Initial First-In-Human Results: CD33-Deleted Hematopoietic Stem and Progenitor Cells Display Normal Engraftment after Hematopoietic Cell Transplantation (HCT) and Tolerate Post-HCT Gemtuzumab Ozogamicin (GO) without Cytopenias

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Background & Method

Relapse is the leading cause of death for patients undergoing allogeneic HCT for acute myeloid leukemia (AML)[1], particularly for patients with high-risk features such as minimal residual disease (MRD)[2,3]. CD33 is an antigen found on the majority of AML cells; however, it is also present on normal myeloid cells[4]. Agents targeting CD33, such as Mylotarg™ (GO), an anti-CD33 antibody-drug conjugate, therefore are associated with extensive cytopenias[5,6]. Preclinical studies have previously shown that CD33 is dispensable for normal biology of human hematopoietic stem and progenitor cells[7]. In order to reduce AML relapse post-HCT, a CRISPR/Cas9 gene-edited, *CD33*-deleted donor allograft, trem-cel (formerly known as VOR33), was developed to enable post-HCT CD33-directed therapies while protecting healthy donor cells from on-target myelosuppression.

VBP101 is a first-in-human (FIH) study, where CD33-positive AML patients at high risk of relapse, i.e., MRD+ or with evidence of persistent bone marrow (BM) blasts, undergo myeloablative HCT with trem-cel followed by treatment with low-dose GO. The objective of this study is to evaluate the safety of trem-cel and GO in AML patients at a high risk of relapse.

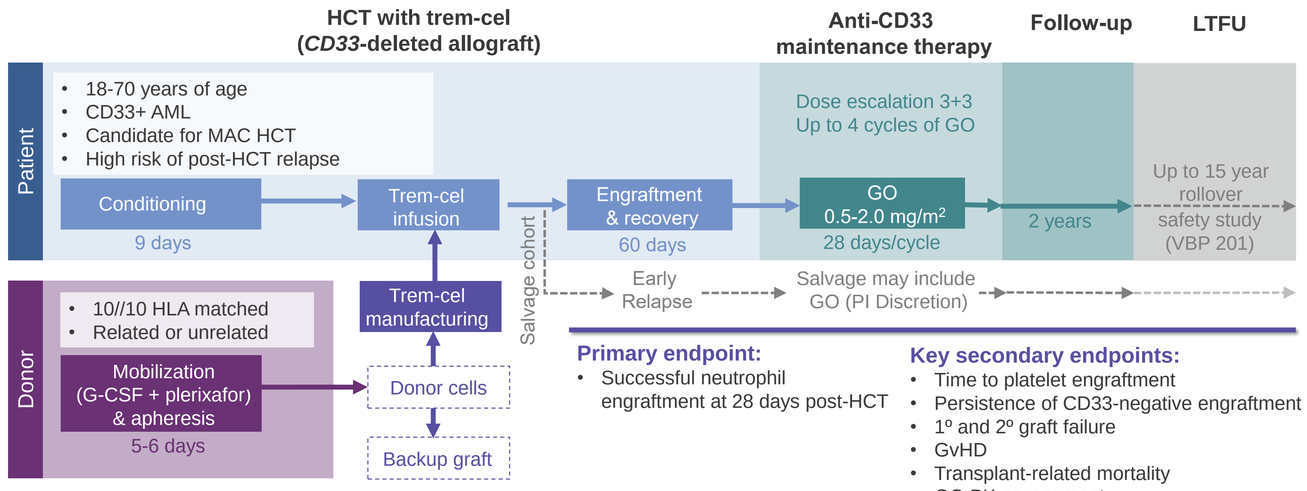

Figure 1: VBP101 Study Design.

Results

Patient 1: 64-year-old female patient diagnosed with AML including high-risk features: highly complex (adverse) cytogenetics, myelodysplasia (MDS)-related changes, and *TP53* mutation. After initial treatment, the patient relapsed and received high-dose cytarabine (HiDAC) + venetoclax + decitabine to achieve a second CR2 with 1.8% MRD in the bone marrow (BM). A 10/10 HLA matched unrelated donor (MUD) was identified and consented. Donor was mobilized using granulocyte colony-stimulating factor (G-CSF) and plerixafor per protocol. Trem-cel was manufactured with 88% *CD33* gene editing efficacy; the patient's trem-cel dose was 7.6 x 10^6 CD34+ cells/kg. The patient received myeloablative conditioning (MAC) with busulfan/melphalan/fludarabine/anti-thymocyte globulin (ATG) prior to trem-cel infusion.

Patient 2: 32-year-old male diagnosed with AML after myeloid sarcoma partially resected from the small bowel and omentum. Initial cytogenetics were Inv 16 and +22. Subsequent t(3;3) was identified (adverse risk). Initial treatment consisted of 7+3 cytarabine/daunorubicin with CR but 1.8% MRD in BM. The patient then received 3 cycles of HiDAC, remaining in CR with persistence of abdominal disease by positron emission tomography (PET). A 10/10 HLA MUD was identified and consented. Donor was mobilized with G-CSF and plerixafor per protocol. Trem-cel was manufactured with 87% CD33 gene editing efficacy; the patient's trem-cel dose was 3.2 x 10^6 CD34+ cells/kg. The patient received MAC with busulfan/melphalan/fludarabine/ATG prior to trem-cel infusion.

Data cutoff date: 06 February 2023.

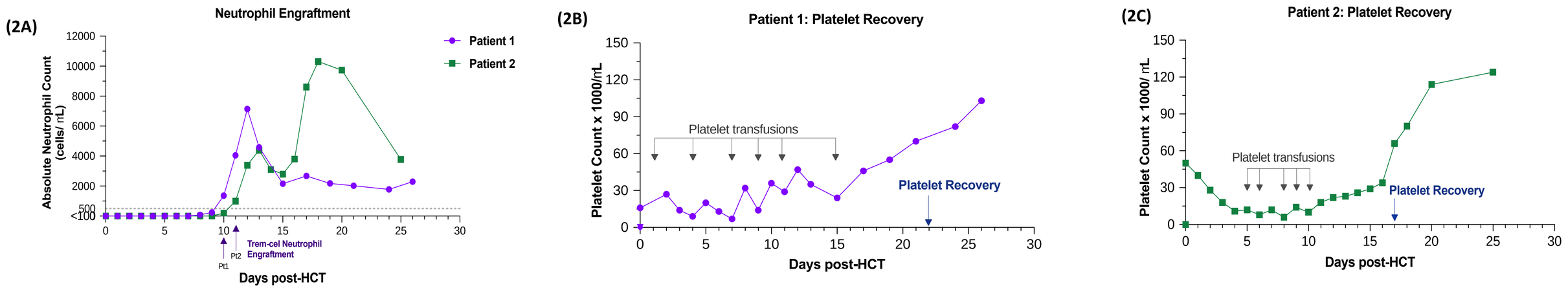

Figure 2: In Patient 1, neutrophil recovery occurred at 10 days (Fig 2A) and platelet recovery at 22 days post HCT with trem-cel (Fig 2B). In Patient 2, neutrophil engraftment occurred at 11 days (Fig 2A) while platelet recovery occurred at 17 days post HCT with trem-cel (Fig 2C).

Transplant Day	D28	D60	D100
Bulk			
Donor Chimerism	100%	100%	100%
CD33 Gene Editing (Indels)	95.2%	95.9%	99.4%
Monocytes (CD14+ CD15+)			
Donor Chimerism	100%	100%	100%
CD33 Gene Editing (Indels)	95.0%	95.6%	99.7%
%CD33-Negative Cells by Flow	95.3%	96.0%	99.9%
NK Cells (CD16+ CD56+)			
Donor Chimerism	100%	100%	100%
CD33 Gene Editing (Indels)	92.1%	94.9%	95.4%
B Cells (CD19+)			
Donor Chimerism	--	100%	100%
CD33 Gene Editing (Indels)	--	95.6%	98.5%
T cells (CD3+)			
Donor Chimerism	--	--	97.0%
CD33 Gene Editing (Indels)	--	--	97.6%**

**97.6% includes all T cells (97% donor T cells and 3% recipient T cells)

Table 1: Assessment of peripheral blood 28 days (D28), 60 days (D60), and 100 days (D100) post HCT with trem-cel showed 100% donor chimerism in whole blood, monocytes, and B cells. At D100 assessment, 97% donor chimerism was observed in T cells. >95% *CD33* editing was observed in bulk cells and monocytes. Enrichment in *CD33* editing was observed after two doses of GO was administered between D60 and D100.

	VBP101	Relapse Refractory AML (GO Phase 1 Study [0903A1-101-US])[9]		
PK Parameters	0.5mg/m²	1 mg/m²	2 mg/m²	5 mg/m²
$T_{1/2}$ (Hr)	64.56	--	--	--
T_{max} (Hr)	2.95	--	--	--
C_{max} (ng/mL)	259	50	411	1,325
AUC_{last} (Hr*ng/mL)	19,049	--	--	--
AUC_{inf} (Hr*ng/mL)	22,924	943	11,110	29,980
Vz (L/m**2)	2.03	--	--	--
CL (L/hr/m**2)	0.0218	--	--	--

Cycle 1 Day 1= C1D1; Cycle 1 Day 8=C1D8; PK=pharmacokinetic.

Table 2: Plasma samples were collected pre-GO dosing and post-GO dosing on C1D1 (1, 2, 3, 4, and 6 hours) and C1D8 and analyzed for hP67.6 by ELISA. Pharmacokinetic (PK) parameters were calculated using a non-compartmental analysis method in Phoenix WinNonlin Version 9.3 using the concentration data measured after drug infusion and the actual dosing and PK sampling information.

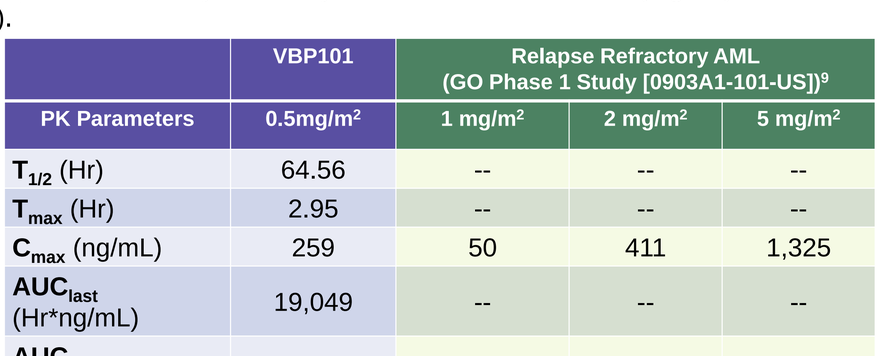

Figure 3: Patient 1 received GO (0.5 mg/m²) every 28 days beginning at D68 post HCT for a total of 3 doses. Neutrophil and platelet counts were stable in the patient during GO cycles through D147. No elevations of liver function tests (LFTS) were observed.

Clinical Course and Safety:

Patient 1:

- Serious adverse events (SAEs) after trem-cel dosing included Grade (Gr) 3 renal colic attributable to nephrolithiasis.
- Infectious adverse events (AEs) included a Gr 1 and Gr 2 skin infection, Gr 2 cytomegalovirus (CMV) reactivation, Gr 2 urinary tract infection, and Gr 2 BK virus (urine); all resolved.
  - Hepatic AEs included Gr 1 and Gr 2 aspartate aminotransferase (AST)/alanine aminotransferase (ALT) elevations; both were attributed to anti-fungal therapy and resolved.
  - No trem-cel-related AEs were reported. GO-related AEs included Gr 1 nausea and Gr 2 vomiting.
  - Gr 2 acute graft-versus-host disease (aGvHD) of the gut was reported and is responding to non-systemic steroids.
- Prior to HCT, the patient had 1.8% MRD in the BM and none was observed post-HCT at D28 and D60. MRD in the BM was detected at D101 (0.3%) and D129 (2.2%). The patient discontinued study treatment after Cycle 3 of GO. D147 BM showed relapsed disease with 7.6% blasts.

Patient 2

- Infectious AEs post-trem-cel dose included Gr 3 febrile neutropenia and *Escherichia coli* (E. coli) bacteremia reported at D8 prior to engraftment.
- No SAEs were reported after trem-cel dose through D18.
- No trem-cel-related AEs were reported.

Conclusions

- In this first-in-human trial, results from the two patients transplanted with trem-cel demonstrated neutrophil engraftment and platelet recovery similar to patients who received non-edited CD34-selected grafts.[8]
- Trem-cel was well-tolerated, with no related AEs and no unexpected AEs reported.
- A high level of CD33-negative hematopoiesis was achieved (>90%) and increased over the course of GO treatment. The pattern of myeloid development derived from the *CD33*-deleted graft was similar to a reference patient with a non-edited graft.
- CD33-negative cells were enriched after GO treatment across lineages. The *CD33* gene deletion was observed at the genetic level across all lineages, suggesting editing of an early progenitor hematopoietic cell.
- In the context of a *CD33*-deleted graft, a 0.5 mg/m² dose of GO demonstrated a maximum GO concentration (C_{max}) and area under the curve (AUC) in plasma comparable to doses of 1-2 mg/m² and 4-5 mg/m², respectively, in AML patients.
- At a GO dose of 0.5 mg/m², neutrophil and platelet counts remained stable through multiple cycles of GO, including in the presence of MRD. This suggests protection from on-target hematotoxicity by GO.
- These initial data support the biological dispensability of CD33 in myeloid development and a potential approach enabling post-HCT treatment with GO and other CD33-targeted therapies.

References

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