Supplementary Appendix

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

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Supplementary Appendix to:

CRISPR-Cas9 Gene Editing for SCD and Transfusion-Dependent β-Thalassemia

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Supplementary Methods

I. Mobilization and Apheresis for Participants with TDT in CLIMB THAL-111 (NCT03655678)

Mobilization

Before starting administration of plerixafor and G-CSF (e.g., filgrastim), participants were assessed by the study investigator to confirm eligibility to proceed with apheresis (as per local guidelines). Decisions on whether a central line was required was made by the apheresis-experienced nurse or physician. G-CSF (e.g., filgrastim) was administered subcutaneously or intravenously at a dose of 5µg/kg/dose every 12 hours for 5-6 days. Plerixafor (0.24 mg/kg, subcutaneous injection) was administered for 4 days before leukapheresis. G-CSF (e.g., filgrastim) could be adjusted based on local practices in the presence of significant leukocytosis (e.g., >70 x 10^9/L).

TDT Apheresis Procedure

CD34+ human hematopoietic stem and progenitor cells (HSPCs) were collected per clinical site protocols.

II. Mobilization and Apheresis for Participants with SCD in CLIMB SCD-121 (NCT03745287)

Mobilization

Red blood cell (RBC) exchange transfusions were conducted for a minimum of 8 weeks prior to first mobilization. Before starting administration of plerixafor, participants were assessed by the study investigator to confirm whether they were eligible to proceed with apheresis (as per local guidelines). Each participant underwent stem cell mobilization with plerixafor (0.24 mg/kg subcutaneous injection) only. Patient 2 (SCD) was admitted to the hospital and observed throughout the mobilization process; hemoglobin was maintained at 10g/dL during mobilization therapy.

SCD Apheresis Procedure

Peripheral blood mononuclear cells (PBMC) were collected by apheresis.
Participants received RBC exchange transfusion 3 (± 1) day before the start of mobilization/ apheresis cycle.

III. Conditioning: Busulfan Administration

Busulfan was administered IV daily at a starting dose of 3.2 mg/kg/day for 4 consecutive days (based on weight collected within 3 to 7 days prior to the first day of busulfan administration). Once-daily dosing was the preferred schedule, but the busulfan dose regimen could be adjusted to be given q6h per site/investigator preference. The average target area under the curve (AUC) for participants at a starting dose of 3.2 mg/kg/day for 4 days was 5000 µM·min (range: 4500 to

5500), equivalent to a target cumulative busulfan exposure of 90 mg·hr/L (range 80 to100 mg ·hr/L). The AUC for participants receiving busulfan q6h for 4 days was 1125 μM·min (range: 900 to 1350) (Table S8; Table S13).

IV. CTX001 Manufacturing and Infusion Procedures

CTX001 is composed of autologous CD34+ HSPCs modified with CRISPR-Cas9 at the erythroid lineage-specific enhancer region of the BCL11A gene. All manufacturing procedures, including gRNA, Cas9, and CD34+ cells, were carried out under GMP manufacturing conditions.

The gRNA was synthesized in the 3' to 5' direction using standard solid support synthesis methods followed by purification. Cas9 protein was produced in recombinant *E.coli* fermentation and purified by cation exchange and hydrophobic interaction column chromatography.

Peripheral patient PBMCs were collected at a qualified clinical site by apheresis, according to the clinical protocol, and shipped to the manufacturing site under controlled conditions. Following platelet reduction, CD34+ HSPCs were isolated from PBMCs using a closed, automated, sterile, micro-bead system (CliniMACS Prodigy System, Miltenyi Biotec). CD34+ enriched HSPCs were sampled for cell count, viability, and post-sort CD34+ HSPC enumeration before incubation in defined culture medium. Following this culture period, the cultures were sampled for cell count and viability, and cells were sampled as unedited controls for allelic editing assays.

The ribonucleoprotein complex (RNP) was prepared in situ prior to electroporation by mixing the gRNA and the Cas9 protein. The electroporation mix containing cells and RNP complex was then loaded into a cassette and electroporated (MaxCyte Gen2 GT). After electroporation, the cells were incubated in defined culture medium prior to cryo-preservation and storage in vapor phase liquid nitrogen. Quality control release assays were performed on CTX001 samples, including CD34+ cell purity analysis by flow cytometry, on-target editing frequency (TIDE; see Section XI), post-thaw cell count and viability, as well as compendial sterility, mycoplasma, and endotoxin testing. CTX001 frozen suspension was shipped to the clinical site where it was stored in liquid nitrogen vapor. CTX001 was thawed just prior to the scheduled infusion utilizing local site SOPs. The single dose of CTX001 was given after the last busulfan dose (Figure S2).

V. Definition and Assessments of Neutrophil and Platelet Engraftment

Neutrophil engraftment was defined as the first day of 3 measurements of absolute neutrophil count (ANC) ≥ 500μL on 3 consecutive days, achieved within 42 days post-CTX001 infusion, without use of the unmodified (back-up) CD34+ cells after reaching the nadir, defined as ANC < 500μL. Platelet engraftment was defined as the first day of 3 consecutive measurements of platelet ≥20,000μL (TDT) or ≥50,000μL (SCD) on 3 different days after CTX001 infusion, without platelet-transfusion support in the past 7 days, after reaching the nadir, defined as platelet <20,000μL (TDT) or <50,000 μL (SCD).

VI. Blood for Biomarker Assessments

Blood samples were collected for evaluation of biomarkers:

- Protein based biomarkers, including but not limited to: (1) hemoglobin fractionation and quantitation in peripheral blood to assess bulk fetal hemoglobin (HbF) levels, and (2) the proportion of circulating erythrocytes expressing HbF (F-cells)
- Proportion of alleles with intended genetic modification present in peripheral blood leukocyte DNA.

VII. Guide RNA Sequence Used in CRISPR-Cas9 Editing of the *BCL11A* Enhancer Region

Guide RNA sequence:

7*5*6 *ACA GUU GCU UUU AUC ACG UUU UAG AGC UAG AAA UAG CAA GUU AAA AUA AGG CUA GUC CGU UAU CAA CUU GAA AAA GUG GCA CCG AGU CGG UGC 5*5*5 *U

Modified positions are shown as numbers (5: 2' OMe-rU; 6: 2' OMe-rA; 7: 2' OMe-rC), phosphorothioate positions are highlighted with an asterisk (*).

VIII. Identification of Candidate Off-target Regions Using GUIDE-seq

We performed GUIDE-seq to nominate potential off-target editing sites in primary CD34+ hematopoietic stem and progenitor cells (HSPCs). We optimized the GUIDE-seq protocol for CD34+ HSPCs to provide high cell viability and sensitivity based on the on-target site. To generate the double-stranded DNA oligo (dsODN), two modified single-stranded GUIDE-seq oligonucletotides (Integrated DNA Technologies) were combined with molecular biology grade water and 10x annealing buffer (New England Biolabs) and heated in a thermocycler. The ribonucleoprotein (RNP) complex (sgRNA and Cas9 protein) and desired concentration of GUIDE-seq dsODN was added to CD34+ HSPCs and electroporated at research scale using the Lonza Amaxa 4D-Nucleofector. All donors had high (> 70%) cell viability at 48 hours post-electroporation of the dsODN and RNP. To generate a DNA sequencing library, genomic DNA (gDNA) was isolated using the DNeasy Blood and Tissue Kit (Qiagen) according to the manufacturers protocol. gDNA was sheared to 200 bp fragments using a Covaris LE220 Focused-ultrasonicator. All remaining steps for sequencing library preparation were completed following GUIDE-seq methods described in Tsai et al.[1] GUIDE-seq libraries were sequenced on an Illumina NextSeq500 sequencer (Illumina). Sequencing reads were analyzed using a version of the GUIDE-seq analysis pipeline adapted for NextSeq sequencing reads[2] with the human genome build hg38 as the reference genome. All sites identified from the step "identify" were considered candidate off-target Cas9 cleavage sites regardless of the degree of sequence homology with sgRNA sequence. Due to the potential for false positive candidate off-target regions, all candidate GUIDE-seq regions suitable for next-generation sequencing were assessed

with hybrid capture sequencing, with low-complexity regions excluded from hybrid capture sequencing. The on-target site was used as an internal positive control and was identified in the edited samples of every donor with > 8,000 GUIDE-seq reads. In this experiment the number of (within sample control) on-target GUIDE-seq reads was higher than that in the original GUIDE-seq publication[1]. Tests of multiple guide RNAs from other projects using this same GUIDE-seq assay detected candidate off-target sites that were confirmed following hybrid capture.

IX. Identification of Candidate Off-target Regions Using Computational Methods

Computational prediction was used to identify regions with high sequence similarity to the sgRNA sequence. Specifically, regions in the reference human genome build hg38 were identified that contained up to 3 mismatches, or up to 2 mismatches with one DNA or RNA bulge, relative to the target sequence. The optimal NGG PAM was used for all algorithms, as well as the alternate PAMs NAG, NGA, NAA, NCG, NGC, NTG, and NGT when possible.

X. Hybrid-capture High-coverage Sequencing of Candidate Off-target Regions

Candidate regions identified by GUIDE-seq and computational methods were used to generate hybrid capture probes. gDNA from edited CD34+ HSPCs was isolated using the DNeasy Blood and Tissue Kit (Qiagen) according to the manufacturer's protocol. gDNA was fragmented by acoustic shearing with a Covaris LE220 instrument, and then end repaired, A-tailed, adapter ligated, and amplified with Agilent's SureSelect^{XT} Target Enrichment System for Illumina Paired-End Multiplexed Sequencing Library kit (Agilent Technologies) according to manufacturer's protocol. Prepared libraries were hybridized to probes (Agilent) and captured using streptavidin-coated magnetic beads (Dynabeads MyOne Streptavidin T1), amplified, and purified. Libraries were sequenced on Illumina's HiSeq sequencing platform, and sequencing data was subsequently aligned, deduplicated, and indels proximal to the cut site were counted. For each donor, indel frequency in sgRNA-RNP-treated samples and indel frequency in untreated negative controls were compared. For sites showing an indel frequency at least 0.2% higher in the treated sample compared to the matched untreated sample in any donor, we assessed whether indels seen at a potential off-target site were significantly enriched in samples treated with sgRNA-RNP as compared to untreated controls. A candidate site was confirmed to have editing if the statistical test resulted in a significant p-value ($p < 0.05$). No correction for multiple hypothesis testing was applied.

XI. On-Target Allelic Editing Frequency

The frequency of on-target allelic editing was assessed using Sanger sequencing, followed by analysis with a version of the Tracking of Indels by DEcomposition (TIDE) algorithm[3], except as specified in the engraftment study in Supplementary Methods Section XIV.

XII. Hemoglobin Tetramer Analysis

Edited or unedited CD34+ HSPCs were put through a three-step in vitro erythroid differentiation as previously described with some modifications.[4] Briefly, cells were cultured in the absence of an adherent stromal layer, without hydrocortisone, and with 20 ng/ml stem cell factor. Hemoglobin tetramers from in vitro differentiated erythroid cells were analyzed by ion-exchange high performance liquid chromatography with multi-wavelength UV detector (Agilent Technologies). Data were calculated from area under the peaks for fetal hemoglobin (HbF, α2γ2) and adult hemoglobin (HbA, α2β2 and α2d2), and presented as percentage of HbF over total hemoglobin [HbF/Total hemoglobin].

XIII. Subpopulation Analysis by Flow Cytometry

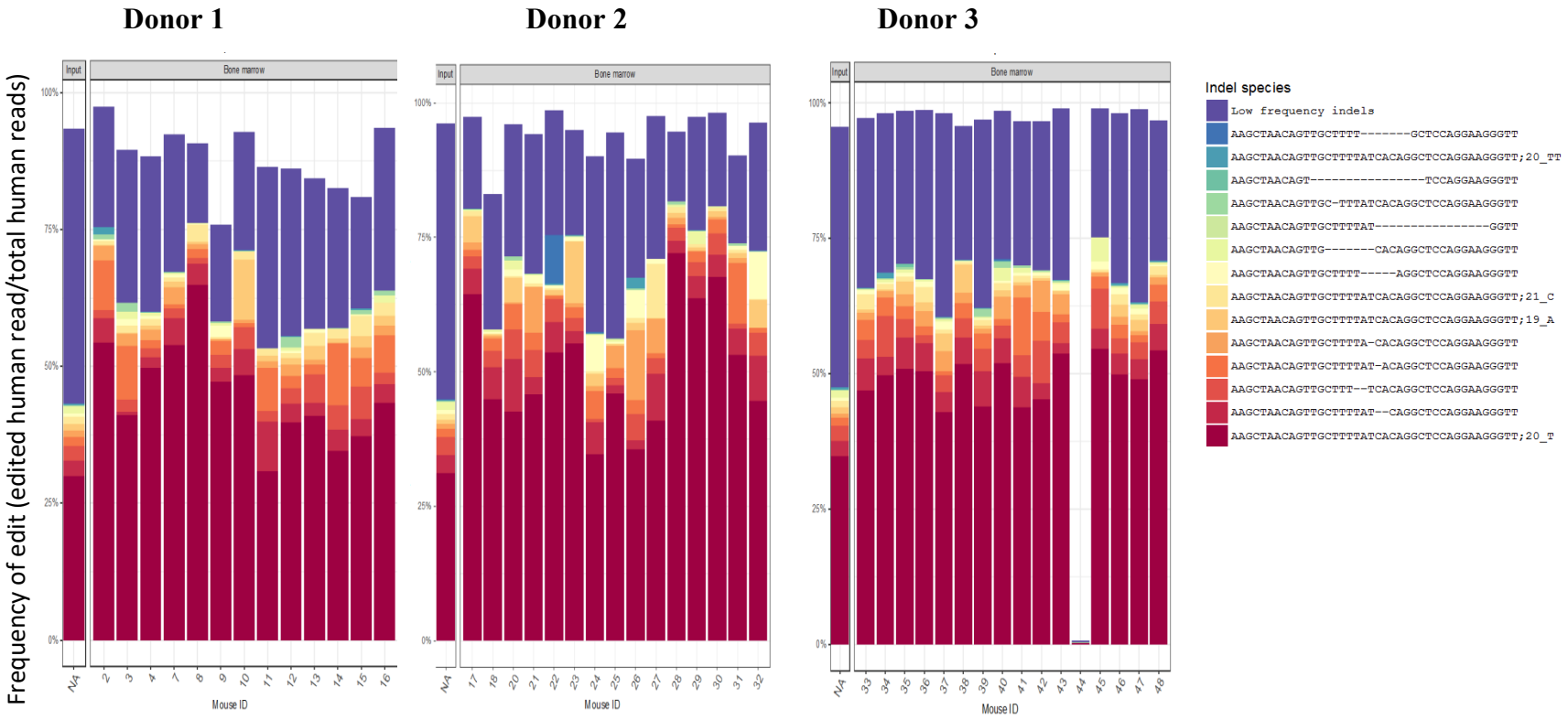
Edited CD34+ HSPCs were stained with CD34 antibody (Clone 581, BioLegend), CD38 antibody (Clone HB7, BioLegend), CD90 antibody (Clone 5E10, BD Biosciences) and CD45RA antibody (Clone HI100, BD Biosciences) and sorted with the BD FACSAria Fusion (BD Biosciences). Subpopulations were sorted based on the cell surface markers: live cells were first divided into two groups based on CD34 and CD38 staining. The CD34+/CD38+ subpopulation was further divided into CD45RA+ (granulocyte-monocyte progenitor cells) and CD45RA– (common myeloid progenitor cells / megakaryocyte-erythroid progenitor cells) populations. The CD34+CD38– subpopulation was further divided into three subpopulations based on CD90 and CD45RA staining patterns: CD90+CD45RA– (LT-HSC), CD90–CD45RA– (multipotent progenitor cells), and CD90–CD45RA+ (multipotent lymphoid progenitor cells). To distinguish between positive and negative staining, fluorescence minus one (FMO) controls were used as references. Allelic editing frequency in each subpopulation was determined as described in Supplementary Methods Section X.

XIV. Mouse Engraftment Study

~0.5 x 10^6 genetically edited or unedited CD34+ human HSPCs were injected into 6-8 weeks old female NOD/SCID/IL2Rγnull (NSG) mice following a total body irradiation (radiation dose 200 cGy). After a 16-week observation period, bone marrow samples were harvested. Percentage of human chimerism was assessed by flow cytometry using antibodies specific for human CD45RA and mouse CD45 (hCD45RA antibody, clone HI100, BD Biosciences; mCD45 antibody, clone 30-F11, BD Biosciences). gDNA was extracted from mouse bone marrow samples. An amplicon sequencing library was generated for each sample with two rounds of PCR: (i) using human genome target-specific primers and (ii) using Illumina sequencing-compatible index primers. Amplicon libraries were sequenced on Illumina's MiSeq sequencing platform with a 2x150bp configuration. Computational analysis was performed to quantify on-target allelic editing frequency and unique indel species. Sequencing reads were demultiplexed, merged, mapped, and aligned to the human reference genome hg38. Sequencing reads were grouped based on the sequence at the cut site to identify unique indel species and corresponding frequencies. To calculate total allelic editing frequency, frequencies of all observed indel species were summed, and a one-sided Mann-Whitney test was used to test for any significant decrease in total percent editing at 16 weeks post-injection. A p-value less than 0.05 was considered statistically significant.

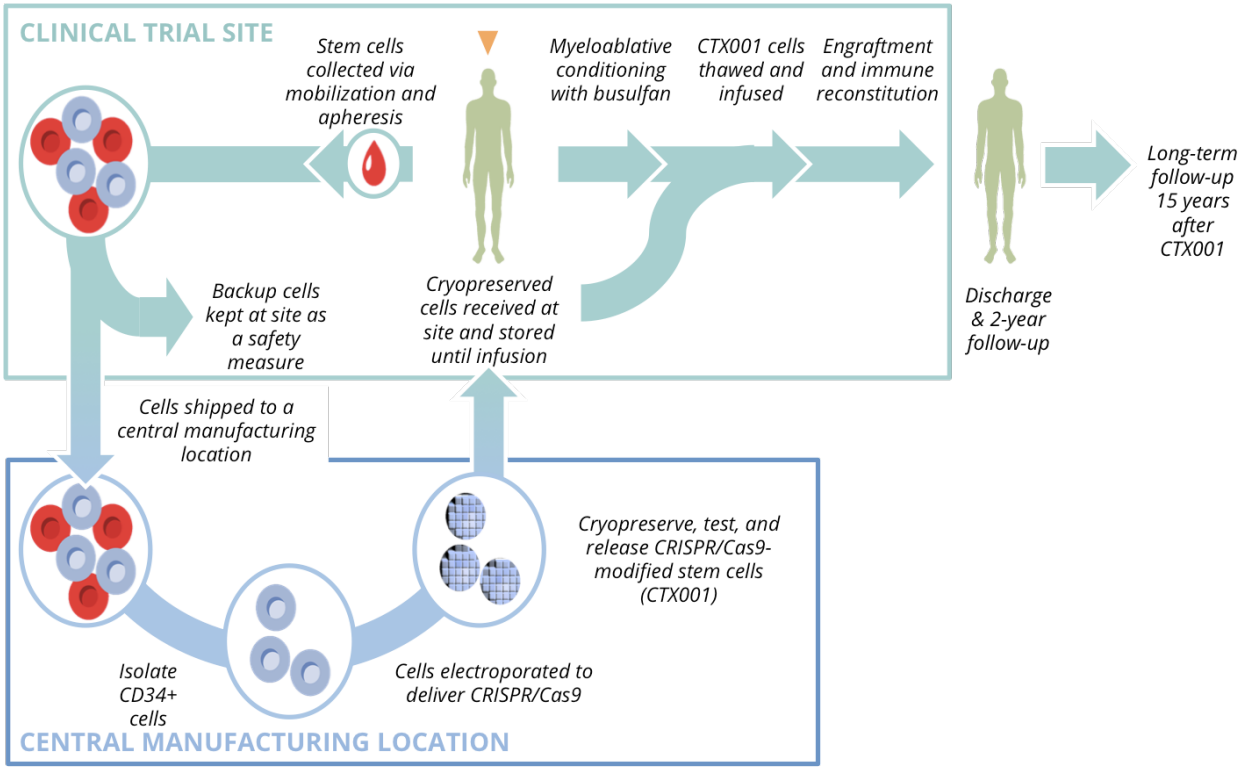
Supplementary Results

Figure S1. Indel Distribution Profile in Input Edited CD34+ Human HSPCs and Engrafted Mouse Bone Marrow



Notes: Indel distributions of CD34^{+} cells in sgRNA-RNP-edited input and mouse bone marrow samples at 16 weeks post-injection for sgRNA-RNP-edited samples from three donors. The top purple stacked bar is a conglomerate of all unique sequences with <8% frequency in all sgRNA-RNP-edited samples. Each color other than the top bar represents a single unique on-target edit (insertion or deletion). Deleted bases are indicated with a dash, whereas insertions are annotated with their position relative to the shown sequence and the sequence inserted, such that "20_T" indicates an insertion of a T at the cut site, centered in the sequence shown (20 bases from the start of the sequence shown). The total proportion of edited sequences is represented by the total height of the stacked bars. Each input edited hHSPC sample and mouse bone marrow sample is represented by a separate bar. Mouse #44 has very low or background levels of allelic editing likely due to experimental error.

Figure S2. Schematic Representation of the CTX001 Manufacturing and Infusion Process



Patients enrolled in CLIMB THAL-111 received a combination of plerixafor and filgrastim for mobilization, while patients enrolled in CLIMB SCD-121 received plerixafor only.

Patients will be followed for 24 months after CTX001 infusion with physical exams, laboratory and imaging assessments, and AE evaluations. All patients who receive CTX001 will be followed for 15 years in a long-term follow-up study (NCT04208529) after completion or withdrawal from CLIMB THAL-111 or CLIMB SCD-121.

Table S1. Allelic Editing Frequency in Subpopulations of Edited CD34+ HSPCs

Sample	Bulk Editing (%)	LT-HSC (%)	MPP (%)	MLP (%)	GMP (%)	CMP/MEP (%)
1	74	78	80	87	85	86
2	77	77	81	79	83	84
3	75	70	71	87	88	87
4	77	65	67	85	83	70
5	80	73	76	90	90	89
6	81	79	80	88	88	79
7	85	85	79	88	89	88
8	86	85	80	89	91	85

LT-HSC: long-term hematopoietic stem cells; MPP: multipotent progenitor cells; MLP: multipotent lymphoid progenitor cells; GMP: granulocyte-monocyte progenitor cells; CMP: common myeloid progenitor cells; MEP: megakaryocyte-erythroid progenitor cells; HSPC: hematopoietic stem and progenitor cells.

Table S2. Persistence of Editing in Mice Engrafted with Edited CD34+ Human HSPCs at 16 Weeks

Sample Type[a]	Number of Samples	Allelic Editing Frequency Mean (Standard Deviation)
Input (Edited CD34+ Human HSPCs)	3	95.1% (1.5%)
Bone marrow from mice engrafted with edited CD34+ human HSPCs	44	91.4% (15.1%)[b]

[a] Genetically edited and unedited CD34+ human HSPCs were injected into NOD/SCID/IL2Rγnull (NSG) mice via a single intravenous (i.v.) injection. After a 16-week observation period, mouse bone marrow samples were collected to assess allelic editing frequency in engrafted human cells.

[b] Using a one-sided Mann-Whitney test to check for a loss of editing after engraftment, the evidence did not reject the null hypothesis that no reduction in editing occurred in bone marrow (P = 0.61), suggesting that there is not a significant reduction in editing over the time span of engraftment.

Table S3. Engraftment of Edited and Unedited CD34+ Human HSPCs at Week 8 and Week 16 in Mice: Analysis of hCD45RA^{+} % Chimerism[a] (Group Averages Across All Donors) Using Flow Cytometry

Tissue	Time Point	Group 1 (No-electroporation) % (SD)	Group 2 (Mock-EP)[b] % (SD)	Group 3 (GFP)[c] % (SD)	Group 4 (sgRNA)[d] % (SD)
Whole Blood	Week 8	43.2 (11.7) n=40	32.0 (10.1) n=47	34.1 (8.6) n=47	32.7 (9.4) n=47
	Week 16	12.9 (7.2) n=41	4.4 (3.5) n=48	4.1 (2.8) n=43	4.4 (3.1) n=44
Bone Marrow	Week 16	64.8 (14.1) n=44	49.5 (12.1) n=45	49.1 (13.0) n=45	46.0 (13.8) n=44
Spleen	Week 16	49.6 (15.5) n=39	28.4 (11.7) n=45	29.5 (11.7) n=43	27.0 (11.2) n=42

Note that all n values refer to the number of flow cytometry samples analyzed.
[a]Percentage of chimerism was determined by flow cytometry and is based on hCD45RA.
% Chimerism = % hCD45RA+ cells in live cells/(% hCD45RA+ cells in live cells + % mCD45+ cells in live cells)
[b] Mock electroporation of cells without any CRISPR/Cas9 editing components
[c] Electroporation of cells with green fluorescent protein (GFP) gRNA and Cas9 protein
[d] Electroporation of cell with sgRNA (used in this study) and Cas9 protein

Table S4. Mobilization Agents and CTX001 Cell Dose Manufactured for Patient 1 (TDT) and Patient 2 (SCD)

Mobilization and cell dose characteristics			
	Mobilization Cycles	**Mobilization Agents**	**Cell dose manufactured, 10^6 cells/kg**
Patient 1, TDT	1	G-CSF/plerixafor	16.6
Patient 2, SCD	2	Plerixafor	3.1

G-CSF, granulocyte colony-stimulating factor; SCD, sickle cell disease; TDT, transfusion-dependent β-thalassemia.

Table S5. Demographics and Pre-Study Characteristics of Patient 1 (TDT)

	Patient 1, TDT
Genotype	β^0/β^+ *β^0 due to the c.118 C>T mutation [also known as Codon 39 (C->T)]in exon 2 of the beta globin gene* β^+ *due to the c.93-21 G>A mutation [also known as IVS-I-110 (G->A)]*
Age at consent, *years*	19
Gender	F
Pre-study pRBC transfusions[a]	
Units/year	34
Transfusion episodes/year	16.5

[a]Annualized number during the 2 years before consenting to study participation. pRBC, packed red blood cells
TDT: transfusion-dependent β-thalassemia; pRBC: packed red blood cells

Table S6. Summary of Adverse Events for Patient 1 (TDT)

Screening to CTX001 infusion	
AEs	12
SAEs	0
After CTX001 infusion	
AEs	32
SAEs	2[a]
Months of follow-up	21.5
AEs by Grade	
Grade 1	31
Grade 2	7
Grade 3	6
Grade 4	0

[a]Pneumonia and venoocclusive liver disease both of which resolved
AE, adverse event; n, number of AE events; SAE, serious adverse event

Table S7. Summary of Laboratory Values for Patient 1 (TDT)[a]

	Normal Range	Baseline	Month 3	Month 6	Month 12
Creatinine (mg/dL)	0.5-1.1	0.54	0.48	0.51	0.68
AST (U/L)	0-34	15	17	21	13
ALT (U/L)	0-34	12	25	19	16
Total bilirubin (µmol/L)	3.4-23.9	85.3	28.6	32.1	27.4
Alkaline phosphatase (U/L)	45-117	80	138	149	158

	Normal Range	Baseline	Month 3	Month 6	Month 12
Hematocrit (%)	34.1-44.9	32.0	33.9	35.5	35.2
Total hemoglobin (g/dL)	11.2-15.7	11.2	11.6	12.5	12.5
MCV (fl)	79.4-94.8	82.9	80.7	80.0	79.6
MCH (PG)	25.6-32.2	29	27.6	28.2	28.3
MCHC (g/dL)	32.2-35.5	35.0	34.2	35.2	35.5
Leukocytes ($*10^6$/L)	3.98-10.00	8.64	4.65	5.14	5.39
Platelets ($*10^9$/L)	182-369	253	115	117	176
$CD19^+$ B-Cells ($*10^6$/L)	100-500	451	304	389	357

[a] Note that all values in table were collected and assayed at local laboratories. ALT: alanine aminotransferase; AST: aspartate aminotransferase; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration

Table S8. Busulfan Administration for Patient 1 (TDT)

Study Day[a]	Busulfan Dose	Observed AUC[b]
Day -7	3.2 mg/kg	3811.40 µmol*min/L
Day -6	5 mg/kg	Not Done
Day -5	5 mg/kg	7490.50 µmol*min/L
Day -4	2.4 mg/kg	Not Done

[a]Study Day 1 is day of CTX001 infusion.
[b]Target range for busulfan dose AUC is 4500-5500 µmol*min/L.
AUC: area under the curve

Table S9. Summary of LDH and Haptoglobin Data for Patient 1 (TDT)

Subject	Serum LDH[a] (U/L)	Haptoglobin[b] (mg/dL)	Reticulocyte Count (10^9/L)
Patient 1	B[c]: 185	B[c]: BDL	B[d]: 13.1
	M1: --	M1: --	M1: 6.3
	M2: --	M2: --	M2: 123.8
	M3: 156	M3: BDL	M3: 83.8
	M4: --	M4: --	M4: 77.2
	M5: --	M5: --	M5: 103.2
	M6: 159	M6: 7.8	M6: 76.8
	M9: 136	M9: 7.8	M9: 105.0
	M12: 159	M12: BDL	M12: 97.2
	M15: 140	M15: 10	M15: 72.7

B: baseline; BDL: below detectable limit; LDH: lactate dehydrogenase; M: Month; NA: not available; TDT: transfusion-dependent β-thalassemia

Note: Measured time points are presented according to study protocol.]

[a] Serum LDH normal range 0-249 U/L.

[b] Haptoglobin normal range 30-200 mg/dL.

[c] Baseline was collected at the most recent nonmissing measurement (scheduled or unscheduled) collected during screening and before the start of mobilization.

[d] Baseline was collected prior to mobilization procedure.

Table S10. Demographics and Pre-Study Characteristics of Patient 2 (SCD)

	Patient 2, SCD[a]
Genotype	β^s/β^s
Age at consent, *years*	33
Gender	F
Pre-study VOCs/year[b]	7
Pre-study hospitilizations/year[b]	3.5
Pre-study pRBC transfusions[b]	
Units/year	5

[a]Patient had received hydroxyurea treatment from 2016 to November 22, 2018 (Study Day −222)

[b]Annualized rate during the 2 years before consenting to study participation.

SCD: sickle cell disease; VOCs: vaso-occlusive crises; pRBC: packed red blood cell

Table S11. Summary of Adverse Events for Patient 2 (SCD)

Screening to CTX001 infusion	
AEs	35
SAEs	11[a]
After CTX001 infusion	
AEs	114
SAEs	3[b]
Months of follow-up	16.6
AEs by Grade	
Grade 1	46
Grade 2	48
Grade 3	53[c]
Grade 4	2[d]

[a]Sickle cell anemia with crisis (n=3), nausea, arthralgia, back pain, pain in extremity, chest pain, neck pain, headache, and abdominal pain, all of which resolved; [b]Sepsis, cholelithiasis, and abdominal pain, all of which resolved; [c]Most common Grade 3AEs occurring ≥2 times after CTX001 infusion: neck pain (n=5), headache (n=5), cholelithiasis (n=4), musculoskeletal chest pain (n=2) and non-cardiac chest pain (n=2), oesophagitis (n=3), and stomatitis (n=2), all of which resolved; [d]Neutropenia and leukopenia, both of which resolved

AE, adverse event; n, number of AE events; SAE, serious adverse event

Table S12. Summary of Laboratory Values for Patient 2 (SCD)[a]

	Normal Range	Baseline	Month 3	Month 6	Month 12
Creatinine (mg/dL)	0.5-1.1	0.73	0.64	0.76	0.45
AST (U/L)	8-46	39	33	70	18
ALT (U/L)	6-50	37	52	95	35
Total bilirubin (µmol/L)	0.0-22.2	15.4	8.6	8.6	8.6
Alkaline phosphatase (U/L)	50-136	203	244	374	186

	Normal Range	Baseline	Month 3	Month 6	Month 12
Hematocrit (%)	36-46	29.9	30.9	34.0	32.3
Total hemoglobin (g/dL)	12-16	10.2	10.3	11.4	11.1
MCV (fl)	80-100	85.3	92	89.8	86.7
MCH (PG)	27-35	29.1	30.6	30.1	29.8
MCHC (g/dL)	31-37	34.1	33.2	33.5	34.4
Leukocytes ($*10^9$/L)	3.9-10.6	11.7	6.2	4.7	3.8
Platelets ($*10^9$/L)	150-450	326	268	256	272
CD-19^+, B-cells ($*10^6$/L)	91-610	595	206	310	392

[a] Note that all values in table were collected and assayed at local laboratories.

ALT: alanine aminotransferase; AST: aspartate aminotransferase; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration

Table S13. Busulfan Administration for Patient 2 (SCD)

Study Day[a]	Busulfan Dose	Observed AUC[b]
Day -6	3.2 mg/kg	5791 μmol*min/L
Day -5	3.2 mg/kg	Not Done
Day -4	2.3 mg/kg	Not Done
Day -3	2.3 mg/kg	Not Done

[a]Study Day 1 is day of CTX001 infusion.
[b]Target range for busulfan dose AUC is 4500-5500 μmol*min/L.
AUC: area under the curve

Table S14. Summary of LDH and Haptoglobin Data for Patient 2 (SCD)

Subject	Serum LDH[a] (U/L)	Haptoglobin[b] (mg/dL)	Reticulocyte Count (10^9/L)
Patient 2	B[c]: 282	B[c]: BDL	B[c]: 251.77
	M1: 155	M1: 377	M1: 29.80
	M2: --	M2: --	M2: 97.92
	M3: 175	M3: 88	M3: 117.60
	M4: --	M4: --	M4: 77.91
	M5: --	M5: --	M5: 56.98
	M6: 193	M6: 90	M6: 94.75
	M9: 173	M9: 124	M9: 94.32
	M12: 138	M12:145	M12: 100.44

B: baseline; BDL: below detectable limit; LDH: lactate dehydrogenase; M: Month; SCD: sickle cell disease
Note: Measured time points are presented according to study protocol.
[a] Serum LDH normal range 100-190 U/L.
[b] Haptoglobin normal range 30-200 mg/dL.
[c] Baseline collected prior to first mobilization procedure.

Table S15. Ratio of Fetal Hemoglobin (HbF) to Sickle Hemoglobin (HbS) in Patient 2 (SCD)

	Screening	Month 3	Month 6	Month 12	Month 15
HbF (g/dL)	0.6552	3.7572	5.3449	4.3672	5.184
HbS (g/dL)	5.3352	3.2926	5.6161	5.4693	6.276
Ratio of HbF/HbS	0.12	1.14	0.95	0.80	0.83

Table S16. Withdrawal from CLIMB SCD-121 Study Prior to CTX001 Dosing

Patient Withdrawn	Reason for withdrawal
001	Early termination: Physician decision, due to worsening SCD, renal disease, and reduction in eGFR that no longer met criteria for having an HSC transplant. Partial drug product (CTX001) was made but not used. Withdrawal was independent of allelic editing.
002	Early termination: Physician decision, after 2 cycles of mobilization and apheresis due to poor collections. Partial drug product (CTX001) was made but not used. Withdrawal was independent of allelic editing.
003	Early termination: Physician decision prior to undergoing mobilization and apheresis due to poor compliance to study visits and poor communication with site.
004	Early termination: Patient personal decision shortly after signing consent prior to undergoing mobilization. No post-enrollment study assessments were conducted with this patient.

Supplementary References

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3. Brinkman EK, Chen T, Armendola M, van Steensel B. Easy quantitative assessment of genome editing by sequence trace decomposition. Nucleic Acids Res. 2014; 4(22):e168.
4. Giarratana M-C, Kobari L, Lapillonne H, et al. Ex vivo generation of fully mature human red blood cells from hematopoietic stem cells. Nat Biotechnol. 2005; 23(1):69-74.

Data Sharing Statement

Vertex Pharmaceuticals Incorporated and CRISPR Therapeutics are committed to advancing medical science and improving patient health. This includes the responsible sharing of clinical trial data with qualified researchers. Proposals for the use of these data will be reviewed by a scientific board. Approvals are at the discretion of Vertex and CRISPR and will be dependent on the nature of the request, the merit of the research proposed, and the intended use of the data. Please contact CTDS@vrtx.com or medicalaffairs@crisprtx.com if you would like to submit a proposal or need more information.