

CRISPR–Cas9 CD33-deleted allogeneic hematopoietic cell transplantation with gemtuzumab ozogamicin maintenance in AML: a phase 1/2 trial

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Patients with high-risk acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) are likely to relapse despite allogeneic hematopoietic cell transplantation (HCT). Post-HCT preventative maintenance can be limited by toxicity toward the normal donor cells. Tremtelectogene empogitemcel (trem-cel) is a CRISPR–Cas9 gene-edited allogeneic HCT product lacking CD33, designed to shield the donor graft from cytotoxicity of subsequent CD33-targeted therapies such as gemtuzumab ozogamicin (GO). In this multicenter, phase 1/2a, open-label study, adult patients with AML/MDS with high relapse risk received trem-cel after myeloablative conditioning followed by GO maintenance (0.5–2.0 mg m⁻² day 1 per 28-day cycles). Patients receiving trem-cel were assessed for the primary safety endpoint of neutrophil engraftment by day 28 and secondary endpoints including time to neutrophil engraftment, incidence of graft-versus-host disease and graft failure, transplant-related mortality, percentage of CD33-negative myeloid cells and survival. Patients receiving trem-cel and GO were assessed for the additional secondary endpoints of safety of maintenance GO with trem-cel and pharmacokinetics of GO after trem-cel transplant. All 30 patients receiving trem-cel achieved the primary safety endpoint of neutrophil engraftment by day 28 with a median engraftment time of 10 days (95% confidence interval: 9–10). Nineteen patients received GO maintenance in phase 1 dose escalation (*n* = 15) and in phase 2 dose expansion (*n* = 4). The trial was stopped early, and this is the final report on the trial including the completed phase 1 portion. GO treatment was safely tolerated up to the recommended phase 2 dose of 2 mg m⁻², and no prolonged high-grade cytopenias were observed. The most common adverse events were cytopenias and infections. Three cases of transplant-related mortality were observed due to renal failure, sepsis and sinusoidal obstruction syndrome, respectively. In summary, trem-cel demonstrated safe, rapid, robust engraftment, and GO maintenance was administered without prolonged hematologic toxicity. ClinicalTrials.gov identifier: [NCT04849910](https://clinicaltrials.gov/ct2/show/study/NCT04849910).

Allogeneic hematopoietic cell transplantation (alloHCT) remains the standard of care for patients with AML who are unlikely to be cured after chemotherapy alone^{1,2}. However, most patients with high-risk features, including adverse molecular/genetic factors, measurable residual disease (MRD) and secondary AML, still relapse after HCT, and long-term survival remains poor^{3,4}. Post-HCT maintenance strategies including hypomethylating agents and targeted inhibitors to prevent relapse are under investigation but are largely limited by hematotoxicity against the transplanted graft and have not demonstrated broad efficacy^{1,2,5,6}.

Therapeutic targeting of AML cells is challenging because AML antigens such as CD33 are also expressed on normal myeloid cells and progenitors^{7,8}. Gemtuzumab ozogamicin (GO; Mylotarg) is a CD33-directed antibody–drug conjugate approved for certain AML indications, but its use after HCT is limited by severe, prolonged cytopenias due to on-target, off-tumor hematotoxicity^{9–12}. CD33 is a candidate for gene editing because its expression is restricted to hematopoietic cells, it is expressed in most AML cases and it appears dispensable for hematopoiesis based on the absence of functional deficiencies in xenotransplantation studies of CD33-deleted human hematopoietic stem cells^{13–15}. These findings are consistent with the observation of non-deleterious homozygous loss-of-function mutations in *CD33* in humans^{16–18}. Tremtelectogene empogeditemcel (trem-cel; VOR33) is a hematopoietic stem and progenitor cell (HSPC) product manufactured from CD34⁺ cells from a human leukocyte antigen (HLA)-matched donor that have been gene edited by CRISPR–Cas9 to target CD33, resulting in a frameshift with premature termination and CD33 protein loss¹⁸. The purpose of trem-cel is to improve patient outcomes by enabling post-HCT targeting of residual, CD33⁺ leukemia with maintenance GO, because the CD33-deleted donor graft should be shielded from the cytotoxic effects of GO. The trem-cel graft was produced using a CD34⁺-selected platform for several key reasons. First, by limiting the CRISPR–Cas9 editing to a single defined cell type, the difficulty characterizing and identifying the confounding effects of editing a highly heterogeneous mixture of lineages was markedly reduced. Second, cell number capacity limits of the manufacturing equipment and reagents precluded using a non-selected graft. Finally, transplantation with CD34-selected grafts has been performed for many years, providing a well-documented baseline for comparison, including a favorable graft-versus-host disease (GvHD) risk profile and similar graft-versus-leukemia (GvL) effect to conventional T-replete transplants¹⁹.

Here we report findings from a study of trem-cel followed by GO maintenance therapy dose escalation/expansion in patients with CD33⁺ AML or MDS at high risk of relapse after alloHCT.

Results

Patient characteristics and disposition

Patients were initially screened ($n = 59$) and underwent assessment for full eligibility and enrollment, including the ability of a suitable 8/8 HLA-matched donor to donate sufficient cells for manufacturing ($n = 32$) (Fig. 1a). Patients with homozygous T alleles for the exon 2 single-nucleotide polymorphism (SNP) rs12459419 in *CD33* were excluded from the study as they lack the GO-binding epitope²⁰. Four screened patients had issues preventing successful trem-cel manufacturing: three had inadequate yields from their donor apheresis to manufacture and one had an equipment failure during manufacture. Patients having a successful manufacturing of trem-cel were allowed to proceed as enrolled in the study ($n = 30$). The first patient to receive trem-cel was consented on 21 June 2022, and last patient to receive trem-cel by the 12 January 2025 data cutoff date was consented on 16 October 2024. The phase 1 dose-escalation portion, where patients, upon meeting criteria after trem-cel HCT, began GO dosing in cohorts of 0.5 mg m⁻², 1 mg m⁻² or 2 mg m⁻² once per 28-day cycle for up to eight cycles ($n = 15$) (Extended Data Fig. 1). Cohorts were escalated in a 3 + 3 strategy after review of each cohort's safety data by a dose escalation committee (DEC). If patients were ready to proceed to GO maintenance and the

last patient of the dosing cohort had not yet been assessed by the DEC, they were allowed to join the lower cohort dose level. After data review of the 2 mg m⁻² cohort, the DEC established the recommended phase 2 dose (RP2D) as 2 mg m⁻², and the phase 2a expansion portion of the trial dosed GO at the RP2D ($n = 4$). Patients relapsing prior to receiving maintenance GO were eligible to move to a disease treatment cohort ($n = 1$). Five patients had been transplanted with trem-cel at the data cutoff but had not yet reached the eligibility for initiation of GO maintenance. The trial was terminated early due to fiscal reasons, and this report, which will serve as the final analysis, primarily describes the completed phase 1 portion.

Patient characteristics are shown in Table 1 and Supplementary Table 1. Twenty-nine patients had AML and one had MDS; 53% patients had more than one adverse risk factor, defined as adverse cytogenetics (European LeukemiaNet (ELN) 2022), $\geq 0.1\%$ blasts at HCT, secondary AML or relapse prior to transplant.

Median trem-cel dose was 8.01×10^6 CD34⁺ cells per kg (range, 2.62–12.44) after manufacturing. Median CD33 gene editing efficiency at manufacture was 90% (range, 71–94). Median CD34 yield of trem-cel manufactured from apheresis input was 34% (range, 15–81).

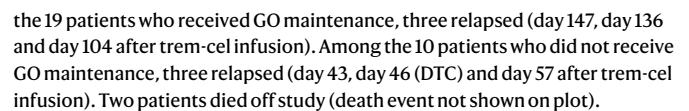
Patient status and treatment received is shown in Fig. 1b. Twenty patients were ongoing in the study, and 13 remained on GO maintenance at the data cutoff. Seven patients died during the study; four deaths were due to progressive disease (two patients received trem-cel only and two received trem-cel plus GO). Transplant-related mortality (TRM) occurred in three patients, all after day 100: renal failure (trem-cel related with adenovirus and cidofovir toxicity contributory), sinusoidal obstruction syndrome/veno-occlusive disease (SOS/VOD) (GO related) and sepsis (unrelated to treatment).

Primary and secondary outcomes

All patients receiving trem-cel were considered as the evaluable population for primary and secondary endpoints, including engraftment, platelet recovery, relapse-free survival (RFS), overall survival, graft failure, GvHD, infections and safety (as detailed in the Methods). The efficacy population (receiving at least one cycle of GO) was additionally evaluated for GO pharmacokinetics, cytopenias during GO treatment and safety. All 30 patients (100%) achieved the primary endpoint of neutrophil engraftment by day 28 after trem-cel infusion; median time to engraftment was 10 days (95% confidence interval: 9–10) (Fig. 2a). Platelet recovery was achieved by 29 patients; median time to recovery was 16 days (95% confidence interval: 15–19) (Fig. 2b). One patient had antiplatelet antibodies identified and was treated for presumptive immune-mediated thrombocytopenia with steroids, intravenous immunoglobulin, rituximab and growth factors and did not achieve platelet recovery until after a CD34 stem cell boost on day 202 after HCT. Secondary graft failure occurred in one patient in the setting of a potential drug toxicity from trimethoprim/sulfamethoxazole and persistent coronavirus hKU1 infection; the patient recovered after receiving an unedited backup graft on day 57 after HCT.

Median duration of follow-up for alive patients was 7.9 months (range, 0.6–23.7). Median RFS and overall survival for all 30 patients receiving trem-cel were both 14.1 months (Extended Data Fig. 2). All cases of relapsed disease were CD33⁺ by flow cytometry. No TRM occurred within the first 100 days. Three patients had TRM after day 100 HCT due to renal failure (trem-cel related with adenovirus and cidofovir toxicity contributory), SOS/VOD (GO related) and sepsis (unrelated to treatment), respectively.

In the 19 patients receiving at least one cycle of GO maintenance, 77 treatment cycles were administered (median number of cycles: 4 (range, 1–8)). GO dose escalation was conducted as planned, and there were no dose-limiting toxicities (DLTs); six patients received the highest GO dose of 2 mg m⁻² in dose escalation. Two patients in the 1 mg m⁻² cohort had their dose reduced to 0.5 mg m⁻² due to thrombocytopenia. The RP2D of GO at 2 mg m⁻² was established based on review of the safety,



Infections occurred in 26 patients (87%); the most common were oral candidiasis ($n = 9$, 30%), BK virus infection ($n = 7$, 23%), pneumonia ($n = 7$, 23%) and COVID-19 ($n = 6$, 20%). Grade 3 or higher infections

Table 1 | Demographic, disease and transplant characteristics

Characteristic	Patients receiving trem-cel (N=30) (%)
Age in years, median (range)	53 (22–68)
≥65 years, n (%)	7 (23)
<65 years, n (%)	23 (77)
Female sex, n (%)	16 (53)
Weight in kg, median (range)	72.6 (48–121)
Race	
White	25 (83)
Asian	2 (7)
Other	2 (7)
Unknown/declined	1 (3)
Ethnicity	
Not Hispanic/Latino	23 (77)
Hispanic or Latino	6 (20)
Unknown/declined	1 (3)
Primary diagnosis, n (%)	
AML	29 (97)
MDS	1 (3)
Cytogenetic risk ELN 2022 (AML) ^a , n (%)	
Favorable	3 (10)
Intermediate	10 (34)
Adverse	16 (55)
IPSS-R score (MDS) ^b , n (%)	
Very high	1 (100)
Other AML risk factors ^a , n (%)	
TP53 mutation	9 (30)
Secondary AML ^c	11 (38)
AML/MDS disease status, n (%)	
Primary induction failure	4 (13)
Complete remission 1	15 (50)
Complete remission 2	7 (23)
R/R	4 (13)
Disease burden at transplant, n (%)	
MRD negative	20 (67)
MRD positive ^d	6 (20)
Blast range, %	0.5–4.4
Active disease ^e	4 (13)
Blast range, %	8–78
Myeloablative conditioning regimen, n (%)	
Busulfan/melphalan/fludarabine/rATG	26 (87)
TBI/cyclophosphamide/thiotepa/rATG	3 (10)
Busulfan/melphalan/fludarabine/equine ATG	1 (3)
Donor type, n (%)	
Unrelated	22 (73)
Related	8 (27)
Minimum 8/8 match	30 (100)

^a n=29 ^b n=1 ^c Defined as AML with myelodysplasia-related change (9/29), prior myeloproliferative disease (1/29) or therapy related (1/29) ^d >0.1% to <5% blasts by flow cytometry ^e ≥5% blasts rATG, rabbit anti-thymocyte globulin.

occurred in 14 patients (54%), with eight patients having grade 3 or higher infections during the first 100 days and eight patients after day 100.

A full listing of all adverse events, TEAEs and TSEAEs in the evaluable population is provided in Supplementary Tables 3–5.

Exploratory objectives

Chimerism analyses showed full donor myeloid, B and natural killer (NK) cell chimerism by day 28 or day 60 in all patients (Fig. 2c). Editing frequency in myeloid, B and NK lineages persisted after HCT and correlated with donor chimerism, demonstrating that CD33-deleted HSPCs can undergo multilineage differentiation without selective disadvantage compared to unedited cells (Fig. 2d). Repair of CRISPR–Cas9-induced DNA double-stranded breaks (DSBs) predominantly occurs via the non-homologous end joining repair (NHEJ) pathway, resulting in small insertions and deletions (InDels) at the target site. Trem-cel drug products typically yield 1-base pair (bp) deletions, 2-bp deletions and 1-bp insertions (Extended Data Fig. 5), all of which lead to frameshift and premature stop codon formation resulting in CD33 protein loss (Fig. 2e), consistent with a previous publication¹⁸. InDel distribution was largely conserved after HCT in myeloid, B and NK cell lineages from that of drug products (Extended Data Fig. 5). However, it is noteworthy that larger insertions (ranging from +2 bp to >+5 bp) were detected in lymphoid lineages, particularly in NK cells, with lesser frequency in B and T cells, during the early timepoints after trem-cel. These InDel species were not detected in the myeloid lineage. The post-HCT myeloid population lacked CD33 surface protein (Fig. 2e), which was associated with no impact on neutrophil function, as evaluated by a qualitative oxidative burst assay at day 28 in 12 of 17 samples (the remainder were indeterminate/non-assessable). The increase in proportion of CD33⁺ cells at day 100 and beyond indicates GO targeting and enrichment at the HSPC level.

In the peripheral blood, NK cells repopulated to a median of 315 per µl (range, 102–717) by day 60. CD4⁺ T cells were measured at a median of 93 per µl (range, 1–195) at day 180 and 368 per µl (range, 229–507) at 12 months. CD8⁺ T cells were at a median of 12 per µl (range, 1–357) at day 180 and 359 per µl (range, 294–425) at 12 months. B cell reconstitution had a median of 217 per µl (range, 1–1,716) at day 180 and 280 per µl (range, 178–383) at 12 months. (Fig. 2f). T cell donor chimerism was variable, even at 12 months (Fig. 2c). This also results in variable editing frequency (Fig. 2d) and InDel distribution, which likely reflects the varying quantities of extractable genomic DNA, thereby potentially affecting accuracy (Extended Data Fig. 5). B and T cell receptor analyses demonstrated the ability of trem-cel to yield de novo clonotypes (that is, HSPC derived) with a diverse repertoire (Extended Data Fig. 6). Clonal tracking revealed that T cells can originate from donor apheresis material and persist beyond 12 months (Extended Data Fig. 6).

Discussion

There is a need for novel strategies to prevent relapse in patients with AML and MDS who are at high risk after alloHCT. There are currently no FDA-approved therapies in this setting. Post-HCT maintenance studies using hypomethylating agents ± venetoclax have yielded mixed results^{5,6}. Targeted therapies, such as gilteritinib for FLT3-mutated disease, have not shown improved RFS except in subgroups²². Other inhibitors, such as IDH or immunotherapies, have also not yet shown to conclusively reduce relapse after HCT^{5,6}.

Trem-cel is a CD33-deleted alloHCT product that enables use of CD33-directed post-HCT maintenance therapies while avoiding limiting hematologic toxicity. In our study, patients receiving trem-cel showed robust and rapid primary neutrophil and platelet engraftment and full donor myeloid chimerism. This is consistent with CD33 being dispensable for engraftment and hematopoiesis and indicates that the trem-cel manufacturing process does not affect engraftment. Time to engraftment/recovery for trem-cel was similar to with patients

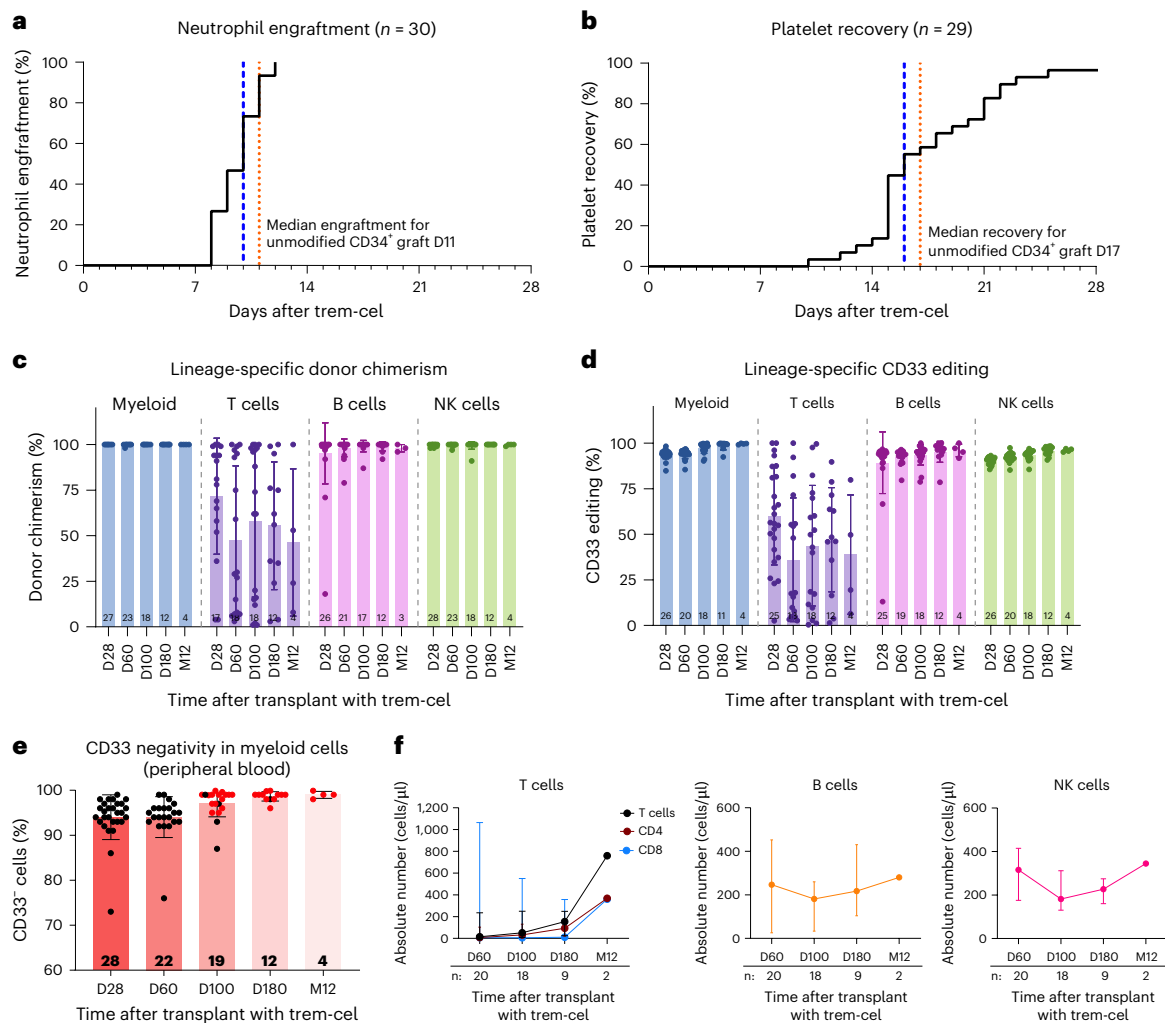


Fig. 2 | Engraftment and immune reconstitution. **a**, Percent of patients achieving neutrophil engraftment, defined as the first of three consecutive days of an ANC ≥ 500 cells per μ l. The orange dotted line indicates median engraftment from a comparator study using unselected CD34-selected graft¹⁹. **b**, Percent of patients achieving platelet recovery, defined as the first day of a sustained platelet count $>20,000$ per μ l with no platelet transfusion in the preceding 7 days. Figure excludes one patient who had antiplatelet antibodies and was treated for immune thrombocytopenia with steroids, intravenous immunoglobulin and a CD34-selected stem cell boost from the backup graft to aid resolution of the thrombocytopenia. The orange dotted line indicates median recovery from a comparator study using unselected CD34-selected graft¹⁹.

c, Donor chimerism after HCT in myeloid, T, B and NK lineages on days 28 (D28), 60 (D60), 100 (D100), 180 (D180) and month 12 (M12) after trem-cel infusion (mean $\pm$ s.d.). **d**, CD33 gene editing efficiency by next-generation sequencing after HCT in myeloid, T, B and NK cell lineages (mean $\pm$ s.d.). Further delineation of donor T and B cell contribution by T and B cell repertoire clonal tracking analysis is described in Extended Data Fig. 6. The number of patient samples analyzed is indicated in each bar. **e**, CD33 protein deletion (as a result of gene editing) measured by flow cytometry (median $\pm$ s.d.). Data points in red indicate sample collection after GO treatment. **f**, Reconstitution of total T, B and NK cell counts over time (median $\pm$ s.d.). Two patients who received backup graft were excluded from analysis beyond day 100 ($n = 1$) and day 180 ($n = 1$).

receiving non-edited, CD34-selected HCTs (median time to neutrophil engraftment 11 days and platelet recovery 17 days)¹⁹.

After trem-cel, the incidences of both acute and chronic GvHD and infections were similar to those observed using standard, unedited CD34-selected alloHCT without GvHD prophylaxis^{19,23}. In comparison to reported immune cell reconstitution data from patients receiving unedited CD34-selected grafts, NK reconstitution displayed similar kinetics to an unedited CD34-selected HCT²⁴; CD4⁺ T cell reconstitution was also similar at 93 per μ l at 180 days and at 368 per μ l at 12 months compared to 135 per μ l and 252 per μ l, respectively. CD8⁺ T cell reconstitution was lower at 12 per μ l at day 180 versus 132 per μ l but fell within the reported range (0–3,643) and increased to a median of 359 per μ l at 12 months compared to 269 per μ l. In the absence of reported B cell reconstitution data from CD34-selected grafts, compared to a T-replete graft using ATG with conditioning, B cell reconstitution compared favorably with 217 per μ l versus 104 per μ l at 180 days and 280 per μ l versus 239 per μ l at 12 months (ref. 25). Both B and T cells displayed

diverse receptor repertoires. Clonal tracking revealed that, although most B cells are generated de novo from trem-cel HSPCs, T cell sources are variable, comprising recipient cells, donor T cell carryover and de novo cells generated from trem-cel HSPCs. However, prolonged mixed T cell chimerism in CD34-selected HCT grafts has not been associated with adverse outcomes^{26,27}. Tracking gene editing profile of trem-cel through InDel distribution both prior to and after HCT is essential for elucidating any potential selective biases in clonality or lineage distribution. Overall, myeloid, B, T and NK cell lineages had all retained similar InDel distribution when assessed against pre-HCT drug products, with the exception of infrequent occurrences of larger insertion species identified specifically within the lymphoid compartment, particularly NK cells. This finding is consistent with the InDel distribution observed in multipotent lymphoid progenitors from several batches of trem-cel drug product (data not shown) where infrequent ranging from +2 bp to +5 bp was detected. This phenomenon is likely attributable to the presence of terminal deoxynucleotidyl transferase

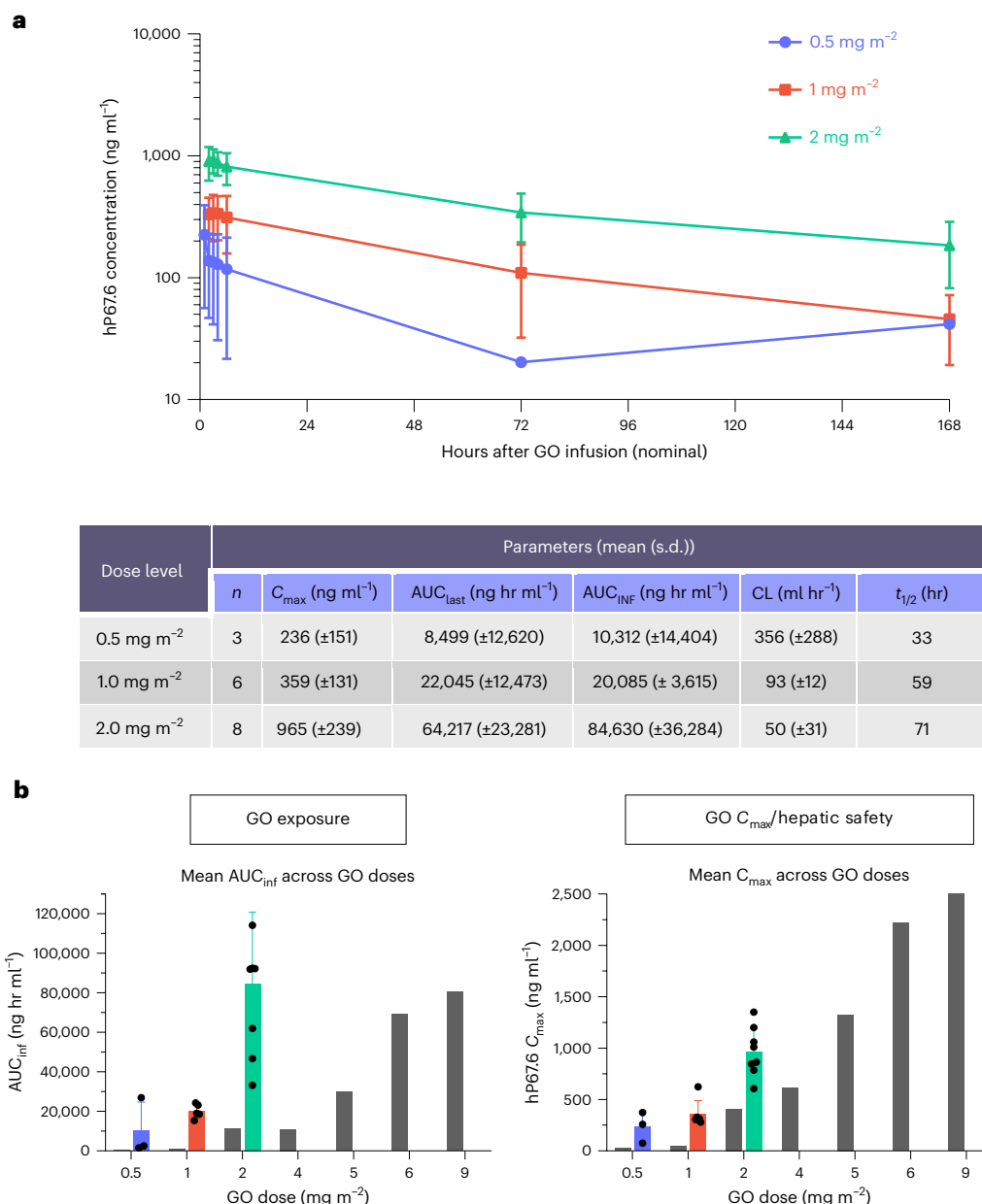


Fig. 3 | Pharmacokinetic analysis. a, Pharmacokinetic profile and parameters for the first GO dose in trem-cel patients receiving 0.5, 1.0 or 2.0 mg m⁻² GO. Error bars denote s.d. and *n* for each cohort reported in the table. **n* = 5. **b**, Area under the curve from 0 to infinity (*AUC*_{inf}) (left) and *C*_{max} (right) for each of the three GO cohorts after trem-cel compared to established GO population pharmacokinetic exposures in a standard R/R AML population²¹. Blue, red and green bars denote

GO doses of 0.5, 1.0 and 2.0 mg m⁻² in patients who received trem-cel; gray bars denote reported GO pharmacokinetic analysis from the standard R/R AML population; s.d. is not provided²¹. Sample sizes are 0.5 mg m⁻² *n* = 3, 1 mg m⁻² *n* = 5 and 2 mg m⁻² *n* = 8. Error bars denote s.d. Some *AUC*_{inf} values may fall outside the 20% extrapolation. CL, clearance; hr, hours; *t*_{1/2}, half-life.

(TdT), which is broadly expressed in lymphoid progenitors and was previously shown to result in longer insertions after Cas9-induced DSB^{28,29}. The prevalence of larger insertions was most notable at day 28 to day 60 after trem-cel, beyond which point multipotent progenitors, which are non-lineage restrictive, begin to contribute to the development of B and NK cells. Neutrophil function in regards to oxidative burst was also intact.

Together, these data suggest that CD33 gene editing in HSPCs persists after HCT, does not confer selective disadvantage in any hematopoietic lineages and does not impact the ability to reconstitute a functional immune system as shown in animal models^{13,14}. These findings are consistent with a recent report detailing human CD33 null individuals, which identify a minimal decrease in total leukocytes but no phenotypic changes in blood lineages nor evidence for pathology¹⁷. The Siglec

family members, including CD33, have been shown to have an inhibitory role in inflammatory response, and a more in-depth investigation into monocyte function may reveal a phenotype, although no clinically overt signs were observed^{30,31}. An alternative approach to complete deletion of CD33 or other potential target antigens to preserve function would be to edit the binding epitope for the targeted therapeutic, as has been shown in preclinical studies^{32–34}. The base editing approach may additionally reduce the theoretical risk of translocations compared to processes using DNA DSBs³².

With deletion of CD33, GO maintenance therapy was feasible up to eight doses using the anticipated administration schedule. No GO-related DLTs were observed in the dose-escalation phase, and GO maintenance therapy was well tolerated. A single event of SOS/VOD was observed distant from GO dosing in the context of

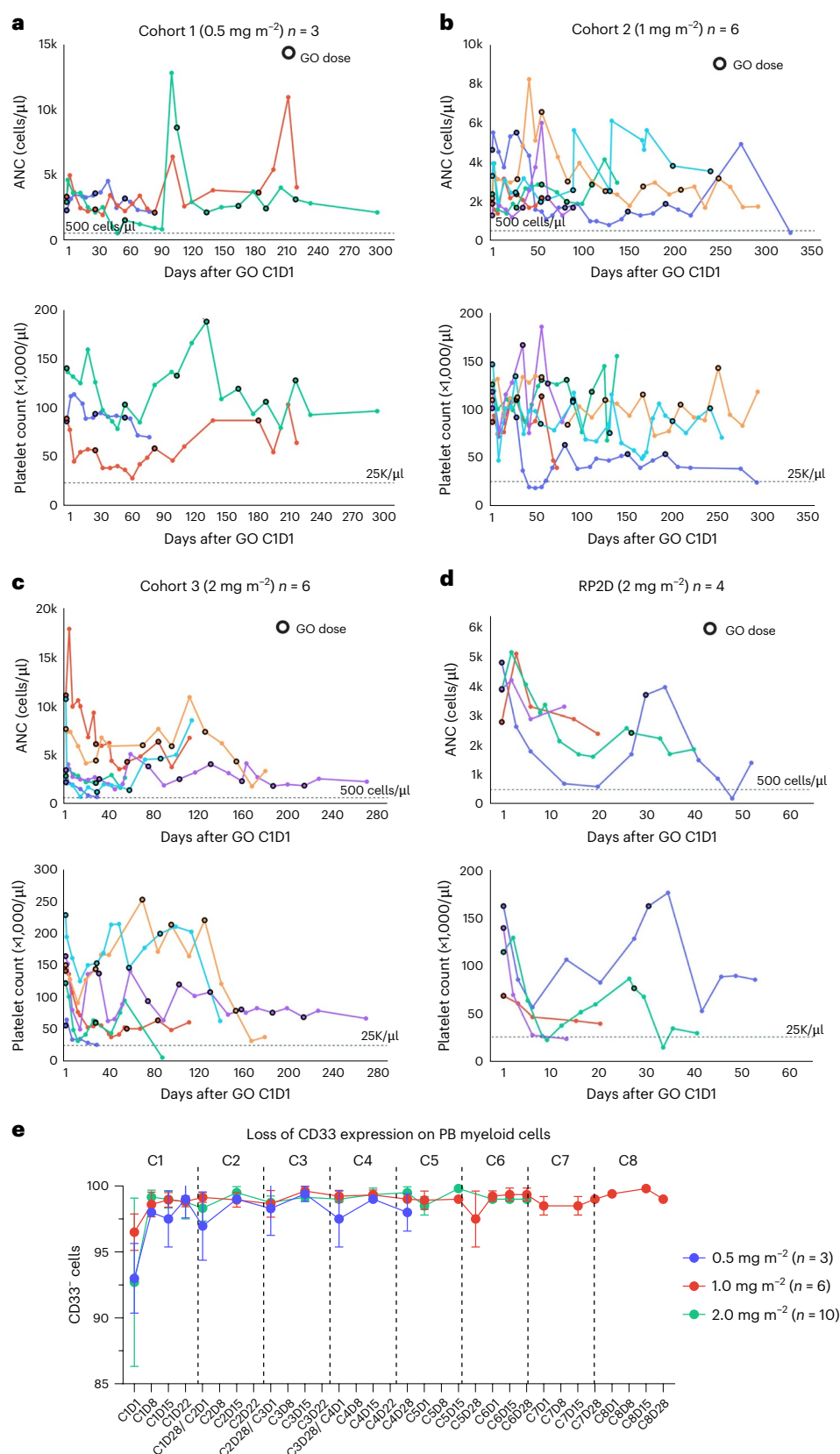


Fig. 4 | Neutrophil and platelet counts and CD33 expression during GO maintenance. **a–d**, Neutrophil and platelet counts in patients receiving GO maintenance at doses of 0.5 mg m^{-2} , 1 mg m^{-2} , 2 mg m^{-2} and the RP2D (2 mg m^{-2}), respectively. Each line represents an individual patient. Patient represented by green line in **d** relapsed after C1 GO. **e**, Percentage of CD33⁺ cells in peripheral

blood myeloid cells measured by flow cytometry during GO maintenance on days 1, 8, 15, 22 or 28 of cycles 1–8 plotted as mean $\pm$ s.d. Day 1 from each cycle was collected prior to GO administration and serves as the baseline of each cycle. 25K, 25,000; C, cycle; D, day; PB, peripheral blood.

Table 2 | Treatment-related TEAEs of grade 3 or higher

System organ class and preferred term	Maximum grade	Patients, n (%)
Trem-cel related		
Any trem-cel-related TEAE grade ≥ 3		9 (30)
Blood and lymphatic system disorders		6 (20)
Anemia	3	2 (7)
Autoimmune hemolytic anemia	3	1 (3)
Neutropenia	3	1 (3)
Thrombocytopenia	4	3 (10)
Gastrointestinal disorders		2 (7)
Diarrhea	3	1 (3)
Oral pain	3	1 (3)
Immune system disorders		1 (3)
Acute GvHD	3	1 (3)
Infections and infestations		2 (7)
Adenovirus infection	4	1 (3)
Cytomegalovirus infection	3	1 (3)
Herpes simplex reactivation	3	1 (3)
Injury, poisoning and procedural complications		1 (3)
Blood stem cell transplant failure	4	1 (3)
Investigations		4 (13)
Neutrophil count decreased	3	2 (7)
Platelet count decreased	4	4 (13)
Weight decreased	3	1 (3)
White blood cell count decreased	4	1 (3)
Metabolism and nutrition disorders		1 (3)
Decreased appetite	3	1 (3)
Renal and urinary disorders		1 (3)
Acute kidney injury	4	1 (3)
Renal failure	5	1 (3)
Respiratory, thoracic and mediastinal disorders		1 (3)
Hypoxia	3	1 (3)
GO related		
Any GO-related TEAE grade ≥ 3 , n (%)		11 (58)
Blood and lymphatic system disorders		8 (42)
Anemia	3	5 (26)
Leukopenia	3	1 (5)
Neutropenia	4	2 (11)
Thrombocytopenia	4	3 (16)
Hepatobiliary disorders		1 (5)
SOS/VOD ^a	4	1 (5)
Infections and infestations		1 (5)
Adenovirus infection	4	1 (5)
Investigations		5 (26)
Electrocardiogram QT prolonged	3	1 (5)
Lymphocyte count decreased	3	1 (5)
Neutrophil count decreased	3	1 (5)
Platelet count decreased	4	3 (16)
White blood cell count decreased	3	1 (5)
Respiratory, thoracic and mediastinal disorders		1 (5)
Epistaxis	3	1 (5)

^a Increased to grade 5 after data cut

complicating gastrointestinal/hepatic issues. Pharmacologic activity of maintenance GO was supported by enrichment of CD33⁺ cells in peripheral blood shortly after the first cycle, reflecting the action of GO on the small portion of residual CD33⁺ unedited donor cells or minute amounts of residual recipient CD33⁺ myeloid cells remaining after engraftment. Enrichment of the CD33⁺ populations after GO was observed for at least 3 months in two patients, suggesting that selection against CD33⁺ cells could be occurring at the HSC or early progenitor stage (Supplementary Table 2). Neutrophil and platelet counts were sustained during GO dosing, demonstrating that trem-cel provides protection from the deep and prolonged cytopenias caused by the known on-target hematologic toxicity of GO^{9–12}. This was supported by low rates of grade 3 or higher neutropenia/thrombocytopenia during GO treatment; consequently, no episodes of febrile neutropenia or hemorrhage were observed, except for a single episode of epistaxis. Trem-cel plus GO maintenance resulted in reduced hematologic toxicity compared to published literature describing GO after standard alloHCT^{11,12,35}.

Given that GO is cleared through binding to the CD33 receptor, it was expected that GO pharmacokinetics would be altered in a CD33⁺ environment. Comparison of GO pharmacokinetic parameters in this study with established GO population pharmacokinetic exposures in a standard R/R AML population indicated a proportionally larger increase in AUC (correlated with efficacy) compared to C_{max} (correlated with hepatotoxicity)²¹. This suggests a broadened therapeutic index for GO after trem-cel. Based upon the relatively steep AUC dose proportionality up to 2 mg m⁻², and the acceptable safety and maintenance responses, further GO dose escalation was not initiated. Compared to the approved 3 mg m⁻² dose for AML, it is predictable that a post-HCT/trem-cel population would require a lower GO dose given the lower CD33 target burden. Thus, 2 mg m⁻² was defined as the RP2D for GO maintenance after trem-cel.

Although median follow-up duration is limited, the median RFS of 14.1 months and approximately 2-year RFS of 40%, respectively, is encouraging considering the high-risk population enrolled, many with multiple risk factors. Although cross-trial comparisons are challenging due to differences in study designs and populations, this RFS is favorable compared to other cohorts of standard HCT in patients with high-risk AML, including those with adverse risk genetics (3-year event-free survival of 30% (ref. 36)), MRD⁺ disease (3-year progression-free survival (PFS) of 12%)³ and *TP53*-mutated AML (median PFS 5 months and 1-year PFS of 25%)³⁷. A major limitation to the study in regards to measuring efficacy in preventing relapse includes a relatively median short follow-up time and relatively small patient numbers, as the study's primary endpoint was to establish safety. In addition, the broad definition of high-risk AML used to enable adequate and timely enrollment of this first-in-human study resulted in a heterogeneous population and made it difficult to precisely identify an established single baseline for overall survival and RFS.

The presence of CD33⁺ disease upon relapse suggests a resistance mechanism other than antigen loss and is consistent with previous studies of GO resistance³⁸. GO is the only approved anti-CD33 therapeutic and has a known toxicity profile and antileukemic activity; however, although importantly establishing proof of concept for hematopoietic protection with trem-cel, other anti-CD33 therapeutics in development, such as antibody–drug conjugates with improved payloads, CD33-directed chimeric antigen receptor (CAR) T or bispecific T cell engagers, may have improved activity, particularly against AML with adverse risk characteristics and lack the known non-hematologic toxicities of GO, such as hepatotoxicity³⁹. Even with a highly effective anti-CD33 therapy, heterogeneity of expression within the AML population or CD33 loss due to selection may enable resistance.

In conclusion, alloHCT with trem-cel resulted in rapid engraftment in patients with AML and MDS at high risk of post-HCT relapse, and GO maintenance was administered without prolonged hematologic

toxicity. Longer follow-up and larger studies are needed to assess the safety and efficacy of this approach. This study provides proof of principle that trem-cel HCT can transform CD33 from a ubiquitous myeloid antigen to an AML-specific antigen that can be engaged by CD33-targeting agents, thus allowing protection of normal hematopoiesis while eliminating residual host AML cells after HCT and providing a foundation for future approaches targeting CD33 or other shared antigens.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41591-026-04362-1>.

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Methods

Study design and trial oversight

This research complies with all relevant ethical regulations, and the study protocol was approved by the Western IRB (WIRB/Copernicus Group), the NMDP IRB and the institutional review boards at Washington University; Miami Cancer Institute; the National Cancer Institute; Hôpital Maisonneuve-Rosemont; Hackensack University Medical Center; the University of California, San Diego; Memorial Sloan Kettering Cancer Center; the University of Kansas Cancer Center; the University of Minnesota; Winship Cancer Center; Emory University; Stanford University; and the University of Utah. All patients signed informed consent, and no compensation aside from study-required travel/lodging reimbursement was provided. Sex and gender were not considered in the study design. This was a first-in-human, phase 1/2a, open-label study in patients with AML or MDS at high risk of relapse who received an HLA-matched alloHCT with trem-cel followed by GO maintenance therapy (ClinicalTrials.gov identifier: [NCT04849910](#), first submitted 21 April 2021; Extended Data Fig. 1). Trem-cel was manufactured as previously described and summarized in the ‘Trem-cell manufacturing’ section¹⁸. Patients underwent busulfan-based or total body irradiation (TBI)-based myeloablative conditioning prior to infusion of trem-cel on day 0. Supportive care was provided per local practice and study guidelines. T cell content of the graft was $<1 \times 10^6$ CD3⁺ cells per kg, and no post-transplant prophylaxis against GvHD was given. On day 60, patients achieving neutrophil engraftment were assessed for disease status and hematopoietic recovery by bone marrow biopsy; eligible patients initiated maintenance GO by infusion. Part 1 (GO dose escalation) followed a standard 3 + 3 design of 3–6 patients per dose cohort (0.5, 1.0 or 2.0 mg m⁻² on day 1 of each 28-day cycle up to eight cycles) to identify maximum tolerated dose (MTD) and RP2D. Part 2 (GO dose expansion) assessed safety and preliminary efficacy of the RP2D. Patients who relapsed or were MRD⁺ could enter the DTC (Extended Data Fig. 1) to receive GO induction and consolidation.

The trial was approved by the institutional review board/independent ethics committee at each site and donor registry and was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice guidelines. All patients and donors provided written informed consent. The study was designed and data were analyzed by the sponsor in collaboration with the investigators.

Eligibility

Inclusion/exclusion criteria (patients and donors)

Full patient eligibility Inclusion criteria

1. Patients must be ≥ 18 and ≤ 70 years of age.
2. Ability to understand and willingness to sign the informed consent form or, if applicable, understanding and willingness of the patient’s caregiver or legally authorized representative prior to initiation of any study-related tests or procedures
3. Patients must have confirmed diagnosis of AML or MDS according to World Health Organization (WHO) classification criteria.
4. The patient’s AML or MDS must have evidence of CD33 expression ($>0\%$) according to institutional flow cytometry criteria.
5. Patients with AML must have one of the following groups of features that are known to be a risk factor for leukemia relapse:
 - Bone marrow in morphological remission ($<5\%$ blasts) with adverse-risk disease-related genetics at presentation (according to ELN guidelines⁴⁰), or
 - Intermediate-risk genetics in morphologic remission ($<5\%$ blasts) with other recognized high-risk criteria, such as MRD⁺ after therapy, or

- Bone marrow with evidence of persistent leukemia 5–10% blasts after induction/salvage therapy. Patients with bone marrow blast count $>10\%$ may participate with Sponsor Medical Monitor approval (note: these patients may have disease-related genetics of any risk criteria at presentation), or
 - Any patient in second or greater remission
- a. Patients with MDS must have all of the following:
 - Previous or current Revised International Prognostic Scoring System (IPSS-R) score of High or Very High risk
 - Previous or current MDS-IB1 or MDS-IB2 per the 2022 WHO criteria
6. Patients must be a candidate (per investigator opinion) for HLA-matched alloHCT using a myeloablative conditioning regimen (see protocol for allowed regimens).
 7. Patients must have a related or unrelated stem cell donor that is an 8/8 match for HLA-A, HLA-B, HLA-C and HLA-DRB1, as assessed by high-resolution DNA-based typing. The selection of related donors is per institutional standards and, for unrelated donors, is per registry standards.
 8. Patients exceeding a weight of 120 kg (265 lbs) may participate with Sponsor Medical Monitor approval.
 9. Patients must have adequate performance status and organ function as defined below:
 - Performance status: Karnofsky score of ≥ 70
 - Cardiac: left ventricular ejection fraction $\geq 45\%$ by echocardiogram or radionuclide scan (that is, multiple-gated acquisition (MUGA) scan)
 - Pulmonary: diffusing capacity of lung for carbon monoxide $\geq 66\%$ (adjusted for hemoglobin levels) and forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV1) $\geq 66\%$
 - Renal: estimated glomerular filtration rate >60 ml min⁻¹ as assessed by Cockcroft–Gault formula and actual body weight
 - Hepatic: total bilirubin $<1.5 \times$ upper limit of normal (ULN) or, if $\geq 1.5 \times$ ULN, direct bilirubin $<ULN$ and alanine aminotransferase/aspartate aminotransferase $<1.5 \times$ ULN (per institutional criteria)
 10. Female patients of childbearing potential must test negative for pregnancy at screening, not be pregnant, nor breastfeeding, nor expecting to conceive within the projected duration of the trial. Sexually active women, unless surgically sterile, must be willing to use a highly effective method of birth control up to 180 days after the last dose of study drug (VOR33 or Mylotarg). Examples of birth control methods are as follows: implants, injectables, combined oral contraceptives, intrauterine devices (IUDs) or vasectomized partner.
 11. Up to 90 days after the last dose of study drug (VOR33 or Mylotarg), male patients with partners of childbearing potential must either be vasectomized or agree to use a condom, in addition to having their partners use another method of contraception, resulting in a highly effective method of birth control such as implants, injectables, combined oral contraceptives or IUDs. Male patients should not have sexual intercourse with females who are either pregnant or lactating without double-barrier contraception. Male patients must not donate sperm during the course of the study.
 12. Currently not requiring systemic corticosteroid therapy (10 mg or less of prednisone or equivalent doses of other systemic steroids are allowed)
- ##### Exclusion criteria
1. Patients with TT genotype (SNP [rs12459419](#), a population variation missing Mylotarg-binding epitope on CD33)
 2. Prior autologous or allogeneic stem cell transplantation
 3. Presence of the following disease-related genetics: t(15;17) (q22;q21) or t(9;22)(q34;q11) or other evidence of acute promyelocytic leukemia or chronic myeloid leukemia

4. Prior treatment with Mylotarg (GO) in the past 3.5 months, except with Sponsor Medical Monitor approval, or any prior Mylotarg-related SOS/VOD
 5. Active central nervous system leukemia
 6. Patients diagnosed with Gilbert's syndrome
 7. Uncontrolled bacterial, viral or fungal infections or known HIV, hepatitis B or hepatitis C infection. (Patients cured of hepatitis C for ≥ 3 years prior to enrollment are permitted.)
 8. Known sensitivity to components of CliniMACS Prodigy CD34 System or VOR33, including iron dextran or murine proteins
 9. Have a history of other active malignancy(ies) unless the patient has been disease free for at least 2 years and is deemed by the treating investigator to be at low risk for recurrence of that malignancy or the only prior cancer is curatively treated cervical cancer in situ or basal cell or squamous cell carcinoma of the skin
 10. Women who are pregnant or who are lactating
 11. Received any investigational drug (that is, an agent that has not received regulatory approval for any indication) within the 30 days prior to the start of conditioning, unless approved by the Sponsor Medical Monitor
 12. History of cardiovascular disease including, but not limited to, myocardial infarction, unstable angina, stroke or transient ischemic attack within the 6 months prior to the initiation of study or congestive heart failure (New York Heart Association class III–IV)
 13. Any serious underlying medical or psychiatric condition that would increase the risk associated with study participation, in the opinion of the investigator or the Sponsor Medical Monitor
- Donor eligibility**
- Inclusion criteria (donor)**
1. Must be ≥ 18 years of age
 2. Must be 8/8 HLA-matched to the patient (HLA-A, HLA-B, HLA-C and HLA-DRB1)
 3. Willing to receive injections of granulocyte colony-stimulating factor (G-CSF) and plerixafor to stimulate the peripheral blood circulation of HSCs and donate hematopoietic progenitor cells by apheresis for up to two consecutive days
 4. Willing and able to donate within the United States. Related donors for patients in Canada may donate at clinical trial site(s) in Canada.
 5. Meet the donor requirements under 21 CFR 1271 and related August 2007 guidance regarding the Eligibility Determination for Donors of Human Cells, Tissues, and Cellular and Tissue-Based Products. Donors considered 'ineligible but suitable' (per CFR § 630.20) may still be considered for donation by the investigator with Sponsor Medical Monitor approval.
 6. Meet additional criteria for donation as per registry or clinical trial site-specific requirements
 7. Willing and able to comply with the protocol procedures, in the opinion of the investigator or registry
 8. Willing and able to provide informed consent
- Exclusion criteria (donor)**
1. For female donors of childbearing potential: pregnancy or interruptible breastfeeding. Pregnancy is an absolute contraindication under this protocol. Women who are breastfeeding must be willing and able to interrupt breastfeeding during G-CSF (filgrastim) and plerixafor administration and for 2 days after the final dose.
 2. Sensitivity to filgrastim or to *Escherichia coli*-derived recombinant protein products
 3. History of autoimmune disorders, including rheumatic diseases and thyroid disorders (although donors with a history of thyroid disease who have undergone successful therapy may be suitable). Exemptions for mild or limited disease may be granted after discussion with the investigator and the Sponsor Medical Monitor.
 4. History of deep vein thrombosis or pulmonary embolism
 5. History of iritis or episcleritis
 6. Platelet count $< 150,000$ per μl at baseline evaluation
 7. Current treatment with lithium. Drug interactions between filgrastim and lithium, which may potentiate the release of neutrophils, have not been fully evaluated.
 8. Positive hemoglobin solubility (for example, SickDex or equivalent) test
 9. Donors receiving experimental therapy or investigational agents within 30 days of the start of mobilization. FDA-approved therapies under investigation for alternative uses are permitted.
 10. Donors deemed not medically suitable per the registry or the clinical trial site or in the opinion of the Sponsor Medical Monitor
 11. Donors who do not meet the eligibility requirements per the registry or clinical trial site-specific procedures, unless otherwise approved to donate by the Sponsor Medical Monitor and the patient's physician.

Donor procedures

Donors were consented after patients consented for the study. Coordination of the unrelated donor process was managed by NMDP Biotherapies and Gift of Life Marrow Registry. Clinical trial sites identified a related donor or initiated a database search for an unrelated donor prior to approaching the patient for consent in this study. The registry or the clinical trial site that consented the donor confirmed that the donor is medically suitable to donate. Donors underwent dual mobilization procedure of G-CSF/plerixafor. After mobilization, up to two apheresis procedures were performed. Collection of apheresis product was obtained sufficient to manufacture trem-cel and provide a minimally manipulated backup graft, both with a minimum of 3×10^6 viable CD34⁺ cells per kg. Apheresis product was shipped fresh to a manufacturing facility, and cryopreserved trem-cel and backup graft were shipped to the clinical sites.

Trem-cel manufacturing

All cell manipulation procedures were performed in accordance with current Good Manufacturing Practices (GMPs) following process-specific procedures and batch records. A detailed description of the gRNA guide selection, off-target analysis using karyotyping and GUIDE-seq, editing efficiency, InDel distribution, colony-forming unit (CFU) analysis and manufacturing process was previously published¹⁸. In brief, the gRNA is manufactured as a 100-mer sequence: [5'-c*c*u*CAC UAG ACU UGA CCC ACG UUU UAG AGC UAG AAA UAG CAA GUU AAA AUA AGG CUA GUC CGU UAU CAA CUU GAA AAA GUG GCA CCG AGU CGG UG C u*u*u*U-3'; * indicates phosphorothioate linkage; lowercase nucleotides indicate 2'-O-Me-rU or 2'-O-Me-rC (Ac)]. The trans-activating CRISPR RNA (tracrRNA) sequence and chemical modification were previously published by Hendel et al.⁴¹. This gRNA targets exon 3 of the human CD33 gene, with the target sequence being 5'-CCTCACTAGACTTGACCCAC-3'. In summary, a minimally manipulated backup graft was removed from the apheresis material for cryopreservation, and the remaining cells were used for trem-cel manufacturing. The donor apheresis material was washed to remove platelets and plasma. The cells underwent positive selection for CD34⁺ cells using magnetic beads on a CliniMACS Prodigy system (Miltenyi Biotec). After selection, CD34⁺ cells were resuspended in MaxCyte electroporation buffer (MaxCyte), combined with a CD33-targeted ribonucleoprotein (RNP) comprising a single precomplexed gRNA and Cas9 protein, and electroporated in a MaxCyte GTx device using the CL1.1 assembly. The Cas9 nuclease is derived from *Streptococcus pyogenes* and manufactured in the form of sNLS-SpCas9-sNLS nuclease, which is commercially available for purchase from Aldevron.

Cells were then resuspended in SCGM media (Cellgenix) containing stem cell factor (SCF), Fms-related tyrosine kinase 3 ligand (FLT-3L) and thrombopoietin (TPO) in a cell culture bag and cultured for up to 3 days. The cells were centrifuged and washed to reduce cellular debris and cytokines. The cells were then resuspended in the final formulation buffer, transferred to an infusion bag and cryopreserved in a controlled rate freezer and stored in liquid nitrogen. Trem-cel and the backup graft were shipped to the clinical sites cryopreserved and thawed per institutional method. A frozen aliquot of trem-cel was thawed, and a viable cell count provided the final predicted CD34⁺ dose calculation based on the weight of the patient.

Conditioning regimens

Targeted busulfan/fludarabine/melphalan/antithymocyte globulin (allowable for all patients) or TBI/cyclophosphamide/thiotepa antithymocyte globulin (option for patients under age 60) were as previously described¹⁹. Busulfan pharmacokinetics were performed per institutional standards.

GO dosing

Maintenance GO. At approximately day 60 after HCT, all patients who had successful neutrophil engraftment had a bone marrow biopsy to assess disease status and hematopoietic recovery. Patients required $\geq 1,000$ cells per μl CD33⁻ absolute neutrophil count (ANC) and platelet count $\geq 50,000$ per μl and were required to be in complete remission prior to receiving maintenance GO at the current dose level. Patients who had evidence of frank leukemia/MDS relapse (defined as either presence of circulating blasts or bone marrow with $>5\%$ leukemic blasts) at any time after trem-cel infusion and recovery of peripheral counts were moved to the DTC.

For all cohorts, dose escalation was executed using a 3 + 3 design. The DLT period was 28 days. The cohort 1 dose was 0.5 mg m^{-2} ; for cohort 2, it was 1 mg m^{-2} ; and for cohort 3, it was 2 mg m^{-2} . Subsequent doses of GO were given a minimum of 28 days between dosing for up to eight cycles. A DEC reviewed pharmacokinetic and toxicity data prior to escalation between cohorts or with cohort expansion. Dose adjustments after the initial GO dose were guided by the FDA package insert. If a patient was ready to begin GO but the prior patient had not cleared their DLT period, they were allowed to backfill into the previous cohort. The DEC was convened to review all available data prior to the expansion of the 2 mg m^{-2} dose and, after the sixth patient at that dose completed their DLT period, to confirm RP2D and the initiation of the expansion RP2D cohort.

DTC. Patients who relapsed were eligible to enter the DTC. Patients were dosed with GO on days 1, 4 and 7 for 1–2 induction cycles every 28 days followed by consolidation dosing on day 1 of up to eight 28-day cycles. Dosing was at 0.5 mg m^{-2} per dose and escalated in a 3 + 3 strategy as with the maintenance cohorts up to 2 mg m^{-2} .

Stopping rules

Stopping rules included the following: increased incidence of patients with primary or secondary graft failure; elevated incidence of grade 3 or higher acute GvHD; non-relapse mortality ≤ 100 days and >100 days after HCT; and elevated incidence of SOS/VOD in patients receiving GO in comparison to an unedited, standard-of-care, CD34-selected HCT. Triggering of a stopping rule required review of the relevant patient data by the Data and Safety Monitoring Board and a safety determination before further dosing of trem-cel and/or GO could proceed.

Endpoints

Primary endpoint

1. Cumulative incidence of patients who achieve neutrophil engraftment (first day of three consecutive days of ANC ≥ 500 cells per μl) by day 28

Secondary endpoints

1. Overall safety on use of VOR33 as a stem cell source in HCT:
 - a. Time to neutrophil engraftment after HCT from day 0, calculated as the first day of three consecutive laboratory values obtained on separate days where the ANC is ≥ 500 cells per μl
 - b. Time to platelet recovery. Platelet recovery is defined as the first day of a sustained platelet count $\geq 20,000$ per μl with no platelet transfusion in the preceding 7 days. The first day of sustained platelet count above this threshold will be designated the day of platelet engraftment.
 - c. Incidence of acute GvHD grade (G) G2–G4 and G3–G4 over the course of the trial
 - d. Incidence of chronic GvHD (all and moderate/severe) assessed at months 12 and 24 after HCT
 - e. Incidence of primary and secondary graft failure measured after HCT. Primary graft failure is defined as no neutrophil recovery to ≥ 500 cells per μl by day 28 after transplant and bone marrow aplasia. Secondary graft failure is defined as initial neutrophil engraftment by day 28 followed by subsequent decline in ANC to <500 cells per μl with bone marrow aplasia, unresponsive to growth factor therapy, but cannot be explained by disease relapse, infections or medications.
2. Safety and tolerability of VOR33 and Mylotarg administered in the maintenance setting after HCT:
 - a. Incidence, severity and causal relationship of adverse events, TEAEs and SAEs grade 3 or higher, graded according to Common Terminology of Adverse Events (CTCAE) version 5.0; SOS/VOD was graded per European Society for Blood and Marrow Transplantation (EBMT) criteria.
 - b. Incidence of DLTs as a function of dose to determine the MTD and RP2D of Mylotarg
 - c. All treatment-related toxicities due to Mylotarg assessed from cycle 1, day 1 up to 30 days after last Mylotarg administration
 - d. Incidence of SOS/VOD after receiving at least one dose of Mylotarg administration
3. Long-term safety of CD33⁻ hematopoiesis:
 - a. Incidence of TRM assessed at day 100 and at months 12 and 24 after HCT
4. Percentage of CD33⁻ myeloid cells in the blood and marrow:
 - a. Percent donor myeloid chimerism and CD33⁻ myeloid cells in peripheral blood at days 28, 60, 100 and 180 and at months 12 and 24
5. RFS, overall survival, MRD and cumulative incidence of relapse (CIR):
 - a. RFS measured from the time of HCT to the date of disease relapse (bone marrow blasts $\geq 5\%$ or reappearance of blasts in the blood or development of extramedullary disease) or the date of death from any cause, whichever comes first. Median RFS and other parameters related to the Kaplan–Meier analysis will be reported if applicable.
 - b. Overall survival is defined as the time from HCT to the date of death from any cause. Median overall survival and other parameters related to the Kaplan–Meier analysis will be reported if applicable.
 - c. Presence of MRD assessed at screening through 24 months/end of study
 - d. CIR is measured from the time of HCT to the date of disease relapse.
6. Pharmacokinetic parameters for Mylotarg in patients who received VOR33:
 - a. Maximal concentration (C_{max}), time to maximal concentration (T_{max}), AUC, volume of distribution and clearance of Mylotarg

Exploratory endpoints

1. Early-onset and late-onset viral, bacterial and fungal infections:
 - a. Incidence of G2–G5 and G3–G5 viral, bacterial and fungal infections that occur up to 100 days after receiving VOR33
 - b. Incidence of G2–G5 and G3–G5 viral, bacterial and fungal infections that occur 100 days after receiving VOR33
 2. Biomarkers for predicting persistence of VOR33 editing and long-term engraftment:
 - a. Percentage of CD33 editing at days 28, 60, 100 and 180 and at months 12 and 24
 - b. Characterization of HSPCs from bone marrow and peripheral blood using assays including CFU and flow cytometry
 3. Immune reconstitution:
 - a. Absolute counts of T cell subsets, B cells and NK cells in the peripheral blood at days 60, 100 and 180 and at months 12 and 24. Assay of T cell function and repertoire.
 - b. Percent donor chimerism of T, B and NK subsets
 4. Donor characteristics:
 - a. Donor safety adverse events after administration of plerixafor and filgrastim for mobilization. Review donor characteristics across all patients for trends. Determine if they have any impact on VOR33 manufacturing, patient outcome and/or safety.
 5. Expression and activity of drug efflux transporters
 - DTC only
 - Primary endpoint
 1. Safety as a function of dose to determine the RP2D of induction and consolidation Mylotarg in patients with R/R or MRD⁺ AML/MDS after alloHCT with VOR33
 - Secondary endpoints
 1. Pharmacokinetic parameters for induction and consolidation Mylotarg, including C_{max}, T_{max}, AUC, volume of distribution and clearance
 2. Percentage of patients who achieve morphologic remission (defined as <5% bone marrow blasts).
 3. Percentage of patients who achieve response by ELN criteria for AML/MDS
 4. Percentage of patients who achieve MRD negativity by flow cytometry (<0.1%) after Mylotarg Induction course
 5. RFS after Mylotarg induction and consolidation course in patients achieving morphologic remission or MRD negativity
 6. Overall survival after Mylotarg induction and consolidation course
 - Exploratory endpoints
 1. Percentage of CD33⁺ myeloid and monocyte populations prior to and after treatment with induction and consolidation Mylotarg
 2. Percentage of CD33⁺ AML/MDS cells prior to and after treatment with induction Mylotarg
 3. Biomarkers for predicting persistence of VOR33 editing and long-term engraftment:
 - a. Percentage of CD33 editing at days 28, 60, 100 and 180 and at months 12 and 24
 - b. Characterization of HSPCs from bone marrow and peripheral blood using assays including CFU and flow cytometry
 4. CD33 antigen density of AML/MDS cells prior to and after treatment with induction Mylotarg
 5. Expression and activity of drug efflux transporters
- Endpoints for the DTC were not assessed as only one patient was enrolled and had not completed treatment at time of the data cut. Due to the relatively short follow-up time of the study at

termination, incidence of chronic GvHD was assessed over the course of the trial instead of 12-month and 24-month post-HCT timepoints, and TRM was assessed to day 100 and over the course of the trial after day 100 (instead of 12-month and 24-month timepoints). Within the exploratory endpoints, data on characterization of HSPCs, assay of T function, donor characteristics and expression and activity of drug efflux transporters were not analyzed.

Study assessments

The ‘evaluable’ population comprised any patient receiving trem-cel and was used for analysis of engraftment, time to engraftment and platelet recovery, GvHD, chimerism, gene editing, immune reconstitution, RFS, overall survival and adverse event reporting. The ‘efficacy’ population comprised patients also receiving at least one cycle of GO and was used for determining GO pharmacokinetics and hematologic toxicity/shift tables. The primary endpoint was cumulative incidence of neutrophil engraftment by day 28, defined as the first day of three consecutive days of ANC ≥ 500 cells per μl . Key secondary objectives were time to neutrophil engraftment and platelet recovery; incidence and severity of GvHD; the safety, tolerability, MTD and RP2D of GO administered after trem-cel; the percentage of CD33⁺ cells and donor myeloid chimerism; RFS; overall survival; and the pharmacokinetic profile for GO after trem-cel.

Patient enrollment. Patient enrollment of the part 1 dose-escalation portion of the trial was within the range prespecified in the protocol (9–18 evaluable patients). The part 2 RP2D expansion cohort was planned for 12 patients and was not fully enrolled due to the early termination of the study. At the time of the data cut, four RP2D patients were evaluable, one patient was in the DTC and five patients had not yet been eligible to receive GO at the RP2D.

Engraftment definitions. Primary graft failure was defined as no neutrophil recovery to ≥ 500 per μl by day 28 after transplant and bone marrow aplasia. Secondary graft failure was defined as initial neutrophil engraftment by day 28 followed by subsequent decline in ANC to <500 per μl with bone marrow aplasia, unresponsive to growth factor therapy, but could not be explained by disease relapse, infections or medications.

Neutrophil engraftment and platelet recovery. Neutrophil engraftment was monitored by daily ANC from infusion to engraftment. Platelet count was monitored until recovery was achieved, defined as the first day of a sustained platelet count $\geq 20,000$ per μl with no platelet transfusion in the preceding 7 days.

GvHD. Grading of acute GvHD was based on the Mount Sinai Acute GvHD International Consortium scale⁴² and grading of chronic GvHD according to the National Institutes of Health Consensus Criteria⁴³. Adverse events were monitored until study end and graded according to CTCAE version 5.0.

Donor chimerism and CD33 editing. Donor chimerism (performed by C3i) and CD33 editing (performed by Microsynth) were evaluated in specific lineages from peripheral blood at each timepoint. In brief, peripheral blood cells were immunostained with anti-human CD45, CD3, CD19, CD16, CD56, CD14 and CD15 and fluorescence-activated cell sorted (FACS) for T, B, myeloid and NK cells. Chimerism was determined with a short tandem repeat (STR) multiplex assay that targeted 15 tetranucleotide repeat loci and the amelogenin gender-determining marker in a single polymerase chain reaction (PCR) amplification. All 13 of the required loci for the Combined DNA Index System (CODIS) used in forensic individual identification are part of the 16-locus multiplex of the AmpFLSTR Identifier Plus PCR Amplification Kit (Thermo Fisher Scientific). After amplification, the single PCR reaction was analyzed

on a SeqStudio 24 Flex Genetic Analyzer (Thermo Fisher Scientific), and the resulting 15-locus STR and amelogenin marker profile of the patient was followed over time, with comparative analysis to the donor and recipient pretransplant profiles carried out with ChimerMarker Automated Chimerism Analysis software version 3.1.8 (SoftGenetics).

For CD33 editing analysis, genomic DNA isolated from the FACS-sorted T, B, NK and myeloid cells was PCR amplified at the CD33-targeted site¹⁸ using the Nextera two-step PCR protocol, followed by deep sequencing of the amplicons using the MiSeq system (Illumina). Small InDels generated by CRISPR–Cas9 editing for each amplicon were analyzed using a bioinformatics analysis pipeline based on CRISPResso2, and the frequencies of all InDels were combined and reported as percent editing.

Bone marrow and peripheral blood AML assessments were done by flow cytometry (Hematologics and Vor Biopharma). CD33 negativity in myeloid populations was also assessed using this method. In brief, samples were immunostained with antibody cocktails containing HLA-DR, CD7, CD11b, CD13, CD14, CD16, CD19, CD33, CD34, CD36, CD38, CD45, CD56, CD64, CD117 and CD123 for 20 minutes at room temperature in the dark. Red blood cells were lysed with the use of 3.5 ml of buffered NHCl (0.83%) at 37 °C for 5 minutes, followed by centrifugation at 300g. The cells were washed with 3 ml of PBS containing 2% fetal calf serum and resuspended to 0.5 ml in 1% paraformaldehyde for analysis on a FACSCalibur flow cytometer (BD Biosciences). A total of 200,000 events were collected for each tube. The flow cytometers were standardized and calibrated with the use of RCP-5 and RFP-5 beads (Spherotech). Patient sample collection for this analysis was performed up to cycle 4 on previous versions of the study protocol but later amended to collect up to cycle 8. For the histogram in Extended Data Fig. 3, the proportion of monocytes expressing CD33 was based on cells expressing high levels of CD14. CD33 expression on myeloid cells was based on CD45 versus log side scatter comparing autofluorescence to cells stained with CD33.

Immune profiling. Immune profiling was determined by mass cytometry based on Maxpar Direct Immune Profiling Assay (Fluidigm). In brief, cells were stained with a 30-marker antibody panel and a viability stain, acquired on a Helios mass cytometer and analyzed in FlowJo software versions 9 and 10 (BD Biosciences).

GO pharmacokinetic assessment. Pharmacokinetics of GO was characterized by measurements of plasma hP67.6, the CD33-targeted humanized antibody portion of GO⁴⁴. Plasma hP67.6 concentration was measured 2, 3, 4, 6, 72 and 168 hours after GO administration on cycle day 1 using a validated sandwich ELISA. In brief, 96-well plates were coated with CD33 antigen, and bound hP67.6 was detected using an HRP-conjugated anti-human IgG4 detection antibody and tetramethylbenzidine peroxidase substrate. Absorbance was read at 450 nm, and the optical density of GO standards was fitted to a four-parameter logistic curve. The lower limit of quantification for this validated assay was 10 ng ml⁻¹. Data collection was performed by Immunologix, and pharmacokinetic parameters were calculated using the actual sampling time. Phoenix analysis software (Certara) was used to calculate pharmacokinetic parameters according to a two-compartment model with nonlinear clearance.

T cell/B cell receptor repertoire assay. Whole blood from patients was sampled on day 60, day 100, day 180 and month 12 (if available) after trem-cel HCT and collected into PAXgene Blood RNA Tubes. T cell receptor/B cell receptor repertoire was identified at iRepertoire using a proprietary multiplex amplification strategy that captures the VDJ rearrangements from immune-specific RNA with the use of unique molecular indices. The resulting libraries generated from input RNA were sequenced using Illumina next-generation sequencing platforms at a read depth of approximately 5 million and a read length of 300-bp paired-end reads.

Unique CDR3 regions of B cells (IgH) or T cells (TCRβ) were used to identify clonotypes. To determine the clonotype origin for samples at any particular timepoint after trem-cel HCT, unedited donor leukapheresis starting material (prior to the trem-cel manufacturing process) and a patient screening sample (prior to transplant) were also processed similarly and used as a reference. To determine the origin of clonotypes, the following criteria were used. For B cells, if a clonotype was found only in patient screening sample or donor leukapheresis starting material, it was assigned as ‘residual recipient’ or ‘donor contaminant’ (carried over into drug product during the trem-cel manufacturing process), respectively. A B cell clonotype was assigned as a ‘donor de novo’ clone when it was exclusively found in timepoints after HCT but not in either donor leukapheresis starting material or patient screening sample. A B cell clonotype was assigned as unknown if none of the above criteria was fulfilled. For T cells, a clonotype was assigned as ‘residual recipient’ or ‘donor contaminant’, similar to B cells. In addition, a T cell clonotype was assumed to be ‘donor de novo’ (that is, trem-cel HSPC derived) only when it was found in none of the following: donor leukapheresis starting material, patient screening, day 60 and day 100 (it is assumed that T cell reconstitution from a CD34-selected graft cannot occur prior to day 60).

Neutrophil oxidative burst assessment. At day 28 after HCT, neutrophil function was assessed by a clinical neutrophil oxidative burst assay that measured the functional ability of neutrophils to produce reactive oxygen species. In brief, neutrophils were stimulated to induce an oxidative burst, and the activity was quantified via flow cytometry. Assays were performed by commercial laboratories and result in a ‘normal’ or ‘abnormal’ readout compared to a control specimen.

Statistics and reproducibility

The ‘evaluable’ analysis population comprised all patients who received trem-cel, and no patients were excluded. As a phase 1/2, non-randomized safety study, no statistical method was used to determine sample size. The investigators were not blinded to allocation during experiments and outcome assessment. Descriptive statistics are reported for all variables. Time-to-event endpoints were estimated using Kaplan–Meier methods. RFS was defined as the time from trem-cel infusion to the date of disease relapse (bone marrow blasts ≥5% or reappearance of blasts in the blood or development of extramedullary disease) or the date of death from any cause, whichever came first. Overall survival was defined as the time from trem-cel infusion to the date of death from any cause. Median duration of follow-up was calculated using a reverse Kaplan–Meier curve. Patients were excluded from immune reconstitution, CD33 negativity and chimerism analysis for any timepoint after receiving the backup graft ($n = 2$).

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

Data will be made available from the corresponding author (jdipersi@wustl.edu) upon reasonable request for non-commercial academic research purposes, subject to institutional review and a data use agreement to protect participant confidentiality and intellectual property considerations. Response will be given within 4 weeks. InDel sequencing data are publicly available at <https://doi.org/10.5281/zenodo.17345128>.

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Author contributions

J.F.D. wrote the original manuscript, conducted the clinical trial and analyzed data. G.K. conducted the clinical trial and reviewed and edited the original manuscript. N.N.S. conducted the clinical trial and reviewed and edited the original manuscript. L.B. conducted the clinical trial and reviewed and edited the original manuscript. H.C.S. conducted the clinical trial and reviewed and edited the original manuscript. D.K. conducted the clinical trial and reviewed and edited the original manuscript. R.T. conducted the clinical trial and reviewed and edited the original manuscript. M.U.M. conducted the clinical trial and reviewed and edited the original manuscript. J.M. conducted the clinical trial and reviewed and edited the original manuscript. J.R. conducted the clinical trial and reviewed and edited the original manuscript. V.E.K. conducted the clinical trial and reviewed and edited the original manuscript. S.S.P. conducted the clinical trial and

reviewed and edited the original manuscript. C.H. analyzed data and reviewed and edited the original manuscript. M.L. analyzed data and reviewed and edited the original manuscript. C.A.S., D.M.L. and D.A.S. designed and conducted the clinical study and reviewed and edited the original manuscript. M.M.L.-S. analyzed data, contributed to the statistical analysis, conducted the clinical study and reviewed and edited the original manuscript. G.M. contributed to the statistical analysis and reviewed and edited the original manuscript. S.T., G.G., H.G.G., B.E.L., J.X.-F., S.L.H. and M.I.L. designed correlative experiments, analyzed data, contributed to the statistical analysis and reviewed and edited the original manuscript. G.D.R. wrote the original manuscript, designed and conducted the clinical trial and analyzed data. B.W.C. conducted the clinical trial and reviewed and edited the original manuscript.

Competing interests

J.F.D.: equity and research funding from Wugen, Magenta Therapeutics and RiverVest Venture Partners; board member or advisory role for SPARC and Vertex; and research funding from NeoImmune Tech, MacroGenics and Bioline. G.K.: consultancy for Vor Biopharma. N.N.S.: research funding from Lentigen, Vor Biopharma and ImmunoACT Sobi; advisory board for Vor Biopharma (unpaid); and royalties from CARGO. D.K.: advisory board for Bristol Myers Squibb and research funding from Iovance Biotherapeutics. J.M.: research funding from Gilead, Atara, CRISPR Therapeutics, Precision Biosciences, Scripps Research Institute, Vor Biopharma and Affimed. V.E.K.: consultancy for Astelles and Servier. S.S.P.: advisory board for CareDx and Sobi; advisory board and speaker for Sanofi; and speaker for Kite and Incyte. L.B., H.C.S., R.T., J.R. and B.W.C. report no conflicts of interest. C.H. and M.L. are employees of Hematologics. C.A.S., D.M.L., D.A.S., M.M.L.-S., S.T., G.M., G.G., H.G.G., B.E.L., J.X.-F., S.L.H., M.I.L. and G.D.R. were employees of Vor Biopharma when the work was conducted.

Additional information

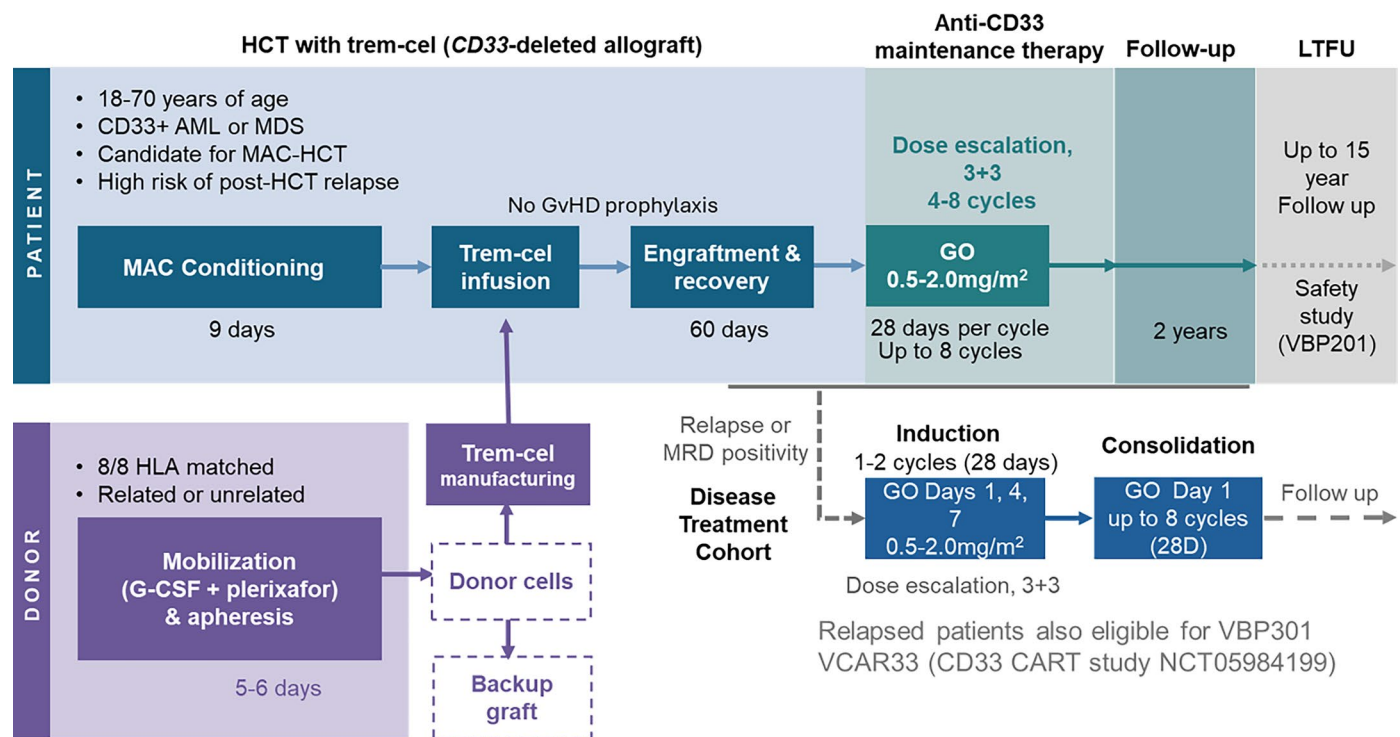
Extended data is available for this paper at <https://doi.org/10.1038/s41591-026-04362-1>.

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41591-026-04362-1>.

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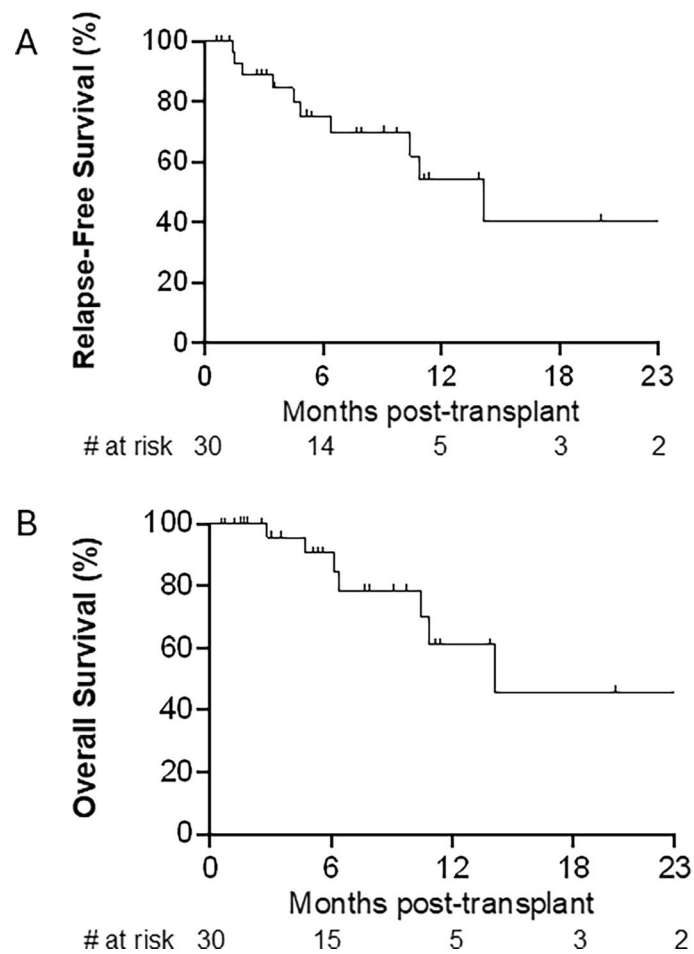
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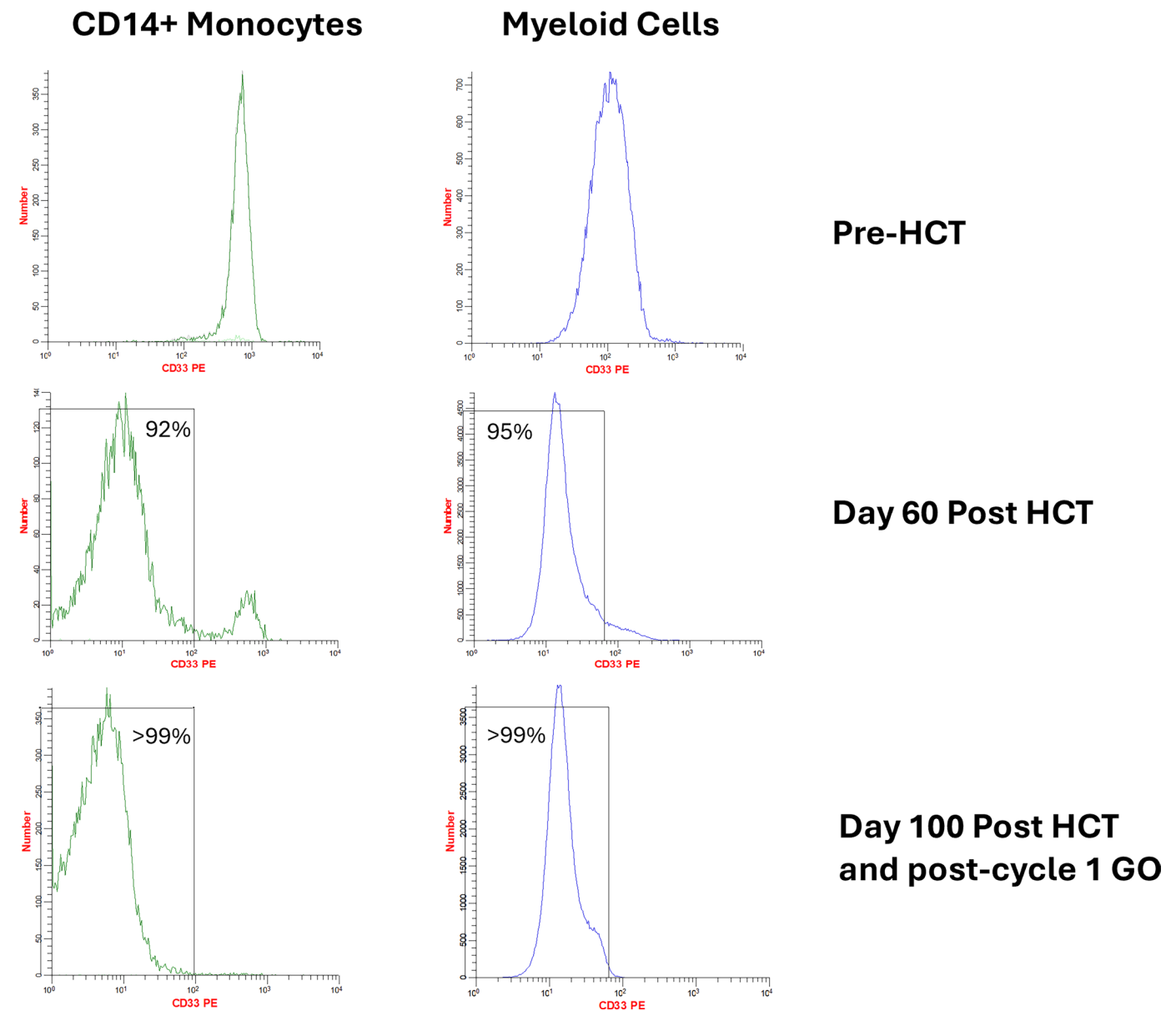


Extended Data Fig. 1 | VBP101 study design. Patients with AML require at least one high risk feature including adverse karyotype, MRD, secondary AML, second remission or greater, or relapsed disease. Following trem-cel infusion, patients are monitored for neutrophil engraftment and platelet recovery, and after 60 days are assessed if they are in remission and have sufficient CD33-negative hematopoiesis to begin maintenance GO. Maintenance GO is given once every 28 days for 8 cycles. Dose escalation of GO follows a 3 + 3 strategy with three cohorts at doses of 0.5, 1, and 2 mg/m². If patients relapse, they may either receive a

fractionated induction dose of GO on the disease treatment cohort or proceed to study VBP301, a CD33 CAR-T trial using cells generated from the matched donor. AML, acute myeloid leukemia; CART T, chimeric antigen receptor T cell therapy; G-CSF, granulocyte colony stimulating factor; GO, gemtuzumab ozogamicin; GvHD, graft versus host disease; HCT, hematopoietic stem cell transplant; HLA, human leukocyte antigen; LTFU, long-term follow-up; MAC, myeloablative conditioning; MDS, myelodysplastic syndrome; MRD, measurable residual disease.

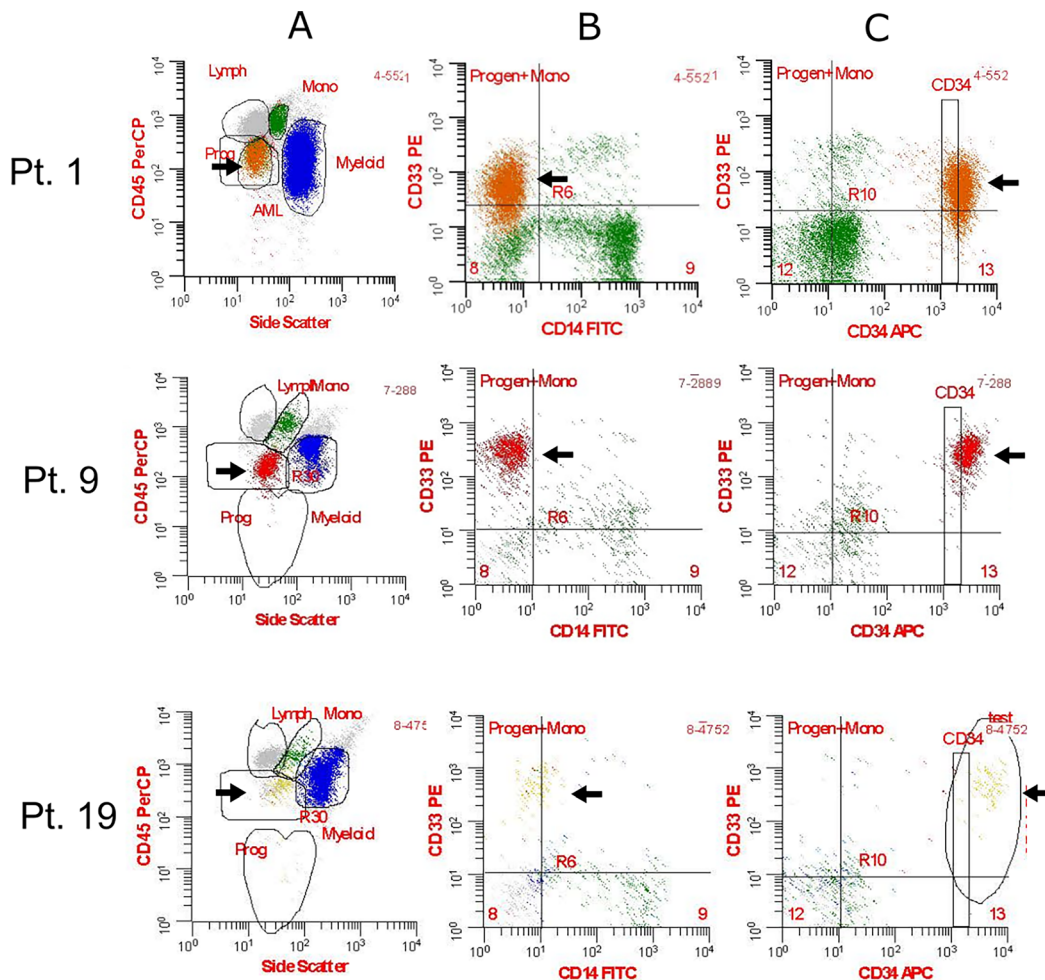


Extended Data Fig. 2 | Relapse-free survival and overall survival in patients receiving trem-cel with or without GO maintenance. Kaplan-Meier curves for (a) relapse-free survival and (b) overall survival. Tick marks represent censored patients.



Extended Data Fig. 3 | Flow cytometry of peripheral blood monocytic and myeloid fractions before and after first cycle of GO. Representative flow cytometry histograms of CD33-positivity from patient peripheral blood pre-HCT,

day 60 (pre-GO) and day 100 (post-GO) post-HCT. Gated for CD14+ monocytic and myeloid populations per **Methods**. GO, gemtuzumab ozogamicin; HCT, hematopoietic cell transplant.



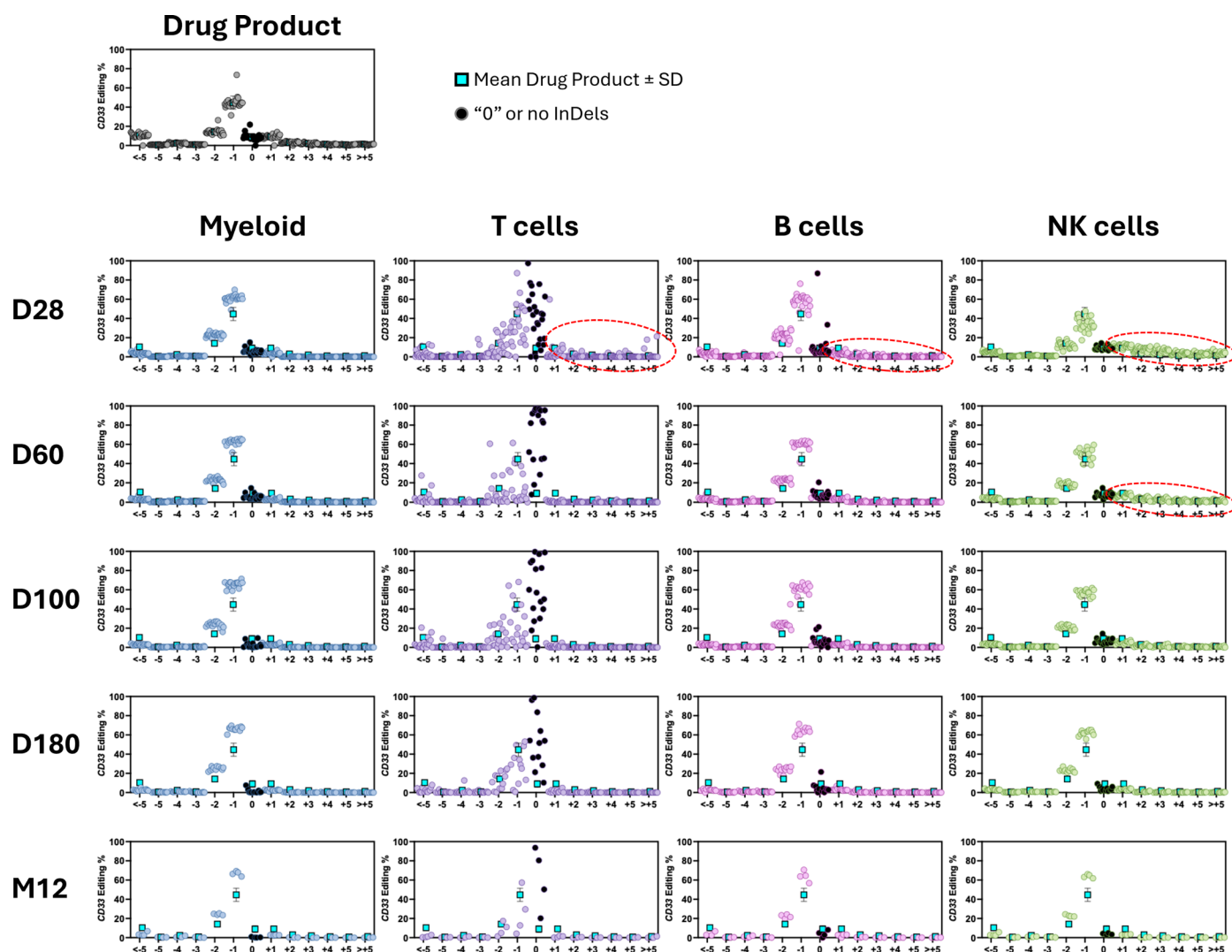
D Pt.1 Pre-Trial: CD45+, CD34+, HLA-DR hetero, CD11b-, CD38hetero, CD36-, CD13hetero, CD16-, CD14-, CD33+, CD56+, CD7-, CD117+, CD64-, CD123+, CD19-
At relapse: CD45+, CD34 (increased), HLA-DR+, CD11b-, CD38+ (dim), CD36+ (dim), CD13+, CD16-, CD14-, CD33+, CD56+ (heterogeneous), CD7-, CD117+, CD64-, CD123+, CD19-

Pt. 9 Pre-trial: CD45+, CD34+, HLA-DRdim, CD11b-, CD38decr, CD36dim/hetero, CD13++, CD16-, CD14-, CD33+, CD56-, CD7+ minor subset, CD117+, CD64-, CD123+, CD19-
At relapse: CD45+, CD34+ incr, HLA-DRdecr (mostly negative), CD11bdim/hetero, CD19-, CD5-, CD38+decr, CD10-, CD117+, CD13+, CD16-, CD14-, CD33+, CD64-, CD36-, CD123+, CD56+ rare subset, CD7+ rare subset

Pt. 19 Pre-Trial: CD45+, CD34++, HLA-DR+, CD11b+subset, CD19-, CD5-, CD38+decr, CD10-, CD117+, CD13++, CD16-, CD14-, CD33++, CD64-, CD36-, CD123+, CD56+, CD7+
At relapse: CD45+, CD34++, HLA-DRdecr, CD11b dim, CD19-, CD5-, CD38 decr, CD10- CD117+, CD13++, CD16-, CD14-, CD33++, CD64-, CD36-, CD123+, CD56+, CD7 hetero

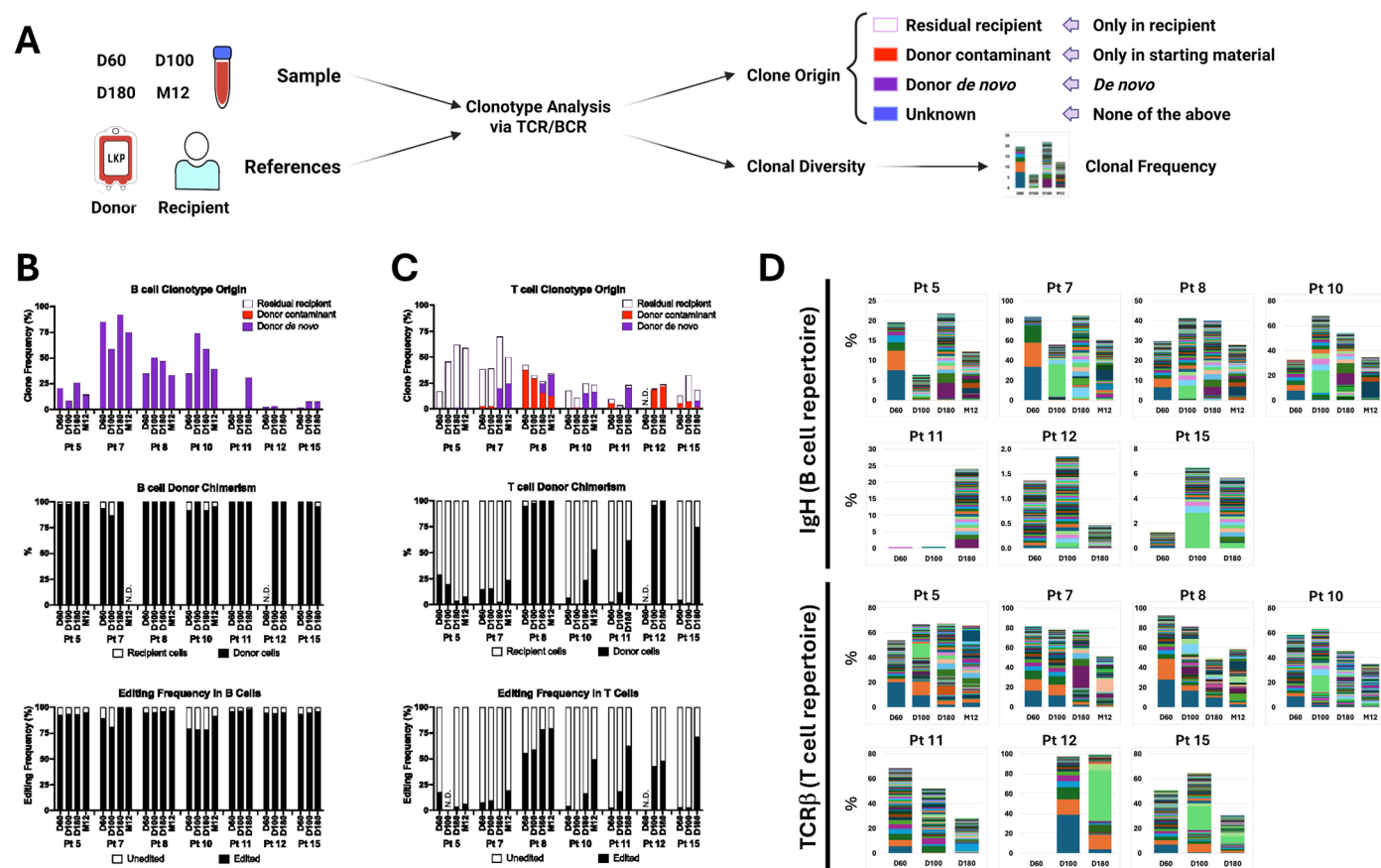
Extended Data Fig. 4 | CD33 expression of AML at relapse. Representative flow cytometry panels from PB of the three patients relapsing after receiving GO maintenance and full flow profiles pre-trial and at relapse. Leukemia populations

indicated by black arrow. **a**, CD45 Y axis vs. Side Scatter X axis. **b**, CD33 Y axis vs. CD14 X axis. **c**, CD33 Y axis vs. CD34 X axis. **d**, Full flow cytometry marker profiles of patient's AML pre-trial and at relapse.



Extended Data Fig. 5 | CD33 on-target gene editing InDel distribution. CD33 gene editing data from drug products prior to each patient’s infusion (topmost panel) or from FACS sorted myeloid, T, B and NK cells post-HCT in Fig. 2d was further delineated by NGS and plotted as frequencies of InDels ranging from -5 (5 base pair deletions) to +5 (5 base pair insertions). The data points represent the InDel distribution for each patient at each timepoint after trem-cel transplant. The black filled data points represent InDel of 0 (no insertions/deletions) and

the cyan filled square data points represent the mean $\pm$ SD InDel frequencies of the drug product from the topmost panel. Red dotted ovals highlight +2 to +5 insertions observed most commonly in NK cells and B/T lineages (to a lesser extent) but not present in myeloid cells during the early timepoints post-HCT. InDel, insertions and deletions; FACS, fluorescence-activated cell sorting; NK, natural killer; HCT, hematopoietic stem cell transplant; NGS, next-generation sequencing; SD, standard deviation.



Extended Data Fig. 6 | TCR/BCR repertoire analysis of immune cells from representative patients receiving trem-cel. **a**, Schematics of clonotype analysis (clone origin and diversity) of reconstituted immune cells. Patient repertoires on D60, day, D100, D180 and M12 post trem-cel HCT (if available) were assessed via NGS to identify clonotypes based on CDR3 region. **b**, Clonotype origin distribution of B cells in selected trem-cel patients. Only the top 100 clonotypes are shown (top), with the majority being de novo clones. **c**, Clonotype origin distribution of T cell clones in selected trem-cel patients. Only the top 100 clonotypes are shown (top, clonotypes of unknown origin are excluded), which agrees with T cell donor chimerism (middle) and CD33 editing in T cells (bottom) for some patients (eg, Patients 5, 7, 10, 11 and 15). Note the discordance between chimerism and editing in certain patients (Patients 8 and 12), indicating donor T cell contribution from sources other than trem-cel HSPCs. Further TCR clonal

tracking analysis traced a portion of donor T cell origin back to the donor leukapheresis starting material prior to the initiation of trem-cel manufacturing defined as donor contaminant and plotted as red bars. De novo T cell clones, likely trem-cel HSPC-derived (purple bars) are detectable only after 180 days post-HST. **d**, TCR and BCR repertoire analysis of representative clones. Top 50 clonotypes from each time points were merged prior to the frequencies of each clonotype plotted for all four timepoints (D60, D100, D180, and M12 if available) and shown in color. Identical clonotypes from different time points are shown in the same color. Top panel: B cell repertoire based on IgH. Bottom panel – T cell repertoire based on TCRβ. N.D. denotes no data due to low cell count. BCR, B cell receptor; D, day; HCT, hematopoietic cell transplant; HSPC, hematopoietic stem and progenitor cell; M, month; NGS, next generation sequencing; TCR, T cell receptor.

Extended Data Table 1 | Shift from baseline in neutropenia grade during GO maintenance

Cohort	Baseline grade*	Highest on-study CTCAE grade, [†] n/N (%) [‡]				
		0	1	2	3	4
Cohort 1 (n = 3)	0	2/3 (67)	0	0	1/3 (33)	0
	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
	4	0	0	0	0	0
Cohort 2 (n = 6)	0	1/6 (17)	1/6 (17)	2/6 (33)	1/6 (17)	0
	1	0	0	0	0	0
	2	0	0	1/6 (17)	0	0
	3	0	0	0	0	0
	4	0	0	0	0	0
Cohort 3 (n = 6)	0	3/6 (50)	0	1/6 (17)	2/6 (33)	0
	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
	4	0	0	0	0	0
RP2D patients (n = 4)	0	2/4 (50)	1/4 (25)	0	1/4 (25)	0
	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
	4	0	0	0	0	0

Extended Data Table 2 | Shift from baseline in thrombocytopenia grade during GO

Cohort	Baseline grade*	Highest on-study CTCAE grade, [†] n/N (%) [‡]				
		0	1	2	3	4
Cohort 1 (n = 3)	0	0	0	0	0	0
	1	0	1/3 (33)	1/3 (33)	1/3 (33)	0
	2	0	0	0	0	0
	3	0	0	0	0	0
	4	0	0	0	0	0
Cohort 2 (n = 6)	0	0	0	0	0	0
	1	0	2/6 (33)	1/6 (17)	2/6 (33)	1/6 (17)
	2	0	0	0	0	0
	3	0	0	0	0	0
	4	0	0	0	0	0
Cohort 3 (n = 6)	0	0	0	2/6 (33)	1/6 (17)	0
	1	0	0	0	2/6 (33)	0
	2	0	0	0	1/6 (17)	0
	3	0	0	0	0	0
	4	0	0	0	0	0
RP2D patients (n = 4)	0	0	0	1/4 (25)	0	0
	1	0	0	0	1/4 (25)	1/4 (25)
	2	0	0	0	1/4 (25)	0
	3	0	0	0	0	0
	4	0	0	0	0	0

Extended Data Table 3 | Summary of treatment-emergent adverse events and serious adverse events

Adverse event, n (%)	Patients receiving trem-cel (N = 30)(%)
Any TEAE	29 (97)
Conditioning-related TEAE	29 (97)
Trem-cel infusion-related TEAE	16 (53)
GO-related TEAE	15 (50)
Grade ≥ 3 TEAE	27 (90)
Grade ≥ 3 trem-cel infusion-related TEAE	9 (30)
Grade ≥ 3 GO-related TEAE	11 (37)
TEAE leading to discontinuation of trem-cel	0
TEAE leading to discontinuation of GO	1 (3)
Any TESAЕ	17 (57)
Conditioning-related TESAЕ	3 (10)
Trem-cel infusion-related TESAЕ	6 (20)
GO-related TESAЕ	3 (10)
Grade ≥ 3 TESAЕ	16 (53)
TESAЕ leading to discontinuation of GO	0
Dose-limiting toxicity	0
SOS/VOD	1 (3)
TEAE leading to death	3 (10)

Extended Data Table 4 | Treatment-emergent adverse events of any grade occurring in > 20% patients by preferred term

Treatment-emergent adverse event, n (%)	Patients receiving trem-cel (N = 30)(%)
Stomatitis	19 (63)
Hypokalemia	17 (57)
Fatigue	14 (47)
Abdominal pain	13 (43)
Anemia	13 (43)
Hypophosphatemia	12 (40)
Neutropenia	12 (40)
Thrombocytopenia	12 (40)
Decreased appetite	11 (37)
Diarrhea	11 (37)
Febrile neutropenia	11 (37)
Pyrexia	11 (37)
Hypomagnesemia	10 (33)
Nausea	10 (33)
Blood creatinine increased	7 (28)
Cough	9 (30)
Dizziness	9 (30)
Oral candidiasis	9 (30)
Vomiting	9 (30)
Blood creatinine increased	8 (27)
Edema peripheral	8 (27)
Oropharyngeal pain	8 (27)
Rash maculo-papular	8 (27)
Alanine aminotransferase increased	7 (23)
Aspartate aminotransferase increased	7 (23)
BK virus infection	7 (23)
Blood bilirubin increased	7 (23)
Dry mouth	7 (23)
Epistaxis	7 (23)
Hypocalcemia	7 (23)
Hypotension	7 (23)
Platelet count decreased	7 (23)
Pneumonia	7 (23)

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (<i>n</i>) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☒	☐ The statistical test(s) used AND whether they are one- or two-sided <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☒	☐ A description of all covariates tested
☒	☐ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☒	☐ For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐ Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection	Immune cell repertoire- iRepertoire, Inc; Chimerism and immune profiling- C3i, Inc; Pharmacokinetics- Immunologix, Inc; CD33 positivity, Disease and MRD analysis and myeloid blood and marrow data collected at Hematologics, Inc, CD33 editing- Microsynth, Inc, Balgach, Switz
Data analysis	Graphing associated statistics- Graphpad Prism v10; Flow cytometry- BD FlowJo v9-10; Chimerism- Softgenetics ChimerMarker v3.1.8; Pharmacokinetics- Certara Phoenix analysis software, CD33 editing- Microsynth, Inc, Balgach, Switz

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

Data will be made available from the corresponding author (jdlpersi@wustl.edu) upon reasonable request for non-commercial academic research purposes, subject

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender	Sex of study participants listed. No analyses performed on basis of sex.
Reporting on race, ethnicity, or other socially relevant groupings	Race (White, Asian, other) and ethnicity reported (Hispanic, non-Hispanic). No analysis based on Race or Ethnicity performed.
Population characteristics	Patients age 18-65 with high relapse risk AML/MDS based on genetics/residual disease/remission status. Reported in Table 1 and Supp Table 1
Recruitment	Patients recruited at trial site transplant centers by Principal Investigators. Enrollment was restricted to patients meeting predefined transplant eligibility criteria, consistent with institutional standards for allogeneic transplantation. These objective criteria minimized discretionary selection and limited variability in baseline risk.
Ethics oversight	Institutional Review Boards at each individual trial site (Washington University School of Medicine, Miami Cancer Institute, National Cancer Institute, Hôpital Maisonneuve-Rosemont/Université de Montréal, Hackensack University Medical Center, UC San Diego, Memorial Sloan Kettering Cancer Center, University of Kansas Cancer Center, University of Minnesota, Winship Cancer Institute- Emory University, Stanford University, University of Utah, University Hospitals Cleveland Medical Center) approved and oversaw trial conduct. NMDP IRB oversaw donor safety for donors they collected.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Part 1, the dose escalation for Mylotarg™ administration, will follow the 3+3 design algorithm to identify the MTD and RP2D. It is expected that approximately 9-18 evaluable patients will be needed for Part 1. The dose expansion in Part 2 will require an additional 12-24 evaluable patients to evaluate safety and preliminary efficacy of the RP2D. No formal statistical comparisons of efficacy will be implemented, but as an illustration, this sample size would have at least 80% power to detect a RFS response of 20% at 2 years, if the true response is 50%, at one-sided $\alpha=0.1$, assuming exact binomial test.
Data exclusions	Patients were excluded from immune reconstitution, CD33 negativity and chimerism analysis for any timepoint after receiving the back-up graft (n=2)
Replication	As this was a first-in-human, open-label, dose-escalation study, the primary objectives were safety, tolerability, and characterization of pharmacokinetics and pharmacodynamics of GO. Reproducibility of experimental findings was ensured through predefined protocol procedures, a statistical analysis plan, validated bioanalytical assays, centralized laboratory testing, and standardized sample handling procedures. For the gene-modified cellular therapy portion, reproducibility was evaluated through manufacturing consistency, validated release testing, standardized clinical management procedures, and safety findings across treated subjects. Due to the small sample size and absence of a randomized comparator arm, formal multivariable covariate adjustment was not prespecified. Baseline demographic and disease characteristics were summarized descriptively. Given the limited cohort sizes, statistical adjustment for baseline covariates was not feasible.
Randomization	non-randomized
Blinding	This first-in-human Phase 1/2 study is designed primarily to evaluate safety, tolerability, pharmacokinetics, and to determine the recommended Phase 2 dose. Given the early exploratory nature of the study, the small cohort size, the need for real-time safety monitoring and dose-escalation decisions, and the objective nature of the primary endpoints, blinding was not considered necessary. An open-label design facilitates rapid safety signal detection and protects participant safety without materially increasing risk of bias.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Clinical data

Policy information about [clinical studies](#)

All manuscripts should comply with the ICMJE [guidelines for publication of clinical research](#) and a completed [CONSORT checklist](#) must be included with all submissions.

Clinical trial registration	ClinicalTrials.gov NCT04849910 https://clinicaltrials.gov/study/NCT04849910
Study protocol	VBP101 Protocol provided in Supplementary Information
Data collection	Data collected at Trial sites and central labs. First treated patient enrolled June 21, 2022 and final patient within January 12, 2025 data cut consented October 16, 2024 .
Outcomes	Primary Endpoint 1. Cumulative incidence of patients who achieve neutrophil engraftment (first day of 3 consecutive days of absolute neutrophil count (ANC) ≥ 500 cells/μL) by Day 28. Secondary Endpoints 1. Overall safety on use of VOR33 as a stem cell source in HCT: a. Time to neutrophil engraftment after HCT from Day 0; calculated as the first day of 3 consecutive laboratory values obtained on separate days where the ANC is ≥ 500 cells/μL. b. Time to platelet recovery. Platelet recovery is defined as the first day of a sustained platelet count $\geq 20,000/\mu$L with no platelet transfusion in the preceding seven days. The first day of sustained platelet count above this threshold will be designated the day of platelet engraftment. c. Incidence of acute GVHD Grade (G) G2-G4 and G3-G4 over the course of the trial. d. Incidence of chronic GVHD (all and moderate-severe) assessed at Months 12 and 24 post HCT. e. Incidence of primary and secondary graft failure measured post HCT. Primary graft failure is defined as no neutrophil recovery to ≥ 500 cells/μL by Day 28 post-transplant and bone marrow (BM) aplasia. Secondary graft failure is defined as initial neutrophil engraftment by Day 28 followed by subsequent decline in ANC to < 500 cells/μL with BM aplasia, unresponsive to growth factor therapy, but cannot be explained by disease relapse, infections, or medications. 2. Safety and tolerability of VOR33 and Mylotarg™ administered in the maintenance setting post-HCT: a. Incidence, severity, and causal relationship of adverse events (AEs), treatment emergent AEs (TEAEs), and serious adverse events (SAEs) G3 or higher, to be graded according to Common Terminology of Adverse Events (CTCAE), version 5.0; sinusoidal obstruction syndrome/veno-occlusive disease (SOS/VOD) graded per European Society for Blood and Marrow Transplantation (EBMT) criteria. b. Incidence of dose limiting toxicities (DLTs) as a function of dose to determine the MTD and RP2D of Mylotarg™: c. All treatment-related toxicities due to Mylotarg™ assessed from Cycle 1, Day 1 up to 30 days post last Mylotarg™ administration. d. Incidence of SOS/VOD after receiving at least one dose of Mylotarg™ administration. 3. Long-term safety of CD33-negative hematopoiesis: a. Incidence of transplant-related mortality (TRM) assessed at Day 100, and Months 12 and 24 post HCT. 4. Percentage of CD33-negative myeloid cells in the blood and marrow. a. Percent donor myeloid chimerism and CD33-negative myeloid cells in peripheral blood at Day 28, 60, 100, 180, and Months 12 and 24. 5. RFS, OS, MRD, and cumulative incidence of relapse (CIR): a. RFS measured from the time of HCT to the date of disease relapse (BM blasts $\geq 5\%$; or reappearance of blasts in the blood; or development of extramedullary disease), or the date of death from any cause, whichever comes first. Median RFS and other parameters related to the Kaplan-Meier (KM) analysis will be reported if applicable. b. OS defined as the time from HCT to the date of death from any cause. Median OS and other parameters related to the KM analysis will be reported if applicable. c. Presence of MRD assessed at Screening through 24 months/EOS. d. CIR is measured from the time of HCT to the date of disease relapse. 6. PK parameters for Mylotarg™ in patients who received VOR33: a. Maximal concentration (C_{max}), time to maximal concentration (t_{max}), area under the concentration-time curve AUC, volume of distribution (V_z), and clearance (CL) of Mylotarg™.

Plants

Seed stocks	Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.
Novel plant genotypes	Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.
Authentication	Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosaicism, off-target gene editing) were examined.

Flow Cytometry

Plots

Confirm that:

- ☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- ☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- ☒ All plots are contour plots with outliers or pseudocolor plots.
- ☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Bone marrow and peripheral blood AML assessments were done by flow cytometry. CD33 negativity in myeloid populations was also assessed using this method. Briefly, samples were immunostained with antibody cocktails containing HLA-DR, CD7, CD11b, CD13, CD14, CD16, CD19, CD33, CD34, CD36, CD38, CD45, CD56, CD64, CD117, and CD123 for 20 minutes at room temperature in the dark. Red blood cells were lysed with the use of 3.5 mL of buffered NHCl (0.83%) at 37°C for 5 minutes, followed by centrifugation at 300g. The cells were washed with 3 mL of phosphate-buffered saline containing 2% fetal calf serum and resuspended to 0.5 mL in 1% paraformaldehyde for analysis on a FACSCalibur™ flow cytometer (BD Biosciences)
Instrument	FACSCalibur™ flow cytometer (BD Biosciences)
Software	FlowJo software v9-10 (BD).
Cell population abundance	A total of 200,000 events were collected for each tube
Gating strategy	Disease status assessed by clinical commercial laboratory, Hematologics, Inc (Pollard JA, Alonzo TA, Loken M, et al: Correlation of CD33 expression level with disease characteristics and response to gemtuzumab ozogamicin containing chemotherapy in childhood AML. Blood 119:3705-3711, 2012). CD33 expression: proportion of monocytes expressing CD33 was based on cells expressing high levels of CD14. CD33 expression on myeloid cells was based on CD45 vs log side scatter comparing autofluorescence to cells stained with CD33.

- ☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.