Supplementary Appendix

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

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Supplementary appendix

Long term safety and efficacy of human FIX gene therapy in hemophilia B

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Supplementary Methods

In vivo transduction inhibition assay. 50µl of test plasma was administered into NOD-SCID mice via a tail vein injection. Mice in the positive control (AAV8 +ve plasma) cohort were injected with 50 µl of plasma from a macaque that had been transduced with AAV8 vector before and was known to have generated a neutralising anti-AAV8 antibody response. Negative control mice (AAV8 –ve plasma) received rhesus plasma from an animal that had been screened previously and found to be AAV8 antibody negative. An additional cohort of mice (PBS control) received 50 µl of PBS instead of plasma. Three hours after injection of plasma, $1x10^{10}$vgs of scAAV2/8-LP1-hFIXco were administered into the tail vein of each mouse. Plasma samples were obtained 5 days after administration of vector and assessed for expression of human FIX antigen. To normalise for variations between experiments, the absolute human FIX antigen levels in the experimental animals were divided by the human FIX values in the PBS control.

Anti-AAV specific ELISA. An immunocapture assay was used to detect anti-AAV8 or Anti-AAV2 specific antibodies in human plasma as described before.[1] In brief, plates were coated overnight with either AAV 8 or AAV 2 viral particles ($2x10^{10}$ vector genomes/5mls of coating buffer) and then washed and blocked with PBST containing 6%. BSA for 1 hour at 37°C. After washing with PBST, 50 µl of dilutions of human plasma (1:50, 1:500, 1:1000) were then applied to these wells in duplicate. Antibodies against AAV were detected with a horseradish peroxidase conjugated anti-human IgG (Sigma, Poole, UK). Results were expressed as the end-point titre in relative units (RU/ml), defined as the reciprocal of the interpolated dilution with an absorbance value equal to five times the mean absorbance background value.

FIX:C measurement. Patient plasma samples were assayed using a standard one-stage APTT based assay on an ACL3000 (Instrumentation Laboratory, Bedford, USA). Briefly, patient samples were diluted 1:5, 1:10, 1:20 in Owrens buffered saline (OBS) and compared to a plasma laboratory standard calibrated against the 3rd international plasma standard (99/826) (NIBSC, Potters Bar, UK) that was diluted 1:5, 1:10, 1:20 and 1:40 in OBS. Patient dilutions were added to lyophilized hereditary FIX deficient plasma (Technoclone, Vienna, Austria) and APTT lyophilized silica reagent (Instrumentation Laboratory, Bedford, USA), incubated 5 minutes then clotted with $CaCl_{2}$. FIX:C results were reported as percentage of normal and IU/dl.

Vector titration. Formulated and vialed vector was titrated by the method of Fagone, et al.[2] Briefly, test article was thawed and mixed 1:1 with an agarose gel loading buffer which included sodium dodecyl sulphate and a quantitative reference DNA. After heating and cooling, samples were electrophoresed on native agarose gels, which were subsequently stained and imaged. The quantity of vector genomic DNA in the test article was evaluated by normalizing the digital signal of the vector genome band with the in-lane reference DNA standard, and comparing that signal with similarly normalized signals from mass standards loaded in separate lanes of the gel.

Detection of rAAV genome in body fluids. Plasma, urine, stool, semen and an oral swab were collected from each subject at regular intervals to assess vector shedding following systemic administration of scAAV2/8-LP1-hFIXco and diluted 1:10 in PBS. 50-100µl of each sample was incubated with 2X volume of digestion buffer (50 mM KCl, 10 mM Tris (pH 8.0), 1% SDS, 2.5 mM $MgCl_2$ and 0.5% Tween 20) containing 200 µg/ml of Proteinase K at 55°C for 3 hours. After 10 minutes at 95°C to inactivate the proteases, 2.5 µL of the sample was used in a quantitative real-time polymerase chain reaction (Q-PCR) with the following primers and probe: 5' primer, 5'GGGCAAGTATGGCATCTACA3'; 3'primer, 5'AAAGCATCGAGTCAGGTCAG3' and probe, TTGGTCTTCTCCTTGATCCAGTT CACA. Negative samples were spiked with vector plasmid and subjected to qPCR to ensure that the sample did not contain inhibitors of PCR. This method could reliably detect 500 vector particles in 1 ml of human plasma as determined by incubating a known concentration of scAAV-LP1-hFIXco with pre-treatment plasma samples.

Cellular Immunology studies. T cell responses were measured using one-color ELISpot assay for IFN-γ as previously described.[3-5] For this assay, ELISpot plates pre-coated with an anti-human IFN-γ antibody were used (Mabtech). The day of the assay, plates were washed four times with 1x PBS and blocked with AIM-V (Invitrogen Gibco) supplemented with 3% heat inactivated (HI) FBS (Hyclone) for at least two hours at room temperature. After the blocking step, plates were washed twice with AIM-V 3% HI-FBS medium and antigens and mitogens were added to wells in 100 µL of volume. Each condition was tested in triplicate unless otherwise specified. Just prior to performing the assay, cryopreserved PBMC were thawed in a 37°C water bath and quickly transferred into culture medium (AIM-V 3% HI-FBS) containing 10U/mL Benzonase (EM Science), washed twice in order to eliminate

residual DMSO and counted using a Countess automated counter (Invitrogen). Trypan blue exclusion was used to evaluate cell viability. After counting, PBMC were adjusted to a concentration of 1-2.5x10^6 cells/mL (depending on the cell recovery) in AIM-V 3% HI-FBS culture medium lacking Benzonase, and 100 µL of the cell suspension was carefully added dropwise to the wells containing antigens and mitogens. The assembled ELISpot plates were then wrapped in foil and placed in a 37°C 5% CO_2 incubator overnight or for at least 20 hours. After incubation, excess medium was removed and 200µL of ice-cold water was added to the ELISpot plates. After 10 minutes incubation on ice, plates were washed 6 times with DPBS containing 0.05% Tween-20 followed by an additional wash with PBS. An anti-human IFN-γ biotinylated antibody (Mabtech) was then added to the plate (100 µL per well of a 1:1000 dilution in PBS 1% BSA) and incubated for 2 hours at room temperature. Plates were then washed 6 times with PBS/Tween as described above and once with PBS only. 100 µL of streptavidin-alkaline phosphatase (Mabtech) diluted 1:1000 in DPBS 1% BSA was then added to each well. After 1 hour at room temperature, plates were washed four times with PBS/Tween and twice with PBS. Spots were detected by adding chromogenic substrate to the plates (BCIP/MBT, KPL). Color development was stopped by multiple washes with distilled water. Plates were then dried overnight at room temperature and stored at room temperature protected from light until analyzed. The spots in each well were enumerated using an ELISpot reader (Cellular Technologies Inc) and analyzed with specific software (Immunospot, Cellular Technologies Inc).

T cell reactivity was tested against the following antigens and controls:
A library of 15-mers overlapping by 10 amino acids in sequence was designed to span the entire AAV8 VP1 protein for a total of 146 peptides (Mimotope). Individual peptides were resuspended in 50% acetonitrile 0.1% acetic acid to a final stock concentration of 5mg/mL. Peptides were organized in 6 pools (AAV-P1-6) and tested at a working concentration of ~3µg/ml per peptide. Similarly, a library of 15-mers overlapping by 10 amino acids in sequence was designed to span the entire human FIX protein for a total of 91 peptides (Mimotope). Individual peptides were resuspended in 50% acetonitrile 0.1% acetic acid to a final stock concentration of 5mg/mL. Peptides were organized in 3 pools (FIX-P1-3) and tested at a working concentration of ~3 µg/ml per peptide. Alternatively, recombinant FIX (Benefix, Wyeth/Pfizer) was used in the assay at a working concentration of ~20µg/ml. Lymphocyte culture medium was used as negative control.

Three positive controls were used in the IFN-gamma ELISpot assay, CEF (a pool of epitopes from CMV, EBV and flu viruses binding to several common HLA alleles from Mabtech), a mix of PMA (0.05 µg/mL) and ionomycin (1 µg/mL), and an anti-CD3 antibody (Mabtech). For the polyfunctional analysis of T cell responses using flow cytometry SEB (Staphylococcus enterotoxin B, Sigma) 1µl/ml (10mg/ml stock) was used.

DNA from the participants enrolled in the study was used for MHC class I and II HLA typing. HLA typing was performed at the University of Pennsylvania Medical Center, Department of Pathology and Laboratory Medicine or at the clinical trial site.

Supplementary Results

Patient demographics: The mean age of the entire cohort of 10 subjects was 36.3 years (range, 22–64) and mean weight was 80.7kg (range = 60-111kg). FIX gene mutation analyses showed that 7 subjects had a missense mutation and 3 had mutations in the coding or the upstream promoter region that resulted in the complete absence of endogenous FIX protein production. Seven subjects received regular prophylaxis with FIX protein concentrate administered between once to three times per week, the rest were on demand treatment. None of the subjects were on interferon or antiviral treatment at the time of or a minimum of six months prior to infusion of scAAV2/8-LP1-hFIXco. All participants had modest levels of anti-AAV2 IgG antibodies before gene transfer but as required by the inclusion criteria, all were negative for neutralizing antibodies to AAV8 as assessed by the in-vivo transduction inhibition assay.

Vector shedding: The magnitude and duration of AAV shedding into plasma, saliva, stools, urine or semen appeared to be dependent on the dose administered with the highest proviral DNA level observed in subjects who received $2x10^{12}$vg/kg with mean plasma scAAV2/8-LP1-hFIXco genome levels that were 20,000 fold higher than observed in the low dose cohort.

Adverse events: Aside from vector dose related elevation in liver enzymes, asymptomatic, grade I-II iron deficiency anemia was observed in 4 subjects (subjects 1, 2, 7 and 8 lowest Hb = 10.5 g/dl) during the first 6 weeks of gene transfer. There was no evidence of blood loss, and the anemia was most likely due to the frequency and volume of blood draws in the initial phase of the study. A prompt resolution of anemia occurred within 4 weeks of commencing supplemental iron therapy in all subjects. Subject 5 suffered from Grade 1 acne at week 12 following vector administration but this resolved spontaneously following cessation of prednisolone therapy. Grade I/II adverse events that were not related to the study agent over the study period included mild unconjugated hyperbilirubinaemia (max 24 µmol/l; [upper limit of normal = 17 µmol/l]) in subject 4 who was known to suffer from Gilbert's syndrome. Subject 5 had evidence of cyclical neutropenia and thrombocytopenia (average trough neutrophil and platelet count = $1.3x10^9$/l and $100x10^9$/l respectively) which preceded gene transfer and was not associated with an increased risk of infection. Subject 1 required treatment iron replacement and local sclerotherapy) for bleeding rectal piles. Subject 2 had a

single episode of raise blood pressure prior to vector administration. His blood pressure during and following vector administration has remained normal and was therefore reported as an AE that was unrelated to the study agent. At 5 weeks after gene transfer Subject 2 suffered from haematuria associated with an increase in creatinine. This resolved promptly following daily treatment with FIX concentrate over a 5 day period. At approximately 7 weeks after gene transfer he developed a staphylococcus epidermidis infection of a loosened left artificial hip joint, implanted 20 years previously. During this time his haematological parameters were abnormal with Grade 1 leucocytosis, thrombocytosis and anaemia. Following a second left hip replacement under antibiotic cover there was a gradual improvement of joint function and haematological parameters. As the hip joint had begun to loosen prior to enrolment, this event was reported as a SAE unrelated to vector administration. Subject 3 suffered a single episode of stomach cramps at 13 weeks after gene transfer that resolved spontaneously. At 16 weeks after gene transfer he suffered a transient period of bradycardia, during anesthesia for left knee replacement surgery. The surgery was undertaken a week later without any adverse events. Several episodes of bleeding into the joints or soft tissue (48 episodes in total) were documented following vector administration. The majority were treated with FIX concentrates for between 1-3 days. Upper respiratory tract infections were reported in Subjects 6 (12 weeks), Subject 7 (12 months), Subject 9 (4 and 6 months) and Subject 10 (10 weeks) at the times after vector administration shown in parenthesis. All of the subjects in the dose escalation arm of the study have had annual abdominal ultrasound scanning to look for the possible development of liver pathology following peripheral vein delivery of scAAV2/8-LP1-hFIXco. The echotexture of the livers in all 6 subjects has remained normal with a follow-up period ranging from 22 to 42 months.

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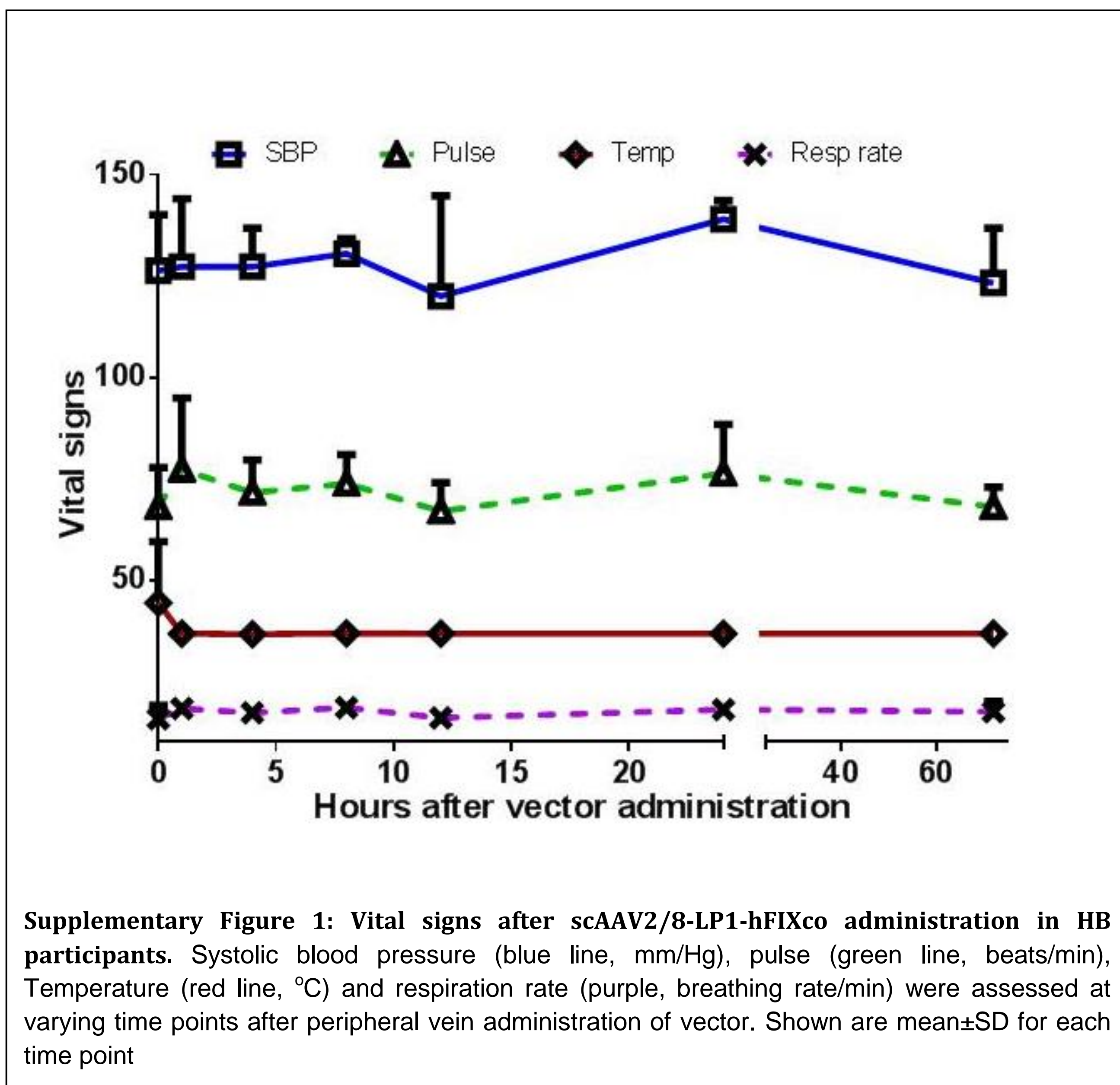
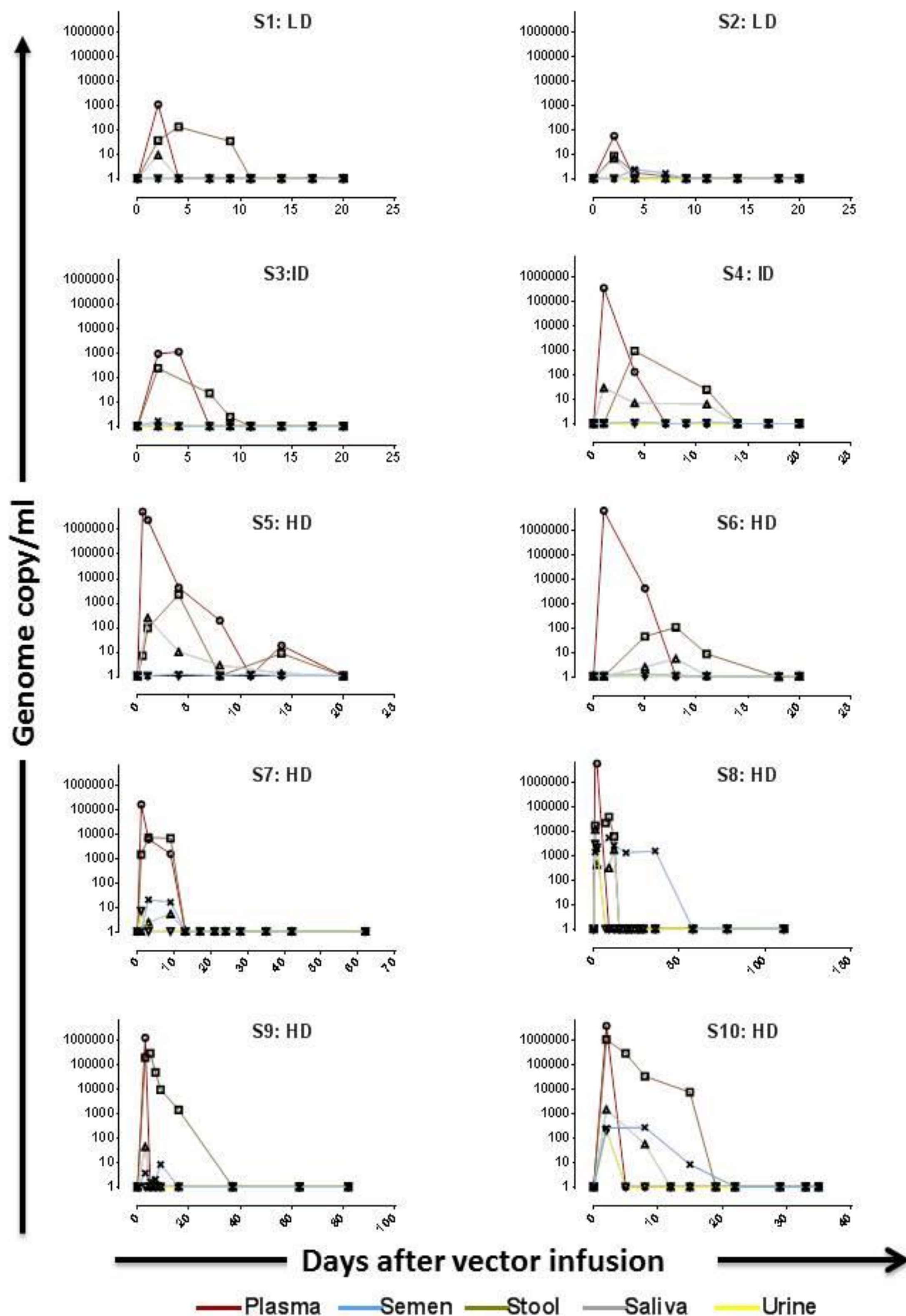
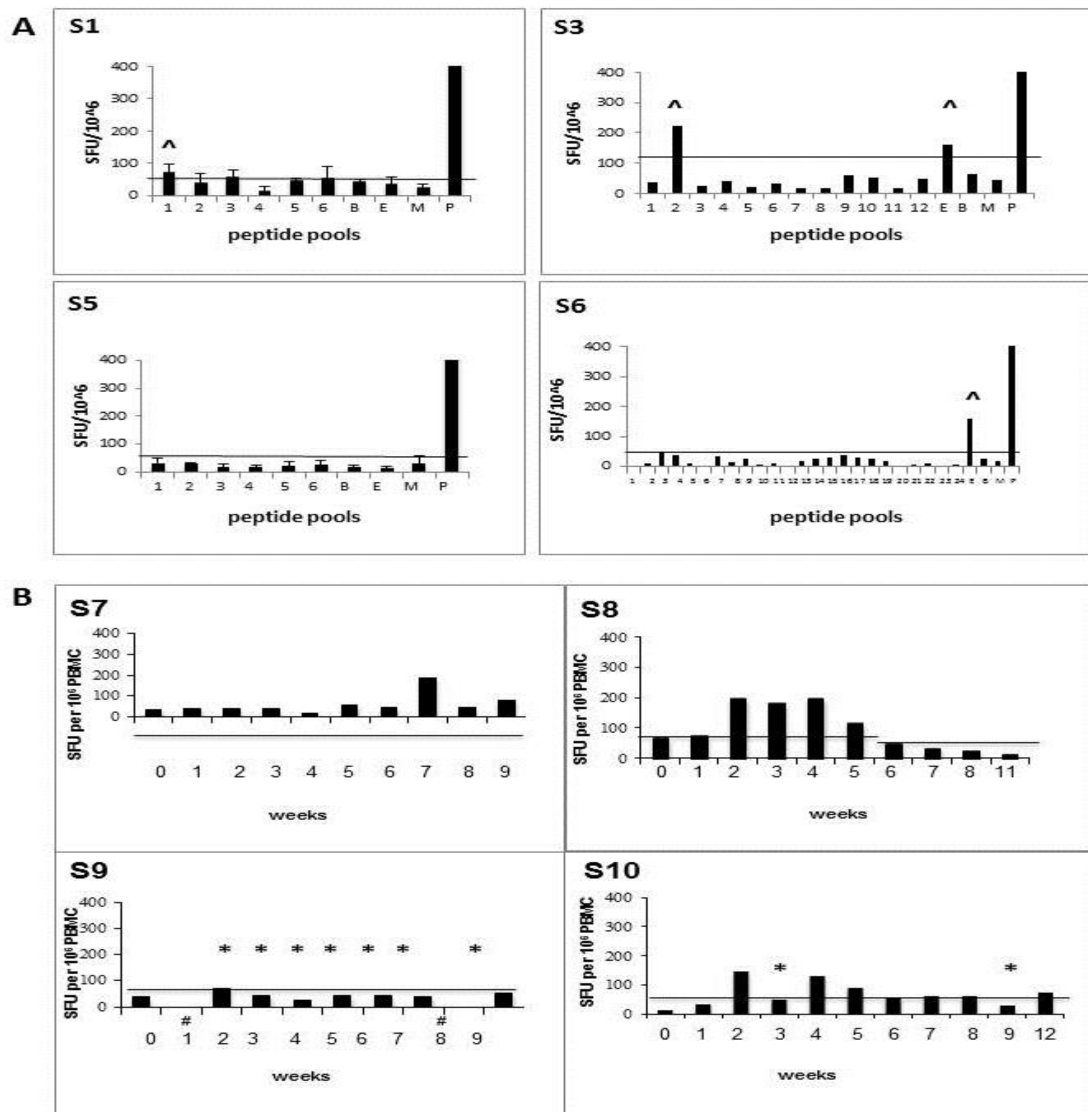
3. Manno CS, Arruda VR, Pierce GF et al. Successful transduction of liver in hemophilia by AAV-Factor IX and limitations imposed by the host immune response. Nat Med 2006;12(3):342-347.
4. Mingozzi F, Maus MV, Hui DJ et al. CD8(+) T-cell responses to adeno-associated virus capsid in humans. Nat Med 2007;13(4):419-422.
5. Mingozzi F, Meulenberg JJ, Hui DJ et al. AAV-1-mediated gene transfer to skeletal muscle in humans results in dose-dependent activation of capsid-specific T cells. Blood 2009;114(10):2077-2086.



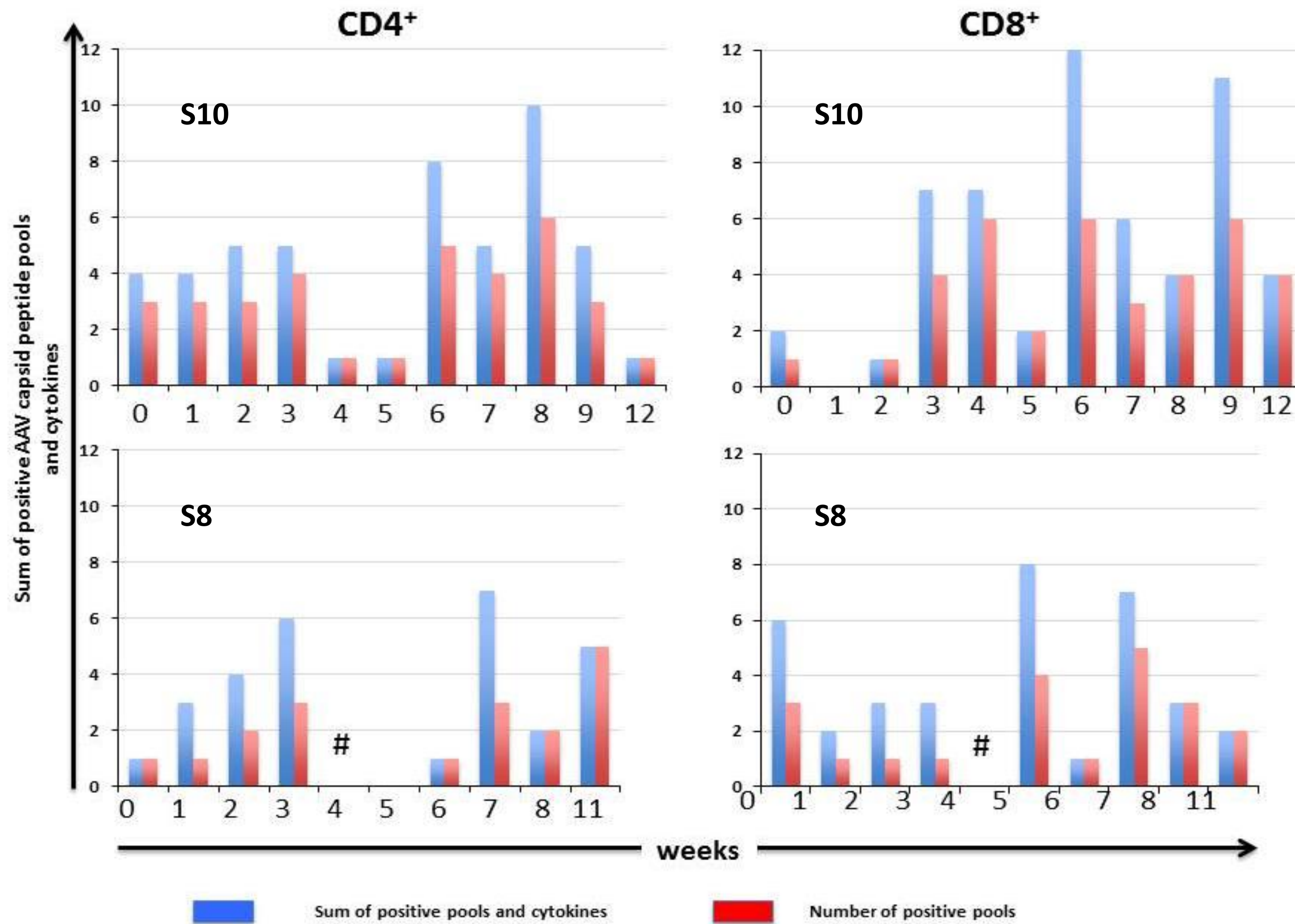
Supplementary Figure 1: Vital signs after scAAV2/8-LP1-hFIXco administration in HB participants. Systolic blood pressure (blue line, mm/Hg), pulse (green line, beats/min), Temperature (red line, °C) and respiration rate (purple, breathing rate/min) were assessed at varying time points after peripheral vein administration of vector. Shown are mean±SD for each time point



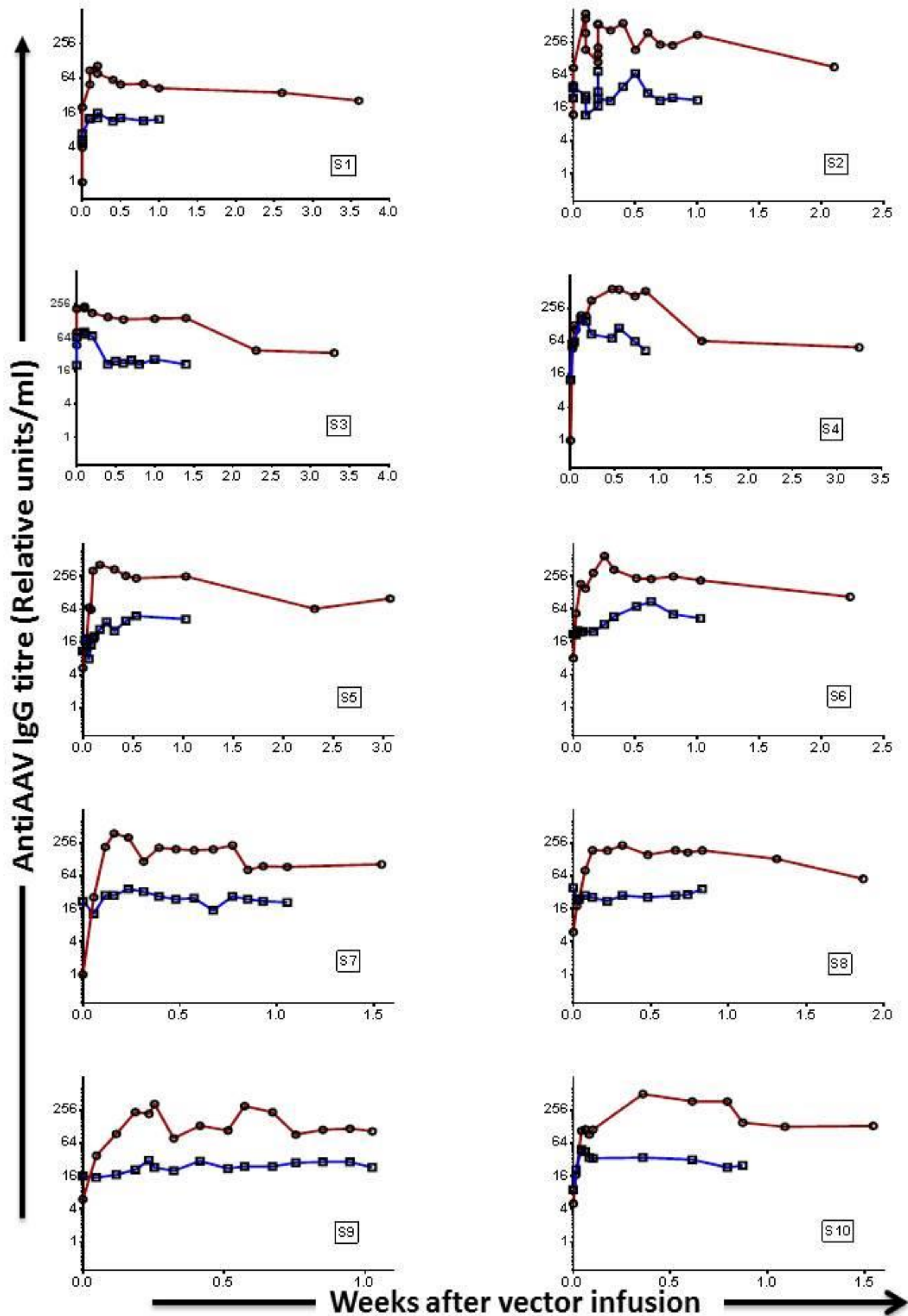
Supplementary Figure 2: Virus shedding post scAAV2/8-LP1-hFIXco administration in HB participants. A qPCR-based assay was used to detect vector sequences in body fluids collected from each subject (S1-S10) on the stipulated day after peripheral vein administration of vector. The PCR primers were specifically chosen to amplify a region within the codon-optimized FIX transgene. Standards consisted of serial dilutions of scAAV2-8-LP1-hFIXco in naïve human plasma. Negative samples were spiked with vector plasmid see methods attached and subjected to PCR to ensure that the sample did not inhibit the PCR reaction. LD = low dose; ID = Intermediate dose; HD = High dose



Supplementary Figure 3: Summary of the cellular immune response after peripheral vein administration of scAAV2/8-LP1-hFIXco. A: IFN-γ ELISPOT at time points >1 year after gene transfer for selected subjects recruited to the dose escalation arm. PBMC were tested against 6-24 pools of peptides derived from the AAV8 capsid protein VP1, empty capsid (E), recombinant FIX Benefix (B), media (M) or PMA/Ionomycin (P). Results are expressed as spot forming units (SFU) per million PBMC. Samples analyzed were drawn at 24 months (S1), 16 months (S3), 14 months (S5) or 16 months (S6) after vector infusion. Threshold for positivity is indicated by the horizontal line and is defined as 3X the medium control spot count and at least 50 SFU per million PBMC. Positive samples are indicated by the symbol ^. **B:** IFN-ɣ ELISPOT assay for capsid-specific T-cell responses over early time points following administration of the high dose of vector in four new subjects (S7-10). Results are shown as the maximum number of spot forming units (SFU) per million PBMC in response to AAV8 capsid peptide pools. The horizontal lines represent the threshold for positivity, defined as three times the SFU/million PBMC of the negative control (medium only) and at least 50 SFU/million PBMC. Subject 7 had T cell reactivity to the AAV8 capsid with a maximum of 180 spot-forming units (SFU) per 1 million PBMCs at week 7, concomitant with an increase in serum ALT levels. Subject 8 showed T cell reactivity starting at week 2 through week 5 with no rise in liver enzymes at that point or subsequently. No T cell reactivity to the AAV capsid was detectable in PBMCs from subject 9 at any time point, although this may have been due to reduced cell recovery and viability. This subject also never had a rise in liver transaminase levels. In subject 10, T-cell reactivity to AAV8 capsid started at week 2 with 143 SFU per million PBMC and remained “low-positive” through week 12. He had an asymptomatic rise in the transaminases at week 9, for which he received steroids. *, poor cell recovery and viability; #, no sample. N.B. The x-axis in panel A represents peptide pools, whereas in panel B represents weeks following vector infusion.



Supplementary Figure 4: Polyfunctional capsid specific T cell analysis of subjects treated at the high dose level: Activation and magnitude of capsid-specific T cell response characterised by the production of multiple cytokines and number of positive T cell clones in a subject with elevation of liver transaminases (top panel) compared with a subject (bottom panel) where the liver enzymes did not rise above baseline values. # = insufficient number of T cells.



Supplementary Figure 5: Summary of the humoral immune response after peripheral vein administration of scAAV2/8-LP1-hFIXco. Shown is the profile of anti-AAV8 (Solid line) and anti-AAV2 IgG antibody response in Subjects 1-10 over a 12 month period after peripheral vein administration of scAAV2/8-LP1-hFIXco at low (LD) intermediate (ID) or high dose (HD) level as determined by ELISA.

Supplementary Table 1: Protocol synopsis

<table>
<tr><td>Study title</td><td>An open label dose-escalation study of a self-complementary adeno-associated viral vector (scAAV2/8-LP1-hFIXco) for gene transfer in subjects with severe hemophilia B.</td></tr>
<tr><td>Clinical Trials.gov Identifier:</td><td>NCT00979238</td></tr>
<tr><td>Study Centers:</td><td>(1) Katharine Dormandy Haemophilia Centre and Thrombosis Unit (KDHCTU) which is part of the University College London, London, UK,
(2) St Jude Children's Research Hospital, Memphis, TN, USA,
(3) Stanford University, Stanford, CA, USA.</td></tr>
<tr><td colspan="2" align="center">Objectives</td></tr>
<tr><td colspan="2"><u>Primary Objective</u>: Assess the safety of systemic administration of a novel self-complementary AAV vector (scAAV2/8-LP1-hFIXco) in adults with severe hemophilia B at three different dose levels consisting of 2 x 10^{11}, 6 x 10^{11} and 2 x 10^{11} vector genomes per kilogram (vg/kg) of body weight as titered by a gel-based method.

<u>Secondary Objectives:</u>

a. Estimate the dose of scAAV required to achieve stable expression of hFIX at or above 3% of normal (≥ 3u/dl).
b. Establish the kinetics, duration and magnitude of scAAV-mediated hFIX expression in individuals with hemophilia B for a given vector dose.
c. Describe the immune responses to the hFIX transgene product and AAV capsid proteins following systemic administration of scAAV2/8-LP1-hFIXco.
d. Assess viral shedding in various body fluids after systemic administration of scAAV2/8-LP1-hFIXco.</td></tr>
<tr><td colspan="2" align="center">Study Design</td></tr>
<tr><td colspan="2">An open label dose-escalation, Phase I/II study entailing peripheral vein administration of a single dose of a novel self-complementary AAV (scAAV2/8-LP1-hFIXco) vector into adult subjects with severe HB following informed consent. Dosing will begin with a low dose (2 x10^{11}vg/kg) and progress to an intermediate dose level (6 x10^{11}vg/kg) and then to the highest dose of 2 x 10^{12} vg/kg. Between 2-6 patients may be enrolled at each of 3 dose levels depending on toxicity and FIX levels. However, only one subject will be treated at a time and observed for at least 42 days prior to the enrolment of the next subject. The dose level will be based on a gel titration method which allows direct visualization and quantitation of the titer by comparison of intensity of bands from the vector to a series of standards. A comprehensive monitoring schedule has been established to assess the primary end point of safety which includes an array of clinical and laboratory evaluations including liver biochemistry, semen analysis for vector genomes, and immunological response to hFIX and AAV capsid. Accrual will be suspended if dose limiting toxicity, including any Grade III-IV adverse events or any Grade II adverse events that persist for more than 7 days and are at least possibly related to the vector product occur in any of the enrolled subjects.</td></tr>
<tr><td colspan="2" align="center">Main Eligibility Criteria</td></tr>
<tr><td>Inclusion criteria:</td><td>1. Males ≥ 18 years of age with established severe HB (FIX:C<1u/dl) resulting from a mutation in the hFIX gene which has not been associated with an inhibitor in the database (http://www.biochem.ucl.ac.uk/pavithra/fix/structure.html.php) with detectable FIX in serum.
2. Treated/exposed to FIX concentrates for at least 10 years,</td></tr>
</table>

	3. A minimum of an average of 3 bleeding episodes per year requiring FIX infusions or prophylactic FIX infusions because of frequent prior bleeding episodes, 4. Able to give informed consent and comply with requirements of the trial, 5. Currently free of inhibitor and have no history of inhibitors to FIX protein, and 6. A negative family history for the development of an inhibitor, 7. Willing to practice a reliable barrier method of contraception.
Exclusion criteria:	1. Evidence of active infection with Hepatitis B or C virus as reflected by HBsAg or HCV RNA positivity, respectively. To be considered negative for active infection, two negative assays at a minimum of a six month interval were required, 2. Exposure to Hepatitis B or C and on antiviral therapy, 3. Serological evidence of HIV or HTLV infection, 4. Significant liver dysfunction as defined by an abnormal ALT (alanine transaminase), bilirubin, alkaline phosphatase or INR. Potential participants who have had a liver biopsy in the past 3 years were excluded if they had significant fibrosis of 3 or 4 as rated on a scale of 0-4 5. Coronary artery disease as a co-morbid condition, 6. Platelet count of <150 x 10^9/l, 7. Creatinine ≥ 1.5 mg/dl, 8. Hypertension with systolic BP consistently ≥ 130mmHg or diastolic BP consistently ≥ 90mmHg, 9. History of active tuberculosis, fungal disease or other chronic infection, 10. History of chronic disease adversely affecting performance, 11. Detectable antibodies reactive with AAV8, 12. Subjects who were unwilling to provide the required semen samples, 13. Poor performance status (WHO performance status score >1) or 14. Received an AAV vector previously or any other gene transfer agent in the previous 6 months
Study Procedures/ Frequency:	Following vector administration, subjects will be evaluated at frequent intervals for local or systemic toxicity, efficacy and biodistribution over a period of 1 year followed by regular follow-up for fifteen years. See Supplementary Table 2.
Criteria for Evaluation:	**Primary Endpoint***:* Safety, defined as the development of dose-limiting toxicity including any Grade III-IV adverse events or any Grade II adverse

	events that persist for more than 7 days and are at least possibly related to the study agent, according to the modified symptom specific NCI Common Terminology Criteria for Adverse Events. **Secondary Endpoint:** Efficacy, defined as persistent expression of functional human FIX at $\geq$ 3% of normal levels in plasma.
Treatment of transaminitis	Prednisolone as the sole medication will be used as per the AASLD guidelines with a slight modification: Prednislonone dose (mg/day) Week 1 — 60 Week 2 — 40 Week 3 — 30 Week 4 — 20 Maintenance until ALT returns to base line and then reduce by 5mg/week. More rapid taper was permitted in subjects where ALT had returned to baseline values within week 1 of steroid therapy.

Supplementary Table 2: Summary of study investigations

Evaluations	Weeks		Weeks													Months								
Study time	-6 to -1	-1 to 0	0	+1	+2	+3	+4	+5	+6	+7	+8	+9	+10	+11	+12	4	5	6	7	8	9	10	11	12
Screening consent	X																							
History/Physical exam	X																							
CXR	X																							
HLA typing	X																							
AAV2 and AAV8 Antibody titers	X																							
FIX antigen (Elisa**)**	X																							
FIX genotype	X																							
Screen for Hep B, C, HIV, HTLV	X																							
Baseline FIX activity level (at least 3 days off therapy)	X	X																						
Infusion consent		X																						
Administration of vector			X																					
History/Physical exam			X	X^0	X^0	X^0	X^0	X^0	X^0	X^0	X^0	X^0	X^0	X^0	X^0	X	X	X	X	X	X	X	X	X
CBC with differential	X	X		X^0	X^0	X^0	X^0	X^0	X^0	X^0	X^0	X^0	X^0	X^0	X^0	X	X	X	X	X	X	X	X	X
Coagulation screen	X	X		X^0	X^0	X^0	X^0	X^0	X^0	X^0	X^0	X^0	X^0	X^0	X^0	X	X	X	X	X	X	X	X	X
CRP	X	X		X^0	X^0	X^0	X^0	X^0	X^0	X^0	X^0	X^0	X^0	X^0	X^0	X	X	X	X	X	X	X	X	X
Urea, Na+. K+, Creat, LDH	X	X		X^0	X^0	X^0	X^0	X^0	X^0	X^0	X^0	X^0	X^0	X^0	X^0	X	X	X	X	X	X	X	X	X
Liver function test (bili, AST, ALT, Albumin, alk phos)	X	X		X^0	X^0	X^0	X^0	X^0	X^0	X*	X*	X*	X*	X*	X*	X	X	X	X	X	X	X	X	X
Cardiac enzymes	X^0	X^0		X^0	X^0	X^0	X^0	X^0	X^0	X^0	X^0	X^0	X^0	X^0	X^0	X	X	X	X	X	X	X	X	X
HFIX activity level	X			X^0	X^0	X^0	X^0	X^0	X^0	X*	X*	X*	X*	X*	X*	X	X	X	X	X	X	X	X	X
FIX inhibitor level	X			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Frozen plasma	X	X		X^0	X^0	X^0	X^0	X^0	X^0	X*	X*	X*	X*	X*	X*	X	X	X	X	X	X	X	X	X
Mononuclear cell collection		X		X^0	X^0	X^0	X^0	X^0	X^0	X^0	X^0	X^0	$\mathbf{X^0}$	$\mathbf{X^0}$	X^0									
AAV-8 antibody titer	X	X		X		X			X			X			X	X	X	X	X	X	X	X	X	X
PCR of vector genomes in blood, saliva, urine, and stools		X		X#		X			X			X			X									
PCR of vector genomes in semen		X		X#		X			X			X			X									
Test for Hep B, C reactivation				X		X			X															
EKG and echocardiogram	X^0																							

*^{0} 1 time per week, * 3 times per week, # 3 times within 7-10 days following vector infusion**,** discontinue after 3 negative samples obtained*

Supplementary Table 3: Limited analysis of the anti-AAV IgG titres following gene transfer in the dose escalation cohort

	Average anti-AAV IgG titres (Relative units)											
Serotype	**2**		**5**		**8**		**9**		**Rh10**		**Rh74**	
Subject	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**
1	5	12	5	7	1	50	4	7	5	6	3	10
2	37	73	7	17	12	319	74	220	41	116	14	248
3	20	81	8	5	37	157	10	43	16	25	10	52
4	13	162	24	33	1	281	14	156	15	88	18	956
5	11	48	12	36	5	182	16	167	25	80	12	261
6	22	87	22	78	8	231	17	81	15	96	14	105
Average titres	18	77	13	29	11	203	22	112	19	68	12	272
Average fold increase in titres over baseline values	4		2		18		5		4		23	
Amino acid sequence homology with AAV8 (%)	82		57		100		82		93		84	
Pre = Anti-AAV IgG level just before administration of AAV2/8-LP1-hFIXco Post = Anti-AAV IgG level at approximately 12 months after administration of AAV2/8-LP1-hFIXco												