AsCas12a gene editing of *HBG1/2* promoters with EDIT-301 (reni-cel) results in rapid and sustained normalization of hemoglobin and increased fetal hemoglobin in patients with severe sickle cell disease and transfusion-dependent beta-thalassemia

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INTRODUCTION

- Sickle cell disease (SCD) and transfusion-dependent β-thalassemia (TDT) are hereditary blood disorders caused by pathogenic variants in the β-globin gene[1,2]
- Clinical evidence has demonstrated that increased fetal hemoglobin (HbF, α2γ2) levels can reduce or prevent SCD and TDT complications[3,4]
- Furthermore, increases in total hemoglobin (Hb) are associated with decreased risk across key clinical outcomes, such as stroke and mortality[5]
- EDIT-301 (renizgamglogene autogedtemcel; reni-cel) is an investigational gene-edited autologous hematopoietic stem cell medicine comprising CD34$^+$ cells from patients that are edited at the γ-globin gene (*HBG1* and *HBG2*) promoters to induce HbF expression
- These edits mimic naturally occurring variants of hereditary persistence of HbF in the *HBG1* and *HBG2* promoters that reactivate γ-globin expression and increase HbF production[6]
- Reni-cel is manufactured with a highly efficient and specific, proprietary gene editing nuclease, *Acidaminococcus sp.* CRISPR-associated protein 12a (AsCas12a)
- In preclinical studies, edited CD34$^+$ cells from patients with SCD or TDT showed improved erythropoiesis (TDT) and generated red blood cells (RBCs) with robust HbF production (TDT and SCD) and reduced sickling (SCD)[7,8]
- Here we report preliminary clinical data (as of November 2023) on reni-cel gene editing, efficacy, and safety in both SCD and TDT patients, which is the first clinical use of AsCas12a

METHODS

- RUBY (NCT04853576) and EdiThal (NCT05444894) are Phase I/II, multi-center, open-label, single-arm studies evaluating the safety, efficacy, and tolerability of reni-cel in patients with severe SCD and TDT, respectively
- Key inclusion and exclusion criteria and primary endpoints for the RUBY and EdiThal studies are summarized in **Table 1**
- Autologous CD34$^+$ hematopoietic stem and progenitor cells (HSPCs) collected by apheresis after plerixafor (RUBY) or plerixafor + filgrastim (EdiThal) mobilization were edited at the *HBG1* and *HBG2* promoters with AsCas12a
- After myeloablative conditioning with busulfan, patients received a single infusion of reni-cel (a minimum of 3 × 10^6 CD34$^+$ cells/kg)
- Neutrophil and platelet engraftment, total Hb, HbF, percentage of F-cells, mean HbF concentration/F-cell (MCH-F/F-cell), markers of hemolysis, transfusion requirement, vaso-occlusive events (VOEs; SCD only), and treatment-emergent adverse events (TEAEs) were assessed for 24 months
- Data included here are based on a cutoff of November 22, 2023 for RUBY patients and November 28, 2023 for EdiThal patients

Table 1. Key study eligibility criteria and primary endpoints for the RUBY and EdiThal trials

	RUBY (NCT04853576)	EdiThal (NCT05444894)
Key inclusion criteria	• 18–50 years • Diagnosis of severe SCD (β^S/β^S, β^S/β^0, or β^S/β^+) • History of ≥2 severe VOEs per year in the 2 years prior to informed consent	• 18–35 years • Diagnosis of TDT • History of at least 100 mL/kg/year or 10 U/year of packed RBC transfusions in the 2 years prior to informed consent
Key exclusion criteria	• Available genetically-matched (10/10 HLA) related donor • Previous or current malignancy or immunodeficiency disorder • Unable to tolerate stem cell therapy or receive RBC transfusion	
Primary endpoints	• Proportion of patients achieving complete resolution of severe VOEs* • Safety and tolerability of reni-cel	• Proportion of participants achieving engraftment • Safety and tolerability of reni-cel

HLA, human leukocyte antigen; RBC, red blood cell; reni-cel, renizgamglogene autogedtemcel; SCD, sickle cell disease; TDT, transfusion-dependent β-thalassemia; VOE, vaso-occlusive event. *A severe VOE requiring medical attention (despite hydroxyurea or other supportive care measures in the pre-treatment period) is defined as: an acute episode of pain with no cause other than a vaso-occlusion, resulting in either a ≥24-h hospital or Emergency Room (ER) observation unit or ≥2 visits to a day unit or ER over 72 h with both visits requiring administration of pain medications; acute priapism lasting >2 h and requiring a visit to a medical facility (with or without hospitalization); acute chest syndrome (ACS), which is defined as chest-wall pain in association with findings of a new pulmonary infiltrate on chest X-ray films associated with fever and/or respiratory symptom; or hepatic or splenic sequestration, which is defined as a sudden increase in organ size associated with pain in the area of the organ, decrease in the hemoglobin concentration of ≥2 g/dL within a 24-h period, and, for liver sequestration, abnormal change in liver function tests, including conjugated bilirubin, not due to biliary tract disease.

RESULTS: BASELINE CHARACTERISTICS, INFUSION, AND ENGRAFTMENT

- The demographics and baseline characteristics of Patients 1–11 with SCD and Patients 1–6 with TDT are described in **Table 2**
- High levels of editing were observed in patient peripheral blood nucleated cells and bone marrow-derived CD34$^+$ cells post-reni-cel infusion

Table 2. RUBY and EdiThal patient demographics, baseline characteristics, and reni-cel infusion and engraftment

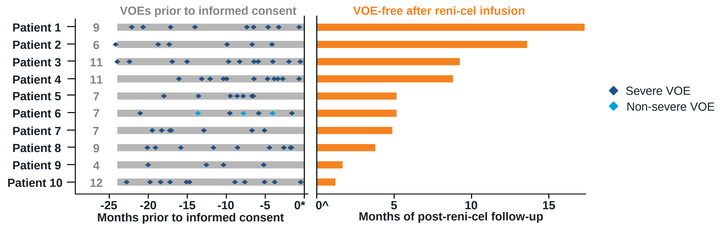
		SCD patients (N=11*)	TDT patients (N=6†)
Demographics and baseline characteristics			
Genotype, n (%)			
SCD	β^S/β^S	11 (100)	-
TDT	β^0/β^0	-	2 (33.3)
	Non-$\beta^0/\beta^{0‡}$	-	4 (66.7)
Sex, n (%)			
Female		6 (54.5)	4 (66.7)
Age, years, mean (SD)		27.6 (4.2)	18.8 (0.9)
Severe VOEs, pre-study annual rate§, mean (SD)		3.9 (1.4)	-
Packed RBC transfusions, pre-study annual rate§, mL/kg/year, mean (SD)		-	162.3 (51.9)
Reni-cel infusion and engraftment			
Total reni-cel dose administered, ×10^6 CD34$^+$ cells/kg, mean (SD)		5.2 (2.5)	7.7 (2.2)
Follow-up duration, months, mean (SD)		6.5 (5.3)	4.1 (2.5)
Time to neutrophil engraftment‖, days, mean (SD)		23.7 (2.8)*	25.5 (3.6)
Time to platelet engraftment#, days, mean (SD)		26.1 (7.7)*	36.6 (11.8)†

RBC, red blood cell; reni-cel, renizgamglogene autogedtemcel; SCD, sickle cell disease; SD, standard deviation; TDT, transfusion-dependent β-thalassemia; VOE, vaso-occlusive event. *One patient had 23 days of follow-up after infusion as of the data cut; neutrophil engraftment and platelet engraftment were not achieved yet; engraftment values are therefore based on n=10. †One patient with TDT had 36 days of follow-up after infusion as of the data cut; neutrophils engrafted, but platelet engraftment was not achieved yet; platelet engraftment values are therefore based on n=5. ‡Non-β^0/β^0 includes β^0/β^+ (n=3) and $\beta E/\beta^0$ (n=1). §The pre-study period is defined as the 2-year period prior to informed consent. ‖Three consecutive measurements with absolute neutrophil count (ANC) ≥0.5 × 10^9/L. #Three consecutive measurements with platelet count ≥50 × 10^9/L (SCD) and ≥20 × 10^9/L (TDT) starting at least 7 days after the platelet transfusion, and 10 days after thrombopoietin (TPO). No TPO was used for patients after reni-cel infusion.

RESULTS: RUBY EFFICACY

- Compared with a mean (SD) of 4.0 (1.4) severe VOEs/year in the 2 years before enrollment (n=10), all patients are completely VOE-free post-reni-cel infusion (**Figure 1**)

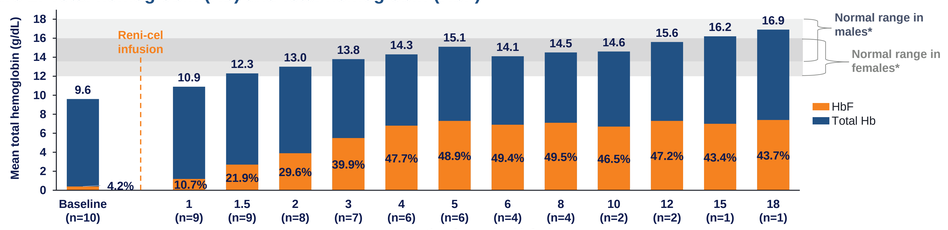
Figure 1: Vaso-occlusive events (VOEs)



Left panel ends at informed consent date. Right panel starts at infusion date. Due to limited follow up period after dosing, Patient 11 was not included. Day 0* is the day of informed consent. Day 0^ is the day reni-cel was infused. reni-cel, renizgamglogene autogedtemcel.

- Following reni-cel infusion, mean total Hb levels rapidly increased from baseline (**Figure 2**)
  - Mean (SD) percentage of HbF was 47.7% (4.2; n=6) by Month 4 and was sustained above 40% through last follow-up
  - All patients achieved normal Hb levels by Month 5

Figure 2: Total hemoglobin (Hb) and fetal hemoglobin (HbF)



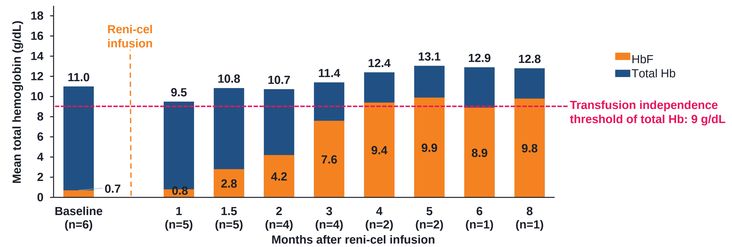
Data cutoff November 22, 2023. Number of male patients = 5; number of female patients = 5. Month 1 is n=9 based on available patient data. Bars show Hb (g/dL). Labels inside / next to the bars indicate mean proportion of HbF as a percentage of total Hb. Total Hb concentrations are shown directly above bars. *Normal total hemoglobin range is 13.6–18.0 g/dL for male patients and 12.0–16.0 g/dL for female patients. Central laboratory reference range. The last RBC transfusion in patients occurred a mean (SD) of 15.4 (6.0) days after reni-cel infusion (n=10). Hb, hemoglobin; HbF, fetal hemoglobin; RBC, red blood cell; reni-cel, renizgamglogene autogedtemcel; SCD, sickle cell disease.

- The mean (SD) MCH-F/F-cell at Month 1 was 18.9 (2.3) pg/F-cell and was sustained above the anti-sickling threshold (10 pg/F-cell) through last follow-up
- The mean (SD) percentage of F-cells was 98.5% (1.3) by Month 5 (n=6)
- After reni-cel infusion, key markers of hemolysis (reticulocyte count, indirect bilirubin, lactate dehydrogenase, and haptoglobin) showed improvements or normalized

RESULTS: EDITHAL EFFICACY

- Following reni-cel infusion, mean total Hb increased (**Figure 3**); all patients maintained Hb levels above the transfusion threshold at last follow-up
- After receiving the last RBC transfusion at 0.5–2.2 months post-reni-cel infusion, all 5 patients have been transfusion-free for a range of 1.8–7.5 months

Figure 3: Total hemoglobin (Hb) and fetal hemoglobin (HbF)



Bars show Hb (g/dL). Labels inside / above the bars indicate mean levels of HbF (g/dL). Total Hb concentrations are shown directly above bars. The last RBC transfusion in patients occurred a mean (SD) of 34.4 (20.9) days after reni-cel infusion (n=5). Hb, hemoglobin; HbF, fetal hemoglobin; RBC, red blood cell.

RESULTS: RENI-CEL SAFETY

- The safety profile of reni-cel in patients with SCD or TDT was consistent with myeloablative conditioning with busulfan and autologous hematopoietic stem cell transplantation
- No serious TEAEs related to reni-cel were reported after reni-cel infusion (**Table 3**)

Table 3. Summary of treatment-emergent adverse events (TEAEs)

TEAE category	RUBY (SCD) (N=10)		EdiThal (TDT) (N=6)	
	No. of patients (%)	No. of events	No. of patients (%)	No. of events
Any TEAE	10 (100)	144	5 (83.3)	103
Any TEAE related to reni-cel*	1 (10)	1	0	0
Any TEAE related to busulfan	10 (100)	66	5 (83.3)	59
Any serious TEAE	2 (20)	3	1 (16.7)	1
Any serious TEAE related to reni-cel	0	0	0	0
Any Grade 3 or 4 TEAE	8 (80)	27	5 (83.3)	42
Any Grade 3 or 4 TEAE related to reni-cel	0	0	0	0
Any TEAE related to reni-cel leading to discontinuation	0	0	0	0
Any TEAE leading to death	0	0	0	0

SCD, sickle cell disease; TDT, transfusion-dependent β-thalassemia; TEAE, treatment-emergent adverse event.
*The only TEAE potentially related to reni-cel was Grade 2 polycythaemia in one RUBY patient. The patient presented asymptomatically and has remained clinically stable. The TEAE has resolved, and total hemoglobin has normalized. The causality of this TEAE to reni-cel has been updated as "not related" as of Dec 18, 2023.

CONCLUSIONS

- Reni-cel showed promising results for gene editing of the γ-globin gene (*HBG1* and *HBG2*) promoters to induce HbF expression in both SCD and TDT patients and is the first clinical use of AsCas12a
- All patients with SCD are VOE-free post-reni-cel infusion with improvements in key markers of hemolysis and increases in the percentage of F-cells, as well as MCH-F/F-cell
- Patients with SCD experienced rapid normalization of Hb, with increases in HbF sustained above 40%
- All patients with TDT maintained Hb levels above the transfusion threshold and are transfusion-free
- Data from patients with >1 month to 18 months of follow up demonstrated early engraftment and a favorable safety profile
- These data from additional treated patients and of longer duration build on strong clinical evidence that support further investigation of reni-cel

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