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

Poster 3974

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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
- Persistently elevated fetal hemoglobin (HbF) is associated with a milder disease course in patients with β–thalassemia[1]
- *BCL11A* is a repressor of γ-globin expression and HbF production in adult erythrocytes. Downregulation of *BCL11A* is a therapeutic strategy for induction of HbF in beta hemoglobinopathies
- In preclinical studies with human hematopoietic stem cells (HSC), zinc finger nuclease (ZFN)-mediated disruption of the GATA-binding region of the intronic erythroid-specific *BCL11A* enhancer (*BCL11A* ESE) decreased *BCL11A* expression and increased HbF production in erythroid cells without disrupting multilineage hematopoiesis[2]
- ST-400 is an investigational cell therapy product comprised of autologous CD34+ cells that have undergone high-precision, ZFN-mediated *ex vivo* editing at the *BCL11A* gene ESE target

Aim

- The aim of this study was to induce HbF expression in edited erythroid cells following infusion of ST-400 in patients with TDT

Methods

Study Design and Patient Population

- The Thales trial (NCT03432364) is a Phase 1/2 study of the safety, tolerability, and efficacy of ST-400 in adult patients with TDT, defined as receiving ≥8 annual red blood cell transfusion events over at least 2 consecutive years before enrollment
- Leukapheresis was performed following mobilization with granulocyte colony-stimulating factor (G-CSF) and plerixafor
- Autologous collections were enriched for CD34+ cells and then transfected with mRNA encoding ZFNs with binding sites flanking the GATA-binding region of *BCL11A* ESE
- The ST-400 product was infused following myeloablative busulfan conditioning
- The study planned to enroll 6 patients to be monitored for safety and efficacy for 3 years post-infusion

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 tracked at the molecular level over time for surveillance of emerging hematopoietic clones
  - Clonal expansion/dominance occurs when the frequency of a unique insertion or deletion is ≥90%, or there is >40% change in the indel frequency from 3 consecutive blood collections
- Patients monitored for the presence of on-target indels in hematopoietic cells, HbF concentration, and transfusion requirements after ST-400 infusion; post-transplantation hemoglobin transfusion thresholds were <8 g/dL, except for Patient 3, which was <7 g/dL
- This updated analysis includes data collected on or before September 14, 2021

Results

- Five patients (average 28 years) have been infused with ST-400 (**Table 1**)
- Patients received an average of 7.3 x 10^6 CD34+ cells/kg (min-max: 4.5-11.4) (**Table 2**)

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^0/β^0	27	Week 130 (36.6 months)
2	30	β$^+$ (severe IVS-I-5: G>C)/β$^+$ (severe IVS-I-5: G>C)	18	Week 117 (34.5 months)
3	23	β^0/β$^+$ (severe IVS-II-654 C>T)	15	Week 104 (27.2 months)
4[a]	18	β^{WT} (αα)/β^0 (ααα)	13	Week 65 (19.4 months)
5	35	β^0/β$^+$ (severe IVS-I-110 G>A)	15	Week 91 (25 months)

[a]Patient 4 conducted the End of Study Visit at Week 65
β^0, absence of β–globin production; β$^+$, decreased β–globin production; β^{WT}, wild type (normal β–globin production); PRBC events, packed red blood cell transfusion

ST-400 Product Characteristics and Hematopoietic Reconstitution

- On-target indels in the ST-400 product ranged from 23% to 80% (**Table 2**)
- Patients achieved neutrophil engraftment in 14 to 24 days and platelet engraftment in 19 to 44 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 Engraftment[c] Day(s)	Received G-CSF Day(s)
1	5.9	91	6.2	23[d]	14	25	5-24
2	4.5	87	4.0	73	15	22	9-22
3	11.4	90	14.8	54	22	35	21
4	5.4	86	7.3	80	24	44	2-35
5	9.5	98	10.4	76	14	19	7-16

[a]Percentage of all *BCL11A* ESE alleles with an indel (not cells with at least 1 edited *BCL11A* ESE allele)
[b]Neutrophil engraftment occurring on the first of 3 consecutive days on which the patient's neutrophil count was ≥500 cells/μL
[c]Platelet engraftment occurring on the first of 3 consecutive measurements over a minimum of 3 days with platelet count ≥20,000 cells/μL and in the absence of platelet transfusion in the preceding 7 days
[d]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
CFU, total colony-forming unit; ESE, erythroid-specific enhancer; G-CSF, granulocyte colony-stimulating factor

- With follow-up ranging from approximately 19 to 37 months, on-target indels at *BCL11A* ESE were present in peripheral blood mononuclear cells (PBMCs) and white blood cells (WBCs) of all 5 subjects (**Table 3**)

Table 3. Mean Percentage of Indels at Targeted Locus (*BCL11A* ESE) in Patients Over Time

Parameter	Day 0	Day 14	Day 28	Day 42	Day 56	Day 90	Week 26	Week 39	Week 52	Week 78	Week 104
ST-400 Drug Product	n=5 61.3%										
PBMC		n=3 2.2%	n=2 15.3%	n=2 13.6%	n=1 6.3%	n=2 8.4%	n=2 9.1%	n=2 10.2%	n=2 8.9%	n=2 7.8%	n=2 14.5%
WBC		n=2 6.5%	n=3 36.8%	n=2 33.1%	n=3 29.5%	n=4 26.2%	n=4 18.2%	n=4 19.4%	n=5 20.1%	n=4 17.3%	n=2 15.9%

PBMC, peripheral blood mononuclear cells; WBC, white blood cells

Safety

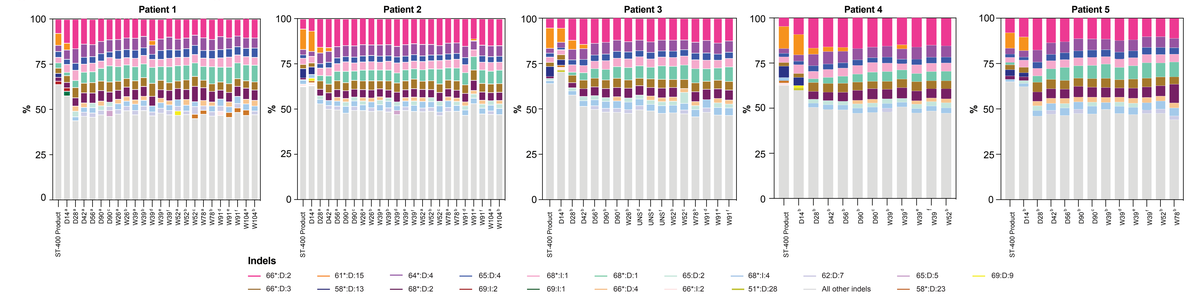
- Only 1 SAE was attributed to the ST-400 drug product (**Table 4**)
- The majority of AEs reported following treatment with ST-400 are consistent with myeloablation

Table 4. Serious Adverse Events

Patient[a]	SAEs	Related to ST-400
1	Hypersensitivity[b]	Related
3	Pneumonia[c]	Not related
5	Drug withdrawal syndrome	Not related

[a]No serious AEs were reported by Patients 2 and 4
[b]Experienced hypersensitivity soon after the initiation of ST-400 infusion, resolved by the end of infusion, and was considered by the investigator most likely related to DMSO
[c]Pneumonia occurred in the time period between the apheresis procedure and the start of chemotherapy conditioning
AE, adverse event; DMSO, dimethylsulfoxide; SAE, serious AE

Figure 1. Monitoring On-target Indel Patterns for Assessment of Hematopoietic Clonality



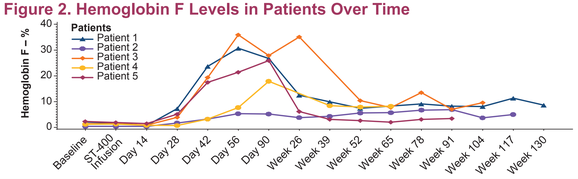
[a]PBMC; [b]WBC; [c]BM; [d]T cells; [e]B cells; [f]myeloid cells
Indel naming convention: I, insertion; D, deletion; first number, start of indel from reference base pair (*=nucleotides flanking indel could align to either side of the indel); number following colon, number of base pairs inserted or deleted.
BM, bone marrow; D, day; PBMC, peripheral blood mononuclear cells; UNS, unscheduled; W, week; WBC, white blood cells

- No emerging clonal hematopoiesis has been observed by on-target indel pattern monitoring over time (**Figure 1**)
- The 20 most frequent indels detected by next-generation sequencing are shown per patient at each time point. There are approximately 200-1200 unique insertion/deletion genotypes detectable on-target in the patient monitoring samples

Changes After ST-400 Infusion

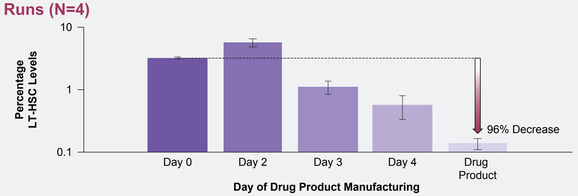
- Peak HbF levels achieved were 23.5 ± 11.4% (min-max: 6.9-35.9%) but were not sustained (**Figure 2**)
- At 65-130 weeks (latest visit), HbF levels were 7.0 ± 2.6% (min-max: 3.4-9.6%) possibly due to low levels of long-term gene-edited progenitors in the final drug product
- A high number of packed red blood cell (PRBC) transfusions were needed in the first month after ST-400 infusion, which is consistent with the time needed for marrow reconstitution
- Patients needed fewer transfusions during the time of the highest observed HbF levels
- Patients resumed PRBC transfusions by 3 months after ST-400 infusion, when HbF levels decreased

Figure 2. Hemoglobin F Levels in Patients Over Time



- A series of experiments were conducted in process development (PD) runs following review of data from the first dosed patients' HbF levels after 3 months. To ensure that the PD runs are representative of clinical manufacturing scale runs, gene editing (indel) levels and CD34+ recoveries were assessed and found consistent with manufacturing runs
- While indels and CD34+ levels show satisfactory levels, a significant drop in long-term HSCs[a] (LT-HSCs) is observed in PD runs using the same manufacturing scale process performed during the ST-400 clinical trials (**Figure 3**)

Figure 3. Decrease of LT-HSC Levels in Manufacturing Representative Runs (N=4)



[a]Include the following phenotypes by flow cytometry: CD34+, CD38-, CD90+, CD45RA-, CD49F+

Conclusions

- All 5 infused patients had rapid hematopoietic reconstitution following myeloablative conditioning, on-target indels in PBMCs, and elevated HbF levels following HSC graft transplantation
- Average HbF increased over 1 to 3 months, with patients requiring fewer PRBC transfusions at the time of the highest observed HbF levels
- After 3 months, the average HbF levels had declined by 63% from peak to last visit, followed by resumption of PRBC transfusions
- The manufacturing process has been shown to result in a loss of the LT-HSC population in the drug product
- The procedure was generally well tolerated with only a transient increase in HbF after reconstitution
- No additional patients will be infused. Infused patients will be asked to participate in a separate long-term safety study

References

1. Musallam KM, Sankaran VG, Cappellini MD, et al. Fetal hemoglobin levels and morbidity in untransfused patients with β-thalassemia intermedia. *Blood*. 2012;119(2):364-367.
2. Chang KH, Smith SE, Sullivan T, et al. Long-term engraftment and fetal globin induction upon *BCL11A* gene editing in bone-marrow-derived CD34(+) hematopoietic stem and progenitor cells. *Mol Ther Methods Clin Dev*. 2017;4:137-148.

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