CD33-Deleted Hematopoietic Stem and Progenitor Cells Display Normal Engraftment after Hematopoietic Cell Transplant (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) or adverse cytogenics[2,3]. CD33 is an antigen found on 85-90% of AML cells[4]; however, it is also present on normal myeloid cells[5]. Antineoplastic agents targeting CD33, such as Mylotarg™ (GO), an anti-CD33 antibody-drug conjugate, therefore are associated with extensive cytopenias[6,7]. Preclinical studies have previously shown that CD33 is dispensable for normal biology of human hematopoietic stem and progenitor cells[8]. 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., AML with myelodysplasia (MDS)-related changes (AML-MRC), evidence of persistent bone marrow (BM) blasts, and/or adverse genetics features, 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 high risk of relapse.

VBP101 Study Design

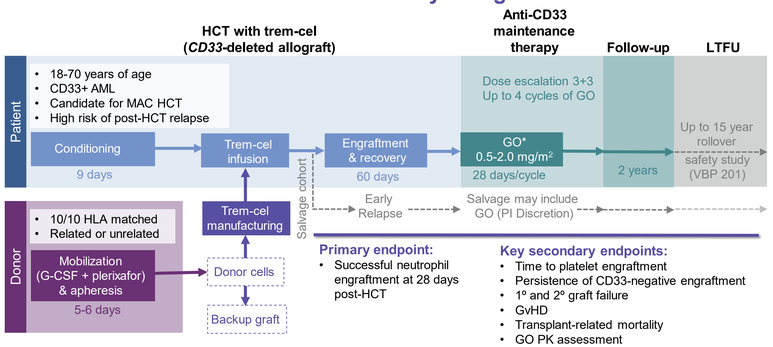


Figure 1: VBP101 Study Design. *Dose escalation is executed using a 3+3 design with a total of three cohorts. The process of dose escalation is continued until maximum tolerable dose (MTD) and recommended phase 2 dose (RP2D) are determined.

Patient and Graft Characteristics

Pt	Age/ Sex	Disease and Genetics	Weight	Donor, Dose, *CD33* gene-editing efficiency
1	64/F	• AML-MRC • Highly complex (adverse) cytogenetics, CR2 • TP53 mutation • MRD: 1.8%	69.9 kg	• 10/10 HLA MUD • 7.6 × 10^6 CD34 cells/kg • 88% *CD33* gene editing
2	32/M	• AML after myeloid sarcoma partially resected from the small bowel and omentum • Initial cytogenetics were Inv 16 and +22; subsequent adverse risk t(3;3) identified • MRD: <0.1%	120.7 kg	• 10/10 HLA MUD • 3.2 × 10^6 CD34 cells/kg • 87% *CD33* gene editing
3	55/F	• AML-MRC • Normal cytogenetics • DNMT3A, IDH2 and SMC1A mutations • MRD: <0.1%	114.1 kg	• 10/10 HLA MUD • 2.6 × 10^6 CD34 cells/kg • 80% *CD33* gene editing
4	68/M	• AML-MRC • Complex cytogenetics, active disease • NRAS, ZRSR2, TET2 mutations • MRD: 16%	72.4 kg	• 10/10 HLA MRD • 5.8 × 10^6 CD34 cells/kg • 89% *CD33* gene editing
5	66/M	• Secondary AML • Normal cytogenetics • KIT D816V, CBL, SRSF2, RUNX1/2, BCORL1 mutations • MRD: <0.1%	102.1 kg	• 10/10 HLA MUD • 4.6 × 10^6 CD34 cells/kg • 85% *CD33* gene editing

Table 1: All patients received myeloablative conditioning with busulfan/melphalan/fludarabine/rabbit anti-thymocyte globulin (ATG), with exception for patient #3, who received equine ATG.

References

[1]Tsirigotis P. et al. Bone Marrow Transplant 2016;51(11):1431–1438; [2]Norkin M. et al. Blood Cancer J 2017;7(12):634; [3]Hourigan CS. et al. J Clin Oncol 2020;38(12):1273–1283; [4]Ehninger A. et. al. Blood Cancer J 2014;4(6):e218. [5]Kenderian SS. et al. Leukemia 2015;29(8):1637–1647; [6]Stein EM. et al. Blood 2018;131(4):387–396; [7]Walter RB. et al. Expert Opin Investig Drugs 2018;27(4):339–348; [8]Borot F. et al. PNAS 2019;116(24):11978–11987; [9]Luznik L. et al. J Clin Oncol 2022;40(4):356–368; [10]Mylotarg ODAC 2017.

Data compiled from EDC, Lab Reports and PI/site reports, Pending full source data verification.

Results

Neutrophil Engraftment and Platelet Recovery Post-HCT with Trem-cel

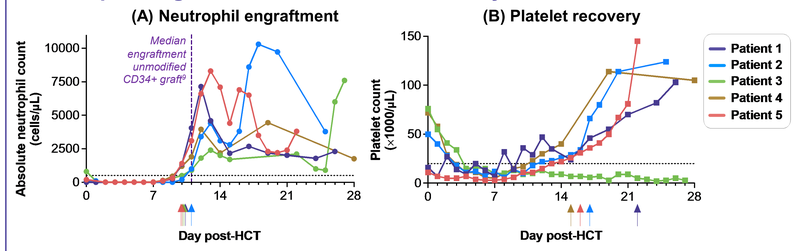


Figure 2: Post HCT with trem-cel, median day of neutrophil recovery is D+10 (10-11) **(A)** and the range of platelet recovery, not inclusive of Patient 3, is from D+15 to D+22 **(B)**. Neutrophil recovery is defined as the first of three consecutive days of an absolute neutrophil count (ANC) ≥500 (dotted line). Platelet recovery is defined as the first day of a sustained platelet count >20,000/μL (dotted line) with no platelet transfusion in the preceding seven days. Arrows under the x-axis indicate day of cell recovery for each patient. Patient 5 data available to D+22 (2 Jun 2023).

Full Donor Chimerism (DC) and Persistent *CD33* Gene Editing Efficiency (CD33GE) in Peripheral Blood Post-HCT with Trem-cel (D+28, D+60)

Patient	Bulk		Monocytes (CD14+ CD15+)		NK cells (CD16+ CD56+)		B cell (CD19+)		T cell (CD3+)	
	Transplant D+28									
	DC%	CD33 GE%	DC%	CD33 GE%	DC%	CD33 GE%	DC%	CD33 GE%	DC%	CD33 GE%
1	100	95.2	100	95.0	100	92.1	QNS	QNS	QNS	QNS
2	94	88.9	100	94.5	99	92.3	100	91.8	4	2.6
3	100	86.6	100	87.9	100	89.0	100	89.4	99	41.6
4	100	pending	100	pending	100	pending	100	pending	52	pending
	Transplant D+60									
1	100	95.9	100	95.6	100	94.9	100	95.6	QNS	QNS
2	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
3	100	87.9	100	90.1	100	90.1	100	88.5	100	48.0

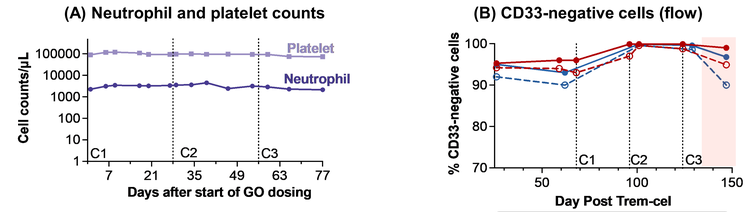
Table 2: Assessment of peripheral blood 28 days (D+28) and 60 days (D+60) post HCT with trem-cel (CD33 GE%= CD33 Gene Editing Efficiency %, DC%= Donor Chimerism %, NC=not collected, QNS = quantity not sufficient).

CD33-negative Cells by Flow Cytometry in Peripheral Blood and Bone Marrow Post-HCT with Trem-cel (D+28, D+60)

Patient	CD33-negative expression by flow (%)							
	Monocyte		Myeloid		Monocyte		Myeloid	
	Transplant D+28				Transplant D+60			
	PB	BM	PB	BM	PB	BM	PB	BM
1	94	92	95	95	94	90	96	91
2	93	91	99	98	NC	NC	NC	NC
3	82	80	86	86	87	85	92	89
4	90	90	95	94	TBD	TBD	TBD	TBD

Table 3: Flow cytometric assessment of peripheral blood and bone marrow 28 days (D+28) and 60 days (D+60) post HCT with trem-cel showed absence of CD33 surface expression in 80-95% of monocytic or myeloid cells. (BM= bone marrow, NC=not collected, PB=peripheral blood, TBD=to be determined).

Patient 1: Heme-protection and Increase in CD33-negative Fraction after GO Dosing

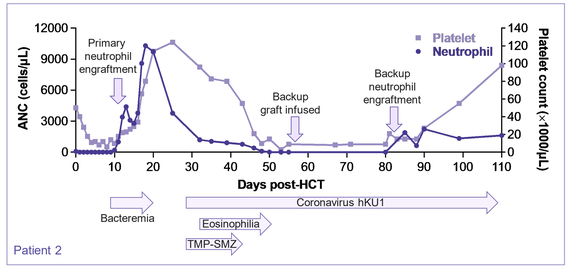


Days Post-HCT (Post-GO)	BM MRD %
D+60 (Pre-GO)	<0.1
D+101 (5d post-C2)	0.3
D+147 (23 post-C3)	7.6

Figure 3: Patient 1 received GO (0.5 mg/m^2) every 28 days beginning at D+68 post-HCT for a total of three doses. **(A)** Neutrophil and platelet counts were stable through GO cycles. No elevations of liver function tests (LFTS) were observed (data not shown). In the context of a *CD33*-deleted graft, a 0.5 mg/m^2 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/m^2 and 4-5 mg/m^2, respectively, in AML patients[10]. **(B)** CD33-negative cells were increased after GO treatment as demonstrated by flow cytometry. Relapsed CD33+ AML overlapped normal cell populations at D+147 time point. Inset table reports BM MRD (AML blast) %.

Patient 2 Clinical Course

After primary neutrophil engraftment at D+11, Patient 2 developed cytopenias after D+28 in the setting of coronavirus hKU1 infection, trimethoprim-sulfamethoxazole (TMP-SMZ) exposure and subsequent eosinophilia. Early sepsis had also developed following initial infusion of the graft. The backup graft was administered for secondary graft failure on D+57 and neutrophil engraftment and platelet recovery were observed 26 and 30 days respectively following back-up infusion. Patient is currently an outpatient.



Patient 3 Clinical Course

Patient 3 had neutrophil engraftment at D+10 however did not have platelet recovery as of D+117. The patient received courses of steroids, IVIg, eltrombopag and romiplostim for treatment of presumed immune thrombocytopenia. The D+60 BM biopsy was hypocellular and showed decreased megakaryocytes. A positive platelet reactive antibody was identified on D+53 and characterized as an anti-HLA Class I Ab. D+100 BM biopsy was normocellular with normal megakaryocytes. As of D+117 the platelet count was 15,000/μL without transfusion in prior 11 days.

Reported Clinical Safety Events

Pt	Related Serious Adverse Events (SAE) or Adverse Events (AE)	Trem-cel related	GO-related	Grade
1	**Gastrointestinal:** Nausea & Vomiting		Y	1-2
2	**Hematologic:** Graft Failure (Secondary; *See Clinical Course*)	Y		4
3	**Dermatologic:** Full-body maculopapular rash (Gr 2 Skin acute GVHD/resolved)	Y		2
	Hematologic: Neutropenia	Y		3
	Other: Dyspnea & Fatigue, Petechia	Y		1 - 2

Table 4: Patient 4 & 5 have no related AEs to report as of data cutoff date: 23 May 2023

Conclusions

- All 5 patients transplanted with trem-cel demonstrated primary neutrophil engraftment (Days 10-11) similar to patients who received non-edited CD34 selected grafts.
- All patients achieved high levels of myeloid donor chimerism by day +28.
  - Patient 2 experienced secondary graft failure in the context of persistent coronavirus hKU1 infection, early bacteremia and possible drug toxicity. Resolved after back-up graft infused.
  - Patient 3 did not achieve platelet recovery by day +117 and is being treated for presumed immune thrombocytopenia.
- A high level of CD33-negative hematopoiesis was achieved (≥80%) in the 4 patients evaluable to day +28.
- In patient 1 (Dosed with GO):
  - CD33-negative cells were increased after GO treatment across lineages, suggesting enrichment of early progenitor hematopoietic cells.
  - At a GO dose of 0.5 mg/m^2, with an AUC corresponding to a dose of 4-5 mg/m^2 , neutrophil and platelet counts remained stable through multiple cycles suggesting protection from GO-induced hematotoxicity.
- 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.