Article:
Chowdary, P., Shapiro, S., Makris, M. orcid.org/0000-0001-7622-7939 et al. (15 more authors) (2022) Phase 1–2 trial of AAVS3 gene therapy in patients with hemophilia B. New England Journal of Medicine, 387 (3). pp. 237-247. ISSN 0028-4793

https://doi.org/10.1056/nejmoa2119913

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Chowdary P, Shapiro S, Makris M et al. Phase 1/2 Trial of a Novel AAVS3 Gene Therapy in Patients with Hemophilia B

Contents

Members of trial management group

- Dr. Pratima Chowdary (Chief Investigator and Chair of TMG), Royal Free Hospital
- Mark Phillips, Trial Manager, Royal Free Hospital
- Jun Pie, Study Nurse, Royal Free Hospital
- Michelle Quaye, Regulatory Manager, UCL Joint Research Office
- Jolanda Neele, Project Director, Syneos Health
- Ana Costa Santos, Project Manager, Syneos Health
- Daniel Mazzolenis, VP Medical Affairs, Medical Monitor, Syneos Health
- Zohra Arab Lead CRA, Syneos Health

In addition to the Sponsor and CRO team, all PI's are part of the TMG:

- Susie Shapiro, Principal Investigator, Oxford
- Ulrike Reiss, Principal Investigator, St Jude Children's Research Hospital
- Jennifer Larkin, Study Coordinator, St Jude Children's Research Hospital
- Niamh O'Connell, Principal Investigator, St James's Hospital
- Sara Boyce, Principal Investigator, Southampton General
- Gerard Dolan, Principal Investigator, St Thomas' Hospital
- Flora Peyvandi, Principal Investigator, University of Milan
- Gillian Evans, Principal Investigator, Kent and Canterbury Hospital
- Sarah Mangles, Principal Investigator, Hampshire Hospitals NHS
- Michael Makris, Principal Investigator, Royal Hallamshire Hospital
- Katherine Talks, Principal Investigator, Newcastle Hospitals NHS

AAVS3 Vector Description

Vector Structure: The AAVS3 capsid consists of a VP1u portion from AAV8 with the remainder (i.e. VP2u and VP3) from AAV3B. The amino acid sequence of the capsid is provided below. Transduction in primary human hepatocyte cultures showed significantly higher hepatic transduction compared to AAV8, AAV5 and AAVrh10. Compared to capsids used in previous clinical trials, the fold increase in transgene expression (average of 4 donor hepatocyte lots) from AAVS3 was 4.2-fold, 12.8-fold, and 5-fold higher compared to AAV8, AAV5 and rh10, respectively. In addition, we have assessed in vivo transduction in a humanized liver mouse model (FRG model) and have shown 24% of human hepatocytes expressing a reporter gene after AAVS3 injection versus 4.3% of human cells after AAV8 transduction.[1-3]

MAADGYLPDWLEDNLSEGIREWWALKPGAPKPKANQQKQDDGRGLVLPGYKYLGPFNGLDKGEPVNAADA
AALEHDKAYDQQLQAGDNPYLRYNHADAEFQERLQEDTSFGGNLGRAVFQAKKRVLEPLGLVEEGAKTAP
GKKRPVDQSPQEPDSSSGVGKSGKQPARKRLNFGQTGDSESVPDPQPLGEPPAAPTSLGSNTMASGGGAP
MADNNEGADGVGNSSGNWHCDSQWLGDRVITTSTRTWALPTYNNHLYKQISSQSGASNDNHYFGYSTPWG
YFDFNRFHCHFSPRDWQRLINNNWGFRPKKLSFKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTDSEYQL
PYVLGSAHQGCLPPFPADVFMVPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFQFSYTFEDVPF
HSSYAHSQSLDRLMNPLIDQYLYYLNRTQGTTSGTTNQSRLLFSQAGPQSMSLQARNWLPGPCYRQQRLS
KTANDNNNSNFPWTAASKYHLNGRDSLVNPGPAMASHKDDEEKFFPMHGNLIFGKEGTTASNAELDNVMI
TDEEEIRTTNPVATEQYGTVANNLQSSNTAPTTRTVNDQGALPGMVWQDRDVYLQGPIWAKIPHTDGHFH
PSPLMGGFGLKHPPPQIMIKNTPVPANPPTTFSPAKFASFITQYSTGQVSVEIEWELQKENSKRWNPEIQ
YTSNYNKSVNVDFTVDTNGVYSEPRPIGTRYLTRNL

Vector Genome: The FRE1 promoter is a transcriptional control unit that consists of truncated elements of the liver-specific human alpha-1 antitrypsin (hAAT) promoter and human apolipoprotein E (ApoE) hepatic control region enhancer. The FIX Padua variant-encoding expression cassette contains the wildtype sequence of hFIX in exon 1 and the 5' portion of exon 2. These exons flank a truncated version of the intron natively present in that position, while the 3' portion of exon 2 and the remaining exons are codon optimized and devoid of CpG dinucleotides. The structure of the expression cassette is provided in Figure S1.

Supplementary Methods

Vector Manufacturing: FLT180a Ph1/2 study manufacturing was performed, in full compliance with cGMP, by Children's GMP, LLC, a company owned by St. Jude Children's Research Hospital.

The vector was produced in an adherent mammalian cell production system. Human embryonic kidney (HEK) 293T cells were thawed from a working cell bank and the culture expanded / propagated in a series of culture vessels of increasing number and / or volume. The vector was then generated by transient cotransfection of Freeline's proprietary 1st generation two-plasmid split-packaging system, with PEIpro® transfection reagent, in a cell stack platform.

Cells were subsequently lysed with ammonium sulphate and the crude lysate harvested. The lysate was filtered through 3 polypropylene filters of decreasing pore size in series and purified by Hydrophobic Interaction Chromatography (HIC). The HIC product was then concentrated by Tangential Flow Filtration and then treated with an endonuclease to digest unencapsulated DNA sequences. At this intermediate stage the product was stored at ≤-60°C to allow pooling of several runs into a single final purification and formulation process.

The batch used in this study pooled 6 runs, each comprising 47 to 52, 10 Stack CELLSTACK®s. The thawed pool was then filtered, and the vector isolated by affinity chromatography (AVB Sepharose High Performance), before final concentration by Ultra Filtration / Diafiltration (to 3.5-6x10^{12} vg/mL) into Phosphate Buffered Saline (PBS), filtration, and addition of a recombinant Human Albumin (rHA) formulation containing Polysorbate 80, resulting in 0.25% w/v rHA.

The drug substance was then transferred to +2-8°C for short term storage ahead of final filling into drug product, to allow an accurate genomic titer to be determined.

The drug substance was then diluted with PBS/0.25% rHA before final filtration through a sterilizing filter (0.2µm) and filling into glass vials with a rubber stopper and aluminum seal. Final storage of the drug product was at ≤-60°C.

The final formulation consists of approximately 2 to 1, empty to full vector particles. However, this figure should be interpreted with caution because there is no standardized, validated method for calculating empty to full ratios.

Update to dose calculation: A new standard manufactured with a higher level of control was introduced to the vector genome titer assay in 2018. During characterization it was noted that reduced vector genome titers were observed with the new standard. Multiple measurements of the clinical drug product during validation of the vector genome titer assay led to the determination that prior nominal doses in the phase 1/2 clinical study (B-AMAZE) should be adjusted by a factor of 0.64 to generate an equivalent numerical dose of product measured with the validated assay. Doses reported in the manuscript are updated to reflect the revised assay. Note that the change to the dose is not reflected in the protocol as the study has ended and retroactive changes to the dose may have caused confusion among patients.

Vector titer: The test sample was evaluated for quantity of FLT180a vector gene by qPCR determination of the FRE1 promoter sequence integral to the FIX expression cassette. Prior to thermocycling, a heat denaturation step was performed. Amplification results in nascent double stranded DNA amplicons that

are detected with the fluorescent intercalator SYBR green monitoring the PCR reaction in real time. Known quantities of the FRE1 genetic material in the form of a linearised plasmid were serially diluted to create a standard of known copies of FRE1 and sample titre interpolated from the standard curve. Each vector titre was the result of three 10-fold serial dilutions of test sample, in 10mM Tris-HCI pH 8.0, from 1 in 1,000 to 1,000,000 plated in triplicate. Results for batch 17-089, used for all patients in the B-AMAZE study, are based on an average of 27 individual determinations performed during assay validation.

Neutralizing antibody (NAb) assay: All patients in this trial were screened by our FACS-based AAVS3 transduction inhibition assay (TIA) using a GFP reporter. After incubation with serially diluted test plasma sample, the percentage of cells with AAVS3-mediated GFP expression is measured by FACS. The screening titre cut-off was 1/100, which is also the minimum required dilution. At a 1/100 titre, a sample is still deemed NAb positive if the percentage of GFP-expressing cells is less than 77%. Therefore, >77% transduced cells at 1/100 titre represent a NAb negative result. Using this assay, we have found that the percent of AAVS3 NAb positive samples in a group of healthy donor plasma samples is >50%, which is similar to the 52% seroprevalence reported for AAV3B in a cohort aged >18.[4] However, the measured seroprevalence of AAVS3, as for other AAV capsid serotypes, is known to vary according to the nature of the sample population, sample size, age, and demography/ethnicity.

Infusion of FLT180a and cessation of FIX prophylaxis: Patients received FLT180a via a single IV infusion into a peripheral vein over a period of one hour. Patients remained at the study center for 12-24 hours until they were deemed fit for discharge by the investigator. Following treatment with FLT180a, patients who were on prophylactic therapy with FIX concentrates continued their usual dosing schedule. If a FIX activity of ≥3% was recorded, prophylaxis was paused and then discontinued if a second FIX activity of ≥3% was recorded within 72 hours.

FIX Activity: Factor IX activity was measured in the patient samples in the central laboratory using a one stage APTT based clotting FIX assay on an ACL TOP coagulometer (Werfen, Barcelona, Spain) calibrated with CRYOcheck® Reference plasma (Precision BioLogic, Dartmouth, NS, Canada), using SynthASil® aPTT reagent and HemosIL FIX deficient plasma (Werfen, Barcelona, Spain).

Changes to the Development Plan: The B-AMAZE study was paused due to the COVID-19 pandemic after 10 patients were enrolled and was subsequently terminated early on 20 October 2020 due to changes in the clinical development plan. The manufacturing process for FLT180a was updated, and a Phase 1/2 dose-confirmation study of FLT180a (B-LIEVE; NCT05164471) with a starting dose of $7.7x10^{11}$ vg/kg began recruitment in January 2022. As with B-AMAZE, B-LIEVE will incorporate dual immunosuppression with glucocorticoids and tacrolimus as prophylaxis for vector-related immune responses.

Statistical analysis: The final study results, as per the planned statistical analysis plan, are reported separately in the clinical study report, and the final study data was used for this article. The long-term follow-up study is ongoing, and preliminary data was used based on a cut-off date of 20 Sept 2021. Data from the two studies were pooled and descriptive statistics were used to summarize the prospectively observed longitudinal data. Analyses were produced ad hoc for this article and are considered exploratory.

Bleeding events and endogenous FIX consumption starting 15 days after FLT180a infusion were compared with retrospective data that were collected at screening from the 3-year period before study enrollment. The Full Analysis Set included all patients who received FLT180a and was the primary population for all analyses of safety, efficacy, and baseline characteristics. Given the dose modifications performed during

the trial, results were presented by dose levels rather than by cohort and given the early termination of the study before the targeted number of patients, no statistical testing was performed.

Supplementary Results

Vector shedding: Plasma, saliva, urine, stool, and semen samples for PCR of vector genome were taken in accordance with the schedule of assessments. Collections continued until three consecutive samples were negative. The median (range) time to unquantifiable results in weeks were as follows for the different matrices: plasma 3.140 (2.14, 5.29); saliva 1.715 (0.57, 2.43); semen 2.070 (0.57, 3.43); stool 3.785 (2.43, 6.14); urine 2.070 (0.57, 2.14).

Immunosuppression regimen by patient

- Patient 1 received a course of prednisolone starting at 60 mg daily on Day 44 and tapering to 10 mg before ending on Day 85.
- Patient 2 received a course of prednisolone starting at 60 mg daily on Day 42 and tapering to 5 mg before ending on Day 90.
- Patient 3 started a course of prednisolone at 60 mg daily on Day 28. He received doses between 40 and 60 mg through Day 112 with a brief interruption to treat increases in liver transaminases, before tapering to 5 mg and ending on Day 140. Patient 3 also received 8 mg daily of tacrolimus from Day 49-69 and 1 g daily IV methylprednisolone for Days 50-52.
- Patient 4 began a course of prednisolone with 70 mg daily on Day 29 and received doses between 70 and 80 mg through Day 79 with a brief interruption to treat increases in liver transaminases. His prednisolone dose was tapered to 2.5 mg by Day 154. On Day 155 he began another tapering course of prednisolone due to increases in liver transaminases from 40 mg to 2.5 mg through Day 196. Patient 4 began tacrolimus on Day 42, starting at 8 mg and tapering to 4 mg before stopping on Day 80. He had a second course of tacrolimus consisting of 10 mg daily from day 157-217. Patient 4 also received 1 g IV infusions of methylprednisolone on Days 37-40 and Days 43-45.

- Patient 5 had a tapering course of prednisolone from 80 mg daily down to 5 mg daily from Days 21-101. He also received tacrolimus at doses of 8 to 12 mg from Day 32 to Day 59 and 1g of IV methylprednisolone on Days 32-34.
- Patient 6 began prednisolone at 90 mg daily on Day 22 and tapered to 2 mg before ending on Day 142, with a brief interruption to treat increases in liver transaminases. He also received a tapering course of tacrolimus from 5 to 2 mg daily from Days 27 to 73 and 500 mg daily IV methylprednisolone from Days 27-30.
- Patient 7 received a tapering course of prednisolone from 100 mg daily to 5 mg daily over Days 22-101 with a brief interruption to treat increases in liver transaminases. He had a second tapering course of prednisolone from 100 mg daily to 5 mg daily from Days 117-151. Patient 7 also had two courses of tacrolimus: one course was 14-18 mg daily on Days 22-87 and the other was 9-18 mg daily on Days 114-164. He received 1 g daily IV methylprednisolone on Days 35-37 and Days 114-116.
- Patient 8 had two tapering courses of prednisolone: one course was between 80 mg daily to 5 mg daily on Days 22-98 and the second course was from 85 mg daily to 5 mg daily over Days 190-253. He had two courses of tacrolimus. The first was a taper from 13 mg to 3 mg over Days 22-140 and the second was a taper from 6 mg to 1 mg daily on Days 190-288.
- Patient 9 had two tapering courses of prednisolone: one course was from 80 mg daily to 5 mg daily on Days 22-93 and the second course was from 80 mg daily to 3 mg daily over Days 182-273. He had two courses of tacrolimus. The first included doses between 4 and 18 mg daily and ran during Days 22-146 and the second was a taper from 6 mg to 1 mg daily on Days 182-246.
- Patient 10 had two tapering courses of prednisolone: one course was from 80 mg daily to 5 mg daily on Days 22-91 and the second course was from 70 mg daily to 2.5 mg daily over Days 175-264. He

had two courses of tacrolimus. The first was a taper from 16 to 4 mg daily and ran during Days 22-147 and the second was a taper from 6 mg to 1 mg daily on Days 175-242.

Narrative of SAEs in Patient 6

The patient presented to the treating team in study Week 10 (Day 73) with increasing dyspepsia of a couple of weeks duration for which he had been taking ranitidine 150 mg twice a day along with Gaviscon® with some relief. He was admitted with worsening symptoms over a day and at admission reported an epigastric discomfort that was sharp, non-radiating, and not affected by food or drink. However, the severity of symptoms had decreased by the time of admission. At admission, his troponin was slightly increased at 17.9 ng/L (ULN 14) along with an increased amylase at 261 IU/L (ULN 100). ECG was performed and did not demonstrate any changes from baseline and there was no evidence of myocardial ischemia. Troponin levels were monitored for one day with no change. The patient was discharged the next day after being started on esomeprazole 40 mg once daily. The troponin elevation was considered non-specific, not an uncommon scenario,[5] and potentially related to prednisolone-induced gastritis.

Five days after being discharged, the patient was admitted again with temp of 38 degrees C, tachycardia of 122 beats/min, saturation of 89% on pulse oximetry and respiratory rate of 26 breaths/min, and rigors for the last couple of days. He was hypotensive with elevated C-reactive protein of around 205 mg/L (ULN 5) and increased erythrocyte sedimentation rate. He was hypoxic, requiring oxygen on the ward, and hypotensive with tachycardia. He was on a decreasing dose of steroids at 5 mg twice a day. He received Tazocin®, clarithromycin, and IV fluids and was reviewed by the critical care team. His chest X-ray was abnormal (differential diagnosis: infectious infiltrate vs. pulmonary edema), with negative

blood cultures and creatinine increased to 140 µmol/L. Oxygen was stopped four days after admission and he was discharged around a week after admission.

Figure S1. Schematic diagram of the FLT180a expression cassette. The FLT180a recombinant vector genome includes ITRs at each end, which are the only AAV DNA sequences included in the vector and are required to enable replication and packaging of the expression cassette during production. The expression construct contains a liver-specific promoter (FRE1) and a partially codon-optimized cDNA encoding FIX with the 'Padua' mutation (replacement of arginine with leucine at residue 338). The FIX-Padua cDNA is interrupted between exons 1 and 2 by a truncated version of the corresponding native intron, which enhances FIX expression.

Abbreviations: AAV=adeno-associated virus; BgPa=bovine growth factor polyadenylation signal; E1=exon 1; E2-8=exons 2 to 8; FIX=Factor IX; FRE1=human liver-specific promoter; ITR=inverted terminal repeat; TI=truncated intron 1.

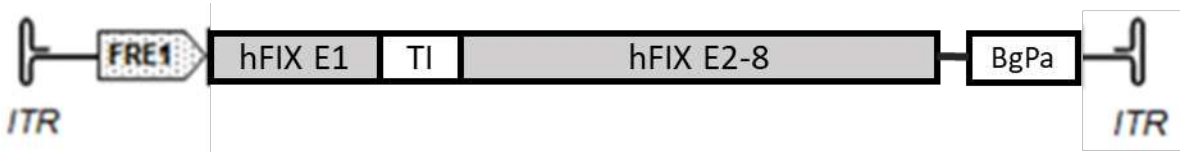

Figure S2. Patient disposition in B-AMAZE. There were 22 screenings for participation in B-AMAZE, but five patients were screened twice to meet requirements of the screening window. Therefore, 17 patients were assessed for eligibility. Among the five patients screened twice, four went on to receive treatment with FLT180a. The reasons the remaining seven patients were not treated with FLT180a are provided. One patient had both a lack of a negative AAVS3 antibody test and a presence or history of a FIX inhibitor and is included in both categories indicated with an asterisk. All treated patients completed the initial 26-week period of B-AMAZE and enrolled in the long-term follow-up study.

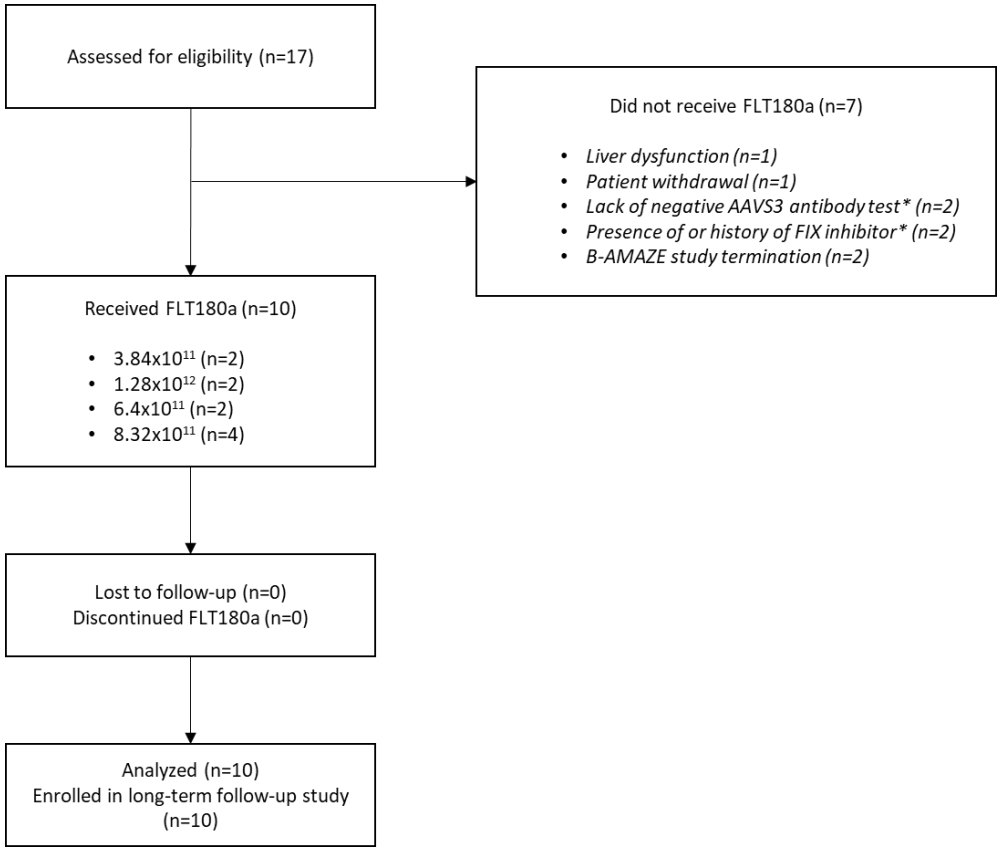

Table S1. Treatment emergent adverse events in B-AMAZE and long-term follow-up by dose level

Adverse Event Preferred Term	3.84 x 10^{11} vg/kg (n=2)		1.28 x 10^{12} vg/kg (n=2)		6.4 x 10^{11} vg/kg (n=2)		8.32 x 10^{11} vg/kg (n=4)	
	Incidence	Number of Events	Incidence	Number of Events	Incidence	Number of Events	Incidence	Number of Events
Transaminases increased[a]			2 (100%)	9	2 (100%)	3	4 (100%)	10
Abdominal distension							1 (25%)	3
Abdominal pain			1 (50%)	1	1 (50%)	1	1 (25%)	1
Abdominal pain upper			1 (50%)	1			1 (25%)	1
Alopecia							1 (25%)	1
Amylase increased			1 (50%)	1				
Anaemia	1 (50%)	1						
Appendicitis					1 (50%)	1		
Arteriovenous fistula thrombosis			1 (50%)	1				
Arthralgia	1 (50%)	1	1 (50%)	1	1 (50%)	2	3 (75%)	6
Arthropathy							1 (25%)	1
Back pain							2 (50%)	2
Bladder pain							1 (25%)	2
Blood bilirubin increased	1 (50%)	1						
Blood creatine phosphokinase increased							2 (50%)	2
Blood glucose increased							1 (25%)	2
Blood lactate dehydrogenase increased							2 (50%)	2
Burning sensation			1 (50%)	1				
Cardiac flutter			1 (50%)	1				
Chapped lips							1 (25%)	1
Chest discomfort							1 (25%)	1
Coagulation factor IX level decreased					1 (50%)	1		
Coagulation factor IX level increased			2 (100%)	2				
Constipation							1 (25%)	2
Cough							1 (25%)	1

Adverse Event Preferred Term	3.84 x 10^{11} vg/kg (n=2)		1.28 x 10^{12} vg/kg (n=2)		6.4 x 10^{11} vg/kg (n=2)		8.32 x 10^{11} vg/kg (n=4)	
	Incidence	Number of Events	Incidence	Number of Events	Incidence	Number of Events	Incidence	Number of Events
COVID-19							1 (25%)	1
Cushingoid					2 (100%)	2		
Decreased appetite							2 (50%)	2
Depression							1 (25%)	1
Dermatitis acneiform					1 (50%)	1		
Developmental hip dysplasia					1 (50%)	1		
Diarrhea	1 (50%)	1	1 (50%)	2	1 (50%)	3	3 (75%)	15
Dizziness	1 (50%)	1					1 (25%)	1
Drug level increased							1 (25%)	1
Dry skin							1 (25%)	1
Dyspepsia	1 (50%)	1	1 (50%)	2	1 (50%)	1	1 (25%)	1
Dyspnoea			1 (50%)	1				
Dysuria							1 (25%)	1
Ear pain							2 (50%)	2
Eczema							1 (25%)	1
Erectile dysfunction							1 (25%)	1
Eructation							1 (25%)	2
Erythema							1 (25%)	2
Fall					1 (50%)	1		
Fatigue			1 (50%)	1	1 (50%)	1	3 (75%)	6
Feeling hot			1 (50%)	1			1 (25%)	1
Folliculitis	1 (50%)	1	1 (50%)	1	1 (50%)	1	1 (25%)	1
Gallbladder polyp	1 (50%)	1						
Gastroesophageal reflux disease					1 (50%)	1	1 (25%)	1
Gingival pain							1 (25%)	1
Haemorrhoids	1 (50%)	1						

Adverse Event Preferred Term	3.84 x 10^{11} vg/kg (n=2)		1.28 x 10^{12} vg/kg (n=2)		6.4 x 10^{11} vg/kg (n=2)		8.32 x 10^{11} vg/kg (n=4)	
	Incidence	Number of Events	Incidence	Number of Events	Incidence	Number of Events	Incidence	Number of Events
Hand fracture							1 (25%)	1
Headache	1 (50%)	1	1 (50%)	2			4 (100%)	15
Hordeolum	1 (50%)	1						
Hypoesthesia	1 (50%)	1						
Hypomagnesaemia							1 (25%)	1
Idiopathic urticaria	1 (50%)	1						
Increased appetite							1 (25%)	1
Influenza like illness					1 (50%)	2	1 (25%)	1
Insomnia					1 (50%)	1	2 (50%)	4
Joint effusion							1 (25%)	1
Joint stiffness					2 (100%)	3		
Ligament sprain	1 (50%)	1					1 (25%)	1
Lower respiratory tract infection							1 (25%)	1
Malaise			1 (50%)	1				
Muscle spasms			1 (50%)	2			3 (75%)	5
Musculoskeletal chest pain					1 (50%)	1		
Musculoskeletal pain							1 (25%)	1
Musculoskeletal stiffness					1 (50%)	1		
Myalgia							3 (75%)	5
Nasal congestion	1 (50%)	1						
Nasopharyngitis	2 (100%)	7					2 (50%)	3
Nausea	1 (50%)	1					3 (75%)	8
Oropharyngeal pain			2 (100%)	2			2 (50%)	4
Pain					1 (50%)	1	1 (25%)	1
Pain in extremity			1 (50%)	1	1 (50%)	1	1 (25%)	1
Paraesthesia							2 (50%)	3

Adverse Event Preferred Term	3.84 x 10^{11} vg/kg (n=2)		1.28 x 10^{12} vg/kg (n=2)		6.4 x 10^{11} vg/kg (n=2)		8.32 x 10^{11} vg/kg (n=4)	
	Incidence	Number of Events	Incidence	Number of Events	Incidence	Number of Events	Incidence	Number of Events
Peripheral swelling			1 (50%)	1				
Petechiae							1 (25%)	2
Pollakiuria							1 (25%)	1
Post procedural discomfort							1 (25%)	1
Post-traumatic pain					1 (50%)	1		
Productive cough	1 (50%)	1					2 (50%)	2
Prostatitis	1 (50%)	1						
Pruritus			1 (50%)	1				
Pulmonary sepsis			1 (50%)	1				
Pyrexia					1 (50%)	1	1 (25%)	1
Radius fracture					1 (50%)	1		
Rash					1 (50%)	1	2 (50%)	2
Rash maculo-papular	1 (50%)	1	1 (50%)	1	1 (50%)	1		
Rhinitis							2 (50%)	2
Rosacea	1 (50%)	1						
Sciatica			1 (50%)	1				
Seasonal allergy			1 (50%)	1	1 (50%)	1	1 (25%)	2
Seborrheic dermatitis	1 (50%)	1						
Sinusitis			2 (100%)	2				
Skin disorder	1 (50%)	1						
Sleep disorder					1 (50%)	2	1 (25%)	1
Somnolence			1 (50%)	1				
Tonsillitis							1 (25%)	1
Tooth fracture					1 (50%)	1		
Toothache							1 (25%)	1
Toxicity to various agents							1 (25%)	2

Adverse Event Preferred Term	3.84 x 10^{11} vg/kg (n=2)		1.28 x 10^{12} vg/kg (n=2)		6.4 x 10^{11} vg/kg (n=2)		8.32 x 10^{11} vg/kg (n=4)	
	Incidence	Number of Events	Incidence	Number of Events	Incidence	Number of Events	Incidence	Number of Events
Tremor			1 (50%)	1			4 (100%)	6
Troponin increased			1 (50%)	1				
Upper respiratory tract infection					1 (50%)	2	1 (25%)	1
Urinary incontinence			1 (50%)	1				
Urinary retention							1 (25%)	1
Urticaria							1 (25%)	1
Visual impairment							1 (25%)	1
Vomiting							2 (50%)	6

Coded data as of 20 Sept 2021 data cut
Adverse events are presented with transaminases increased listed first, then alphabetically.
The duration of follow up varies between dose levels.

a. This category includes events of transaminases increased, alanine aminotransferase increased, and aspartate aminotransferase increased

Table S2. Serious adverse events in B-AMAZE and long-term follow-up

Patient Number	Preferred Term	Reported Term	Severity	Start Day / End Day	Causality (relationship to FLT180a or immunosuppression)
3	Alanine aminotransferase increased	Increased ALT	Grade 3	50 / 52	Related to FLT180a
4	Alanine aminotransferase increased	Raised ALT	Grade 2	37 / 63	Related to FLT180a
4	Coagulation factor IX level decreased	Drop in Factor IX	Grade 2	154 / Not resolved	Related to FLT180a
4	Alanine aminotransferase increased	Rise in ALT	Grade 2	154 / 157	Related to FLT180a
4	Appendicitis	Acute appendicitis	Grade 3	381 / 384	Not Related
5	Alanine aminotransferase increased	Sudden increase in ALT	Mild	28 / 59	Related to FLT180a
6	Alanine aminotransferase increased	Raised ALT	Grade 1	27 / 30	Related to FLT180a
6	Abdominal pain upper	Epigastric Pain	Grade 1	73 / 74	Related to Prednisolone or Prednisone
6	Troponin increased	Raised Troponin	Grade 1	73 / 74	Related to Prednisolone or Prednisone
6	Amylase increased	Raised Amylase	Grade 2	73 / 74	Related to Prednisolone or Prednisone
6	Pulmonary sepsis	Chest Sepsis	Grade 3	79 / 85	Related to FLT180a
6	Arteriovenous fistula thrombosis	Right arm AV fistula thrombosis	Grade 2	243 / 246	Related to FLT180a
7	Transaminases increased	Transaminitis	Grade 2	35 / 44	Related to FLT180a

Patient Number	Preferred Term	Reported Term	Severity	Start Day / End Day	Causality (relationship to FLT180a or immunosuppression)
7	Transaminases increased	Transaminitis	Grade 2	114 / 123	Related to FLT180a
9	Toxicity to various agents	Tacrolimus Toxicity	Grade 2	102 / 103	Related to Tacrolimus
9	Transaminases increased	Transaminitis	Mild	182 / 189	Related to FLT180a
10	Transaminases increased	Transaminitis (early signs)	Mild	175 / 183	Related to FLT180a

ALT denotes alanine aminotransferase
Coded data as of 20 Sept 2021 data cut

Table S3. Adverse events related to immune suppression

Patient	Adverse Event Preferred Term	CTC Grade / Severity	Prednisolone	Methlyprednisolone	Tacrolimus
1	Blood bilirubin increased	Grade 1	Related	N/A	N/A
2	Rash maculo-papular	Grade 1	Related	N/A	N/A
3	Coagulation factor IX level increased	Grade 1	Related	N/A	N/A
3	Folliculitis	Grade 1	Related	N/A	Not Related
4	Insomnia	Grade 1	Related	Not Related	Not Related
4	Dermatitis acneiform	Grade 1	Related	Not Related	Not Related
4	Folliculitis	Grade 1	Related	Not Related	Not Related
4	Dyspepsia	Grade 1	Related	Not Related	Not Related
4	Diarrhea	Grade 2	Not Related	Not Related	Related
4	Diarrhea	Grade 1	Not Related	Not Related	Related
4	Cushingoid	Grade 1	Related	Not Related	Not Related
4	Fatigue	Grade 1	Related	Not Related	Not Related
4	Arthralgia	Grade 1	Related	Not Related	Not Related
4	Diarrhea	Grade 1	Not Related	Not Related	Related
5	Sleep disorder	Grade 1	Related	N/A	N/A
5	Sleep disorder	Grade 1	Related	Related	Not Related
5	Rash maculo-papular	Mild	Related	Related	Not Related
5	Cushingoid	Grade 1	Related	N/A	N/A
6	Amylase increased	Grade 2	Related	N/A	Not Related
6	Troponin increased	Grade 1	Related	N/A	Not Related
6	Abdominal pain upper	Grade 1	Related	N/A	Not Related
7	Tremor	Grade 2	Not Related	Not Related	Related
8	Nasopharyngitis	Grade 1	Related	N/A	Not Related
8	Lower respiratory tract infection	Grade 2	Related	N/A	Not Related
8	Blood glucose increased	Grade 1	Related	N/A	Not Related
8	Headache	Grade 1	Not Related	N/A	Related
8	Headache	Grade 2	Not Related	N/A	Related

Patient	Adverse Event Preferred Term	CTC Grade / Severity	Prednisolone	Methlyprednisolone	Tacrolimus
8	Tremor	Grade 1	Related	N/A	Not Related
8	Blood glucose increased	Grade 2	Related	N/A	Not Related
9	Diarrhea	Mild	Not Related	N/A	Related
9	Nausea	Mild	Not Related	N/A	Related
9	Hypomagnesaemia	Mild	Not Related	N/A	Related
9	Tremor	Grade 1	Not Related	N/A	Related
9	Diarrhea	Mild	Not Related	N/A	Related
9	Fatigue	Mild	Related	N/A	Not Related
9	Depression	Grade 1	Related	N/A	Related
9	Insomnia	Grade 1	Related	N/A	Not Related
9	Drug level increased	Mild	Not Related	N/A	Related
9	Diarrhea	Grade 1	Not Related	N/A	Related
9	Eructation	Mild	Not Related	N/A	Related
9	Abdominal pain	Mild	Not Related	N/A	Related
9	Feeling hot	Mild	Not Related	N/A	Related
9	Toxicity to various agents	Mild	N/A	N/A	Related
9	Headache	Mild	N/A	N/A	Related
9	Toxicity to various agents	Grade 2	N/A	N/A	Related
9	Alopecia	Mild	N/A	N/A	Related
9	Decreased appetite	Mild	N/A	N/A	Related
9	Paresthesia	Mild	N/A	N/A	Related
9	Fatigue	Moderate	Related	N/A	Related
9	Insomnia	Mild	Related	N/A	Related
9	Tremor	Mild	Not Related	N/A	Related
9	Nausea	Mild	Not Related	N/A	Related
9	Eructation	Mild	Not Related	N/A	Related
9	Diarrhea	Mild	Not Related	N/A	Related
9	Headache	Mild	Not Related	N/A	Related
9	Insomnia	Mild	Related	N/A	N/A

Patient	Adverse Event Preferred Term	CTC Grade / Severity	Prednisolone	Methlyprednisolone	Tacrolimus
10	Myalgia	Grade 1	Related	N/A	Not Related
10	Paresthesia	Grade 1	Not Related	N/A	Related
10	Tremor	Grade 1	Not Related	N/A	Related
10	Increased appetite	Grade 1	Related	N/A	Not Related
10	Diarrhea	Grade 1	Not Related	N/A	Related
10	Folliculitis	Grade 1	Related	N/A	Not Related
10	Paresthesia	Grade 1	Not Related	N/A	Related
10	Diarrhea	Grade 1	Not Related	N/A	Related
10	Muscle spasms	Grade 1	Related	N/A	Not Related
10	Musculoskeletal pain	Grade 1	Related	N/A	Not Related
10	Tremor	Grade 1	Not Related	N/A	Related
10	Insomnia	Grade 1	Related	N/A	Not Related

Coded data as of 20 Sept 2021 data cut.

N/A in the table indicates the patient was not receiving the agent at the time of the event.

Events may be listed multiple times if they occurred more than once.

Table S4. Representativeness of the study participants to the hemophilia B population

Disease	Severe and moderately severe hemophilia B
Special considerations related to:	
Sex and gender	Hemophilia B is an X-linked recessive disease and is therefore much more common in males, especially the severe and moderately severe phenotypes.[6-8]
Age	Severe/moderately severe hemophilia B is an inherited condition present at birth that affects patients for life. The age distribution of patients with severe hemophilia B varies by region as mortality risk increases with less access to care.[6,8]
Race or ethnic group	There is no known difference in the prevalence of severe/moderately severe hemophilia B by race or ethnicity.[8]
Geography	Hemophilia B is a global disorder, but the number of known patients varies by region due to variability in diagnosis and mortality rates across regions.[6,8]
Overall representativeness of this trial	This was a Phase 1/2 trial in 10 adult male patients performed at sites in the United Kingdom. Patients reported their age and race. Ninety percent of patients (9/10) were white, and ages ranged from 25 to 67 at screening. Racial and ethnic diversity of the patient population was lower than that observed globally, but the age distribution among the adult patients was broad.

References

1. Dane A, Lee D, McIntosh JH, et al. Pre-clinical evaluation of an engineered AAV capsid designed to mediate higher transduction of human hepatocytes. Hum Gene Ther 2017;28:A103.

2. Dane A, McIntosh J, Lee D, et al. Preclinical Evaluation of an Engineered AAV Capsid in Non-Human Primates for the Treatment of Haemophilia B. Blood 2018;132:2197-.

3. Dane A, Khinder J, Miranda CJ, et al. RNAScope® analysis of non-human primate liver after AAVS3 delivery shows widespread liver transduction and periportal transgene expression. Hum Gene Ther 2021;32:A108.

4. Perocheau DP, Cunningham S, Lee J, et al. Age-Related Seroprevalence of Antibodies Against AAV-LK03 in a UK Population Cohort. Hum Gene Ther 2019;30:79-87.

5. Alcalai R, Planer D, Culhaoglu A, Osman A, Pollak A, Lotan C. Acute Coronary Syndrome vs Nonspecific Troponin Elevation: Clinical Predictors and Survival Analysis. Archives of Internal Medicine 2007;167:276-81.

6. Report on the Annual Global Survey 2020. World Federation of Hemophilia. (Accessed March 23, 2022, at https://www1.wfh.org/publications/files/pdf-2045.pdf.)

7. Srivastava A, Brewer AK, Mauser-Bunschoten EP, et al. Guidelines for the management of hemophilia. Haemophilia 2013;19:e1-47.

8. Iorio A, Stonebraker JS, Chambost H, et al. Establishing the Prevalence and Prevalence at Birth of Hemophilia in Males: A Meta-analytic Approach Using National Registries. Ann Intern Med 2019;171:540-6.