Preliminary Safety and Efficacy Results from PRECIZN-1: An Ongoing Phase 1/2 Study on Zinc Finger Nuclease-Modified Autologous CD34+ HSPCs for Sickle Cell Disease (SCD)

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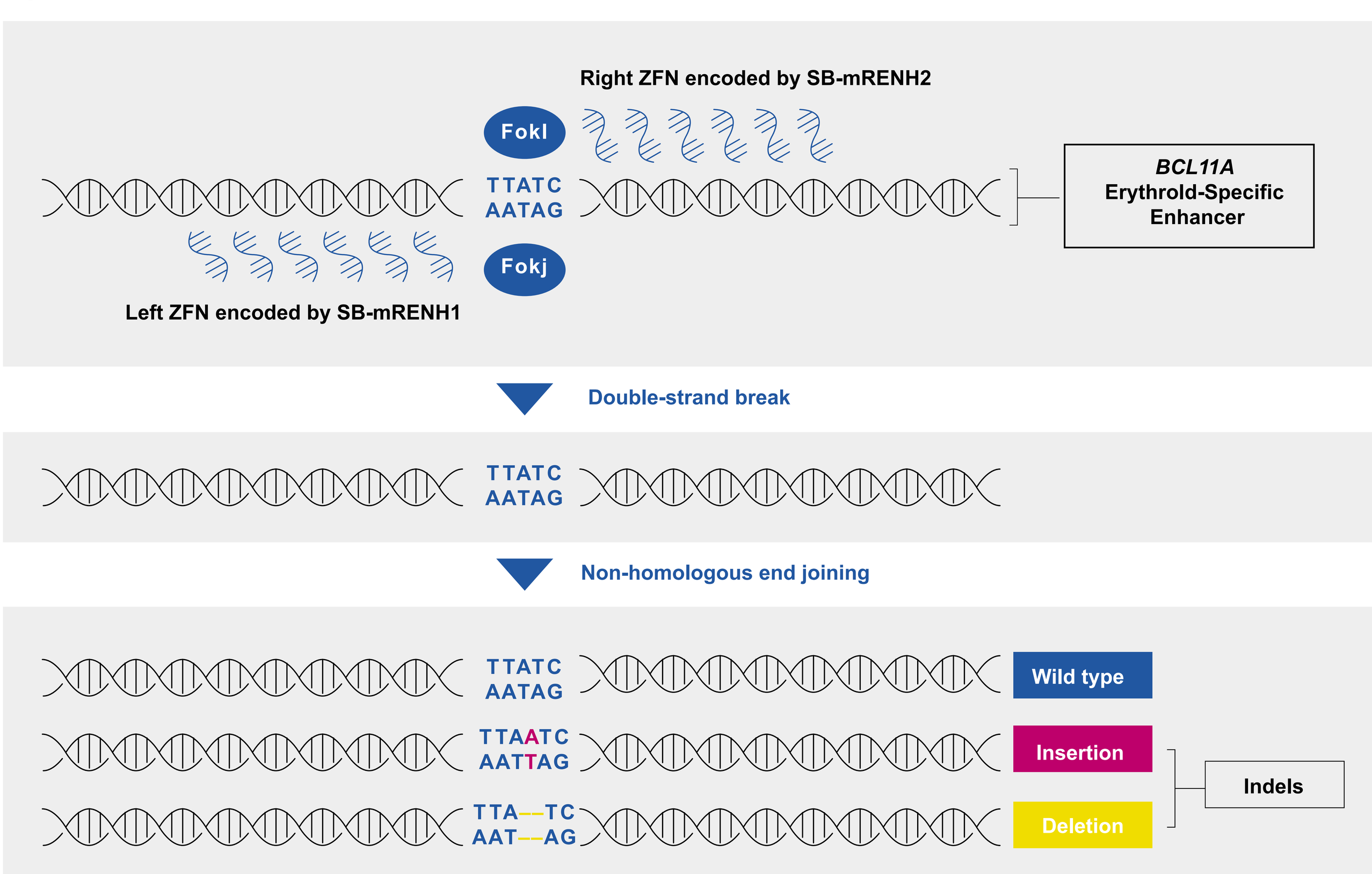
Introduction

- Sickle cell disease (SCD) is an autosomal recessive disorder characterized by a substitution of glutamic acid to valine in the sixth amino acid of the β-globin peptide, generating sickle hemoglobin (HbS). HbS polymerizes into inflexible polymers under hypoxic conditions, altering red blood cells (RBC) to a 'sickle' shape and other protean cellular perturbations[1,2]
- SCD affects ~100,000 patients in the US, and while almost all affected persons survive into adulthood in the US and UK, lifespan is shortened by 2–3 decades compared with the general population[3]
- Clinical phenotypes of SCD include hemolytic anemia and cycles of microvascular vaso-occlusion, leading to injury of virtually all organs[3,4]
- Elevated fetal hemoglobin (HbF) levels in patients with SCD are shown to ameliorate multiple symptoms and improve survival in patients with SCD[3,5]
- The transcription factor B-cell lymphoma/leukemia 11A is encoded by the *BCL11A* gene and is the master regulator of the HbF to adult hemoglobin (HbA) switch at birth[6,7]
- A natural erythroid-specific enhancer (ESE) in *BCL11A* serves as a key regulator of its expression in RBC precursors. It is hypothesized that modification of the *BCL11A* gene ESE will reactivate HbF in erythroid lineage without affecting *BCL11A* function in non-erythroid cells[6]
- Zinc finger nucleases (ZFN) are engineered proteins for DNA site-specific editing. They are created via attachment of *FokI* to a pair of zinc finger proteins (ZFP), naturally occurring human DNA-binding proteins, combining the DNA recognition specificity of ZFPs with the nuclease domain of *FokI* to create double-strand breaks at precisely defined target sites in the genome[8,9,10] **(Figure 1)**
- Heterodimerization of the ZFN pair results in precise and specific cutting and editing of key nucleotides in the *BCL11A* ESE. Repair of the double-strand breaks typically results in the introduction of insertions and deletions (indels), which can cause functional disruption of the target gene sequence[8,9,10]
- SAR445136 (BIVV003) is a novel therapeutic product comprising autologous CD34+ hematopoietic stem and progenitor cells (HSPCs) modified *ex vivo* by ZFNs to generate precise on-target edits at the *BCL11A* gene ESE. The aim is to increase endogenous HbF production in patients with severe SCD

Figure 1. Proposed mechanism of action of ZFN-mediated disruption of the BCL11A enhancer

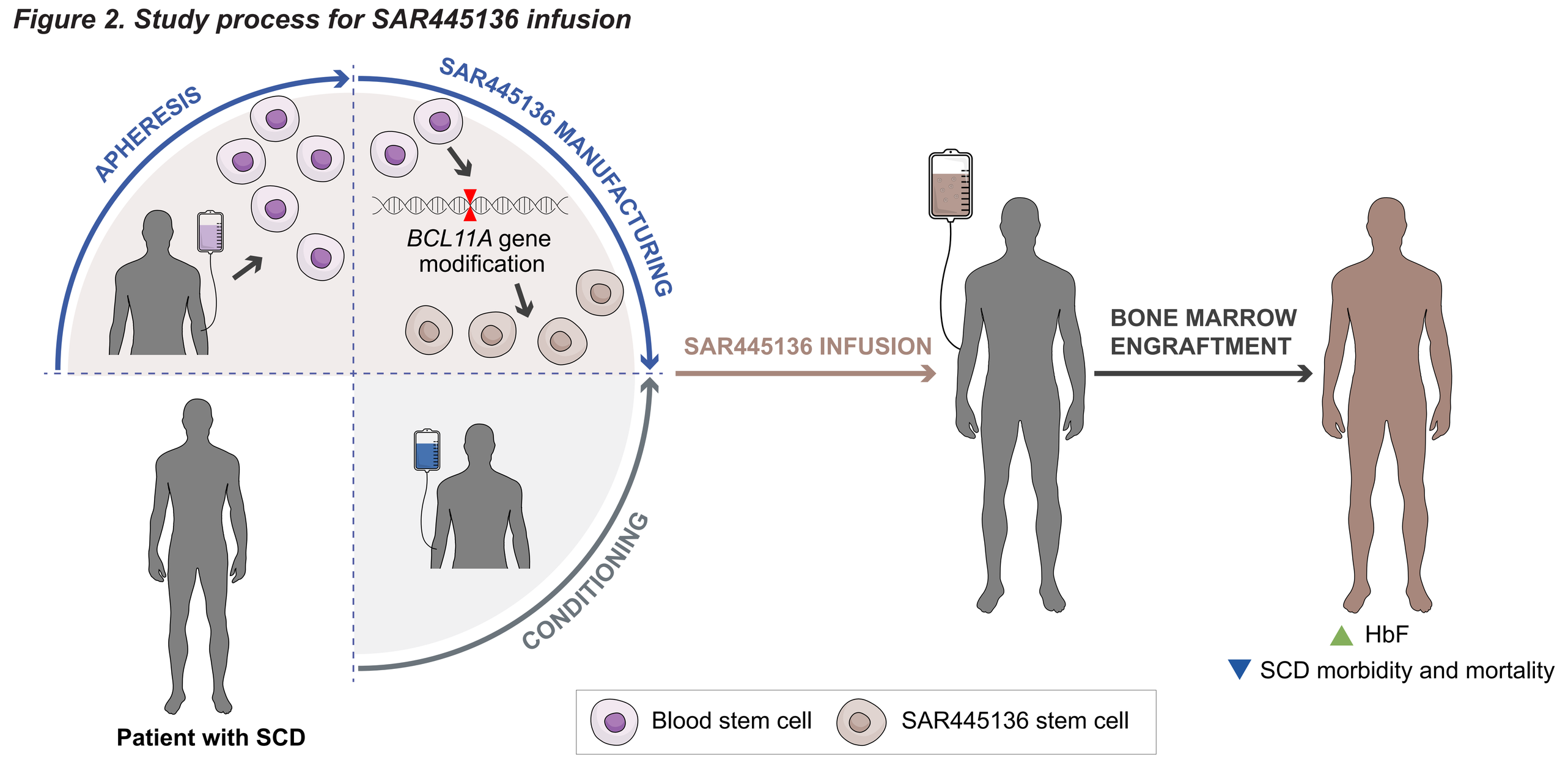


A, adenine; C, cytosine; G, guanine; T, thymine; ZFN, zinc finger nuclease

PRECIZN-1: SAR445136 Phase 1/2 study design and methodology

- PRECIZN-1 (NCT03653247) is an ongoing, first-in-human, open-label, single arm, multi-site, US-based study to evaluate safety, tolerability, and efficacy of SAR445136 in patients with severe SCD (n=8; age 18–40 years)
- There was an initial 12-week screening period to assess participants' suitability for the study
- Enrolled participants underwent plerixafor mobilization (240 μg/kg/day for up to 3 days) and apheresis to collect autologous CD34+ HSPCs with a target of 10 × 10^6 CD34+ HSPC/kg for manufacturing SAR445136. Additional apheresis cycles were allowed to achieve the minimum cell dose and unmodified rescue aliquots
- Autologous HSPCs were transfected *ex vivo* with ZFN messenger ribonucleic acids (mRNAs) targeting the ESE region of the *BCL11A* locus to manufacture SAR445136
- Myeloablation of the patient's bone marrow was performed using busulfan. At least 72 hours after the final busulfan dose, a single IV infusion of 3–20 × 10^6 CD34+ HSPC/kg was administered **(Figure 2)**

Figure 2. Study process for SAR445136 infusion



- Participants were monitored for hematopoietic engraftment and recovery, adverse events (AE), clinical and laboratory hemolysis markers, total hemoglobin (Hb) and HbF, percentage of F cells, and sickle-cell-related events post-SAR445136 infusion. Health-related quality of life (HRQoL) was assessed by the PROMIS-57 survey at screening, Weeks 26 and 52, and early termination visit
- Patients will be followed for a total of 104 weeks

Key study endpoints

- Primary endpoints (to evaluate safety and tolerability of SAR445136):
  - Survival at post transplantation Day 100, Week 52, and Week 104 (last study visit)
  - Successful engraftment and occurrence of AEs and serious adverse events (SAE)
- Secondary endpoints (to assess the success and kinetics of stem-cell collection, manufacturing, and engraftment) include:
  - CD34+ HSPC yield from stem-cell mobilization
  - Yield of ZFN-edited CD34+ HSPC
  - Time to initial neutrophil recovery following infusion (first of three consecutive days with absolute neutrophil count ≥500/μL)
  - Time to platelet recovery post infusion (first of three consecutive measurements with a platelet count ≥50,000/μL at least 7 days after last platelet transfusion)
  - Clinical assessments after SAR445136 infusion, including quality of life (QoL) measures

Baseline characteristics

- Nine subjects have been enrolled to date (September 22, 2021). Of the eight subjects who have completed mobilization and apheresis, five achieved successful cell target yields ranging from 3.4–13.8 x 10^6 CD34+ HSPC/kg per apheresis day (mean: 6.73 x10^6 CD34+ HSPC/kg per apheresis day) in one apheresis cycle (4.45–10.9 x 10^6 CD34+ HSPC/kg per 2-day cycle). Two subjects failed to mobilize and one discontinued due to intercurrent cholangitis. One subject is scheduled for infusion. Baseline patient characteristics of the four subjects infused are shown in **Table 1**

Table 1. Baseline characteristics and clinical history

Subject	103-002	100-001	102-001	103-003
Genotype	HbSB0	HbSS	HbSS	HbSS
Gender	Female	Female	Male	Male
Race	African American	African American	African American	African American
Age at consent, years	35	20	18	25
Pain crises, #events/2 years	10	22	0	6
Disease modifying medications, Y/N	N	Y*	Y*	N
Chronic RBC transfusion therapy, Y/N	N	Y	Y	Y

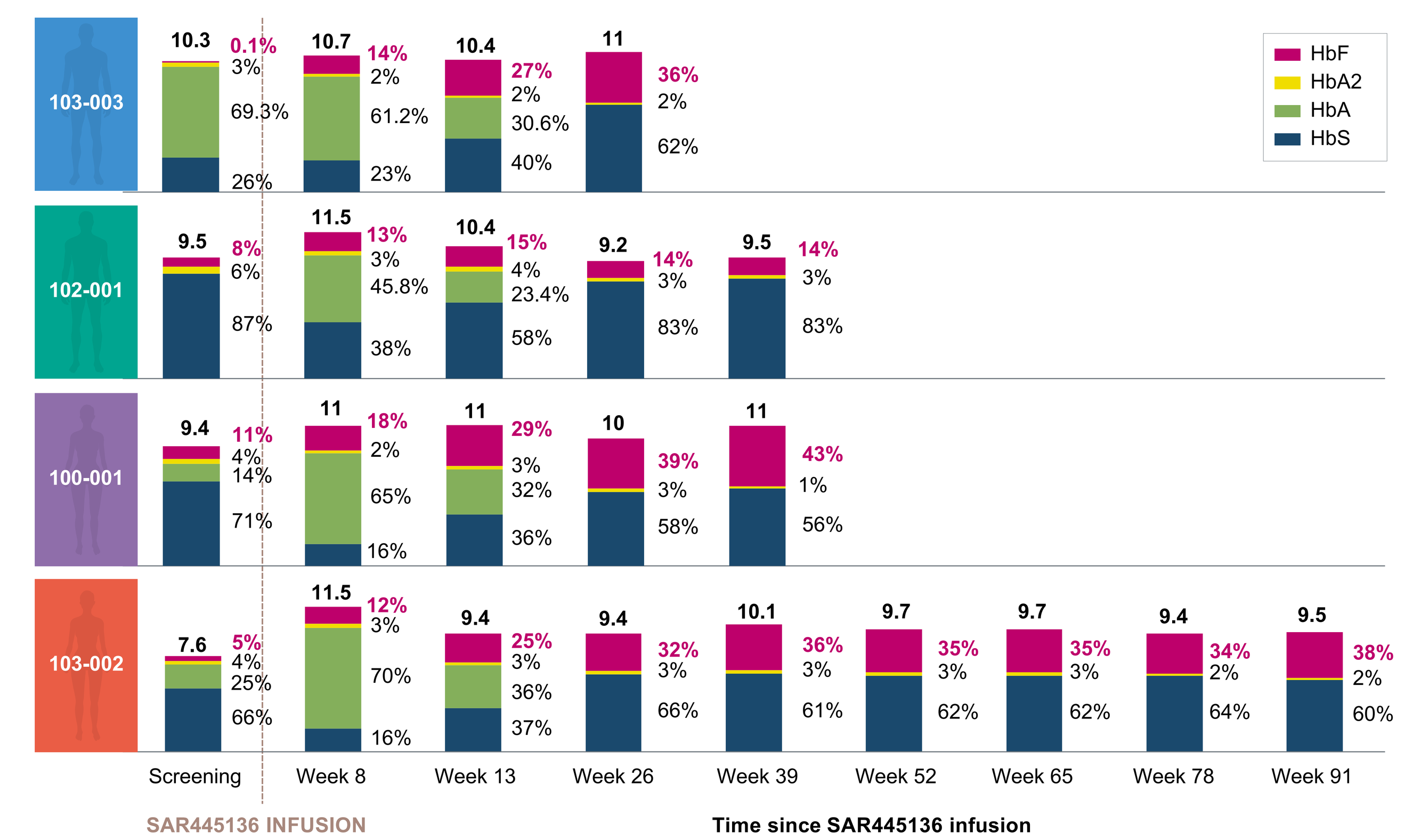
*Hydroxyurea
RBC, red blood cell

- Pre-apheresis peripheral blood white blood cells ranged from 23–36.9 x 10^3/μL (mean: 28.7 x 10^3/μL) and % CD34+ was 0.09–0.36% (0.22%) with absolute CD34+ counts of 20–80/μL (mean: 60/μL)
- Four of the mobilized subjects were successfully infused with SAR445136 at a single dose ranging from 3.2–9.7 x 10^6 CD34+ HSPC/kg (mean: 5.17 x 10^6 CD34+ HSPC/kg). All four subjects engrafted with a median time to neutrophil and platelet recovery of 21.5 and 24.5 days, respectively. No rescue doses were given

Hb fractionation following SAR445136 infusion

- Total Hb and clinical markers of hemolysis stabilized by Week 26 postSAR445136 infusion in all four subjects
- Percent HbF level (1–11% at screening) increased to 14–39% by Week 26 in all four subjects, and was 38% in one subject at 91 weeks' follow-up **(Figure 3)**

Figure 3. Total Hb and Hb fractionation in all patients after SAR445136 infusion



HbA, adult hemoglobin; HbA2, variant adult hemoglobin; HbF, fetal hemoglobin; HbS, sickle hemoglobin

- Percent F cells increased to 48–94% by 26 weeks' follow-up in all four infused subjects, persisting at 99% in the subject with 91 weeks' follow-up. The fourth subject had 94% F cells at 26 weeks' follow-up **(Figure 4)**
- By week 26 all patients reached a level of >/= to 10 pg of HbF/F cells, and this level was sustained in 2/3 patients with longer follow-up (Figure 5)
- The SAR445136 investigational drug product had on-target *BCL11A* gene modification (61–78% indels) in all four subjects. At 26 weeks' post-SAR445136 infusion, the indel frequency ranged from 17–34% (mean=25%) in unsorted bone marrow in all four subjects. In the subject with 91 weeks' follow-up, the marrow indel frequency was 28% and 26% at Weeks' 26 and 52, respectively

Figure 4. F cells over time for treated participants

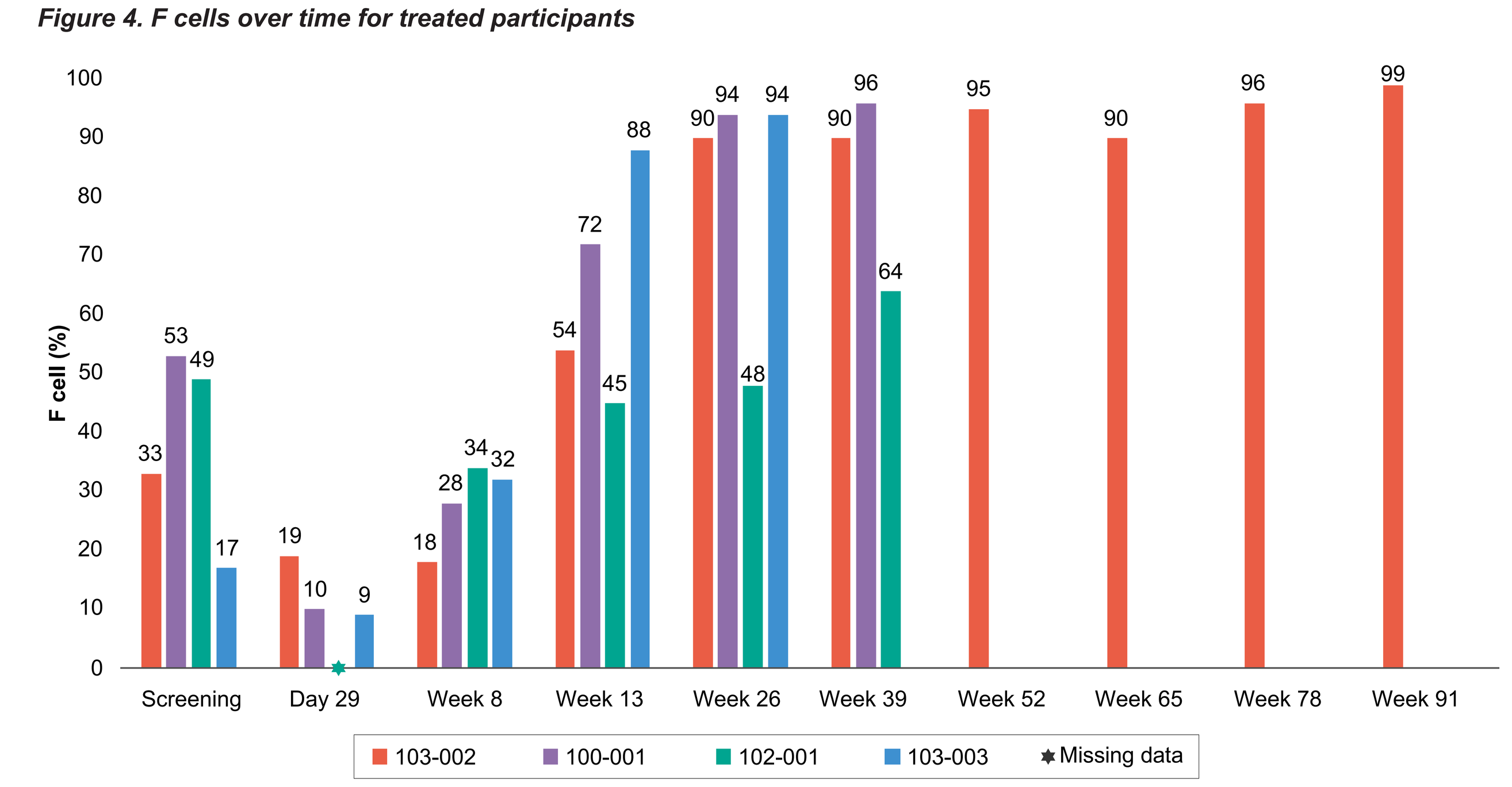
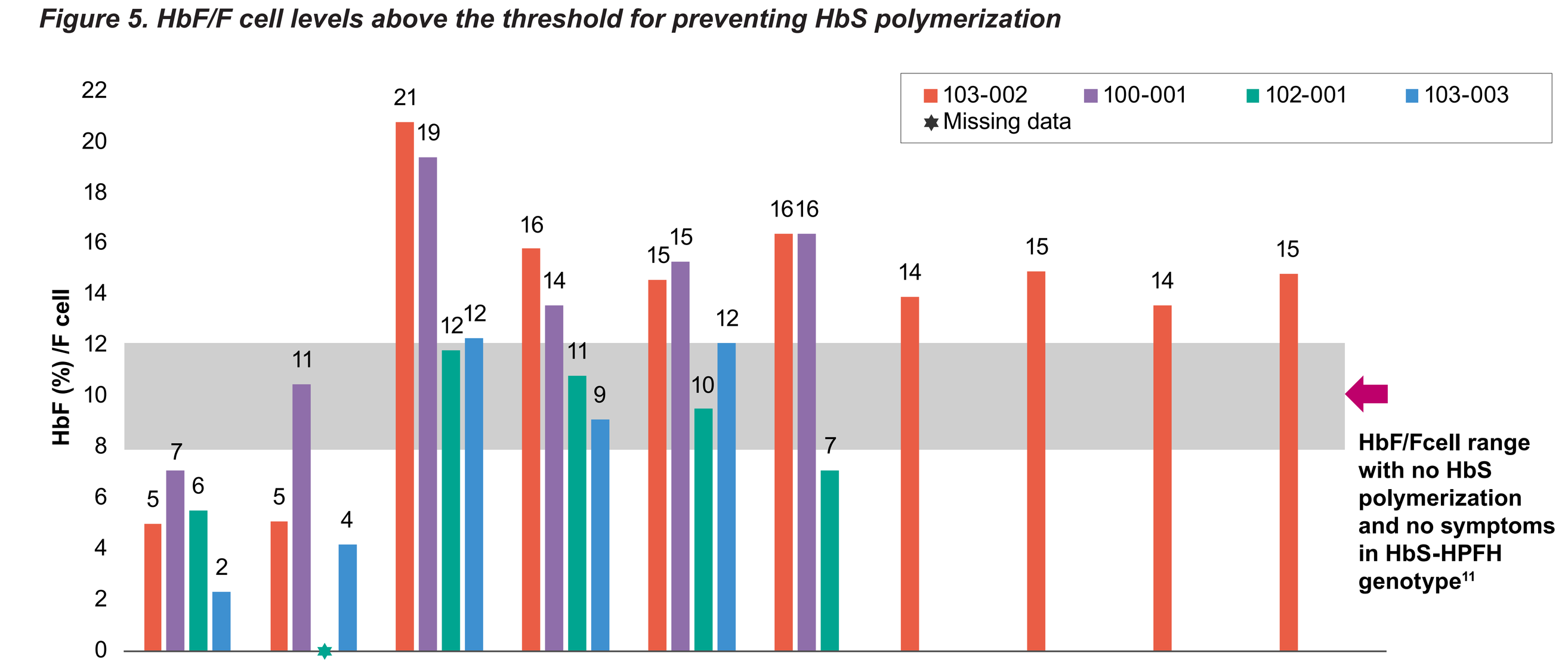


Figure 5. HbF/F cell levels above the threshold for preventing HbS polymerization

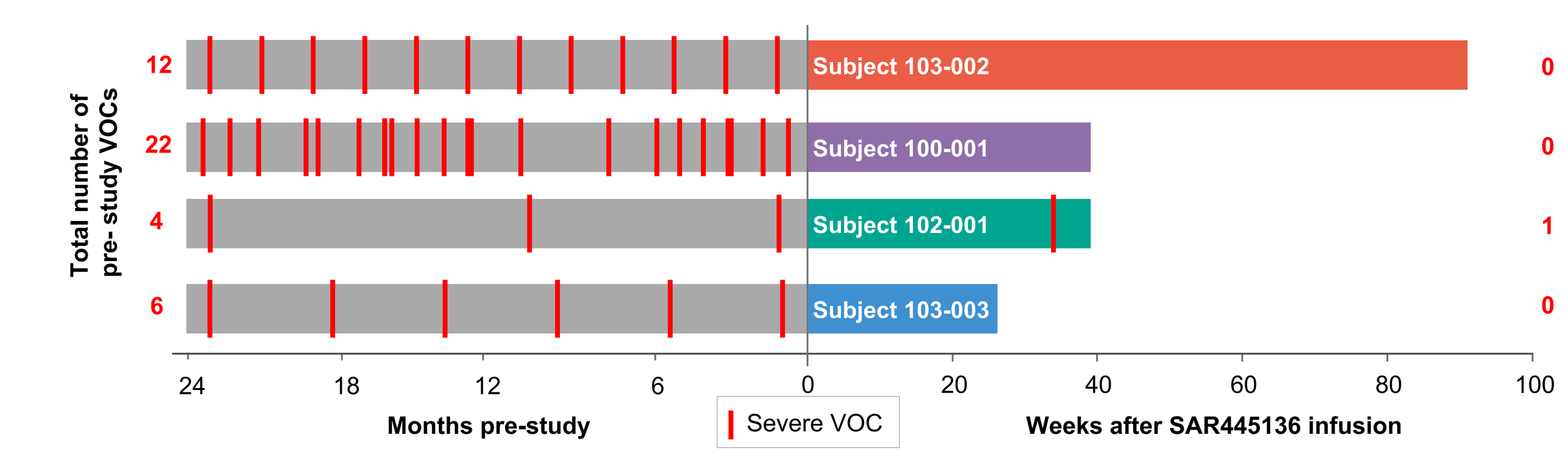


HbF, fetal hemoglobin; HPFH, Hereditary persistence of fetal hemoglobin

Safety and tolerability

- Plerixafor and busulfan were generally well tolerated in patients with SCD, and most AEs reported in the screening, mobilization, apheresis, and conditioning periods were SCD-related events **(Figure 6)**
  - Two SAEs of sickle cell anemia with crisis in two patients were reported by the investigator as related to plerixafor
  - One SAE of nausea was reported by the investigator as related to busulfan
- Most AEs reported after SAR445136 infusion were related to busulfan
- One SAE of sickle cell anemia with crisis vaso-occlusive crisis (VOC) was reported ~9 months after SAR445136 infusion in one patient; no other SCD-related events were reported in the four patients
- There were no AEs assessed as related to SAR445136 by the investigator or sponsor

Figure 6. Number of VOCs reported pre- and post-SAR445136 infusion



VOC, vaso-occlusive crisis

QoL

- Although preliminary and based on a small sample size with limited follow-up, a trend of improvement in all PROMIS-57 HRQoL domains except sleep disturbance and pain interference was observed for patients whose scores were worse than the normal at baseline

Conclusion

- These preliminary proof-of-concept efficacy and safety results confirm the potential therapeutic value of ZFN-mediated modification of the *BCL11A* ESE region and SAR445136 infusion to address current unmet needs of patients with SCD
- All four infused patients showed increases in total Hb, HbF, and Percent F cells
- SAR445136 was well tolerated in all four patients infused to date, with a single post-infusion VOC reported in one patient ~9 months after treatment
- The manufacturing process has been shown to result in a loss in the LT-HSCs population in the drug product
- Despite this manufacturing challenge, there was natural enrichment of modified erythroid lineage cells in the blood with elevated HbF, sufficient for a clinical benefit
- This may support the prediction that "correction-modifcation" of even a minority of HSCs is sufficient for a clinical benefit, following the principal of mixed chimerism after allo-HSCT

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Isobelle Galeon, **David Reiner**, **Sharon E. Smith**, **Lin Wang**, **Anne Ramezi**, and **Pablo Rendo** are employees of Sanofi, and may hold shares and/or stock options in the company