprehensively assessed the safety and tolerability of BBM-H901 in adolescent patients with hemophilia B. The study design is shown in Extended Data Fig. 1. Detailed inclusion and exclusion criteria are listed below.

## Inclusion criteria

1. Participants and/or their legal guardians must fully understand the purpose, nature, procedures and potential risks of the study; provide written informed consent; and voluntarily agree to participate.
2. Requirements for age, sex, and body weight:
  - (a) Male participants aged  $\geq 12$  years but  $< 18$  years
  - (b) Body weight of 45 kg or above for participants aged  $\geq 16$  years but  $< 18$  years
  - (c) Body weight of 40 kg or above for participants aged  $\geq 12$  years but  $< 16$  years
3. Participants clinically diagnosed with hemophilia B with endogenous FIX:C levels of  $\leq 2$  IU  $\text{dl}^{-1}$  ( $\leq 2\%$ ); if a FIX:C level of  $> 2\%$  at screening is determined to be due to insufficient elution of FIX protein products, the test can be repeated once after sufficient elution.
4. Titers of nAbs and binding antibodies to BBM-H901 capsid of  $\leq 1:4$  and  $1:200$ , respectively.
5. A history of bleeding events and/or injection of a FIX product (recombinant or plasma-derived) within 12 weeks before screening, noted in the participant's medical records.
6. No history of allergic reactions or allergies related to the administration of FIX or intravenous human immunoglobulin.
7. Participants who had received any recombinant or plasma-derived FIX products for at least 75 exposure days, had a negative FIX inhibitor test result, and had no clinical signs or symptoms suggestive of a reduced response to FIX products at

screening; or participants with a history of FIX inhibitor positivity who had remained inhibitor-negative for at least 2 years after ITI and had received FIX products for at least 100 cumulative exposure days during the inhibitor-negative period (participants whose previous inhibitor positivity was not treated with ITI or whose inhibitors recurred after successful ITI were considered ineligible).

8. Acceptable laboratory test values obtained during screening and before study drug administration (day  $-1$ ):
  - (a) Hemoglobin  $\geq 11$  g  $\text{dl}^{-1}$  or greater than or equal to the age-specific ULN
  - (b) Platelets  $\geq 100 \times 10^9$  cells per liter
  - (c) AST and ALT levels  $\leq 1.5 \times$  the ULN
  - (d) Total bilirubin  $\leq 1.5 \times$  the ULN
  - (e) Estimated glomerular filtration rate  $\geq 60$   $\text{ml min}^{-1}$
9. Older underage participants and their statutory guardians should clearly understand the requirements for contraception and know that participants must either avoid sexual intercourse or use reliable contraceptive methods.
10. For good compliance, participants and their statutory guardians must be willing to participate in the gene therapy clinical study, able to attend regular follow-ups and able to accurately complete the participant's diary during the follow-up period with assistance from family members or caregivers.

## Exclusion criteria

1. Positive for hepatitis B surface antigen, hepatitis B virus DNA or hepatitis C virus RNA; those with a history of hepatitis B or C should have two previous sample collections at intervals of at least 3 months and are considered negative if negative results are obtained for the above indicators in both assessments.
2. Receiving antiviral therapy for hepatitis B or C.
3. Individuals with coagulation disorders other than hemophilia B.
4. Individuals taking any systemic immunosuppressive agents within 30 days before screening, except GCs and immunosuppressive agents recommended by the investigator; individuals allergic to GCs, tacrolimus or other immunosuppressive agents.
5. History of vaccination within 30 days before screening, or with a planned vaccination during immunomodulatory therapy.
6. Individuals with potential liver diseases, such as a previous diagnosis of portal hypertension, splenomegaly, hepatic encephalopathy or liver fibrosis stage 3 or higher; nodules and cysts detected by B-ultrasound; or an increase in  $\alpha$ -fetoprotein levels identified by laboratory tests and determined clinically relevant by the investigator.
7. Individuals with scheduled surgery during the 52-week study period following BBM-H901 infusion.
8. Presence of an acute or chronic infection that may increase the risk associated with study participation, or other chronic diseases that may render the individual unsuitable for participation as determined by the investigator.
9. Individuals who have received gene therapy before screening, other study drugs within 4 weeks before screening, or within five half-lives of the investigational product (whichever is longer).
10. Intolerable anaphylaxis to prior treatment with FIX or nephrotic syndrome during prior treatment with FIX.
11. Use of any plant preparation (herbal supplements or traditional Chinese medicines derived from plants, minerals or animals) that may affect liver function within 4 weeks before or during the study, except for topically administered drugs; use of Chinese herbal medicines that may affect the study results, as determined by the investigator.

12. Life-threatening bleeding, such as intracranial hemorrhage within 2 years before enrollment, sequelae after previous intracranial hemorrhage, severe hemorrhage or potential causes of gastrointestinal bleeding—including, but not limited to, gastrointestinal ulcers.
13. Individuals with serious diseases or other conditions that the investigator deems unsuitable for study participation.
14. Family members of study site staff directly involved in the implementation of the study, family members of study site staff who are otherwise supervised by the investigator, and family members of employees of related companies directly involved in the implementation of the study.

## Procedures

All eligible participants received prophylactic oral prednisone at 1 mg kg<sup>-1</sup> (for body weight <60 kg) or 60 mg (for body weight ≥60 kg) daily for 1–3 days, followed by intravenous administration of BBM-H901 at a dose of 5 × 10<sup>12</sup> vg kg<sup>-1</sup> with an infusion rate of 2.5 ml min<sup>-1</sup> (4 × 10<sup>12</sup> vg ml<sup>-1</sup>). The initial prednisone dose lasted 2 weeks and was tapered off over approximately 8 weeks (the tapering schedule may be adjusted according to clinical manifestations). The use of prednisone could be resumed based on liver function and FIX:C levels during follow-up. The recommended tapering regimen and the regimen for additional immunosuppressive therapy are provided in the Supplementary Methods.

## Endpoints

Primary endpoints:

1. Incidence of DLT events, as determined by the safety review committee, within 10 weeks after BBM-H901 infusion
2. Incidence of AEs and SAEs within 52 weeks after BBM-H901 infusion
3. Changes in liver function (ALT and AST) within 52 weeks after BBM-H901 infusion compared to before treatment

Secondary endpoints:

1. Pharmacokinetic parameters of Padua-FIX and recombinant AAV vectors after infusion of BBM-H901:
  - (a) Padua-FIX:C levels within 52 weeks after BBM-H901 infusion, including peak activity, steady-state activity and mean activity levels
  - (b) Changes in AAV vector shedding (plasma, urine, feces, saliva and PBMCs) within 52 weeks after BBM-H901 infusion
2. Efficacy endpoints for BBM-H901:
  - (a) ABR within 52 weeks after BBM-H901 infusion
  - (b) FIX:C levels at each visit within 52 weeks after BBM-H901 infusion
  - (c) Number and volume of infusions of other FIX protein products (including recombinant and plasma-derived FIX protein products, such as recombinant human coagulation FIX for injection and PCC) within 52 weeks after BBM-H901 infusion
  - (d) Number of target joints within 52 weeks after BBM-H901 infusion
  - (e) Number of joint bleeds within 52 weeks after BBM-H901 infusion
  - (f) Proportion of participants without bleeding events within 52 weeks after BBM-H901 infusion
  - (g) Changes in joint health score, joint ultrasound score and quality of life from baseline to 4, 12, 26 and 52 weeks after BBM-H901 infusion, assessed using the following scales related to hemophilia evaluation: HJHS, HEAD-US-C and CHO-KLAT<sup>33</sup>
3. Other safety endpoints for BBM-H901:
  - (a) Incidence of FIX inhibitors (detected by the Bethesda or Nijmegen-Bethesda method) within 52 weeks after BBM-H901 infusion

- (b) Titers of nAbs and binding antibodies to the AAV capsid
- (c) Clinically relevant changes in vital signs, physical examination findings, clinical laboratory test values, 12-lead electrocardiogram results and α-fetoprotein levels within 52 weeks after the infusion of BBM-H901
- (d) Cellular immune status of the body before and after AAV vector infusion, assessed by scRNA-seq analysis
- (e) Changes in bone mineral density before and after treatment in adolescent participants

## Statistical analysis

The sample size calculation for this study did not involve formal statistical hypothesis testing. With three age groups planned, a total of 11 participants (3 + 4 + 4) were enrolled. For continuous variables, descriptive statistics are reported, including sample size (*n*), mean ± s.d., median, minimum and maximum values. Categorical variables are summarized as frequency counts (*n*) or percentages (%). Clinical data collected after infusion (for example, ABR, number of target joints and number of FIX concentrate treatments) were compared to data collected during the 52 weeks before enrollment. The change in FIX:C levels from baseline was statistically summarized, and the mean and s.d. of the differences were reported. A negative binomial regression model was used to estimate the mean ABR (and its 95% CI) within 52 weeks after infusion. The Wilcoxon signed-rank test was used to analyze the differences in infusions of other FIX protein products, the number of target joints and the number of joint bleeds.

## Immunomodulatory therapy

In this study, prophylactic steroids—that is, oral GCs such as prednisone or prednisolone—were administered from day –1 before BBM-H901 infusion to 8 weeks after the infusion. The initial regimen of oral GCs and the recommended hormone tapering schedule during the study are shown in Supplementary Table 1.

By referring to the guidelines issued by the American Association for the Study of Liver Diseases, the data from the SJ-UCL (St. Jude Children's Research Hospital–University College London) study, and the experience with the product reported in previous studies—in the context of no other etiologies—we initiated immunomodulatory therapy when the following were observed during the follow-up period after gene vector infusion:

1. Liver aminotransferase (ALT and/or AST) levels greater than 1.5× the baseline value or above the ULN
2. A continuous decrease in the vector Padua-FIX:C levels in the absence of evidence of FIX inhibitor production
3. Laboratory parameters and clinical assessment results that require the initiation of immunomodulatory therapy as determined by the investigator

Treatment may include GCs such as prednisone or prednisolone. If GCs are ineffective, the investigator may consider alternative or combination therapy with other immunosuppressive agents (for example, tacrolimus). The specific initial dosing regimen and dose reduction plan for GCs are provided in the Supplementary Table 1. The initial dose and tapering schedule could be adjusted by the investigator based on the participants' clinical manifestations, laboratory results and examination findings.

## Study vector production

BBM-H901 is a new vector composed of an engineered liver-tropic AAV capsid (AAV843) and FIX-901 (a fully synthesized liver-specific promoter and CpG-reduced FIX Padua coding sequence), packaged in an AAV particle as a double-stranded AAV genome.

The BBM-H901 cassette (FIX-901) is a codon-optimized cassette expressing FIX Padua driven by a liver-specific promoter. It consists of a mini liver-specific promoter, LXP2.1, and an optimized cDNA encoding

the hyperactive FIX Padua (R338L). The LXP2.1 promoter is only 188 bp long and contains several hepatocyte nuclear factor transcriptional elements, a TATA box and specificity protein 1 (SP1) transcriptional elements. The FIX coding sequence was codon-optimized by removing the TCG and CGT motifs from the *F9* sequence without altering the amino acid sequence, and the Padua mutation was introduced. The BBM-H901 cassette sequence and the AAV843 capsid sequence are detailed in the Supplementary Methods.

The vector was produced by triple-plasmid transient transfection of suspended HEK293 cells in a 200-liter single-use bioreactor. Vector particles were released from collected cells by adding detergent directly to the bioreactor. Pool lysates were filtered through a depth filter, followed by concentration and diafiltration using tangential flow filtration (TFF). Post-TFF samples were purified through a centrifugation-free downstream process that included affinity and anion exchange chromatography, followed by diafiltration through TFF into a neutral formulation. The percentage of full particles was >80%, as determined by analytical ultracentrifugation. The final product was assayed for purity, safety and potency, including genome titer by qPCR and droplet digital PCR (ddPCR), SDS-PAGE purity, cell-based potency, high-performance liquid chromatography–size exclusion chromatography purity, residual plasmid DNA, residual host cell DNA, residual host cell protein, endotoxin, sterility, pH and osmolality.

### Vector titration by ddPCR

Initially, 10  $\mu$ l of a tenfold diluted AAV sample was mixed with 190  $\mu$ l of a DNase I mixture (comprising 184  $\mu$ l of 1 $\times$  PBS, 4  $\mu$ l of Mg<sup>2+</sup> and 2  $\mu$ l of DNase I) and incubated at 37 °C for 1 h. Following digestion, 10  $\mu$ l of 0.5 M EDTA and 210  $\mu$ l of an optimized dilution buffer were added to the mixture. The sample was then subjected to a 5,000-fold gradient dilution using the optimized dilution buffer. Reaction mixtures were prepared using the recommended ddPCR mix (EvaGreen) from APEXbio, incorporating the same primers as those used in the qPCR assay targeting the FIX Padua coding sequence and 3  $\mu$ l of the diluted sample, in a final reaction volume of 7  $\mu$ l. Each reaction mixture was loaded into a sample well of a 16-well consumable chip from Stilla Technologies. Amplification was performed using a conventional thermal cycler with the following protocol: initial denaturation at 95 °C for 10 min, followed by incubation at 37 °C for 20 min, and then 45 cycles of 95 °C for 10 s and 60 °C for 45 s. To calculate the genome copy number per milliliter (vg ml<sup>-1</sup>), the concentration of positive droplets was multiplied by the sample dilution factor and the volume of sample added to each reaction (3  $\mu$ l), then divided by the total reaction volume (7  $\mu$ l).

### Anti-AAV843 capsid nAb assay

A cell-based assay was used to detect nAbs to the BBM-H901 capsid in serum. The AAV vector with the BBM-H901 capsid packaged the Gaussia luciferase gene. Serum from participants, at a range of dilutions, was mixed with 2  $\times$  10<sup>9</sup> vg ml<sup>-1</sup> AAV-luciferase. A hepatocyte-derived carcinoma cell line, Huh-7, was transduced with a mixture of serum and AAV-luciferase. After 72 h, luciferase in the cell culture medium was measured using a luminometer. The lowest dilution that inhibited luciferase expression by  $\geq$ 50% was reported as the nAb titer.

### Anti-AAV843 capsid binding antibody assay

AAV843 empty capsid virus (2  $\times$  10<sup>9</sup> viral particles per well) was coated onto 96-well microplate wells (Ag<sup>+</sup>), with uncoated wells as negative controls (Ag<sup>-</sup>). Gradient-diluted human serum (minimum required dilution 1:50) was incubated and washed, then incubated with an HRP-conjugated secondary antibody, followed by TMB substrate for color development. The signal-to-noise ratio was calculated using the following formula: ((OD<sub>450</sub> Ag<sup>+</sup>) – (OD<sub>450</sub> Ag<sup>-</sup>))/(OD<sub>450</sub> Ag<sup>-</sup>). The maximum dilution factor corresponding to a ratio of  $\geq$ 2 was considered the titer of the test serum. A titer of  $\leq$ 1:200 was considered negative.

### Coagulation tests

FIX:C was measured using an OS method with both Actin FSL (Siemens Healthcare Diagnostics) and SynthASil (Instrumentation Laboratory) aPTT reagents on the corresponding instruments. FIX inhibitors were measured using the Bethesda method during the screening period and at weeks 2, 4, 8, 12, 16, 26, 32, 42 and 52.

### IFN $\gamma$ ELISpot

AAV843 capsid-derived peptides were predicted using the Immune Epitope Database<sup>34</sup> and subsequently synthesized. The peptide list is provided in Supplementary Table 2.

On day 0, cryopreserved PBMCs were thawed and cultured in RPMI-1640 medium supplemented with 10% FBS in 96-well polyvinylidene fluoride-bottom plates precoated with an anti-IFN $\gamma$  capture antibody. Cells were stimulated with either a peptide mixture (0.1  $\mu$ g) or PBS and incubated at 37 °C for 72 h. IFN $\gamma$  ELISpot (Elabscience, cat. no. ESP-H0002) assay was then performed according to the manufacturer's protocol. The peptides used are listed in Supplementary Table 2.

### Single-cell sequencing

The 10x Genomics Chromium system was used to construct the cDNA library from single cells for sequencing.

**Cell isolation.** We collected 5 ml of peripheral blood from the participants each time. PBMCs were freshly isolated using density gradient centrifugation with Ficoll-Paque, followed by lysis of red blood cells using an ammonium chloride–potassium (ACK) buffer. Cell samples were stored at 4 °C for the subsequent generation of an scRNA-seq library.

**scRNA-seq analysis.** The 10x Genomics Chromium system was used to construct the cDNA library from single cells for sequencing. This system uses a network of microfluidic channels to create a single-cell encapsulation system that produces gel beads in emulsion (GEMs) surrounded by oil droplets. The reverse-transcribed and amplified cDNA libraries were constructed using 5' gene expression chemistry and sequenced with 150-bp paired-end (PE150) sequencing on the NovaSeq 6000 platform.

**scRNA-seq data processing.** The raw sequencing data from the participants were processed using Cell Ranger (version 6.1.2) with reference to the human genome (GRCh38) and default parameters. Data from different participants and stages were then combined using Seurat (version 4.3.0) with the merge function. First, a total of 102 genes associated with mitochondria (13 genes) and ribosomes (99 genes) were excluded, and cells with detected gene numbers between 200 and 7,500, as well as total RNA read counts between 0 and 50,000, were retained. Subsequently, the gene counts were normalized for each cell using the NormalizeData function in Seurat. The top 30 principal components were used to perform data integration by invoking the RunHarmony function in Harmony 2. Finally, the clustering algorithm in Seurat was applied to cluster the cells, identifying 28 clusters (resolution = 0.5).

**Cell subset annotations.** In total, 20 cell types were identified: naive B cells (CD79A, TCL1A<sup>high</sup>); transitional B cells (CD79A, TCL1A<sup>low</sup>); activated B cells (CD24, SWAP70); plasmablasts (JCHAIN); CD4<sup>+</sup> naive T cells (CD3D, CCR7); CD4<sup>+</sup> effector memory T cells (IL-7R, TNFRSF4); regulatory T cells (FOXP3); CD8<sup>+</sup> naive T cells (CD3D, CCR7, CD8A); CD8<sup>+</sup> T<sub>eff</sub> cells (CD3D, CD8A, CST7, NKG7);  $\gamma\delta$  T cells (TRDV2); mucosal-associated invariant T cells (SLC4A10); proliferating T cells (MKI67); natural killer cells (CST7, NKG7, GNLY); CD14<sup>+</sup> monocytes (CD14); CD16<sup>+</sup> monocytes (FCGR3A); activated monocytes (FCN1, LGALS3); conventional dendritic cells (FCER1A, CD1C); plasmacytoid dendritic cells (CLEC4C, LILRA4); hematopoietic stem cells (CD34); and platelets.

**Immune score-related subtype analysis.** Gene sets termed ‘Hallmark interferon alpha response’, ‘Hallmark interferon gamma response’ and ‘Hallmark TNFA signaling via NFkB’ from MSigDB3 were selected for functional analysis. The immune score was evaluated using the `AddModuleScore` function in Seurat. Cell types with notably higher immune scores were defined as hyperinflammatory. A *t* test was used for differential analysis.

**Differential expression analysis.** Differential expression analysis was performed using the `RunDEtest` function implemented in the SCP R package, a dedicated tool for scRNA-seq data analysis<sup>35</sup>. Pairwise comparisons were conducted between one designated group and each of the remaining groups. The union of the resulting differentially expressed genes was then compiled across all comparisons.

**GO analysis.** Gene Ontology (GO) enrichment analysis for biological processes was performed using the `compareCluster` function (integrated with `enrichGO`) implemented in the `clusterProfiler` R package, with parameters set as `pAdjustMethod = BH`, `pvalueCutoff = 0.05`, `qvalueCutoff = 0.05` and `redundancy reduction cutoff = 0.7`. Differentially expressed genes for analysis were identified using the `FindMarkers` function in Seurat with `min.pct = 0.25`, `logfc.threshold = 0.25`, and the Wilcoxon rank-sum test.

**Cell–cell communication analysis.** Thirteen immune cell types were filtered and retained, and cell–cell communication probabilities were calculated for each experimental group based on the human ligand–receptor database in the `CellChat v2` R package<sup>36</sup>. Low-confidence communications were filtered out (with a threshold of `min.cells = 5`), and communication networks were generated for each group and subsequently integrated. To visualize the statistical significance of cell–cell communication interactions, raw *P* values recorded as NA (not available) were replaced with 1 to avoid undefined results when calculating  $-\log_{10}(P)$ . A constant offset of 0.001 was then added to each resulting *P* value before logarithmic transformation to prevent undefined results when *P* = 0. Twenty ligand–receptor pairs were selected, and multigroup bubble plots were generated in which bubble size represents the statistical significance of cell–cell communication, and bubble color indicates the communication probability.

**Interpretation of single-cell sequencing results.** To validate the accuracy of cell type annotation in our scRNA-seq dataset, we generated a heat map (Supplementary Fig. 3a) displaying the normalized expression levels of canonical cell type-specific marker genes across all identified cell populations. The NF- $\kappa$ B p65 subunit and FOS can exert a synergistic transcriptional activation effect through physical interaction, thereby markedly enhancing the expression of downstream proinflammatory genes. FOS activation can be attributed to experimental batch effects, a technical artifact that frequently occurs in specimens subjected to tissue dissociation<sup>37</sup>. Notably, our study used fresh PBMCs directly isolated from peripheral blood, which does not involve tissue dissociation and therefore fundamentally reduces this type of technical variation. To further confirm the robustness of our results, we performed targeted analyses in the CD8<sup>+</sup>T<sub>eff</sub> subset, where NF- $\kappa$ B pathway activation was most pronounced. We observed substantial upregulation of FOS expression at the 10 mg GC and Stop GC time points, accompanied by increased expression of the downstream interferon-responsive gene *IFNG* and the T cell survival-associated gene *BCL2*. Conversely, the expression of heat shock protein (HSP) family genes was substantially downregulated. Taken together, these data indicate that the increased NF- $\kappa$ B enrichment score is a genuine biological consequence of immune activation, rather than a technical artifact arising from experimental procedures (Supplementary Fig. 3c). For each of the three participants, we separately extracted the upregulated genes at the 10 mg GC time point compared to week 4 and performed

GO enrichment analysis. Taking the type I interferon production pathway as the reference standard, participant 2 showed notably stronger pathway activity than the other two participants. This may be attributed to the fact that participant 2 experienced a decrease in activity at week 6, while prednisone was tapered to 10 mg at week 8, leading to a residual immune response effect (Supplementary Fig. 4 and Supplementary Data 1). We conducted GO enrichment analysis on genes upregulated at the Stop GC stage compared to the 10 mg GC time point. Importantly, although participant 7 underwent GC withdrawal considerably later (32 weeks after AAV injection) compared to participants 2 and 4 (12 and 11 weeks after AAV injection, respectively; Extended Data Fig. 3), the underlying biological response to GC cessation remained highly conserved. In participants 4 and 7, we observed a pronounced enhancement of activation across multiple immune cell subsets among the top 10 GO terms, dominated by T cell activation. Notably, notable activation of T cell pathways was also detected in non-T cell subsets, indicating that the global immune interaction network might be fully activated upon GC withdrawal regardless of the duration of administration. Visualization also detected neutrophil-associated activation terms, which can be explained by the shared transcriptional profiles and regulatory circuits of monocytes and neutrophils, resulting in cross-mapping during functional annotation. Conversely, in participant 2, following the discontinuation of GC therapy, the molecular alterations were restricted to the regulatory programs governing metabolism, T cell signal transduction and antigen presentation, with no robust T cell activation observed. This pattern may explain the distinct treatment response displayed by participant 2 compared to the other two participants (Supplementary Fig. 5 and Supplementary Data 2). Specifically, participant 2 exhibited a distinct clinical phenotype following GC withdrawal: transaminase levels remained persistently elevated for 4 weeks before a delayed reduction in FIX:C. Following GC cessation, participant 7 exhibited both a marked decline in FIX:C and a concurrent elevation in transaminase levels. Therefore, rather than confounding the results, the data from participant 7 may help support a potential core biological finding: GC withdrawal might trigger specific immunological and clinical outcomes—either a rapid immune-mediated reduction in FIX:C (as observed in participants 4 and 7) or an elevation in transaminase levels (as seen in participants 2 and 7). The temporal delay in GC withdrawal for participant 7 may further suggest that this biological outcome is functionally conserved regardless of when GC withdrawal occurs.

### Protocol amendments

During the trial, the protocol was amended to make it more reasonable and operationally feasible, taking into account the clinical risks and benefits for the participants. We provide the main protocol amendments here and detail them in the Protocol Change Records section of the Supplementary Information.

1. The start time of prophylactic steroid administration was changed from 1 week before infusion to 1 day before infusion (day –1); the initial dosing regimen was established based on body weight (prednisone or prednisolone 1 mg per kg per day for participants with a body weight of <60 kg; prednisone or prednisolone 60 mg per day for participants with a body weight of ≥60 kg). The dose reduction regimen was adjusted accordingly based on the initial dosing schedule.
2. The cellular immune status of the body before and after AAV vector infusion, assessed using scRNA-seq analysis, was included as a safety endpoint.
3. The original inclusion criterion 8 (no FIX inhibitors; no past medical history of developing FIX inhibitors after 75 exposure days to FIX products; no clinical signs or symptoms of reduced response after infusion of FIX products) was removed and revised as the current inclusion criterion 7, as follows: participants

- who had received any recombinant or plasma-derived FIX products for at least 75 exposure days, had a negative FIX inhibitor test result, and had no clinical signs or symptoms suggestive of a reduced response to FIX products at screening; or participants with a history of FIX inhibitor positivity who had remained inhibitor-negative for at least 2 years after ITI and had received FIX products for at least 100 cumulative exposure days during the inhibitor-negative period (participants whose previous inhibitor positivity was not treated with ITI or whose inhibitors recurred after successful ITI were considered ineligible).
4. Exclusion criterion 10 (intolerable anaphylaxis to prior treatment with FIX or nephrotic syndrome during prior treatment with FIX) was added.
  5. The original exclusion criterion 11 (participants with a history of life-threatening bleeding, such as intracranial hemorrhage, including those with gastric ulcers posing a risk of severe gastrointestinal bleeding) was deleted and revised as the current exclusion criterion 12, as follows: life-threatening bleeding, such as intracranial hemorrhage within 2 years before enrollment, sequelae after previous intracranial hemorrhage, severe hemorrhage or potential causes of gastrointestinal bleeding—including, but not limited to, gastrointestinal ulcers.
  6. Bone mineral density was included as a safety indicator.

### Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

### Data availability

Deidentified individual participant data are available in the text, tables and figures. The detailed trial protocol and statistical analysis plan are available in the Supplementary Information.

The single-cell sequencing data generated in this study have been deposited in the Genome Sequence Archive (GSA) at the National Genomics Data Center (<https://ngdc.cncb.ac.cn/gsa-human/browse/>) under accession number **HRA017585**. Requests for more information on the trial should be directed to the corresponding author, L.Z., and will be responded to within 120 days. Source data are provided with this paper.

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### Author contributions

L.Z., R.Y., Q.H. and X.X. conceived and designed the clinical study. F.X., M.J. and B.X. executed the research, collected and interpreted data, and drafted the original paper. A.Z., Y.W., W.L., A.L., X.D., Y.C., X.L., R.F., T.S. and W.G. executed the research, collected data and reviewed the paper. X.P., X.W., W.J., J.Z., Z.D. and C.Y. were involved in data interpretation and critically reviewed the paper. All authors (except the employees of Belief Biomed; X.W., W.J., J.Z., Z.D., C.Y. and X.X.) had full access to the raw data, and all authors had final responsibility for the decision to submit the work for publication. All authors reviewed and approved the final version of the paper.

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### Competing interests

Belief Biomed provided the research drug and was involved in the study design. Shanghai Couragen Medical Laboratory Co., Ltd. serves as a central laboratory for anti-AAV antibody and vector shedding testing. X.W., W.J., J.Z., Z.D., C.Y. and X.X. are employees of Belief Biomed. The other authors declare no competing interests.

### Additional information

**Extended data** is available for this paper at <https://doi.org/10.1038/s41591-026-04636-8>.

**Supplementary information** The online version contains supplementary material available at <https://doi.org/10.1038/s41591-026-04636-8>.

**Correspondence and requests for materials** should be addressed to Xiao Xiao, Renchi Yang, Qun Hu or Lei Zhang.

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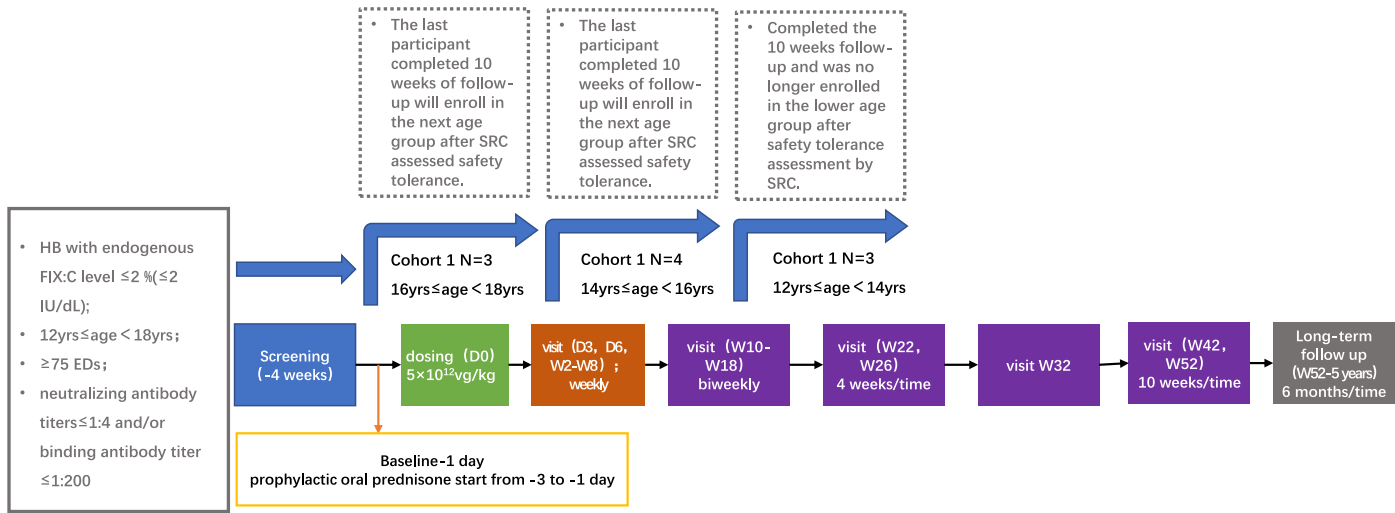
**Extended Data Table 1 | Bleeding events in phase 1 study**

| Participant with bleeding episodes | Bleeding time (after treatment, weeks) | FIX:C at bleeding event (or pre- and post-visit viewpoint data)                                       | Summary of the situation                  | Cause analysis       | Treatment measures                                        | FIX level outcome                                                                                                                    |
|------------------------------------|----------------------------------------|-------------------------------------------------------------------------------------------------------|-------------------------------------------|----------------------|-----------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------|
| 1                                  | W103                                   | W78 FIX activity was 9.8IU/dL                                                                         | Bleeding from gastrointestinal hemorrhage | Spontaneous bleeding | FIX 3000IU 1 time dose as needed                          | FIX activity 12.8IU/dL at W104;                                                                                                      |
| 7                                  | During W45                             | W42 FIX activity 21.6 IU/dL before bleeding; And W46 FIX activity 8.6 IU/dL, W50 FIX activity 4 IU/dL | left back                                 | Bleeding from trauma | Observation                                               | After prednisone treatment from W52, FIX activity was slightly higher, and W56 FIX activity 25.5 IU/dL. W78 FIX activity 12.9 IU/dL. |
|                                    | During W46                             |                                                                                                       | left inguinal region                      | Bleeding from trauma | Use FIX 3 time as needed, 1500, 2000, 1000IU respectively |                                                                                                                                      |
|                                    | During W47                             |                                                                                                       | left buttock                              | Spontaneous bleeding | Use FIX 1500IU 1 time as needed                           |                                                                                                                                      |
|                                    | During W49                             |                                                                                                       | left thumb                                | Spontaneous bleeding | Use FIX 1500IU 1 time as needed                           |                                                                                                                                      |
|                                    | During W61                             |                                                                                                       | oral mucosa                               | Bleeding from trauma | 1 time dose of PCC 1500IU as needed                       |                                                                                                                                      |

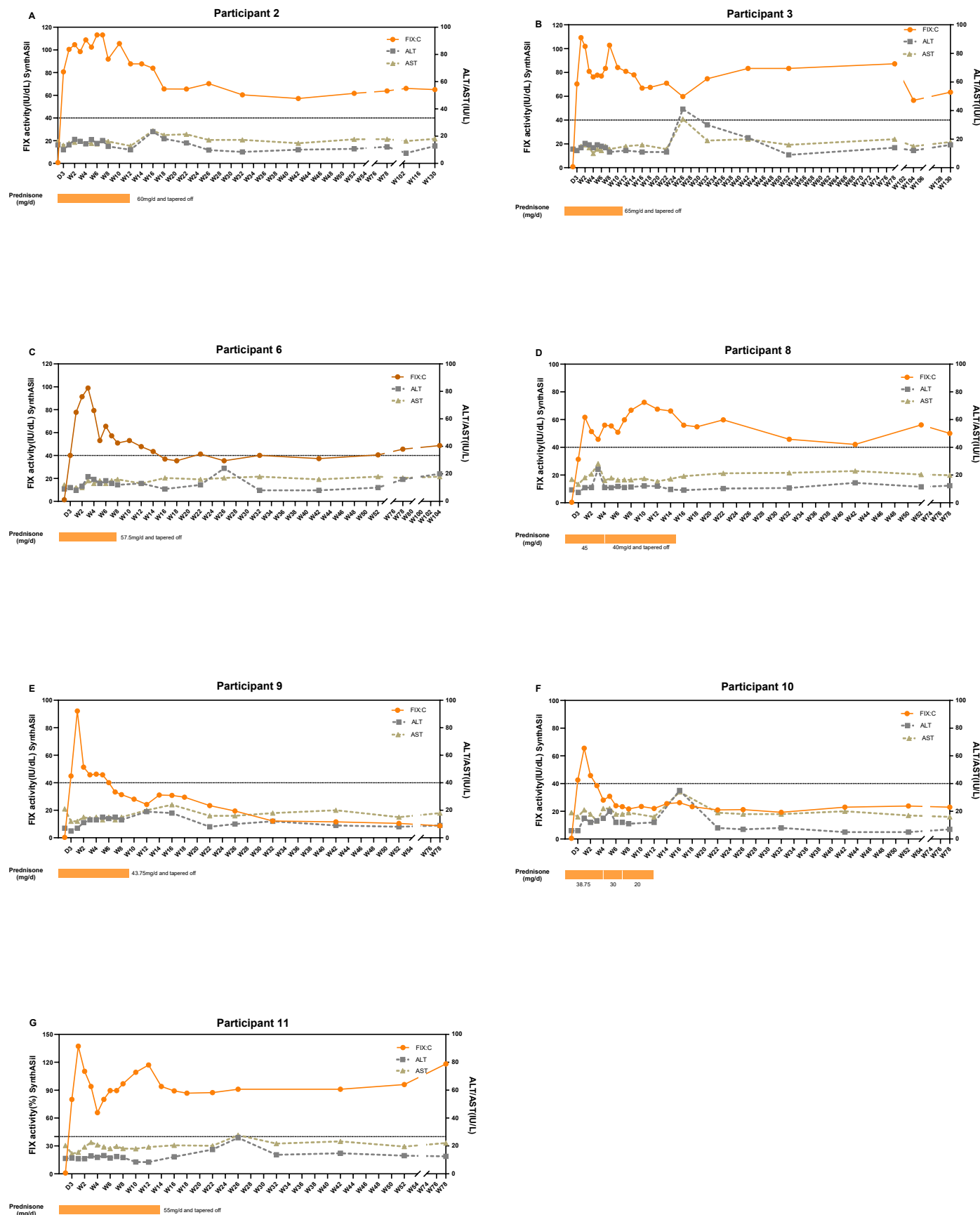
**Extended Data Table 2 | Comparison between adult phase 3 study and adolescent phase 1 study**

|                                     | Adult phase 3 study (n=26) | Adolescent phase 1 study (n=11) |
|-------------------------------------|----------------------------|---------------------------------|
| <b>Safety</b>                       |                            |                                 |
| AE (Grade 3 or above)               | 0                          | 5                               |
| SAE                                 | 0                          | 1                               |
| Liver transaminases                 | 7(26.9%)                   | 1(9%)                           |
| <b>Efficacy</b>                     |                            |                                 |
| Peak FIX:C <sup>ab</sup>            | 97.7 (SD 57.0) IU/dL       | 106.4 (SD 25.1) IU/dL           |
| FIX:C <sup>a</sup> at W52           | 55.1 (SD 35.9) IU/dL       | 41.8 (SD 30.1) IU/dL            |
| FIX:C <sup>a</sup> above 40% at W52 | 65.4%                      | 60%                             |
| ABR [mean(95%CI)]                   | 0.6 (0.2–2.0)              | 0.5 (0.1–2.9)                   |

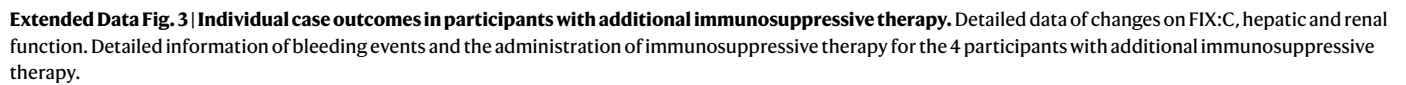
<sup>a</sup>Use One Stage SynthASil method <sup>b</sup>The peak value here was determined by selecting the highest value observed within the 52-week period for each individual participant and then performing the analysis on those selected values. It does not refer to the data from the week with the highest overall mean value across all participants.

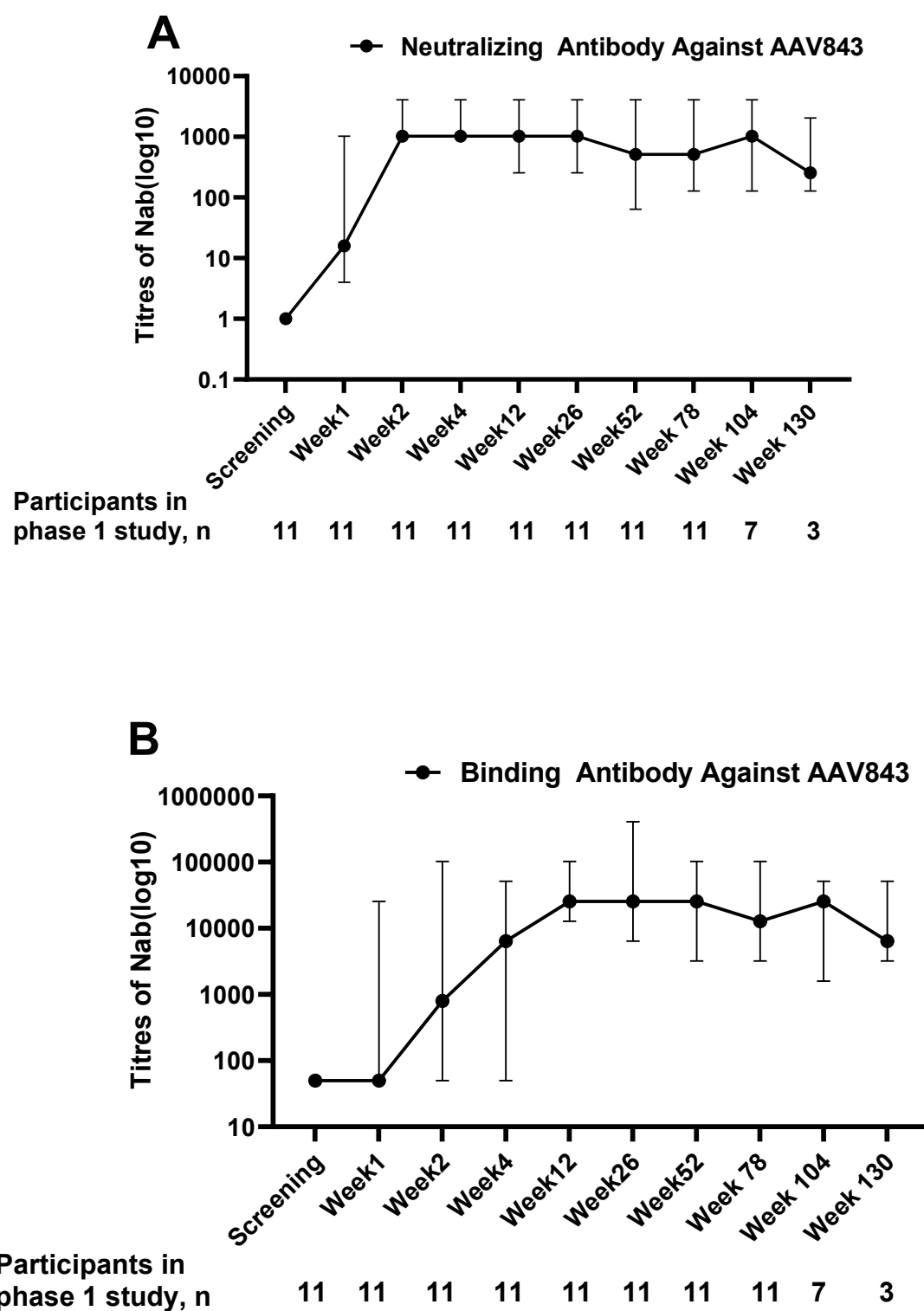


**Extended Data Fig. 1 | Diagram of study design.** Study design of phase 1 study.



**Extended Data Fig. 2 | Individual case outcomes in participants without additional immunosuppressive therapy.** Detailed data of changes on FIX:C, hepatic and renal function, and the tapering process of immunosuppressive therapy for the 7 participants without additional immunosuppressive therapy.





**Extended Data Fig. 4 | Neutralizing and Binding antibody Against AAV843. a.** Neutralizing antibody against AAV843 pre and post gene therapy. **b.** Binding antibody against AAV843 pre and post gene therapy. The dot depicting median, lower and upper bounds depicting minima and maxima.

Reporting Summary

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| <input type="checkbox"/>            | <input checked="" type="checkbox"/> A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly                                                                                                                                    |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> The statistical test(s) used AND whether they are one- or two-sided<br><i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>                                                               |
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| <input checked="" type="checkbox"/> | <input type="checkbox"/> For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes                                                                                                                                                |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i> ), indicating how they were calculated                                                                                                                                                          |

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

- |                 |                                                                                                                                                                                                                            |
|-----------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Data collection | The study used EDC (Electronic Data Capture) to collect data from the participants.                                                                                                                                        |
| Data analysis   | All statistical analyses were performed using SAS software 9.4. Figures were made using Graphpad Prism 9. scRNA analysis using Cell Ranger(v6.1.2) ,Seurat (v4), Harmony v1.0, SCP, CellChat v1.6.1, R v4.0.5, Python v3.8 |

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

- All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
  - A description of any restrictions on data availability
  - For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

De-identified individual participant data are available in the text, tables and figures of the article. The detailed trial protocol, statistical analysis plan is available in Protocol and Statistical Analysis Plan section of supplementary data.  
The single-cell sequencing data generated in this study have been deposited in the Genome Sequence Archive (GSA) at the National Genomics Data Center under

## Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

|                                                                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
|--------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Reporting on sex and gender                                        | We enroll male hemophilia B patients because female is rare as a x-linked recessive disease.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| Reporting on race, ethnicity, or other socially relevant groupings | We enroll Chinese hemophilia B patients for the study was a multicenter study in China.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| Population characteristics                                         | 12 ≤ age < 18 years                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| Recruitment                                                        | Patients could access the enrollment information through advertisements posted in the hospital, inquiries during the consultation, and multimedia platforms. Patients were screened and enrolled based on the related inclusion/exclusion criteria at baseline. Written informed consents were obtained from parents or legal guardians of the children before enrollment. Participants will not receive any compensation for participating in the study, and the sponsor will cover the cost of transportation and nutrition for participating in the biosample collection. Full inclusion/exclusion criteria were provided in the trial protocol. No self-selection bias and other biases. |
| Ethics oversight                                                   | This study was reviewed and approved by the Institutional Review Board of the Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Science and Peking Union Medical College, informed consent was obtained by each participant and their parents or legal guardians.                                                                                                                                                                                                                                                                                                                                                                                              |

Note that full information on the approval of the study protocol must also be provided in the manuscript.

## Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://nature.com/documents/nr-reporting-summary-flat.pdf)

## Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

|                 |                                                                                                                                                                                                                                                                                    |
|-----------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Sample size     | The sample size calculation for this study does not involve formal statistical hypotheses. With three age groups planned, a total of 9 participants (3+3+3) will be enrolled (the exact number of enrolled participants may be adjusted based on efficacy and safety assessments). |
| Data exclusions | No data were excluded from analyses.                                                                                                                                                                                                                                               |
| Replication     | Since antibodies against AAV develop after gene therapy, each patient currently has only one opportunity to receive the treatment. For any given participant, gene therapy cannot be administered repeatedly.                                                                      |
| Randomization   | The trial is non-randomized. Data analysis was performed between before and after gene therapy.                                                                                                                                                                                    |
| Blinding        | This trial was a single-arm trial. Blinding is not applicable for this study.                                                                                                                                                                                                      |

## Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

## Materials &amp; experimental systems

|                                     |                                                           |
|-------------------------------------|-----------------------------------------------------------|
| n/a                                 | Involved in the study                                     |
| <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> Antibodies            |
| <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> Eukaryotic cell lines |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Palaeontology and archaeology    |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Animals and other organisms      |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Clinical data         |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Dual use research of concern     |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Plants                           |

## Methods

|                                     |                                                 |
|-------------------------------------|-------------------------------------------------|
| n/a                                 | Involved in the study                           |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> ChIP-seq               |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Flow cytometry         |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> MRI-based neuroimaging |

## Antibodies

## Antibodies used

1. goat anti-human IgG H&L(HRP) (abcam,ab81202)
2. mAb002 anti-mouse IgG 30467 Belief Biomed
3. Anti-CD3 mAb CD3-2 Mabtech 3605-1-S
4. anti- IFN- $\gamma$  mAb 7- B6-1HRP?Mabtech?3420-9H?
- 5.CMV (CD4 andCD8) Mabtech 3619-1

## Validation

goat anti-human IgG H&L(HRP) was purchased from commercial vendor;  
 ELISpot Pro: Human IFN- $\gamma$  (Pro )Kit (MABTECH, Code: 3420-2HPT-10) was purchased from commercial vendor (<https://www.mabtech.com/products/elispot-pro-human-ifn-g-hrp-3420-2hpt>). The antibodies ?anti-CD3 mAb and anti- IFN- $\gamma$  mAb 7- B6-1HRP?were included in the ELISpot Pro kit;  
 CMV was purchased from commercial vendor <https://www.mabtech.com/products/peppool-cmv-cd4-and-cd8-human-3619-1-0>  
 All antibodies were validated in the methodological validation.

## Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

## Cell line source(s)

Homo sapiens, human

## Authentication

HEK293 cells were sourced from the American Type Culture Collection (ATCC), and CoA showed that the cells were free of adventitious agents contamination. The self built Master Cell Bank (MCB) and Working Cell Bank (WCB) have been verified to be free of endogenous and adventitious agents contamination.

## Mycoplasma contamination

Mycoplasma contamination negative.

Commonly misidentified lines  
(See [ICLAC](#) register)

*Name any commonly misidentified cell lines used in the study and provide a rationale for their use.*

## Clinical data

Policy information about [clinical studies](#)

All manuscripts should comply with the ICMJE [guidelines for publication of clinical research](#) and a completed [CONSORT checklist](#) must be included with all submissions.

## Clinical trial registration

NCT05709288

## Study protocol

The full trial protocol was provided in the Supplementary information.

## Data collection

The treatment and follow-up visit were performed at Institute of Hematology & Blood Diseases Hospital, Chinese Academy of Medical Sciences and Tongji Hospital affiliated to Tongji Medical College of Huazhong University of Science & Technology. The patients were enrolled between April, 2023 and April, 2024.

## Outcomes

The primary objective of this study was to assess the safety of BBM-H901 in adolescent participants. The secondary objective was to assess the pharmacokinetic characteristics, and efficacy of BBM-H901.  
 Safety assessments included incidence of DLT within 10 weeks post-infusion, incidence of adverse events and severe adverse events, liver function after infusion.  
 Pharmacokinetic characteristics assessments included the activity of Padua-FIX and vector shedding within 52 weeks post-infusion.  
 Efficacy assessments included annualized bleeding rates (ABR), FIX activity, usage of FIX products (time and dosage) and number of target joints after BBM-H901 infusion.

## Seed stocks

Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.

## Novel plant genotypes

Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.

## Authentication

Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosaicism, off-target gene editing) were examined.