VOR33: A Clinic-Ready CRISPR/Cas9 Engineered Hematopoietic Stem Cell Transplant for the Treatment of Acute Myeloid Leukemia

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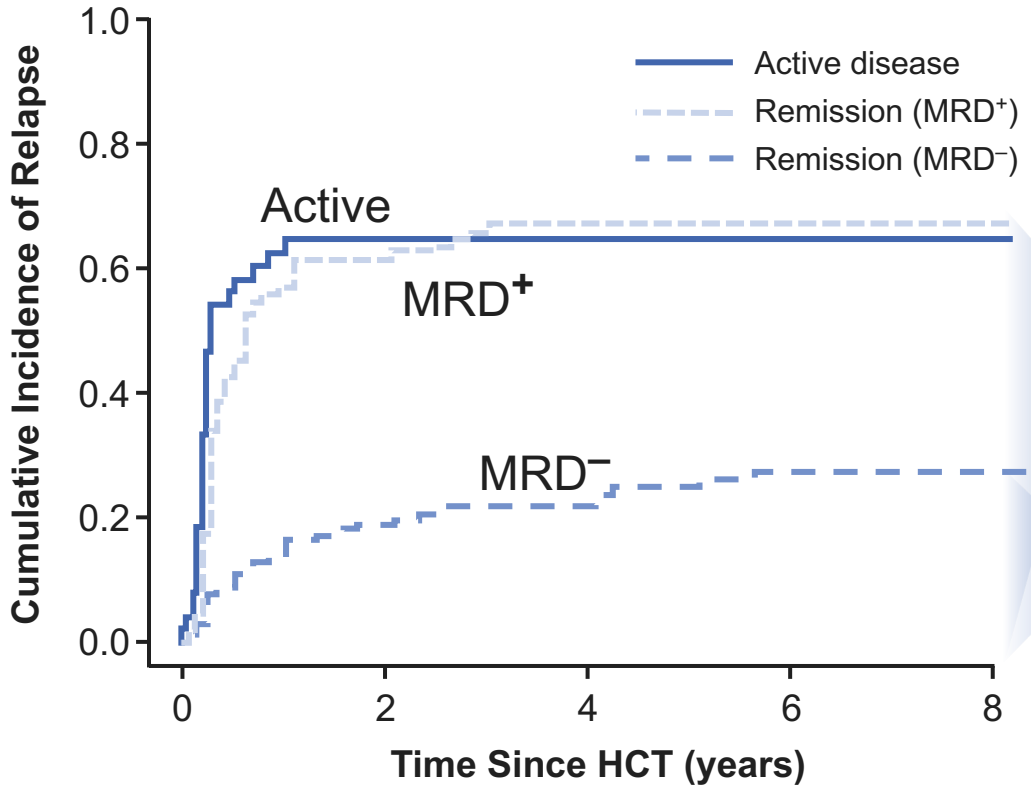
Disclosures

John Lydeard is a salaried employee of Vor Biopharma and hold an equity interest in the company.

>60% of Patients With AML With MRD+ Remission Will Relapse Within 1 Year After Transplantation

Cumulative Incidence of Posttransplant Relapse



Cumulative Risk of Relapse After Transplantation

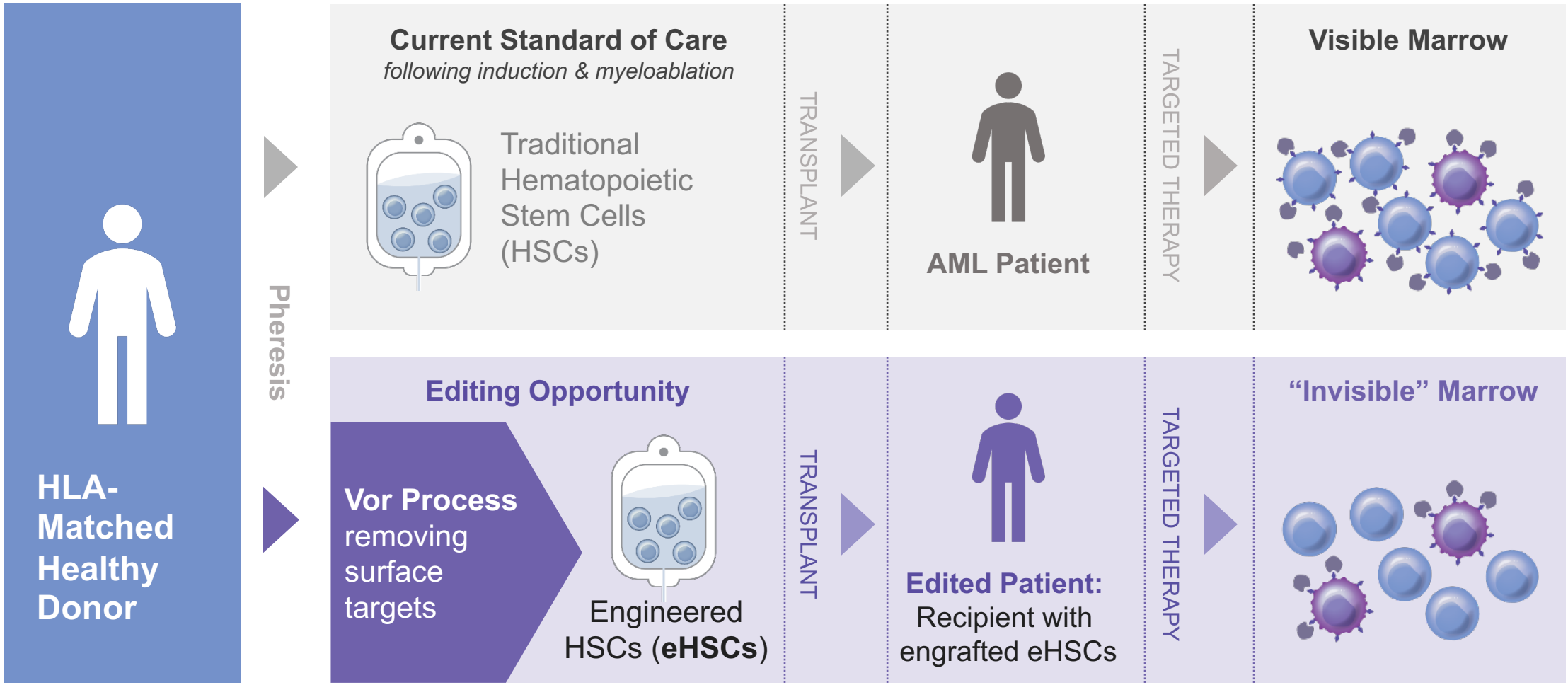
Highest unmet need and initial focus — **>60%** of patients with active disease and MRD+ relapse within 3 years

Substantial need remains in "low risk" — **~25%** of MRD− cases relapse in 5 years

In relapsed patients, the 2-year survival rate is <20%

AML, acute myeloid leukemia; HCT, hematopoietic cell transplantation; MRD, measurable residual disease.
Araki D, et al. *J Clin Oncol.* 2016;34(4):329-336.

VOR33: Engineering the Patient to Make Treatment-Resistant Transplant



CD33 as a Therapeutic Target in AML

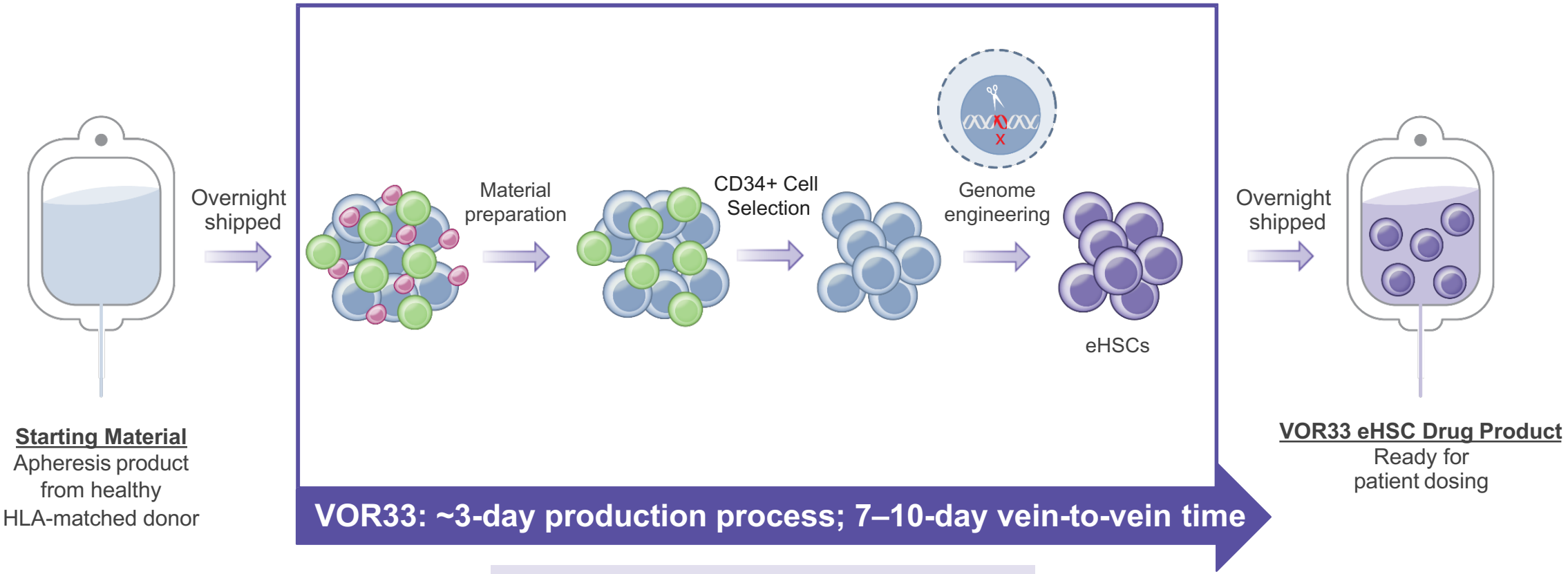
- Broadly expressed in AML Blasts and Leukemic Stem Cells
  - Expressed on normal myeloid cells
- Member of Siglec family (Siglec3), role unknown
- CD33 KO well tolerated in mouse and human
  - 65 individuals with homozygous loss-of-function (LOF) in gnomAD database[1]
- CD33 KO POC is well established
  - Borot et al, 2019[2]; Kim et al, 2018[3]; Humbert et al, 2019[4]

HSPC, hematopoietic stem and progenitor cell; POC, proof of concept; siglec, sialic acid-binding immunoglobin-like lectin.

1. Genome Aggregation Database. gnomAD v2.1.1. Accessed May 7, 2021. https://gnomAD.broadinstitute.org **2.** Borot F, et al. *Proc Natl Acad Sci USA*. 2019;116(24):11978-11987. Erratum in: *Proc Natl Acad Sci USA*. 2019 Jul 16;116(29):14780-14781. **3.** Kim MY, et al. *Cell*. 2018;173(6):1439-1453.e19. **4.** Humbert O, et al. *Leukemia.* 2019 Mar;33(3):762-808.

Efficient Deletion of CD33 at Clinical Scale Manufacturing

VOR33: Streamlined Cell Manufacturing Process



Starting Material
Apheresis product from healthy HLA-matched donor

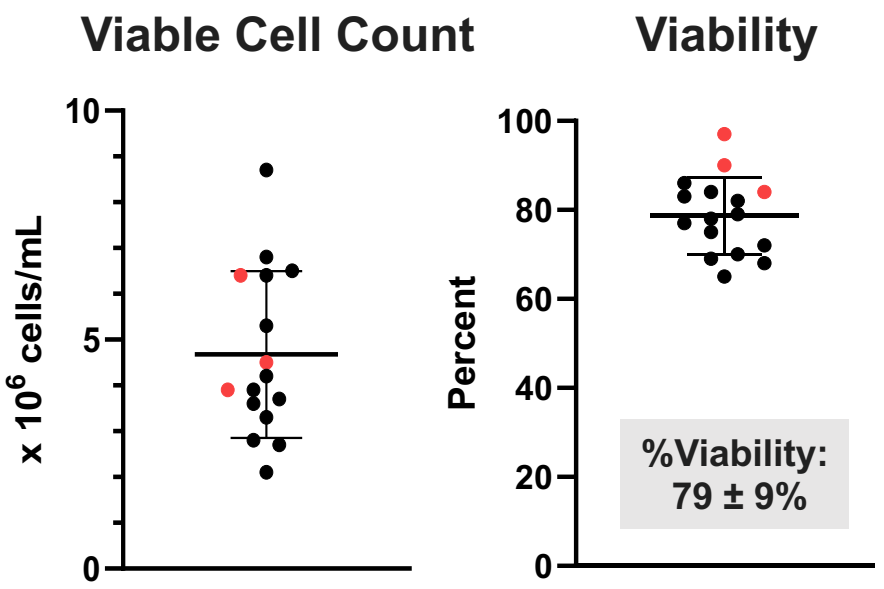
VOR33 eHSC Drug Product
Ready for patient dosing

- ✓ No new genetic material nor viral vectors
- ✓ No cell expansion

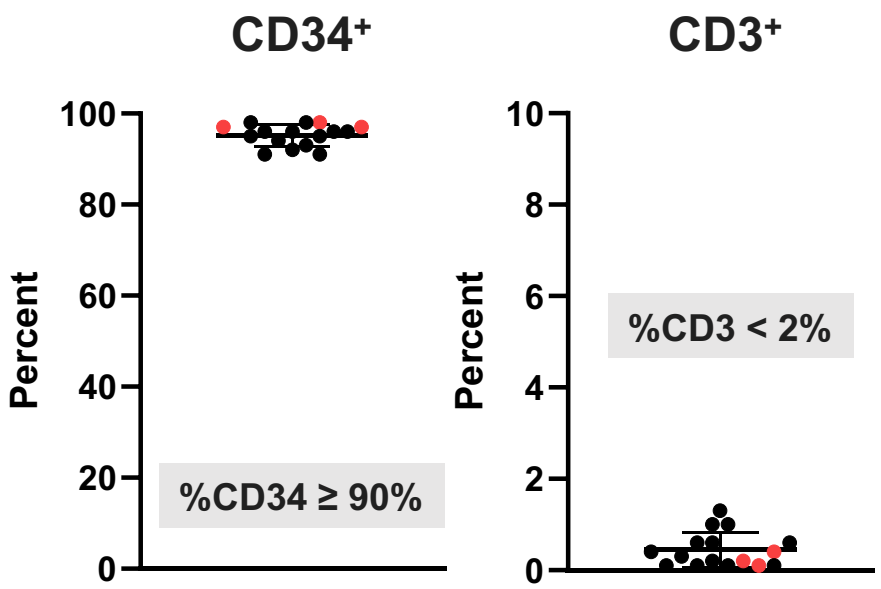
CAR-T, chimeric antigen receptor T-cell therapy.

VOR33 Manufacturing At Scale is Reproducible and Robust

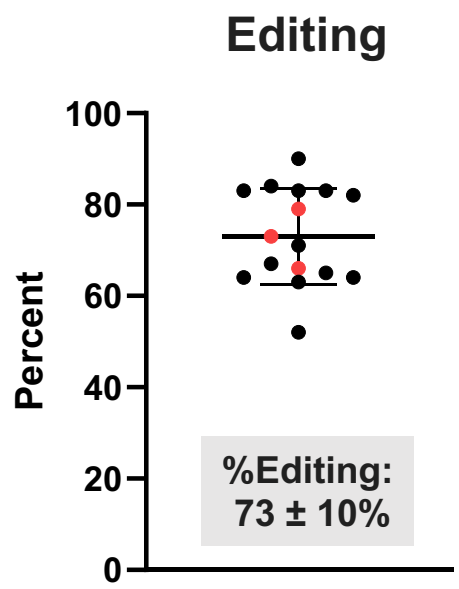
Cell Yield



Cell Purity (T-Cell Removal)



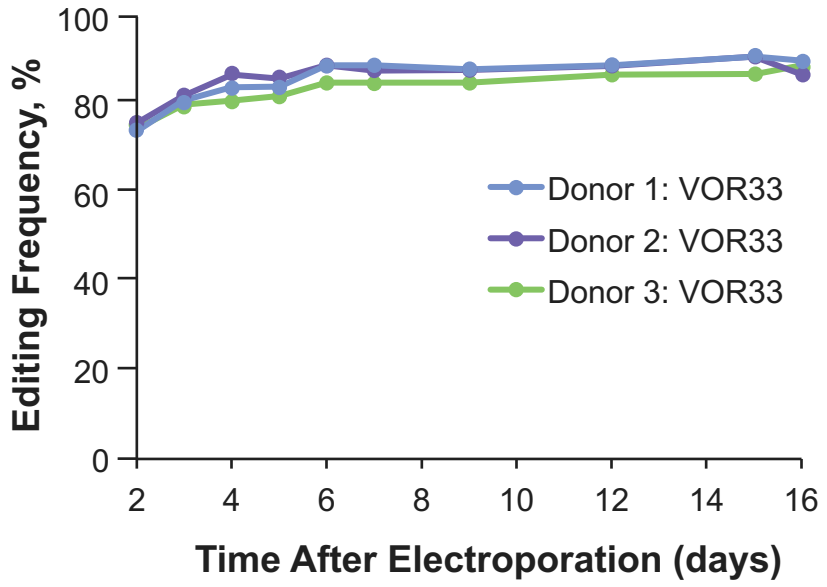
Editing ~73%



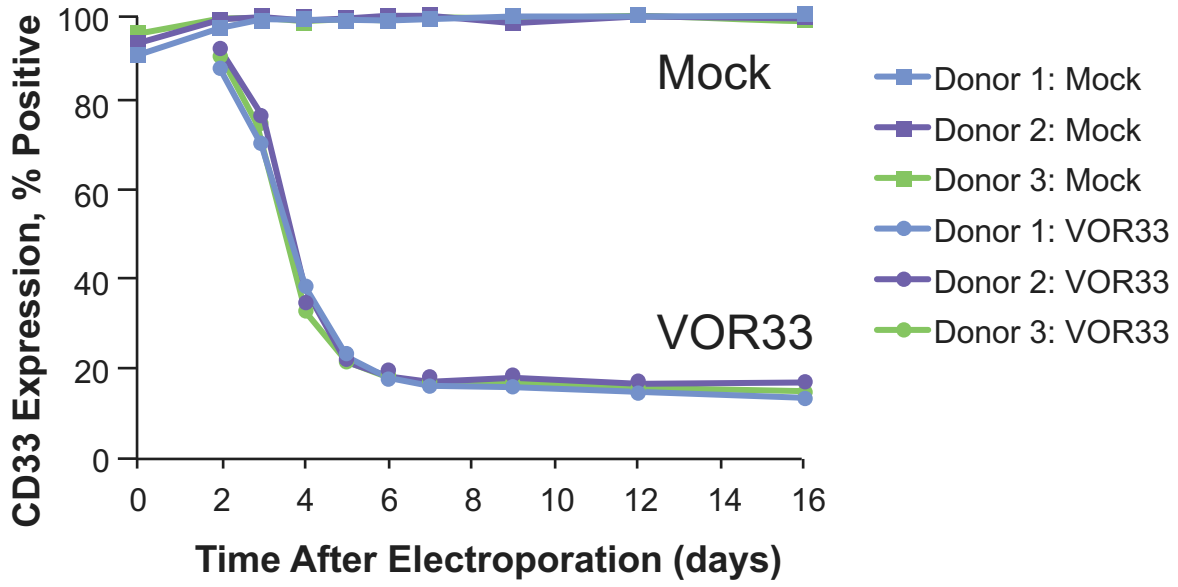
Data represents the results of at-scale manufacturing from 16 batches produced at 2 manufacturing sites. Symbols in red (●) indicate batches used in pharmacology and toxicology studies.

VOR33: CD33 Expression Protein Loss Upon Gene Editing

Gene Editing



Protein Expression



- Efficient gene editing stabilizes 4 days after delivery of the Cas9/gRNA RNP via electroporation
- Protein loss stabilizes 5 days after electroporation

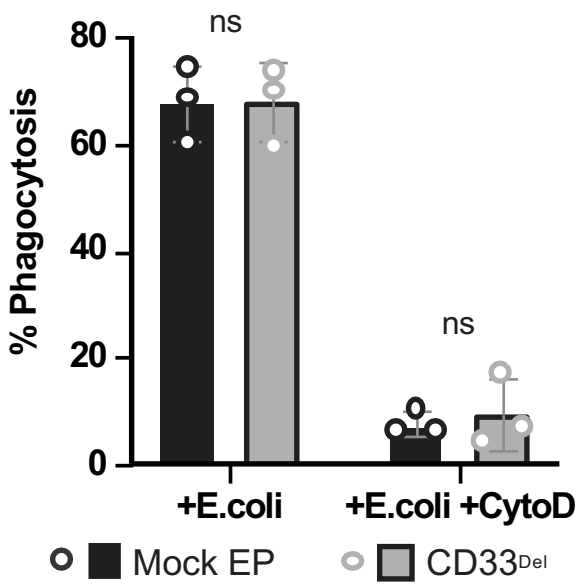
RNP, ribonucleoprotein.

Maintenance of Hematopoietic Function in CD33 Gene-Edited Cells

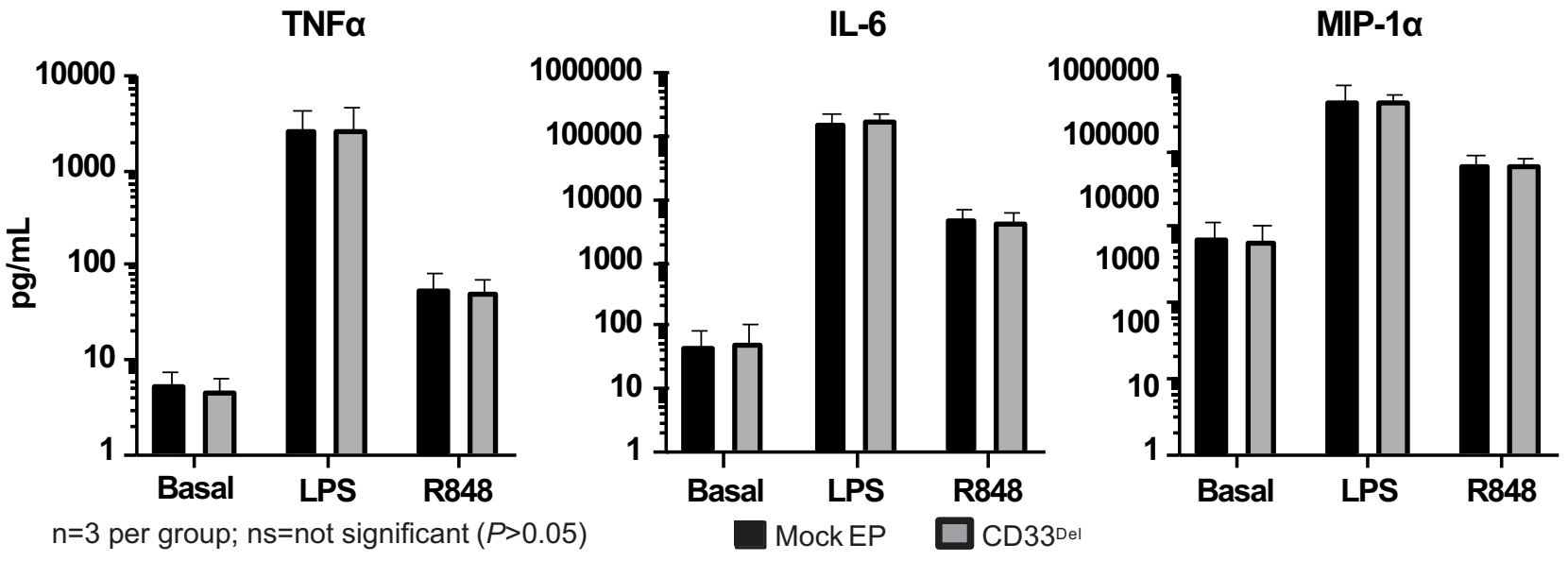
VOR33: No Observed Impact on Cell Function

In Vitro Cell Function Assays

Phagocytosis

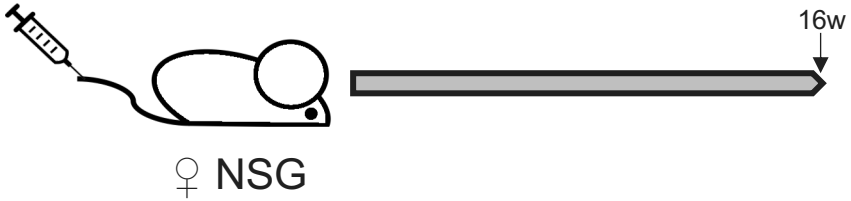


Cytokine production



CD33DEL, CD33 deleted; eHCS, engineered hematopoietic stem cell.; EP, electroporation; IL, interleukin; LPS, lipopolysaccharide; MIP, macrophage inflammatory protein; ns, not significant; TNF, tumor necrosis factor.

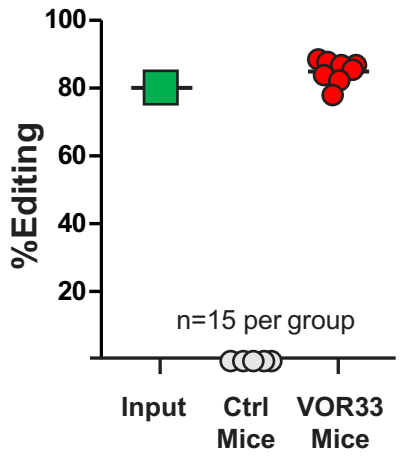
VOR33: No Impact on HSC Engraftment and Differentiation *in vivo*



- Multilineage engraftment
- Bone Marrow Editing %
- Hematopoietic progenitor potential

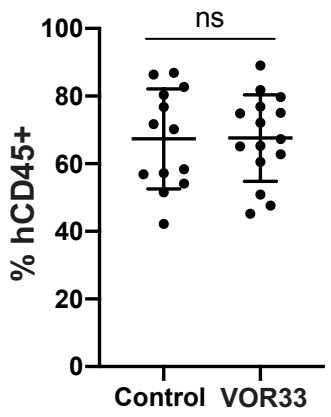
High Editing Maintained for 16-Weeks of Engraftment

Editing Efficiency

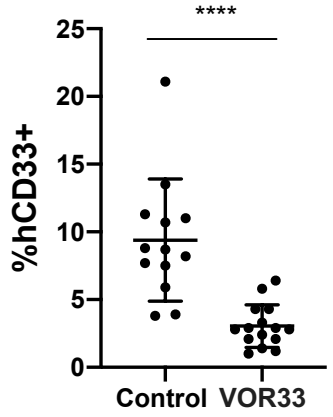


Xeno-Transplant in NSG Mouse Model: 16-Week Bone Marrow No Impact on Differentiation

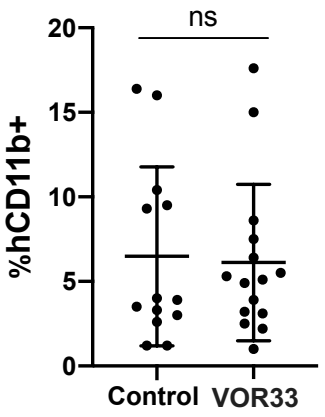
Human Chimerism



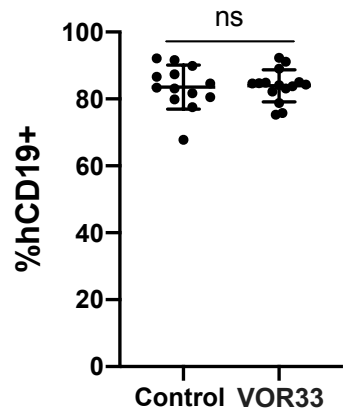
CD33 Frequency



Myeloid cell frequency



B-cell frequency



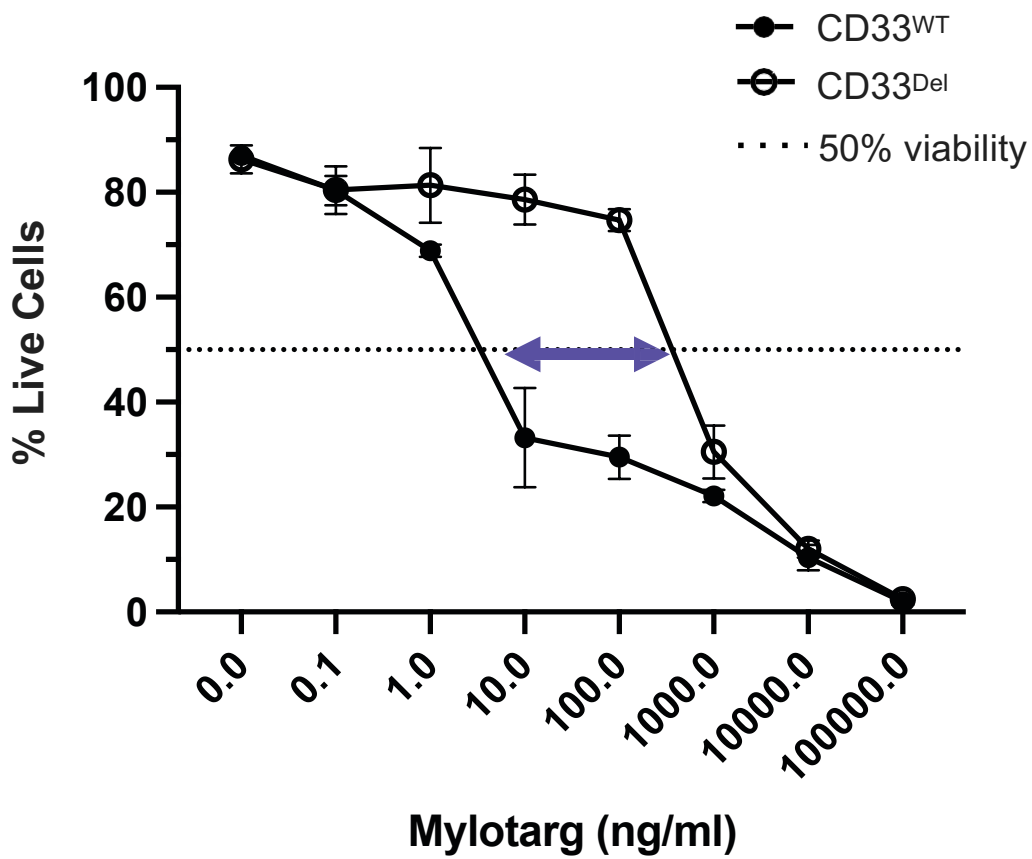
n=15, ns, not significant ($p > 0.05$); ****$p < 0.0001$ (ordinary one-way ANOVA)

HCS, hematopoietic stem cell; NSG, NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ; Ctrl,control

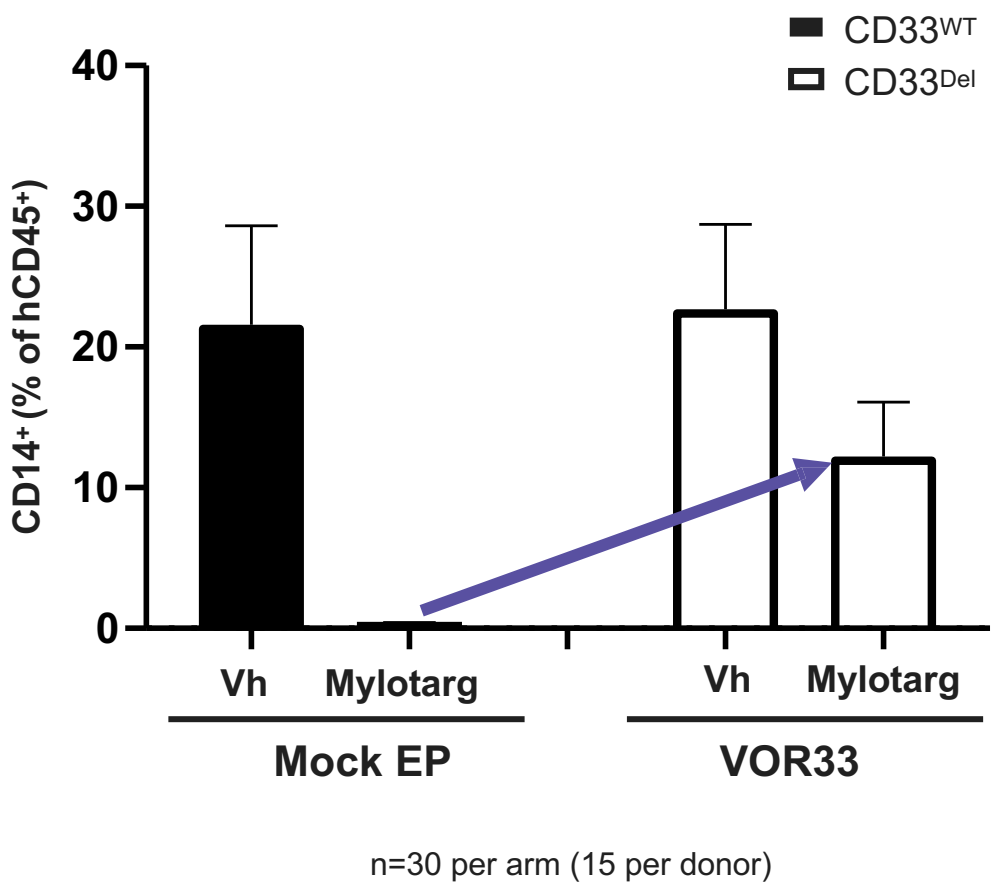
Pharmacology: CD33 Null Cells Are Protected From CD33-Directed Therapy

VOR33: Protected from CD33 Directed Therapy

In Vitro: Monocytes



In Vivo: 16-Week Engraftment



n=30 per arm (15 per donor)

EP, electroporation; Vh, vehicle; WT, wild-type.

Safety: Toxicology and Off-Target Results

Toxicology Assessment Did Not Reveal Significant Adverse Findings for VOR33

In life observations (every 3–6 days)

- Mortality
- Clinical signs
- Body weights
- Food/water consumption

Hematology (12)

- Cell morphology
- Hematocrit
- Hemoglobin
- Mean corpuscular hemoglobin
- Mean corpuscular volume
- Mean corpuscular hemoglobin concentration
- Platelet count & platelet/thrombocrit
- Red blood cell count
- Red cell distribution width
- Reticulocyte counts (absolute & relative)
- WBC
- WBC differential (absolute & relative)

Organ weights (11)

- Adrenals
- Brain
- Heart
- Kidneys
- Liver
- Ovaries
- Prostate
- Spleen
- Testes
- Thymus
- Uterus

Clinical Chemistry (19)

- Sample Appearance (when abnormal)
- A/G ratio (calculated)
- Alanine aminotransferase
- Albumin (A)
- Alkaline phosphatase
- Aspartate aminotransferase
- Bilirubin (total)
- Calcium (total)
- Chloride
- Cholesterol (total)
- Creatinine
- Globulin (G; calculated)
- Glucose
- Phosphorus (inorganic)
- Potassium
- Protein (total)
- Sodium
- Triglycerides
- Urea

Histopathology (43)

- Adrenals
- Aorta (thoracic)
- Brain
- Cecum
- Colon
- Duodenum
- Epididymis
- Esophagus
- Eyes
- Femur with marrow
- Gallbladder
- Heart
- Injection site
- Ileum
- Jejunum
- Kidneys
- Liver (2 lobes)
- Lungs with bronchi
- Lymph nodes (iliac, mesenteric and inguinal)
- Olfactory bulb
- Optic nerves
- Ovaries
- Pancreas
- Pituitary
- Prostate
- Rectum
- Salivary gland (mandibular)
- Sciatic nerve
- Seminal vesicles
- Skeletal muscle (thigh)
- Skin, subcutis & mammary gland (inguinal)
- Spinal cord (cervical)
- Spleen
- Sternum with marrow
- Stomach
- Testes
- Thymus
- Thyroids with parathyroids
- Tongue
- Trachea
- Urinary bladder
- Uterus
- Vagina

All assessment performed in compliance with GLP

WBC, white blood cell; GLP, good laboratory practice.

Assessment of Off-Target Edits: VOR33 Has No Significant Off-Target or Safety Concerns

Unbiased Assessment of Off-Target Sites	**GUIDE-seq** Low frequency genomic sites identified with no perceivable safety risk
In Silico Predicted Off-Target Sites	**Hybrid Capture NGS** No reproducible and reliably edited sites
Gross Chromosomal Abnormalities	**Karyotyping** No chromosomal abnormalities

See poster #858, Hazelbaker et al

NGS, next-generation sequencing.

Summary

- **Clinical ready manufacturing process**
- **HSC engraftment and function are unaltered despite loss of CD33**
- **CD33 null cells are protected from CD33-directed therapy**
- **No toxicology or genomic off-target findings**
- **VOR33 IND and CTA have been cleared by FDA and Health Canada, respectively**
  - Multicenter first-in-human Phase 1/2a clinical trial initiating in 2021 (NCT04849910)

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Mike Pettiglio

Kienan Salvadore

Gabriella Angelini

Azita Ghodssi

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Kush Parmar

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Appendix

ASGCT Abstract

VOR33: A Clinic-Ready CRISPR/Cas9 Engineered Hematopoietic Stem Cell Transplant for the Treatment of Acute Myeloid Leukemia

Introduction: AML is the most common form of adult acute leukemia, with median 5-year survival rate <30%. Allogeneic hematopoietic cell transplant (HCT) has long been the standard of care for high-risk patients (pts), with >3500 transplants performed annually in the US. There is unmet need for new treatments in ~40% of pts who relapse. With existing targeted therapies, cell surface marker expression between cancer and normal cells is not differentiated enough to limit "on-target, off-tumor" toxicity. Antigens (Ag) (eg, CD33) expressed on normal myeloid cells and/or progenitors (Levine et al. 2015) confer dose-limiting toxicity of antigen-directed therapies in AML. To unlock the full potential of targeted treatments, we create treatment-resistant hematopoietic stem cells (HSCs) by genetically ablating CD33 from healthy, HLA-matched (10/10) donor HSCs followed by HCT. This process results in a target Ag-negative hematopoietic system. The reconstituted hematopoietic compartment of pts receiving CD33-null cells is expected to be resistant to cytotoxicity induced by Mylotarg™, an anti-CD33 monoclonal antibody conjugated with cytotoxic calicheamicin. Human HSCs with CD33 removed, as well as their progeny, show no impairment of hematopoietic function and display resistance to CD33-targeted therapies (Borot et al. 2019; Humbert et al. 2019; Kim et al. 2018). Notably, this is consistent with natural human genetic evidence of CD33 null individuals with no deleterious phenotype (https://gnomad.broadinstitute.org/). Here, we describe the preclinical data and process scale-up of the CD33-null HSC graft (VOR33) for a first-in-human clinical trial.

Methods/Results: The manufacturing process yielded clinically relevant doses of VOR33 (>3x10^6 viable CD34+ cells/kg) under GMP-like conditions with GMP-appropriate reagents. CD34+ cells, isolated from G-CSF and plerixafor mobilized peripheral blood leukapheresis products, were edited using CRISPR/Cas9 to disrupt *CD33* gene. At scale, we routinely achieved gene knockout efficiency >70% (90% biallelic). Importantly, cell viability was unaffected by loss of CD33 and cells differentiated from VOR33 displayed normal myeloid markers, phagocytosis potential and induction of inflammatory cytokines equivalent to unedited (CD33+) control cells. Phenotypic and functional characterization revealed no difference in frequency of long-term HSCs in VOR33 vs unedited controls. Pharmacology studies using NOD/SCID-gamma mice, with VOR33 cells manufactured under GMP-like conditions, showed normal long-term engraftment (16-week bone marrow chimerism of 83.1±9.0% vs 87.9±7.3% in control group) and multilineage differentiation. In addition, we observed persistence of VOR33 gene editing and preservation of indel species distribution after 16 weeks, indicating no counterselection or clonal expansion of CD33-null cells. Importantly, we found loss of CD33 protein conferred selective protection to VOR33-derived myeloid cells vs Mylotarg *in vitro* (>65-fold) and *in vivo* (>60-fold). In our GLP toxicology study of >40 tissues, we saw no tumorigenicity or notable changes in toxicology parameters. In-depth genotoxicity analyses were carried out with a subset of scaled-up manufactured lots of VOR33, including those used for toxicology and pharmacology studies. Deep sequencing of 2369 genomic sites, by homology-dependent and independent methods, revealed no off-target editing.

Conclusion: These studies set the stage for initiation of, as well as evaluation of safety and efficacy in, a multicenter first-in-human clinical trial of VOR33 in pts with AML.