Preliminary Results of a Phase 1/2 Clinical Study of Zinc Finger Nuclease-Mediated Editing of *BCL11A* in Autologous Hematopoietic Stem Cells for Transfusion-Dependent β-Thalassemia

Poster# 3544

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Background

- Transfusion-dependent β-thalassemia (TDT) is an inherited, severe anemia characterized by absent or reduced β-globin chain synthesis resulting in excess α-globin chains, ineffective erythropoiesis, and hemolysis. TDT is treated with lifelong blood transfusions.
- Allogeneic hematopoietic stem cell transplantation (HSCT) can be curative in TDT but is often limited by donor availability and the risks of graft failure and graft-vs-host disease. Autologous HSCT using *ex vivo* gene-modified hematopoietic stem progenitor cells (HSPC) circumvents these limitations.
- Persistently high expression of fetal hemoglobin (HbF) can ameliorate TDT.[1] Thus *BCL11A*, a master regulator of the fetal-to-adult hemoglobin switch, is a rational target for disruption by gene editing to increase HbF for patients with TDT.
- In pre-clinical studies with human HSPC, zinc finger nuclease (ZFN)-mediated disruption of a GATA-binding region in the intronic erythroid-specific enhancer (ESE) of *BCL11A* increased endogenous HbF production in erythroid cells without adversely affecting normal, multi-lineage hematopoiesis.[2]
- ST-400 is an investigational cell therapy consisting of autologous CD34+ HSPCs that have undergone high-precision, ZFN-mediated *ex vivo* gene editing to disrupt the *BCL11A* ESE.

Aim

- To evaluate safety, tolerability, and efficacy of ST-400 transplantation in patients with TDT in a study using ZFN-mediated, *ex vivo* gene editing targeting *BCL11A* ESE (THALES study, NCT03432364).

Methods

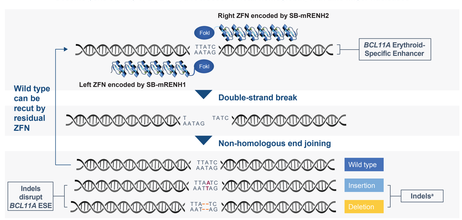
Study Design and Patient Population

- This is a Phase 1/2 single-arm clinical trial enrolling a total of 6 patients.
- Inclusion criteria include patients aged 18 to 40 years with molecularly confirmed β-thalassemia, treated with ≥8 packed red blood cell (PRBC) transfusion events per year for ≥2 consecutive years, and medically able to complete all aspects of the autologous HSCT procedure.
- Exclusion criteria include γ-globin allelic variants associated with clinically significant high oxygen affinity.

Targeted Gene Editing and ST-400 Manufacturing

- Mobilized CD34+ HSPCs were selected from apheresis products and transfected *ex vivo* via electroporation with SB-mRENH1 and SB-mRENH2, engineered mRNAs encoding 2 ZFNs that use Fok1 nuclease heterodimers for site-specific cleavage at a GATA-binding region of the *BCL11A* ESE.
- After double-strand break by the engineered ZFNs, endogenous DNA repair introduces a variety of small insertion or deletion mutations (collectively called indels) disrupting the ESE region (**Figure 1**), resulting in decreased expression of *BCL11A* and allowing de-repression and increased expression of endogenous γ-globin.
- Single-cell erythroid differentiation *in vitro* experiments have previously revealed skewing toward bi-allelic editing following HSC transfection with SB-mRENH1 and SB-mRENH2, with around 90% of all modified cells demonstrating indels at both *BCL11A* ESE targets.

Figure 1. Targeted Gene Editing at the Erythroid Specific Enhancer of *BCL11A*

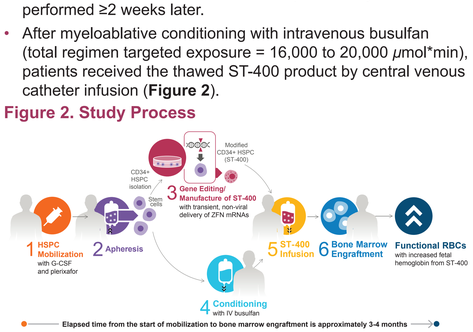


*Example sequences shown. Many other indel sequences are possible.
ESE, erythroid specific enhancer; ZFN, zinc finger nuclease.

Study Procedures

- After mobilization with granulocyte colony-stimulating factor and plerixafor, CD34+ cells were collected via apheresis on 2 consecutive days and submitted for ST-400 manufacturing; unmanipulated backup grafts were collected on the third day.
- If needed, a second mobilization and apheresis cycle was performed ≥2 weeks later.
- After myeloablative conditioning with intravenous busulfan (total regimen targeted exposure = 16,000 to 20,000 μmol*min), patients received the thawed ST-400 product by central venous catheter infusion (**Figure 2**).

Figure 2. Study Process



G-CSF, granulocyte colony-stimulating factor; HSPC, hematopoietic stem progenitor cells; IV, intravenous; mRNA, messenger RNA; RBC, red blood cells; ZFN, zinc finger nuclease.

- For this first-in-human study of ST-400, the protocol directed that patients 2 and 3 could not begin chemotherapy conditioning until the previous patient demonstrated neutrophil and platelet engraftment; after successful engraftment of patient 3, patients 4-6 may begin chemotherapy conditioning.
- Patients were monitored for safety and efficacy. The current study will encompass follow-up for 3 years, after which patients will be offered participation in a long-term safety follow-up study.

Outcome Measures

- Safety and tolerability were assessed by incidence of adverse events (AEs) and serious AEs (SAEs).
- Success and kinetics of hematopoietic reconstitution were assessed by neutrophil (absolute neutrophil count ≥500 cells/μL) and platelet (≥20,000 cells/μL unsupported by transfusion) engraftment.
- On-target indel patterns were tracked at the molecular level over time for surveillance of emerging hematopoietic clones.
- Patients were monitored for presence of on-target indels in hematopoietic cells, HbF concentration, and transfusion requirements after ST-400 infusion; post-transplantation hemoglobin transfusion thresholds were per clinical sites' standard practice (Patients 1 and 2: <8 g/dL; Patient 3: <7 g/dL).
- This preliminary analysis includes safety data available on-or-before October 10, 2019, and laboratory and clinical data available on-or-before November 4, 2019.

Results

- To date, autologous ST-400 product has been manufactured for 5 patients, 3 of whom have received ST-400 (**Table 1**).
- Safety and efficacy data reported in this analysis are preliminary. Adverse events, HbF production, indel markings, and PRBC transfusion requirements for these patients are expected to evolve over time, potentially for 12 months or longer.

Table 1. Patient Demographics and Disease Characteristics

Patient	Age at Consent (Years)	Genotype	Annualized PRBC Events Pre-enrollment	Most Recent Study Visit
1	36	β° β°	27	39 weeks
2	30	β⁺ (severe IVS-I-5: G>C) β⁺ (severe IVS-I-5: G>C)	18	26 weeks
3	23	β° β⁺ (severe IVS-II-654 C>T)	15	12 weeks
4	18	β^{WT} (αα) β° (αααα)	13	Pre-infusion
5	35	β° β⁺ (severe IVS-I-110 G>A)	15	Pre-infusion

β°, absence of β-globin production; β⁺, decreased β-globin production; β^{WT}, wild type (normal β-globin production); PRBC events, packed red blood cell transfusions.

Mobilization and Apheresis Outcomes

- Peripheral blood CD34+ counts before daily apheresis varied from 25 to 118 cells/μL.
- Patient 1 underwent 2 cycles of mobilization and apheresis due to low cell dose and CFU potency in the first ST-400 lot. The backup graft was cryopreserved from the first cycle.
- Patients 2, 3, 4, and 5 each underwent 1 cycle of mobilization and apheresis from which their ST-400 lots were manufactured, and back-up grafts cryopreserved.

ST-400 Product Characteristics and Hematopoietic Reconstitution

- On-target indels in the ST-400 product ranged from 23% to 80% (**Table 2**). The lowest indel value was seen in Patient 1, for whom editing efficiency was near 25% in 2 separately manufactured lots.
- Using the same manufacturing process at clinical scale, CD34+ cells from 12 healthy donors were efficiently edited: median on-target indels, 71%; range, 59% to 83%.
- ST-400 viable nucleated cell doses were 4.5 to 11.4 x 10^6 cells/kg.
- Patients demonstrated neutrophil engraftment in 14 to 22 days and platelet engraftment in 22 to 35 days.

Table 2. ST-400 Product Characteristics and Hematopoietic Reconstitution

Patient	Cell Dose (10^6/kg)	CD34+ (%)	CFU Dose (10^5/kg)	On-target Indels[a] (%)	Neutrophil Engraftment[b] Day(s)	Platelet Recovery[c] Day(s)
1[d]	5.9	91	6.2	23[e]	14	25
2[d]	4.5	87	4.0	73	15	22
3[f]	11.4	90	14.8	54	22	35
4	5.4	86	7.3	80	Pre-infusion	Pre-infusion
5	9.5	98	10.5	76	Pre-infusion	Pre-infusion

[a]Percentage of all *BCL11A* ESE alleles with an indel; this is not equivalent to the percentage of all cells with at least 1 edited *BCL11A* ESE allele. [b]Neutrophil engraftment was defined as occurring on the first of 3 consecutive days on which the patient's neutrophil count was ≥500 cells/μL. [c]Platelet engraftment was defined as occurring on the first of 3 consecutive measurements spanning a minimum of 3 days (in the absence of platelet transfusion in the preceding 7 days) on which the patient's platelet count was ≥20,000 cells/μL. [d]Patients 1 and 2 received G-CSF from Day +5 through neutrophil engraftment per site's standard operating procedure. [e]Patient 1 underwent 2 cycles of apheresis and manufacturing of ST-400; on-target indel percentage for the lot not shown was 26%. All other patients underwent only 1 cycle of apheresis and manufacturing. [f]Patient 3 received G-CSF from Day +21 through neutrophil engraftment per site's standard operating procedure. CFU, total colony-forming unit; ESE, erythroid-specific enhancer; G-CSF, granulocyte colony-stimulating factor.

Safety

- One SAE attributed to ST-400 drug product was reported to date; this SAE of hypersensitivity occurred during ST-400 infusion, resolved by the end of infusion, and was considered by the investigator to be likely related to the product's cryoprotectant excipient, dimethyl sulfoxide (DMSO).
- Otherwise, reported AEs have been consistent with the known toxicities of mobilization, apheresis, and myeloablative busulfan conditioning.

Table 3. Serious Adverse Events

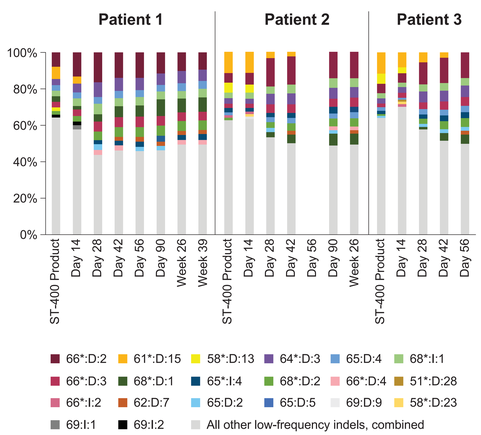
Patients[a]	Serious Adverse Events	Related to ST-400
1	Hypersensitivity[b]	RELATED
3	Pneumonia[c]	NOT RELATED

[a]No serious AEs have been reported by Patients 2, 4, or 5. [b]Occurred during infusion of ST-400 and rapidly resolved with medical management; considered by the investigator to be likely related to the product's cryoprotectant excipient, DMSO. [c]Pneumonia occurred in the time period between the apheresis procedure and the start of chemotherapy conditioning. DMSO, dimethyl sulfoxide.

Changes After Infusion

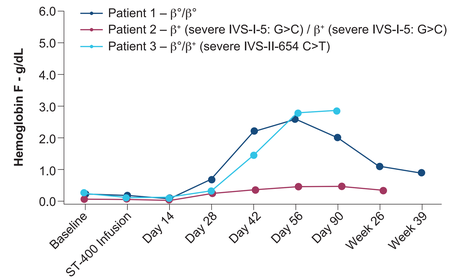
- No emerging clonal hematopoiesis has been observed by on-target indel pattern monitoring over time in the 3 dosed patients (**Figure 3**). Through most-recent observations in Patient 1 (Month 9), Patient 2 (Month 6), and Patient 3 (Day 56), the maximum frequency of a unique indel at any time point has been 16%, 16%, and 14% of all indels detected, respectively.
- After ST-400 transplantation, HbF concentration increased compared with baseline in all 3 patients (**Figure 4**), with Patients 1 and 3 showing greater induction than Patient 2. In this very early experience, no definite patient- or ST-400 lot–related characteristics are determined to be causative of these findings, though it is noteworthy that Patient 2 received ST-400 product with the lowest cell dose and CFU potency.

Figure 3. Monitoring On-target Indel Patterns for Assessment of Hematopoietic Clonality



The 10 most frequent indels detected by next-generation sequencing of nucleated blood cells (bone marrow aspirates, circulating leukocytes, or peripheral blood mononuclear cells, as available) are shown per patient at each time point.
No emerging dominance worrisome for hematopoietic clonality has been observed over time. Indel naming convention: I, insertion; D, deletion; first number, start of indel from reference base pair (*=nucleotides flanking indel could align to either side of the indel); number following colon, number of base pairs inserted or deleted. Day 56 data are not available for Patient 2.

Figure 4. Fetal Hemoglobin (g/dL) Over Time in Evaluable Patients



- In Patient 1, indels have persisted in peripheral leukocytes through Month 9. After an initial transfusion-free period of 6 weeks, this patient has resumed intermittent PRBC transfusions, with a 33% reduction in annualized PRBC units transfused at approximately 8 months since engraftment.
- In Patient 2, indels have persisted in peripheral leukocytes through Month 6. The patient is receiving intermittent PRBC transfusions.
- In Patient 3, indels have persisted in peripheral leukocytes through Day 56. After an initial transfusion-free period of 7 weeks, the patient has received 2 PRBC transfusions beginning at 62 days post-infusion.

Conclusions

- In early experience, ST-400 has been well tolerated.
- One SAE attributed to ST-400 drug product was reported to date: this SAE of hypersensitivity occurred during ST-400 infusion, resolved by the end of infusion, and was considered by the investigator to be likely related to the product's cryoprotectant excipient, DMSO.
- Other AEs were consistent with those observed during myeloablative autologous HSCT.
- Because these data are preliminary, additional patients and further follow-up are needed to understand the potential long-term risks and benefits of ST-400, including analysis of the effects of patient- and ST-400 product–related characteristics.
- Enrollment is ongoing. Infusion with ST-400 is planned for a total of 6 patients in this study.

References

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