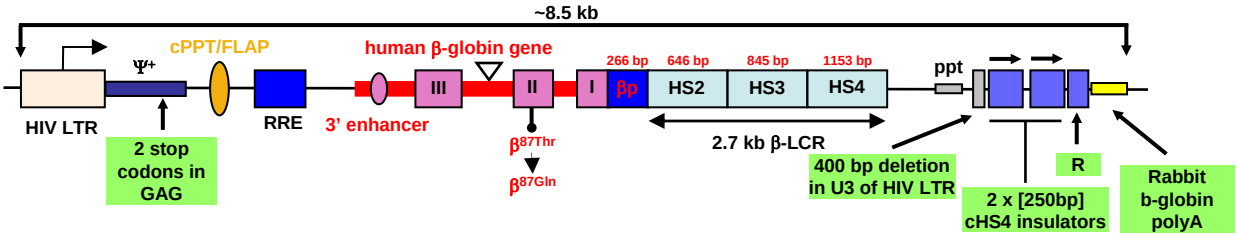
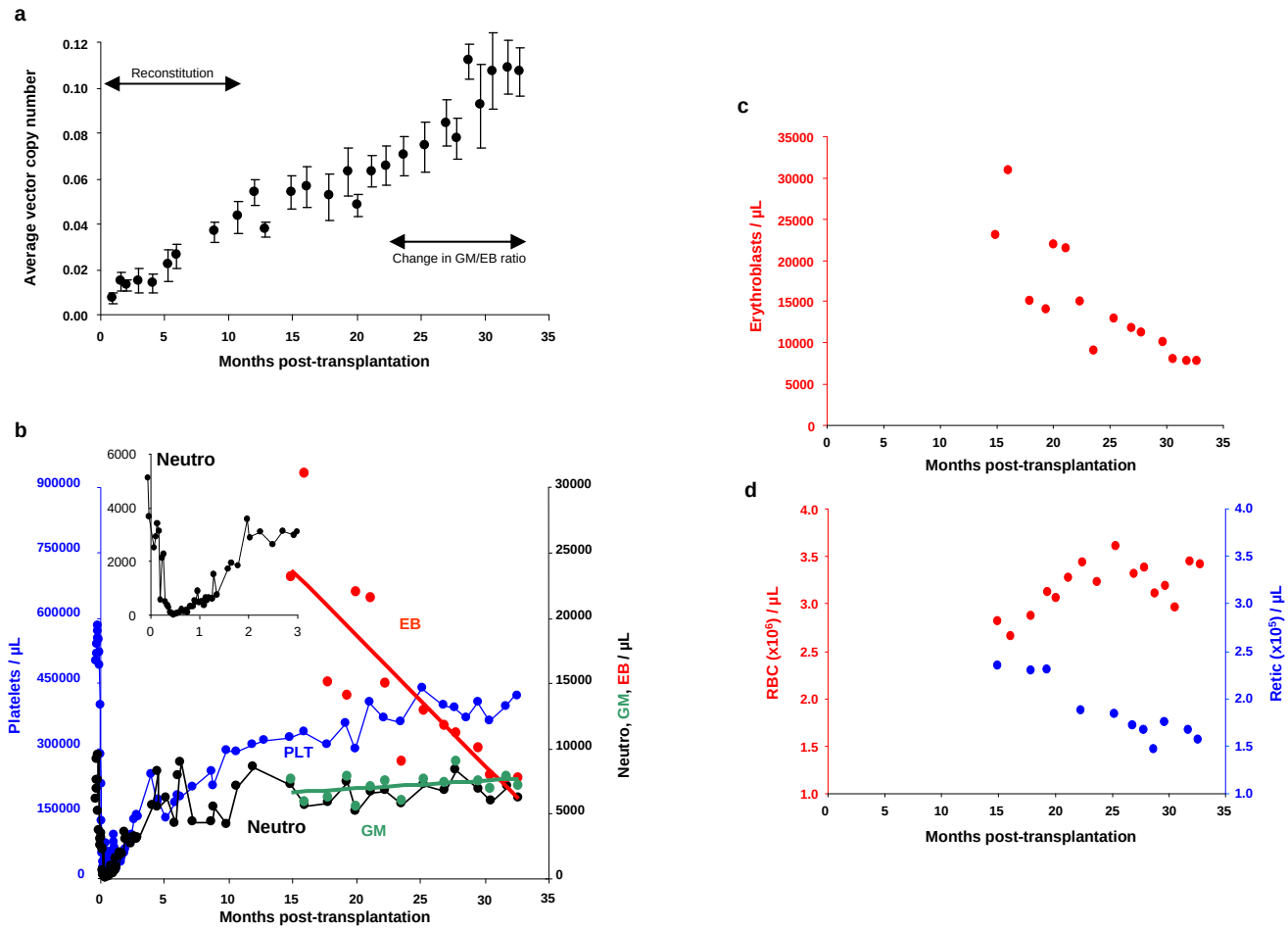
Supplementary Fig. 1



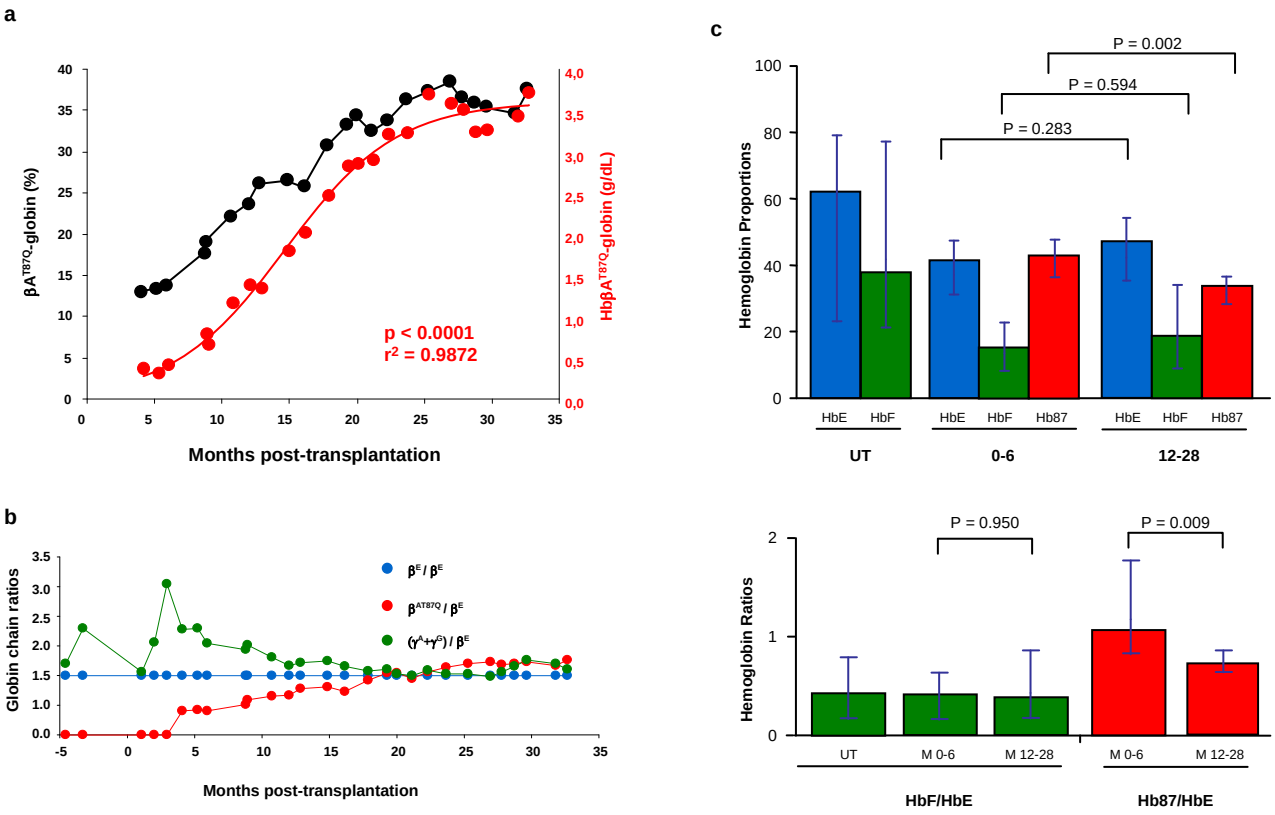
Supplementary Fig. 1. Diagram of the LentiGlobinTM vector. The 3' β-globin enhancer, the 372bp IVS2 deletion, the $\beta^{A\text{-}T87Q}$ mutation (ACA Thr to CAG Gln) and DNase I Hypersensitive Sites (HS) 2, HS3 and HS4 of the human β-globin Locus Control Region (LCR) are indicated. Safety modifications including 2 stop codons in the Ψ+ signal, the 400bp deletion in the U3 of the right HIV LTR, the rabbit β-globin polyA signal and the 2 x 250 bp cHS4 chromatin insulator are indicated. HIV LTR, human immunodeficiency type-1 virus long terminal repeat; Ψ+, packaging signal; cPPT/flap, central polypurine tract/DNA flap; RRE, Rev-responsive element; βp, human β-globin promoter; ppt, polypurine tract. Southern blot analysis of transduced cells indicate that up to ≈ 1/3 of integrated vector copies contain a deletion of one of the cHS4 cores within the right U3, and ≈ 2/3 of integrated vector copies contain a deletion of one of the cHS4 cores within the left U3.

Supplementary Fig. 2



Supplementary Fig. 2. Kinetic relationship of blood average vector copy numbers and blood cell counts. a, qPCR-based kinetics of average vector copy numbers in nucleated peripheral blood cells at any IS. Mean values and standard deviations are shown. Alignment with blood cell count kinetics here below (Supplementary Fig. 2b) suggests that the first phase of increase in blood average vector copy numbers is concurent with the "Reconstitution" phase following engraftment, while the second phase "Change in GM/EB ratios" is likely to reflect the decrease in the blood EB / GM count ratio considering that vector copy numbers in blood GMs are ~ 6-fold higher than in blood EBs (Table 1). qPCR performed on pooled *in vitro* colonies forming cells (CFCs) (from blood 27 mPT) gave an average readout of 0.14 copy / cell while scoring of the number of individual CFC positive by PCR for the presence of a vector was 13.8%, indicating that the vector copy number per transduced cell was ~ 1 (0.14 / 0.138). **b**, Kinetics of blood cell counts. PLT, platelets; Neutro, neutrophiles; linear regression of EB (erythroblasts, red) and GM (granulocytes and monocytes, green). (**Inset**) Kinetics of neutrophile count recovery during the first 3 mPT (ANC became > 500 /µL at Day 27 post-transplant); the patient also required 7 platelet transfusions. **c,** Kinetics of blood EB counts after reaching transfusion independence. **d**, Kinetics of RBC (red) and blood reticulocyte cell (blue) counts after reaching transfusion independence.

Supplementary Fig. 3



Supplementary Fig. 3. Differential ratios of Hb species and lack of influence of *HMGA2* IS on β^{AT87Q}-globin expression. a, HPLC-based percentages of β^{AT87Q}-globin chain to the sum of all non α-globins (black) and concentrations of $Hb\beta^{AT87Q}$ (red) in RBCs. **b,** Kinetics of differential contributions of fetal (γG- and γA) and β^{AT87Q}-globin chains relative to β^{E}-globin in whole blood. **c**, (**Top**) Median percentages of fetal (F), therapeutic (87) and E hemoglobins (histograms) in individual blood BFU-E colonies, as assessed by HPLC. The three groups of histograms are the median values for untransduced (UT, n=16) and transduced colonies isolated from blood 0 to 6 mPT (M 0-6, n=8), when *HMGA2* IS contribution was negligible, vs. 12 to 28 mPT (M 12-28, n=64) when *HMGA2* IS was predominant. Error bars define the 25th and 75th percentiles. (**Bottom**) Median ratios of Hb species. HbF/HbE ratios (histograms) are unchanged between the three groups, whereas $Hb\beta^{AT87Q}$ expression was substantially higher when vector integration was not at *HMGA2* IS. The differences between the median values are statistically significant by the non parametric Mann-Whitney rank sum test (p = 0.002 for percentages of $Hb\beta^{AT87Q}$ and p = 0.009 for the ratio $Hb\beta^{AT87Q}$/HbE).

Supplementary Tables

Supplementary Table 1. Primers and probes used for qPCR and RT-qPCR

Assays	Amplicons	Name	Sequence (5' to 3') or TaqMan gene expression assay number*	Modification	Conc.
Vector copy number in human and mouse cells	LG vector	GAG F	GGAGCTAGAACGATTCGCAGTTA	-	720 nM
		GAG R	GGTTGTAGCTGTCCCAGTATTTGTC	-	720 nM
		GAG P	ACAGCCTTCTGATGTCTCTAAAAGGCCAGG	5'FAM 3'TAMRA	140 nM
	hβ-actin	hbAct F	TCCGTGTGGATCGGCGGCTCCA	-	900 nM
		hbAct R	CTGCTTGCTGATCCACATCTG	-	900 nM
		hbAct P	CCTGGCCTCGCTGTCCACCTTCCA	5'FAM 3'TAMRA	250 nM
	mβ-actin		Mm00607939_S1*	5'FAM 3'NFQ-MGB	1X
Vector copy number at the HMGA2 locus	LG/HMGA2 junction	U5F	ACCCTTTTAGTCAGTGTGGAAA	-	720 nM
		HMGA2R1	CCAGTTTAGGAGAAAAAGCAAA	-	1200 nM
		HMGA2P1	ACTGCATTCAACTAGACTGAACTTGGTCACCA	5'VIC 3'TAMRA	200 nM
Vector copy number at the RFX3 locus	LG/RFX3 junction	U5F	ACCCTTTTAGTCAGTGTGGAAA	-	720 nM
		RFX3R2	AAGGTTGCCCATTTTTGAA	-	1200 nM
		RFX3P1	AAGCTTTCCCAAATCAAACTAGCTTACCGTGA	5'FAM 3'TAMRA	200 nM
Vector copy number at the ZZEF1 locus	LG/ZZEF1 junction	U5F	ACCCTTTTAGTCAGTGTGGAAA	-	720 nM
		ZZEF1R1	GACAGGTTTCACCGTGTTGGT	-	1200 nM
		ZZEF1P1	CTCTAGCAGTCAGGAGTTTAAGACCAGTC	5'VIC 3'TAMRA	200 nM
HMGA2 expression and "run-on"	Exons 1-2		HS171569_m1*	5'FAM 3'NFQ-MGB	1X
	Exons 3-4		HS00971724_m1*	5'FAM 3'NFQ-MGB	1X
	Exons 4-5		HS00971725_m1*	5'FAM 3'NFQ-MGB	1X
	hGAPDH		HS99999905_m1*	5'FAM 3'NFQ-MGB	1X
	Exon 1 - Intron 1	H1F5	TCAGAAGAGAGGACGCGGCC	-	900 nM
		HMGA2i1R1	GACTGGGCTCCGCACTC	-	900 nM
		Hi1P1	CGCCCTCGTACTGACTTGCTG	5'FAM 3'TAMRA	250 nM

FAM: 6-carboxyfluorescein ester, TAMRA: tetramethyl-6-carboxyrhodamine, NFQ: non fluorescent quencher, MGB: minor groove binder, Conc: concentration. *Applied Biosystems.

Supplementary Table 2. Primers used for PCR on DNA of CFC colonies.

Assays	Amplicons	Size (bp)	Name	Sequence (5' to 3')
Detection of the vector in human colonies	LG vector	431	LGF10	GAGAGCGTCGGTATTAAGC
			LGR11	TGGCCTGATGTACCATTTGC
	human Epo	322	Epo24	CGCTTTGGAGGCGATTTACC
			Epo26	CATTTCCCGGACCTGGACC
Detection of the vector at the HMGA2 junction in colonies	LG vector and LG/HMGA2 junction	258 and 411	LGF14	GCCTTGAGTGCTCAAAGTAG
			LGR16	CCCTACTCTCTCCTTCTAGC
			HMGA2i3R2	CAGCAACTCAGTATCCATTCTG

Supplementary Notes

1. Clinical trial protocol

1.1. Protocol synopsis. This is a Phase I/II, open label, safety and efficacy study with anticipated clinical benefit of the administration of autologous bone marrow or peripheral blood CD34+ cells transduced with a lentiviral vector encoding $\beta^{A\text{-}T87Q}$-globin (LentiGlobin™) to 5 patients with severe, transfusion-dependent beta-thalassemia (β-Thal) and 5 patients with sickle cell anemia (SCA). Patients will be selected from a population with at least 2 years of follow-up at a specialized center. Selected patients must meet the inclusion criteria and have none of the exclusion criteria and have given their informed consent. Following a screening period ranging from 7-28 days, patients will undergo a period of hypertransfusion followed by either peripheral blood mobilization or bone marrow harvest at one of the hospital centers under general anesthesia. The bone marrow will be processed for $CD34^+$ cell selection. If sufficient cells are not obtained, additional mobilizations and/or a second bone marrow harvest may be performed. A portion of $CD34^+$ cells will be cryopreserved for rescue therapy, and a portion transduced with the LentiGlobin^{TM} vector (i.e., the gene therapy product or GTP). A sample of transduced cells (i.e., the genetically modified organism or GMO) will undergo release testing while the remainder is cryopreserved for approximately 8 weeks. Genetically altered cells will not be infused into patients until all release testing, including extensive screening for replication competent lentivirus (RCL), is completed and meets specifications (see GTP and GMO release testing below). Patients will be admitted to the Bone Marrow Transplant Unit of the hospital and undergo conditioning with Busulfex^{TM} to induce myelosuppression at a dose in compliance with the "Autorisation de Mise sur le Marché (AMM)" of France. If the absolute neutrophil

count is <500/μL, patients will be kept in isolation. The transduced cells will be transported to the patient's hospital, washed, counted and checked for viability. The LentiGlobin^{TM} transduced CD34^{+} stem cells will be infused into the patients via a central catheter at a dose of at least 3 x 10^{6} CD34^{+} cells/kg over 20 minutes while vital signs are monitored. Patients will be followed daily in the transplant unit for adverse events and laboratory parameters to monitor bone marrow engraftment. Once engraftment occurs and patients are stable, they will be discharged from hospital and followed monthly for 6 months then at least every 3 months for a total of 24 months. Evaluations will include routine hematology and chemistry safety laboratory assessment and special hematologic testing, bone marrow examination, collection of adverse events and concomitant medications, and evaluation of disease-specific hematologic and clinical parameters. The primary endpoints are safety and tolerability of the LentiGlobin^{TM} transduced cell infusion and time to engraftment of the autologous, manipulated CD34^{+} cells. Additional endpoints include biological and biochemical measures of the presence of the transduced gene and gene product in hematopoietic and blood cells, transfusion requirements, and the number of hospitalizations and clinical events occurring at various time periods during the course of the 2 year follow-up period. All patients will be followed at least yearly for a total of 15 years post-transplant for serious adverse events, RCL testing and banking of blood cells for insertional mutagenesis testing in the event that a malignancy develops.

1.2 Inclusion criteria for β-thalassemia patients.

1. Patients must be between 5 and 35 years of age. The first 3 patients enrolled must be at least 15 years of age and must be sexually mature at the time of study entry to allow for the option of sperm or ovarian, oocyte and/or embryo preservation banking. Sexual maturity in females is defined as the onset on menses. Male patients must be

able to donate or undergo sperm extraction; the resulting sample for banking must have a density of at least 20 x 10^6/ml and >40% motility. After analysis of the first 3 patients, enrollment of younger patients may be considered.

2. Patients must have one of the genetic forms of β-thalassemia confirmed by Hb studies and genomic DNA analysis.
3. Patients must have permanent transfusion dependency defined as requiring at least 100 mL/kg/year of packed red blood cells and a requirement for iron chelation therapy. The diagnosis must be confirmed by Hb studies.
4. Patients must be candidates for allogeneic bone marrow transplant and not have a suitable, willing HLA-identical sibling donor.
5. Patients must meet Lansky Performance Status ≥70% for children or Karnofsky Index ≥80% for adults.
6. Patients must have survival expectancy >6 months.
7. Patients must have been treated and followed-up for at least the past 2 years in a specialized center where they have undergone full evaluation of their disease, including psychiatric evaluation. Detailed medical records of this care for at least the past 2 years must be available, as each patient will serve as their own control.
8. Patients ≥18 years of age must be able to provide written informed consent and have had a psychiatric evaluation to establish their motivation, the clarity of their consent, and the absence of severe psychiatric disease. For patients <18 years of age, both parents or legal guardian must be able to provide written informed consent and must be willing to undergo a psychiatric evaluation to establish their motivation for enrolling the minor, the clarity of their consent, and the absence of severe psychiatric disease. When possible, involvement of the child in the decision is highly recommended, and should be clearly documented.

9. Discontinuation of any hydroxyurea or erythropoietin therapy at least 3 months prior to infusion of stem cells.

1.3 Exclusion criteria for β-thalassemia patients.

1. Having a suitable, willing HLA-identical sibling.
2. HIV seropositivity.
3. Positivity for Hepatitis B surface antigen.
4. Positivity for Hepatitis C antibody.
5. Active bacterial, viral (e.g., hepatitis B or C), or fungal infection.
6. A contraindication to anesthesia for bone marrow harvesting.
7. A history of prior malignancy (excluding basal cell carcinoma of the skin or in situ carcinoma of the cervix, curatively resected), even if considered cured.
8. A white blood cell (WBC) count <3,000/μL and/or platelet count <120,000/μl.
9. A family member with a known or suspected Familial Cancer Syndrome (including but not limited to breast, colorectal, ovarian, prostate and pancreatic cancers).
10. Prior bone marrow transplantation.
11. A history of psychosis, any psychiatric disorder, severe mental retardation, or current drug or alcohol abuse, which, in the opinion of the investigator, would make the patient unsuitable for participation in the study.
12. A history of malaria relapses in the absence of recent infestation.
13. The presence of antibodies against Vesicular Stomatitis Virus (VSV).
14. A history of complex allo-immunization which could cause difficulty administering transfusions.
15. Pregnancy or breastfeeding in a postpartum female or absence of adequate contraception for fertile patients. Females of child-bearing potential must agree to use

a medically acceptable method of birth control such as oral contraceptive, intrauterine device, barrier and spermicide, or contraceptive implant/injection throughout the 26 month study period. Pre-menarchal females must agree to use a medically acceptable method of birth control if their child-bearing status should change during the study period. A female will be considered post-menopausal if it has been at least 12 months since her last menstrual cycle or if she has undergone surgical sterilization.

16. A history of major organ damage including:
17. Cerebral vascular disease with severe neurological or cognitive-mental sequelae, excluding isolated hemiplegia
18. Liver disease with ALT >3 x upper limit of normal or the presence of histopathological evidence of liver cirrhosis on liver biopsy.
19. Heart disease with ejection fraction <25%.
20. Kidney disease with a calculated creatinine clearance <30% normal value.
21. Lung disease with a substantial alteration in pulmonary function tests (i.e., pO_2 <90 and/or carbon dioxide diffusion coefficient <60%).
22. The presence of pulmonary fibrosis or pulmonary hypertension.
23. Hormonal disorders including insulin-dependent diabetes, hyper-or hypo-thyroidism, or hypo- or hyper-parathyroidism.
24. Participation in another clinical study with an investigational drug within 30 days of screening.
25. Presence of chromosomal abnormalities in bone marrow detected after bone marrow harvest.
26. Absence of informed consent.
27. Presence of Lucarelli Class III (inadequacy of iron chelation therapy, presence of hepatomegaly and portal fibrosis on liver biopsy).

28. Patients who are compliant with iron chelation therapy and who have young children and/or a desire to become a parent in the short term.

Patients whose transfusion requirement is decreased by erythropoietin treatment.

1.4. cGMP lentiviral vector production. Clinical grade LentiGlobin™ vector (the gene therapy product, GTP) was produced in a cGMP facility (Indiana University Vector Production Facility, IN, USA) by transfection of HEK293T cells expanded from a qualified master cell bank. The 5 LentiPak™ plasmids described in[1] i.e., HPV569 (Lentiglobin™ transfer vector containing the modified globin gene), HPV275 (gag-pol), ΨN 15 (VSV-G env), p633 (rev), and HPV601 (tat)) were manufactured and purified to qualify for use in cGMP manufacturing (Puresyn, PA, USA). The calcium phosphate mediated transient transfection (Promega, WI, USA) in DMEM media (Invitrogen) supplemented with New Zealand sourced 5% fetal bovine serum (HyClone) was followed by harvest in serum-free media (OptiPRO; Invitrogen). Clarification, concentration, Benzonase™ treatment (Novagen®) and diafiltration (TFF/UF, Spectrum) into X-Vivo 20 medium (Lonza) produced the Lentiglobin™ GTP which was subsequently cryopreserved until release testing was completed. As for the specific vector batch used to transduce the CD34+ cells harvested from Patient P2, an infectious titer of $1.1x10^8$ TU/ml was determined by transducing NIH 3T3 mouse fibroblasts with serial dilutions of the viral supernatant in the presence of polybrene (8 µg/ml). After seven days in culture, the total DNA was prepared and integrated copies were quantified by TaqMan assay using GAG primers and normalized by Southern blot analyses.

1.5. cGMP transduction of $CD34^+$ cells (Patient P2). Pre-stimulation of $1.9x10^8$ cells (92% $CD34^+$) was conducted for 34 hours in RetroNectin™ coated (50µg/mL, 2h RT; Takara) X-fold (Miltenyi) cell culture bags in X Vivo-20 media (Lonza) containing the recombinant

human cytokines SCF, Flt-3L, IL-3, and TPO (Peprotech,) at 300, 300, 100, and 10 ng/mL respectively as well as protamine sulfate at 4μg/mL (Choay; AMM# 310 117.3 (1955/98)). The transduction of $2.05x10^8$ cells (100% $CD34^+$) was performed in undiluted GTP (titer: $1.1x10^8$ TU/mL;) for 18 hours at ~$2x10^6$ $CD34^+$ cells per mL in the same media with cytokines. The post-transduction cells, $2.96x10^8$ (99.6% $CD34^+$), were then washed and cryopreserved until release testing was completed. After cyropreservation, $2.53x10^8$ viable cells were recovered for transplant to the patient.

1.6. GTP and GMO release testing. The LentiGlobin™ GTP is tested and released according to specifications agreed upon for potency (transducing titer of the GTP on NIH3T3 cells followed by qPCR analysis, and GTP particle concentration by p24 Elisa), identity ($\beta^{A\text{-}T87Q}$-globin protein production using HPLC analysis of erythroid cells differentiated from GTP-transduced human $CD34^+$ cells, and restriction digest/Southern blot analysis of GTP-transduced cells), safety (sterility, endotoxin, mycoplasma, adventitious viruses and replication competent lentivirus using amplification/coculture with readout assays as described in[2]), purity (residual host and plasmid DNA, residual Benzonase™ and residual EGF), appearance and pH. The LentiGlobin™ GMO is also tested and released according to specifications agreed upon for potency and identity (transduction efficiency of the GTP-transduced patient $CD34^+$ cells using qPCR analyses and restriction digest/Southern blot analysis of GTP-transduced patient $CD34^+$ cells, the expression of $\beta^{A\text{-}T87Q}$-globin in patient erythroid progenitors using BFU-E culture followed by HPLC analysis, and the level of hemoglobin containing $\beta^{A\text{-}T87Q}$-globin in bulk erythroid cell progeny using HPLC analysis), safety (sterility, endotoxin, mycoplasma, and replication competent lentivirus using amplification/co-culture of 1% of the patients GTP-transduced $CD34^+$ cells with readout assays as described in[2] as well as RT-PCR for gag sequences), and pre-infusion viability.

2. Patient P1 transplant history.

Patient P1 was transplanted with cells that appear - after the fact - to have been compromised during the *in vitro* transduction procedure because of a issue with a lot of plastic bag used in CD34+ cell culture and vector transduction. Presumably because of this, the patient underwent a prolonged phase of pancytopenia/aplasia post-conditioning/transplantation that lasted over 5 weeks. Because G-CSF injections were ineffective and the bone marrow cellularity was low, untransduced autologous CD34+ cells (back-up cells) were injected. She had a transient rise in peripheral blood cells bearing a vector, but, as expected from the poor quality of the GMO and the dilution with untransduced CD34+ cells, the transduced cells rapidly decreased to very low levels. The patient continues to be monitored, according to the trial protocol. Patient P1's current clinical status is good, though still transfusion dependent with extremely low vector copies at the limit of quantification by qPCR assay.

3. Bone marrow cytology (Patient P2).

Bone marrow was harvested from sternum 20 mPT. Cellularity was high with erythroid to non erythroid ratio of 0.65. Granulocytes, monocytes, lymphocytes and plasmocytes percentages were 31, 0, 3 and 1% respectively. Erythroid cell percentage of 65% included proerythroblasts (0%), basophilic erythroblasts (6%), polychromatophilic erythroblasts (7%) and acidophilic erythroblasts (52%). Megakaryocytic cells were rare and mastocytes abundant. No blasts were detected.

Supplementary References

1. Westerman, K. A., Ao, Z., Cohen, E. A. & Leboulch, P. Design of a trans protease lentiviral packaging system that produces high titer virus. *Retrovirology* **4**, 96 (2007).

2. Sastry, L. *et al.* Certification assays for HIV-1-based vectors: frequent passage of gag sequences without evidence of replication-competent viruses. *Mol Ther* **8**, 830-839 (2003).