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POSTER ABSTRACTS

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One Year Follow-up on the First Patient Treated with Nula-Cel: An Autologous CRISPR/Cas9 Gene Corrected CD34+ Cell Product to Treat Sickle Cell Disease

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Introduction

Sickle cell disease (SCD) is the most common serious genetic disease in the world with over 500,000 births per year globally. It is estimated that there are >100,000 patients in the US with SCD and at least 20% have severe disease. Patients with SCD have at least one *HBB* gene with an adenine to thymidine variant at position 6 of the coding region resulting in a glutamic acid to valine amino acid change (*HbS*). The success of allogeneic hematopoietic stem cell transplantation demonstrates that reconstitution of the hematopoietic compartment with HSCs that contain at least one non- *HbS* allele can cure the disease. Homology directed repair gene editing (HDR-editing) has achieved high frequencies of conversion of the thymidine to the non-pathologic adenine in CD34+ hematopoietic stem and progenitor cells from patients with SCD. Nulabeglogene autogedtemcel (nula-cel) is an investigational drug product in which HDR-editing of plerixafor-mobilized CD34+ HSPCs corrects the underlying variant that causes SCD. This is the first drug to directly correct a disease-causing variant. We report on the 1 year follow up of the first and only patient to receive nula-cel.

Results

Patient 1 is now a 23 year old female with homozygous SCD. She was consented and enrolled on a Phase I//II trial to test the safety and efficacy of nula-cel. In the 2 years prior to enrollment, the patient averaged 6 VOC's and 4 hospitalizations per year. Two aphereses of plerixafor-mobilized CD34+ cells were performed followed by fresh CD34+ cell purification. The purified CD34+ cells were pooled and cryopreserved. A 5-day manufacturing process starting with 9.3 x 10^6 cells/kg of cryopreserved CD34+ cells resulted in a yield of 8.75 x 10^6 CD34/kg with an on-target allele correction frequency of 33%. In August, 2022 the thawed drug product was infused after the patient received AUC-adjusted busulfan myeloablative conditioning chemotherapy. The initial cell viability was 77% giving a viable infused cell dose of 6.74 x 10^6 CD34/kg. Follow-up exploratory apoptois studies, however, showed the viable CD34+cell dose may have been as low as 3.5 x 10^6 CD34+/kg. Neutrophil engraftment occurred at transplant Day +40. Because of the lack of platelet recovery and continued platelet and

RBC transfusion requirements, the patient was started on eltrombopag on day +106 (marrow cellularity of 5%). Since eltrombopag usage was not part of the protocol, its use prompted the reporting of an SAE but the trial was never put on FDA hold. The last dose of GCSF was D+140, the last platelet transfusion was D+181 and the last RBC transfusion was D+263. With rising platelet counts and hemoglobin and a stable ANC >1500, eltrombopag was discontinued on D+322. The blood counts have continued to slowly increase after discontinuation of the eltrombopag. On D+307, with a Hgb of 8.5 g/dl, hemoglobin electrophoresis demonstrated HgbA=12.5%, HgbF >78% and HgbS=4.5%. On D+349 the blood tests show Hgb=9.1 g/dl, Platelet=77,000/ul, ANC=1788/ul with an absolute reticulocyte count of 119,130/ul. There are no signs or symptoms of hemolysis. Table 1 shows the time course of gene marking with stable allele gene correction and INDEL frequencies since D+49. No change in off-target INDEL frequency nor change in on-target INDEL spectrum has occurred and no evidence of oligoclonal or clonal hematopoiesis has been observed. Clinically, the patient has shown marked improvement in quality of life with zero VOE's or other manifestations of SCD. The patient still has not reached a steady state and updated results will be presented.

Conclusion

We describe the clinical results of the first patient treated with an autologous cell product (nula-cel) in which the pathologic variant was directly corrected. The mechanism of action is fundamentally different than other clinical gene editing and gene therapy programs as the level of the pathologic HgbS is directly decreased thereby removing the dominant negative effect that HgbS has on red blood cells. The high HgbF is an unexpected but potentially effective mechanism of action for the clinical benefit and is being further studied. Longer follow up to assess the durability of the curative clinical impact is underway. Improvements in cell manufacturing are being implemented to shorten the duration of transfusion needs and increase the level of gene correction of engrafted cells. Plans to treat the next patients with an improved nula-cel product are in place.

Disclosures Lowsky: *Orca Bio:* Research Funding. **Miller:** *Graphite Biologics:* Ended employment in the past 24 months. **Schroeder:** *Incyte:* Honoraria; *Novo nordisk:* Consultancy; *GSK:* Consultancy; *Kura:* Membership on an entity's Board of Directors or advisory committees; *Sorrento therapeutics:* Membership on an entity's Board of Directors or advisory committees; *Marker Therapeutics:* Membership on an entity's Board of Directors or advisory committees. **Buchholz:** *Graphite Biologics:* Ended employment in the past 24 months. **Intondi:** *Graphite Biologics:* Ended employment in the past 24 months. **Lehrer:** *Graphite Biologics:* Current Employment, Membership on an entity's Board of Directors or advisory committees. **Lerner:** *Graphite Biologics:* Ended employment in the past 24 months. **Amoury:** *Graphite Biologics:* Ended employment in the past 24 months. **Treusch:** *Graphite Biologics:* Ended employment in the past 24 months. **Chew:** *Graphite Biologics:* Ended employment in the past 24 months. **Silva:** *Graphite Biologics:* Ended employment in the past 24 months. **Mashhedi:** *Graphite Biologics:* Ended employment in the past 24 months. **Siu:** *Graphite Biologics:* Ended employment in the past 24 months. **Perrone:** *Graphite Biologics:* Ended employment in the past 24 months. **Vijay:** *Graphite Biologics:* Ended employment in the past 24 months. **Jain:** *Graphite Biologics:* Ended employment in the past 24 months. **Krassovsky:** *Graphite Biologics:* Ended employment in the past 24 months. **Matern:** *Graphite Biologics:* Ended employment in the past 24 months. **Lahiri:** *Graphite Biologics:* Ended employment in the past 24 months. **Rodriguez:** *Graphite Biologics:* Ended employment in the past 24 months. **Skowronski:** *Graphite Biologics:* Ended employment in the past 24 months; *Kamau Therapeutics:* Current Employment. **Flores:** *Graphite Biologics:* Ended employment in the past 24 months. **Lee:** *Graphite Biologics:* Ended employment in the past 24 months. **Roncarolo:** *Graphite Biologics:* Current equity holder in publicly-traded company, Membership on an entity's Board of Directors or advisory committees; *TR1X:* Current equity holder in private company, Membership on an entity's Board of Directors or advisory committees; *Atara:* Membership on an entity's Board of Directors or advisory committees; *Kamau Therapeutics:* Current equity holder in private company, Membership on an entity's Board of Directors or advisory committees. **Feldman:** *MicroFluidX:* Membership on an entity's Board of Directors or advisory committees; *FreshWind Bio:* Membership on an entity's Board of Directors or advisory committees; *Autolomous:* Membership on an entity's Board of Directors or advisory committees; *Alaunos Therapeutics:* Membership on an entity's Board of Directors or advisory committees. **DiPersio:** *Magenta:* Current holder of *stock options* in a privately-held company, Other: Ownership Investment, Patents & Royalties; *Macrogenics:* Research Funding; *WUGEN:* Current holder of *stock options* in a privately-held company, Other: Ownership Investment, Patents & Royalties, Research Funding; *Bioline:* Consultancy; *Rivervest:* Consultancy; *Vertex:* Consultancy. **Porteus:** *Graphite Biologics:* Current equity holder in publicly-traded company, Membership on an entity's Board of Directors or advisory committees; *Allogene Therapeutics:* Current equity holder in publicly-traded company, Membership on an entity's Board of Directors or advisory committees; *Alaunos Therapeutics:* Current equity holder in publicly-traded company, Membership on an entity's Board of Directors or advisory committees; *Kamau Therapeutics:* Current equity holder in private company; *CRISPR Tx:* Current equity holder in publicly-traded company.

Table 1: Frequencies of Genome Editing Outcomes over Time

Day After Infusion	Sample	INDELs (%)	Unedited (%)	Corrected (%)
Pre-Infusion	Drug Product (nula-cel)	21.4	43	33.1
Day 37	PB CD15+	66.92	18.93	9.31
Day 49	PB CD15+	69.93	20.43	6.68
Day 63	PB CD15+	70.66	20.78	5.89
Day 91	PB CD15+	70.61	20.72	5.93
	BMMC	69.36	25.25	3.39
	CD34+	71.31	21.15	5.17
Day 126	PB CD15+	68.13	23.38	6.05
Day 135	BMMC	66.54	28.87	3.32
	CD34+	74.31	18.80	5.27
Day 182	PB CD15+	70.2	21.3	5.8
Day 184	BMMC	73.6	21.4	2.3

Abbreviations: PB CD15+=Peripheral Blood CD15+ Purified Cells; BMMC=Bone marrow mononuclear cells; CD34+=Bone Marrow CD34+ Purified Cells

Figure 1

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