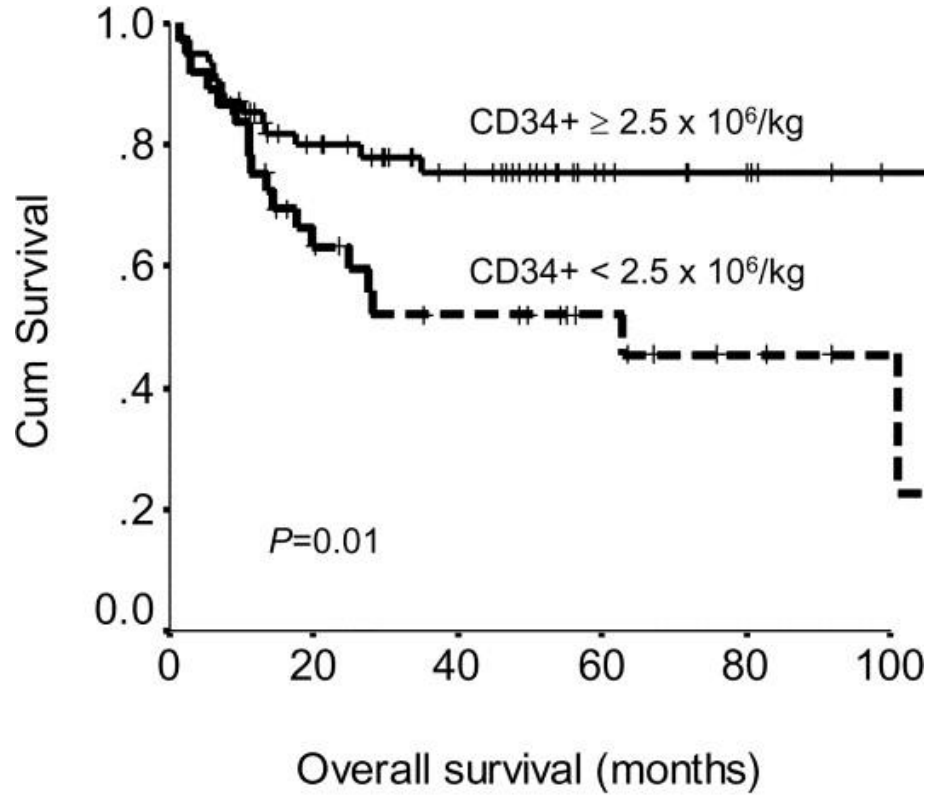
Expansion with E478 Significantly Increases the Rate of CRISPR-Mediated Homology Directed Repair (HDR) and Improves Engraftment of Human Hematopoietic Stem Cells

Kevin A. Goncalves, PhD
Magenta Therapeutics

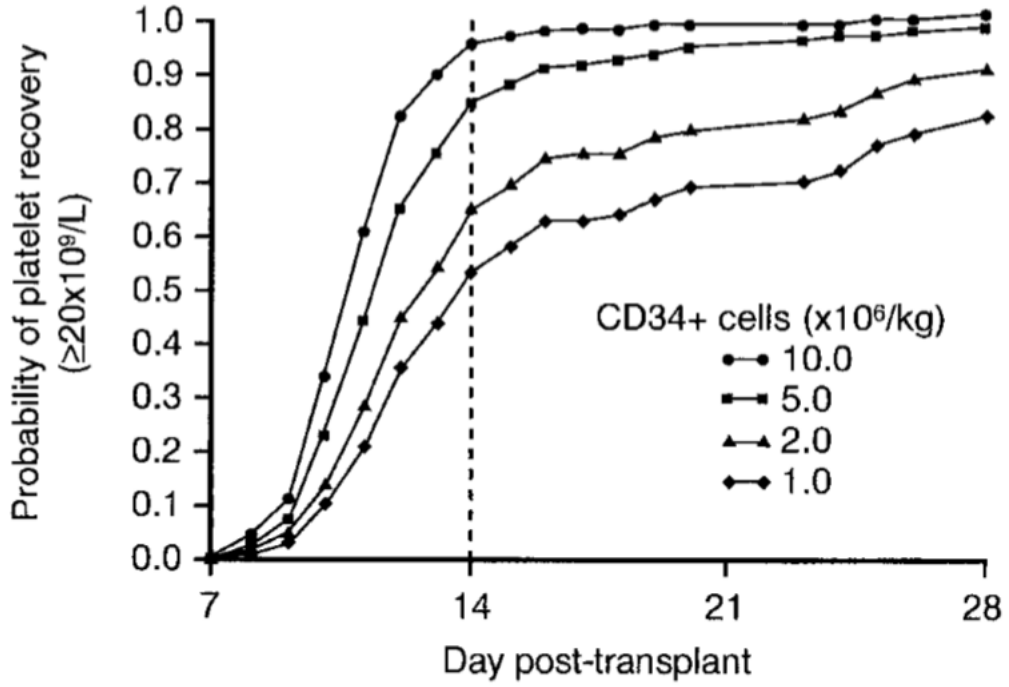
Increased CD34+ Cell Dose Leads to Improved Survival and Engraftment

ALLOGENEIC HEME-ONC



Lee SH et.al. Biol Blood Marrow Transplant. 2005 Feb;11(2):122-8

AUTOLOGOUS HEME-ONC

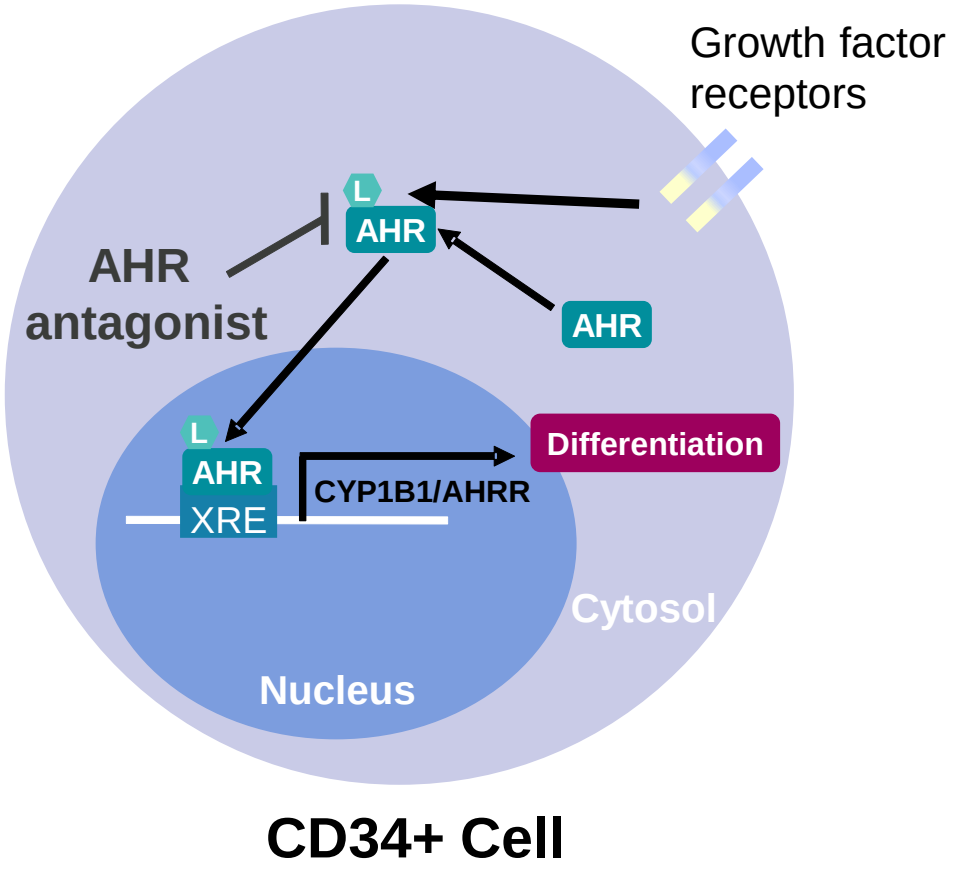


Siena S, et. al.; J Clin Oncol. 2000 Mar;18(6):1360-77.

Aryl Hydrocarbon Receptor (AHR) Antagonists Drive HSC Renewal By Blocking Differentiation via a Defined Mechanism

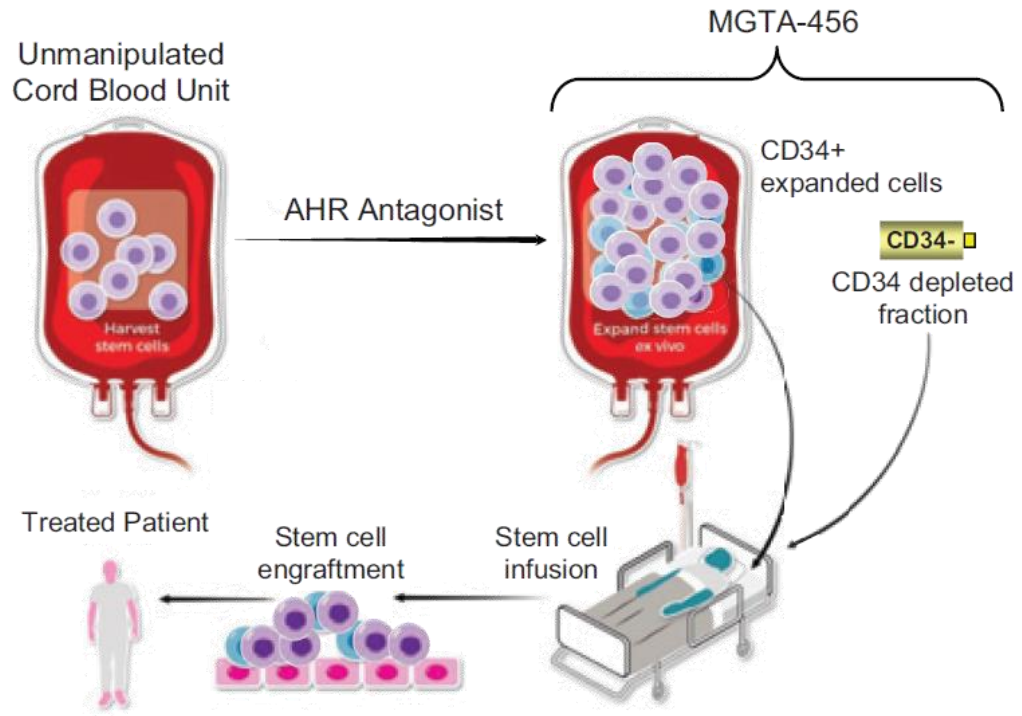
- Identified in a screen of compounds that promoted CD34/CD133 expansion: StemRegenin 1
- Acts via antagonism of AHR which plays a prominent role in HSC differentiation
- AHR antagonist-mediated AHR inhibition is reversible

Boitano et al., Science, 2010



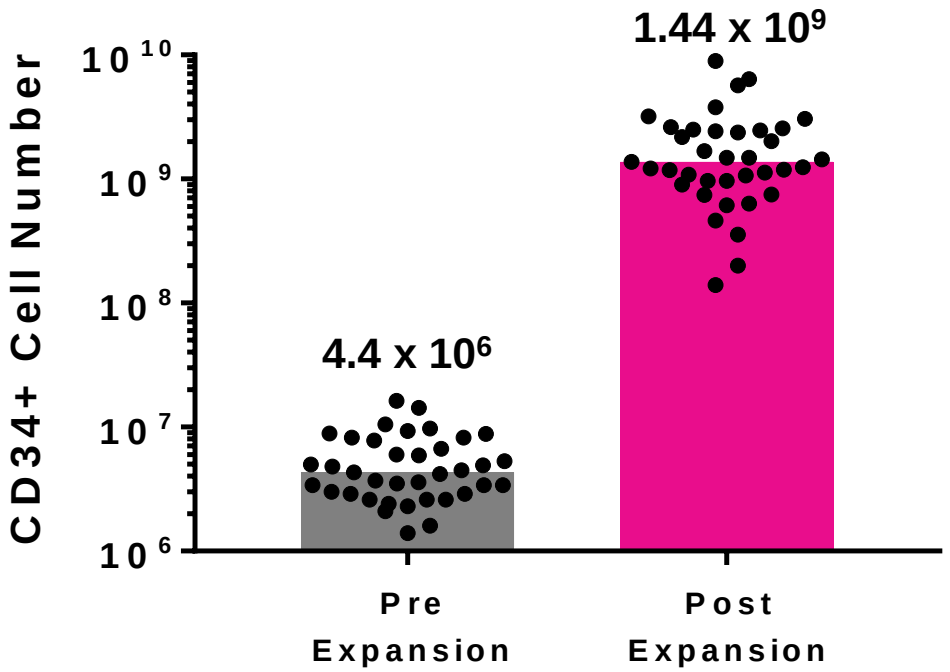
During Ph1/2 Trial, AHR Antagonist Used to Manufacture MGTA-456 Expanded CD34+ Cord Blood Cells More Than 300-Fold

MGTA-456: ALLOGENEIC STEM CELL THERAPY



36 Patients Treated in Phase I/II Heme-Onc Study
5 Patients Treated in Phase II Study in Inherited Metabolic Diseases (IMDs)

CD34+ CELL EXPANSION OVER 15 DAYS



Wagner et al. Cell Stem Cell 2016; 18:144-155

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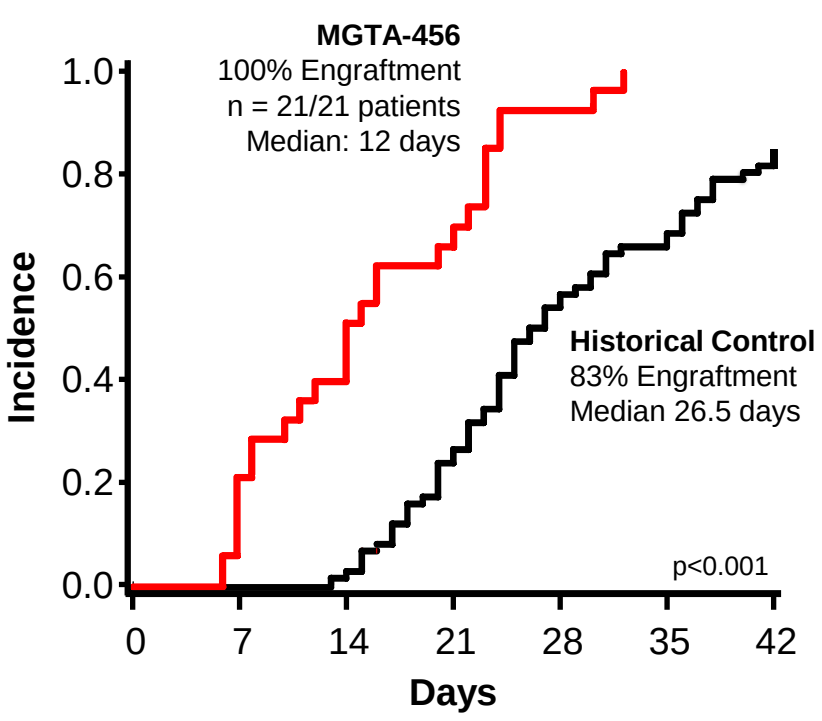
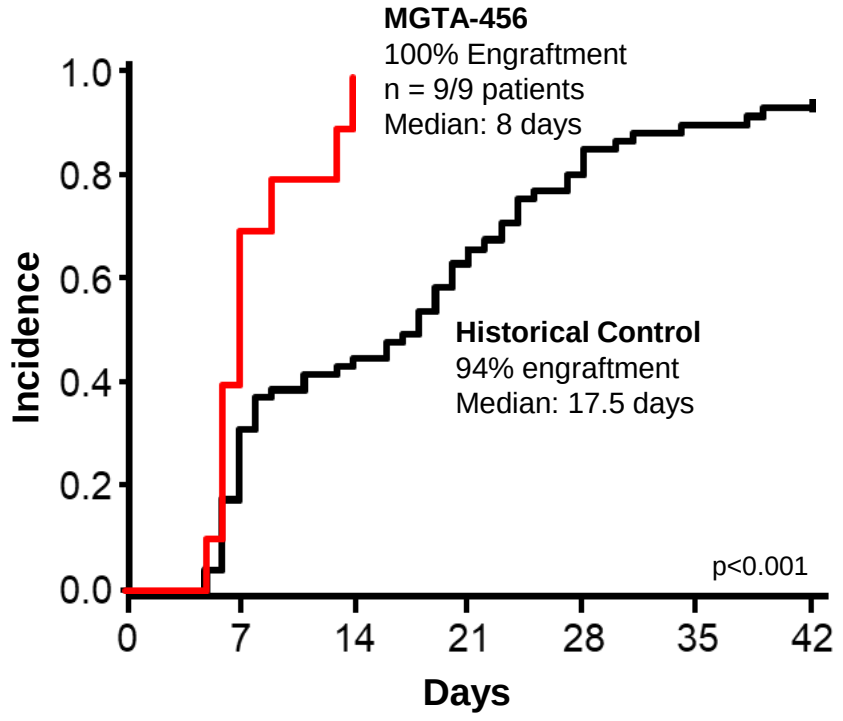
MGTA-456 Has Been Clinically-Validated in Hem/Onc Patients

MYELOABLATIVE CONDITIONING
Flu / Cy / TBI 1320



NON-MYELOABLATIVE CONDITIONING
Flu / Cy / TBI 200



MGTA-456
ASGCT PRESENTATIONS

ABSTRACT #120
MGTA-456 Heme-Onc Ph2 Study
John Wagner, MD
May 12th 3:45-5:30 PM

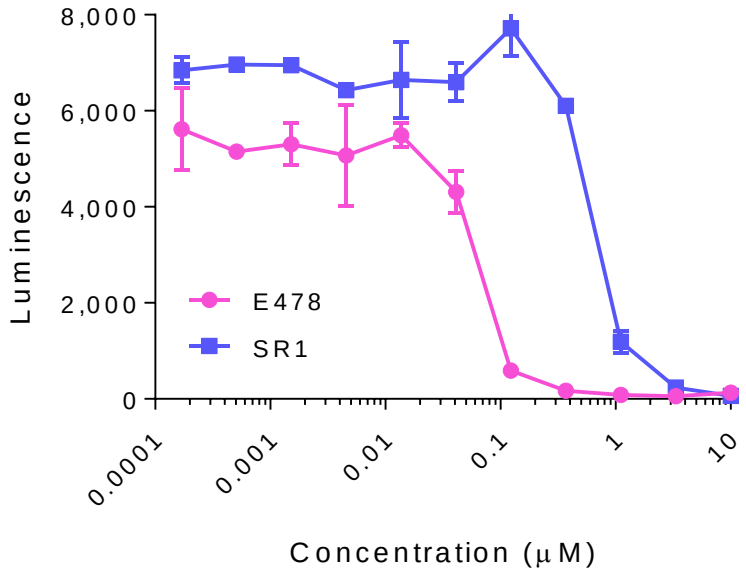
ABSTRACT #1302
MGTA-456 IMD Ph2 Study
John Wagner, MD
May 15th 8-9:45 AM

ABSTRACT #248
MGTA-456 Non-Clinical IMD Study
Sharon Hyzy, MS
May 12th 5:30-6:30 PM

Wagner et al., ASH 2017

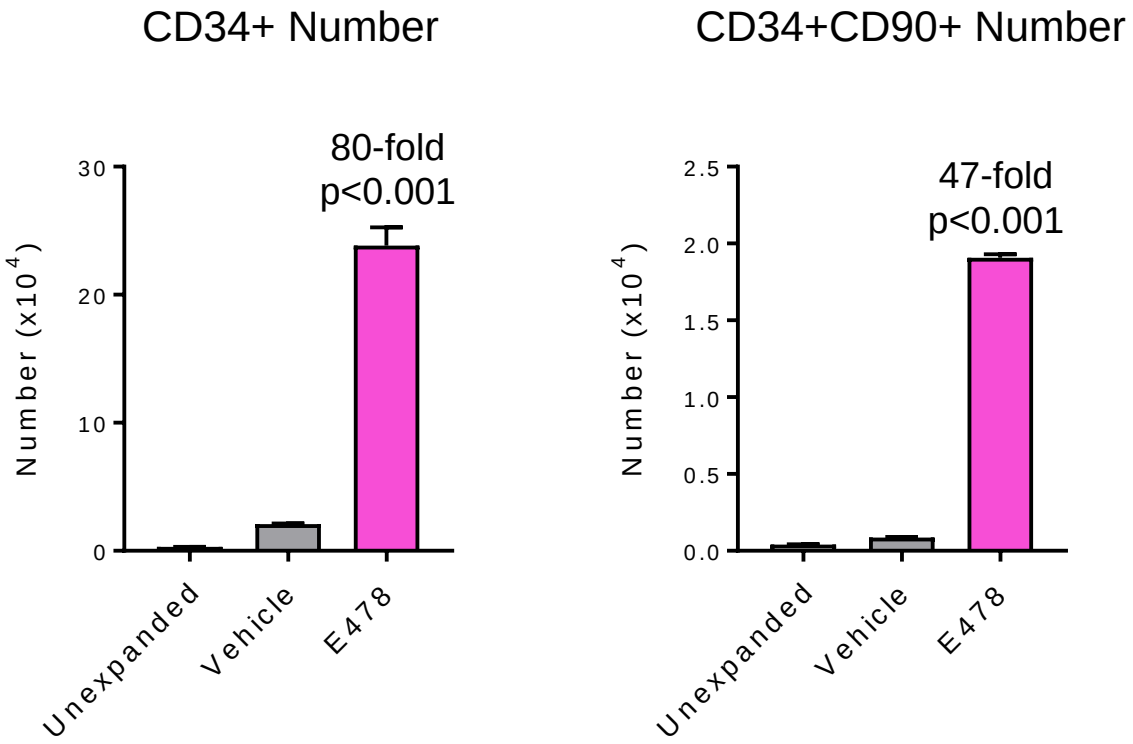
E478 Is a Novel, Potent AHR Antagonist

AHR ANTAGONIST ACTIVITY



Mean ± SD (n=2)

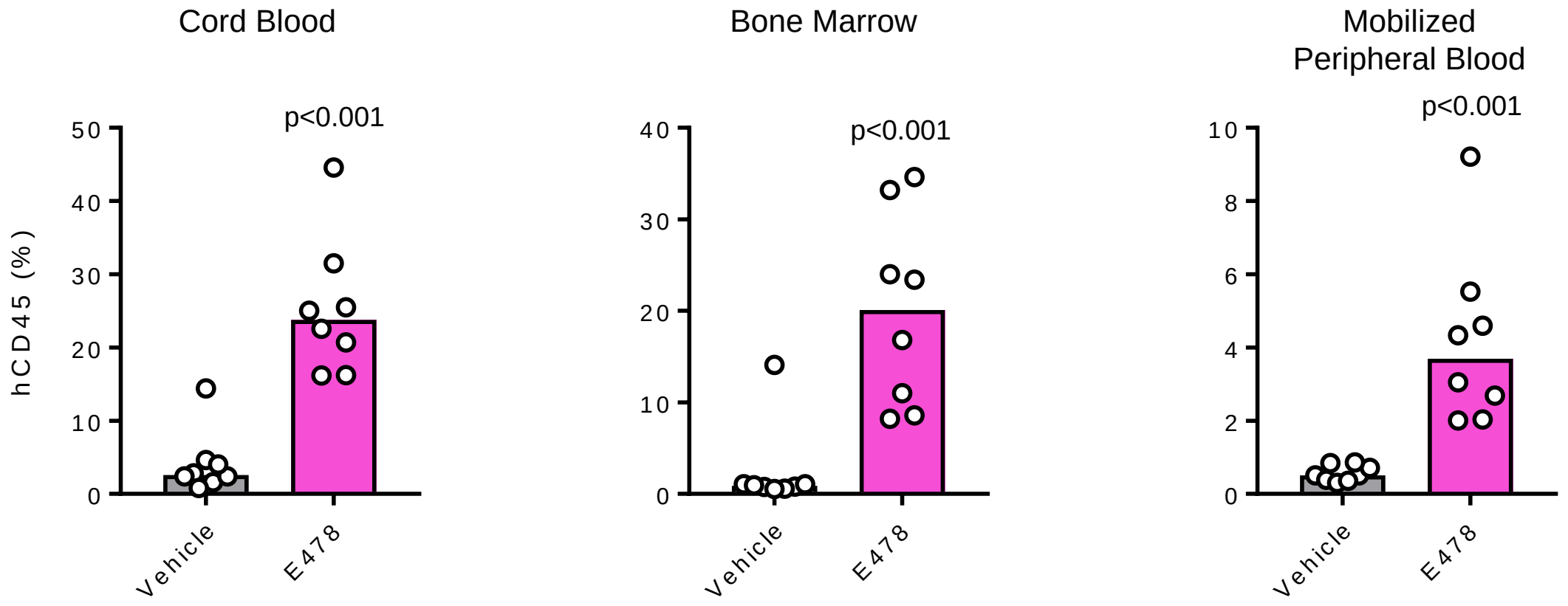
IN VITRO EXPANSION AFTER 10 DAYS



Mean ± SD (n=2)

All Human Hematopoietic Stem Cell Sources are Expandable with E478

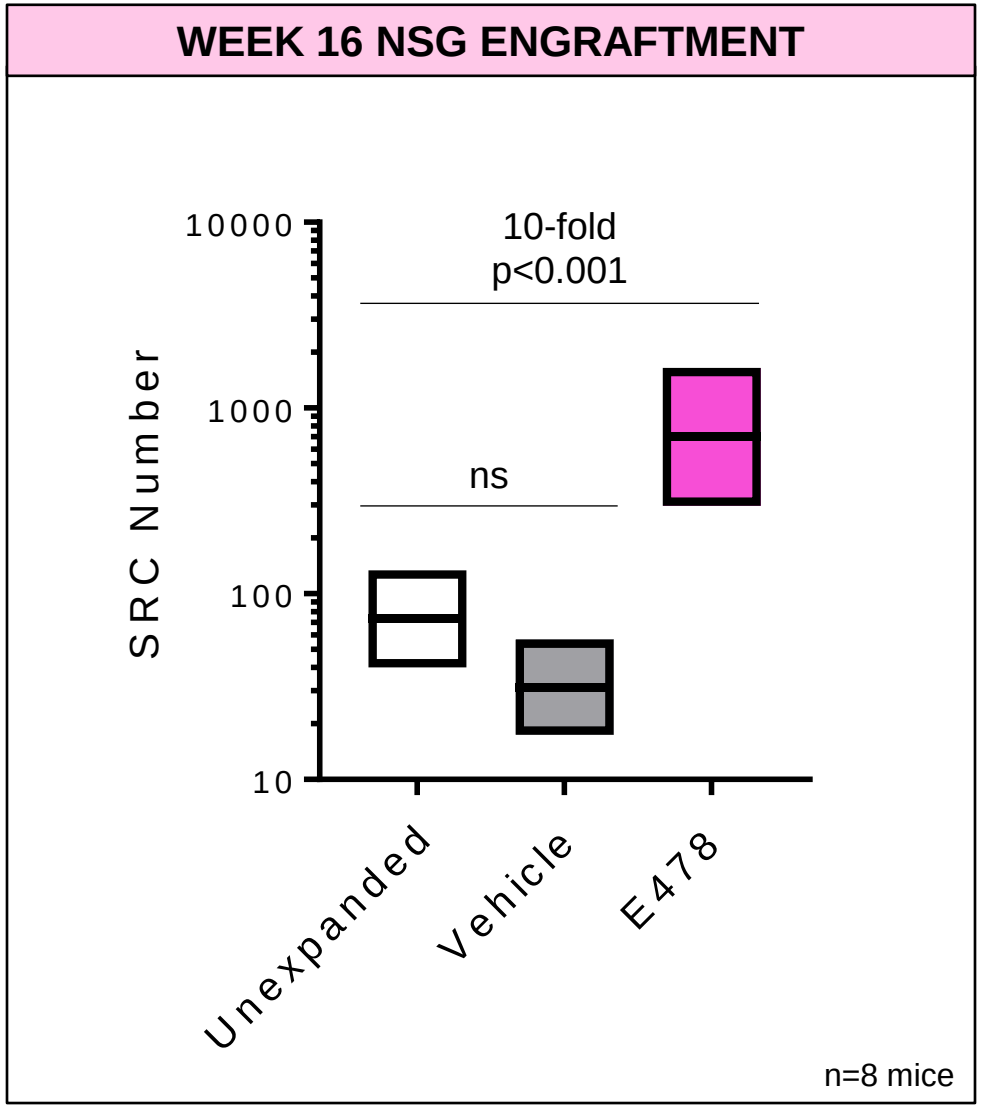
WEEK 16 NSG ENGRAFTMENT



10 day cultures were started with the following CD34+ cell numbers:
Cord Blood: 7,000, Bone Marrow: 200,000, Mobilized Peripheral Blood: 100,000

n=8 mice

E478 Significantly Increases HSCs in a Limit-Dilution Study Using Mobilized Peripheral Blood



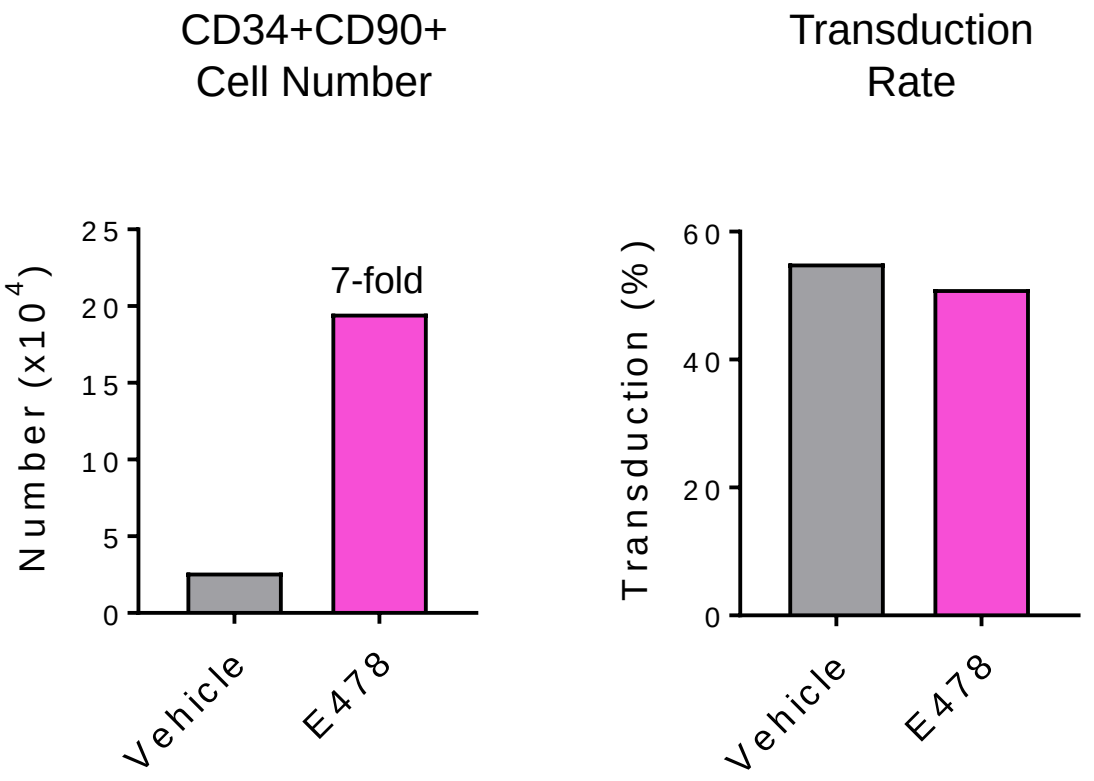
E478 Enables a Variety of Gene Therapy Applications

E478 expands number of gene-modified HSCs:

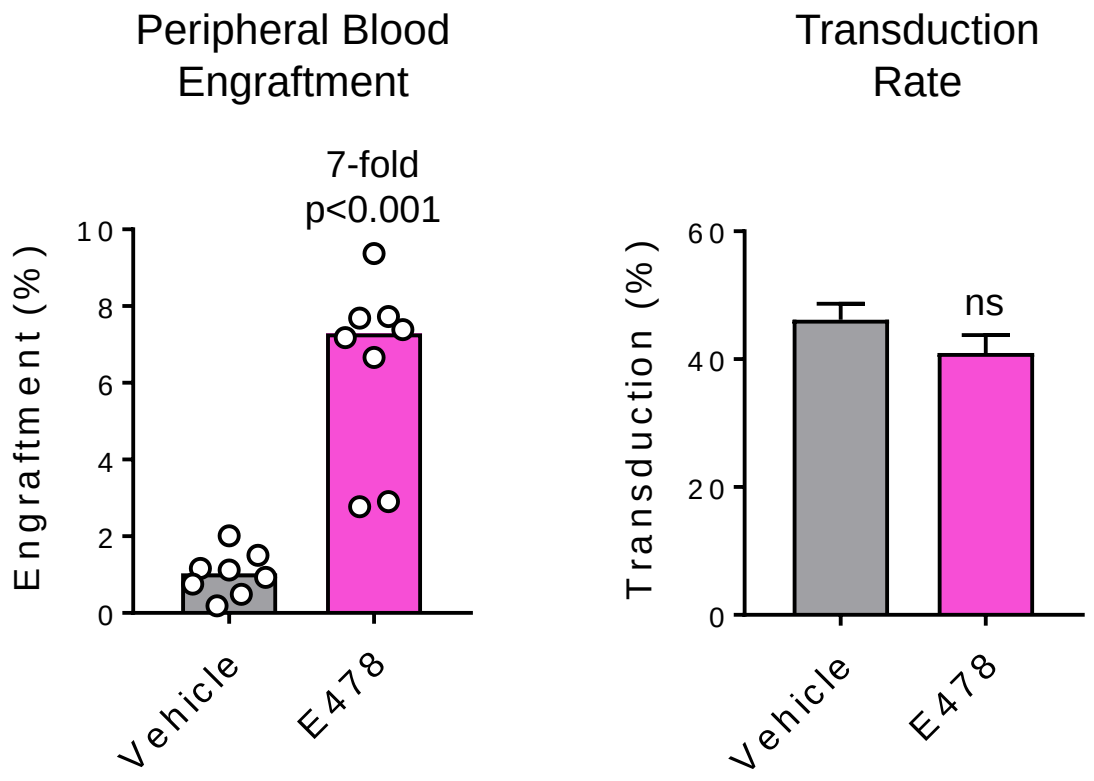
1) Lentiviral Transduction
2) CRISPR-Cas9 Knockout
3) Gene Correction / Insertion

E478 Provides Higher Dose of Lenti-Transduced HSCs

IN VITRO EXPANSION AFTER 7 DAYS



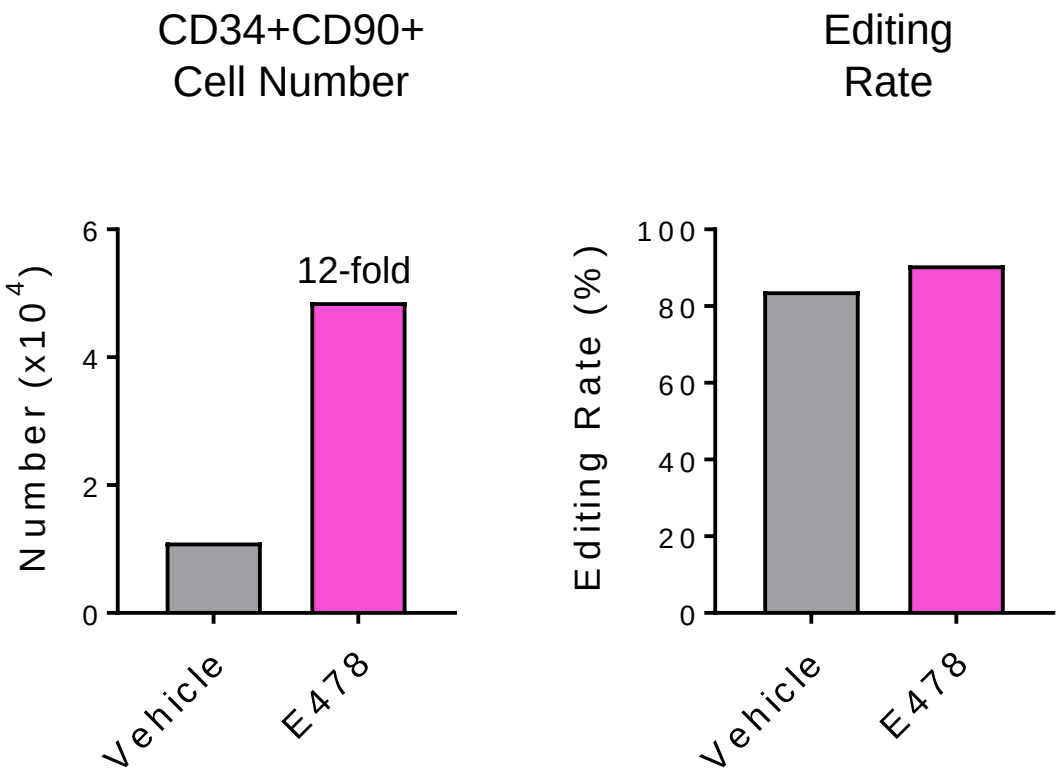
WEEK 16 NSG ENGRAFTMENT



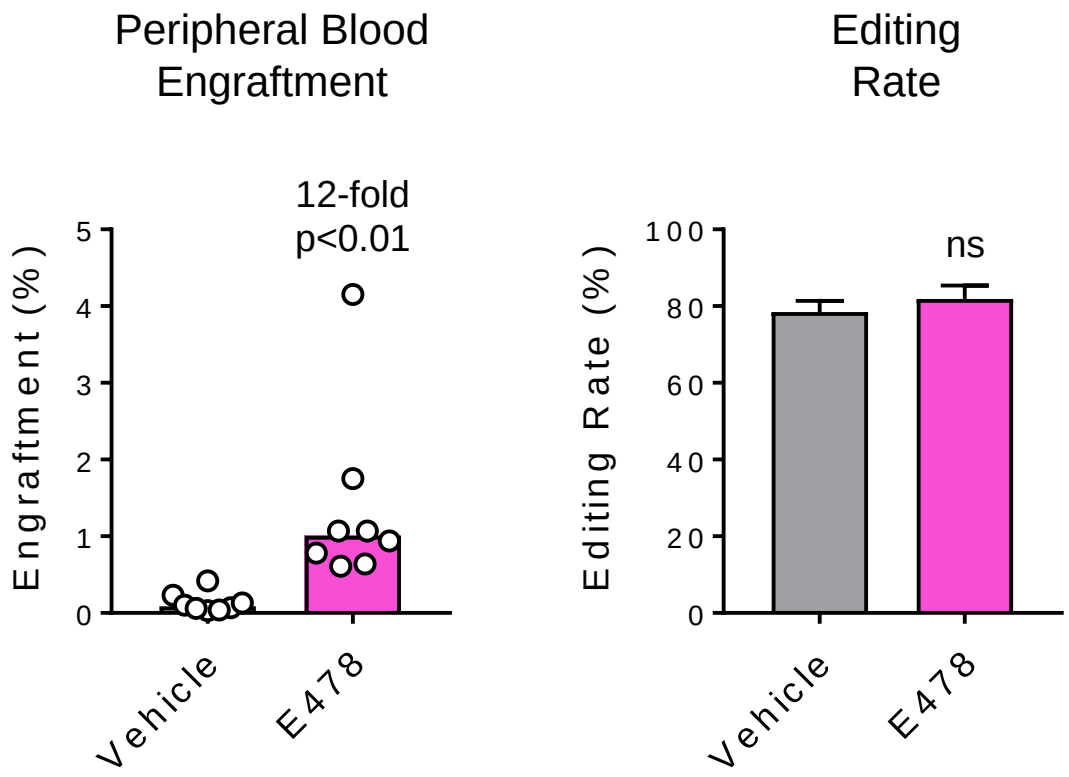
n=8 mice

E478 Provides Higher Dose of CRISPR-Edited HSCs

IN VITRO EXPANSION AFTER 7 DAYS



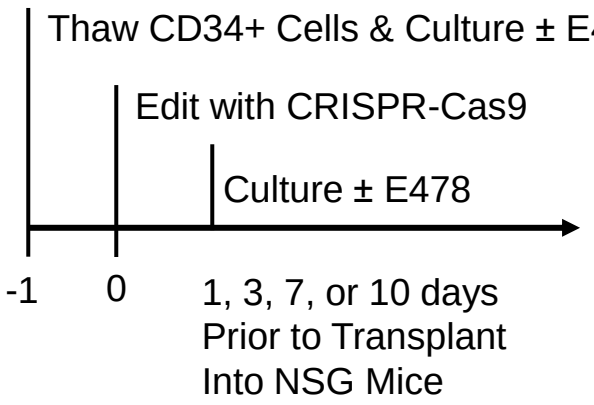
WEEK 16 NSG ENGRAFTMENT



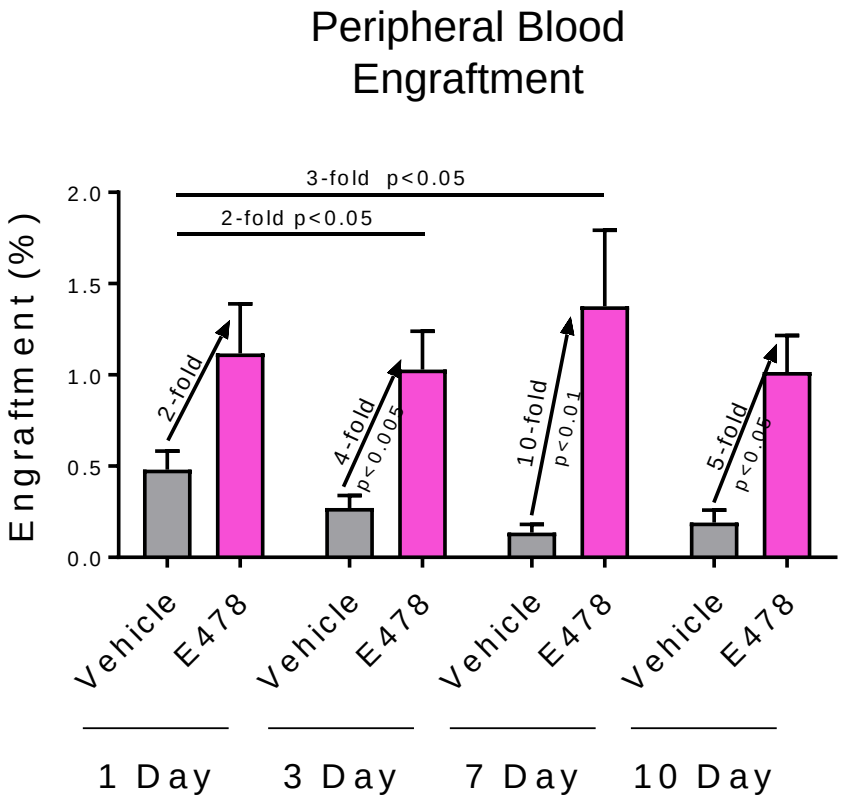
n=8 mice

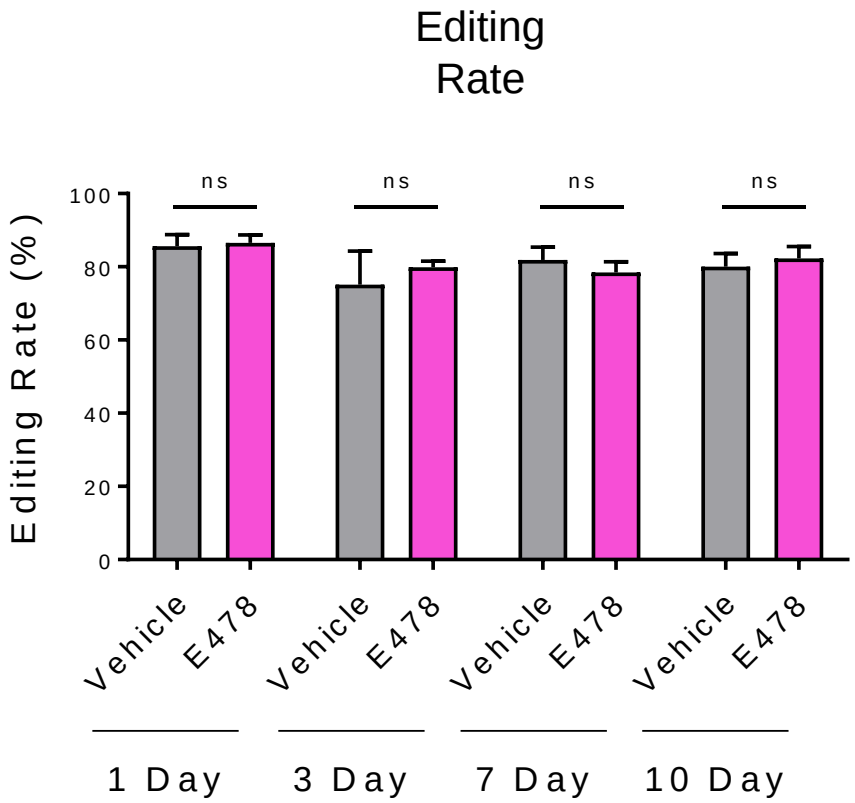
E478 Results in Higher Engraftment at All Timepoints Evaluated

EXPERIMENTAL SCHEMA



WEEK 16 NSG ENGRAFTMENT



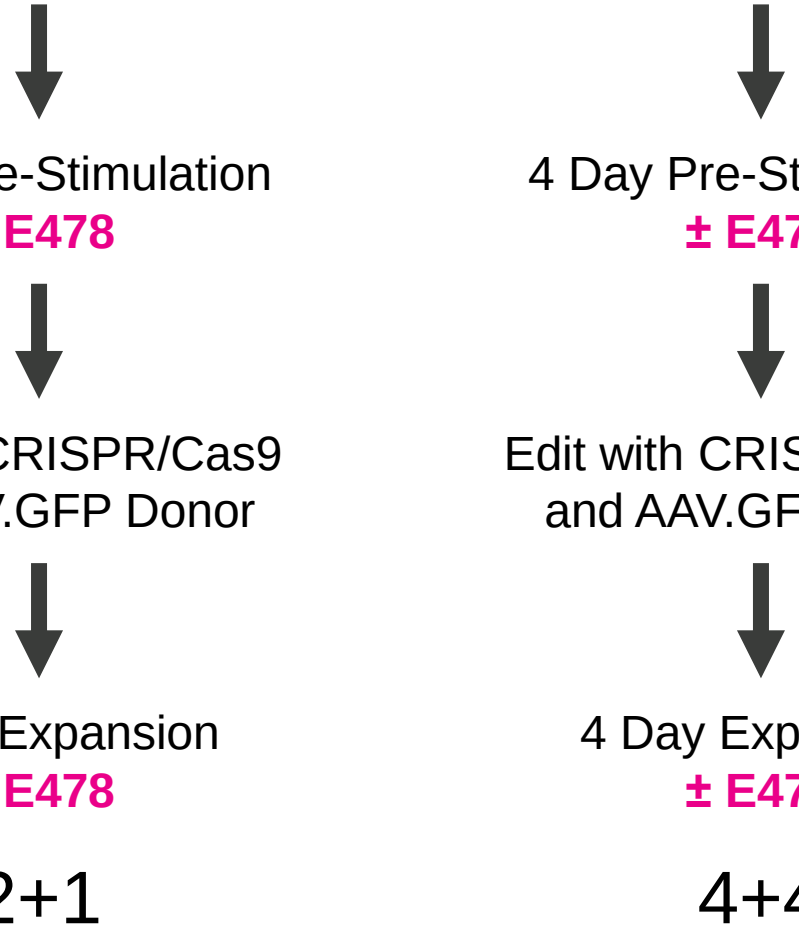


n=8 mice

Magenta Therapeutics

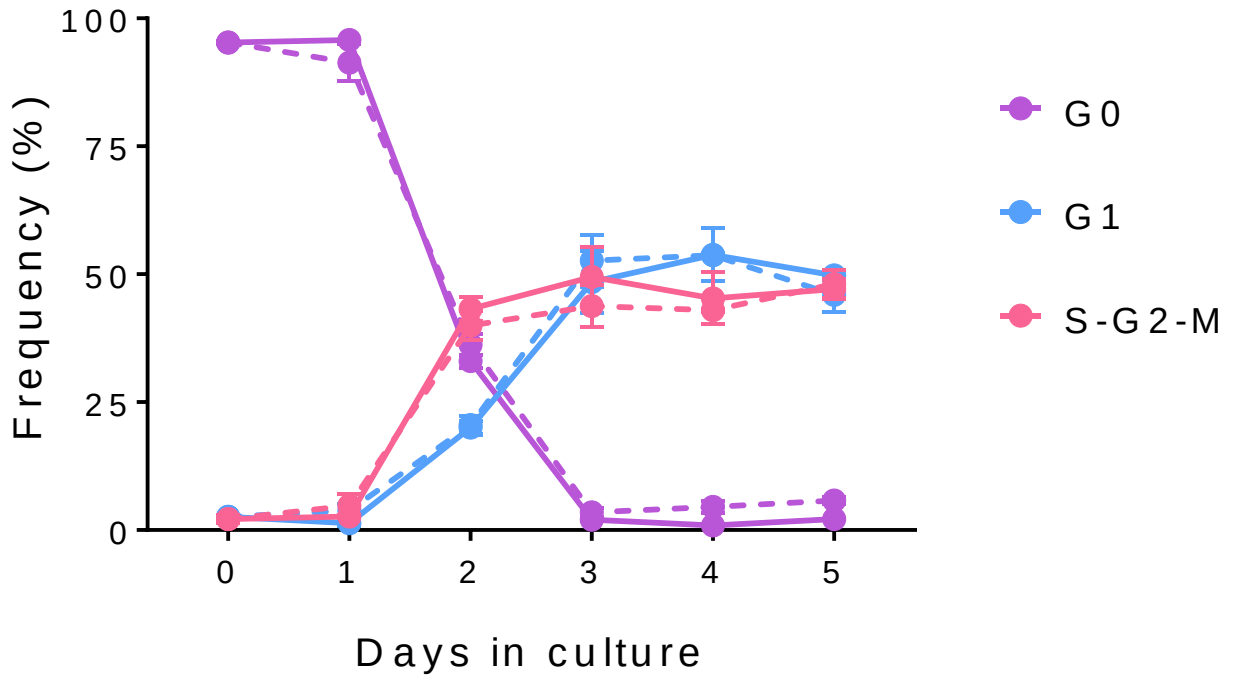
Does E478 Expand The Number of Gene-Corrected HSCs?

Experimental Design with Mobilized Peripheral Blood CD34+ Cells



All CD34+CD90+ Cells Are Actively Cycling After Three Days in Culture

CELL CYCLE PROFILE OF CD34+CD90+ MOBILIZED BLOOD CELLS

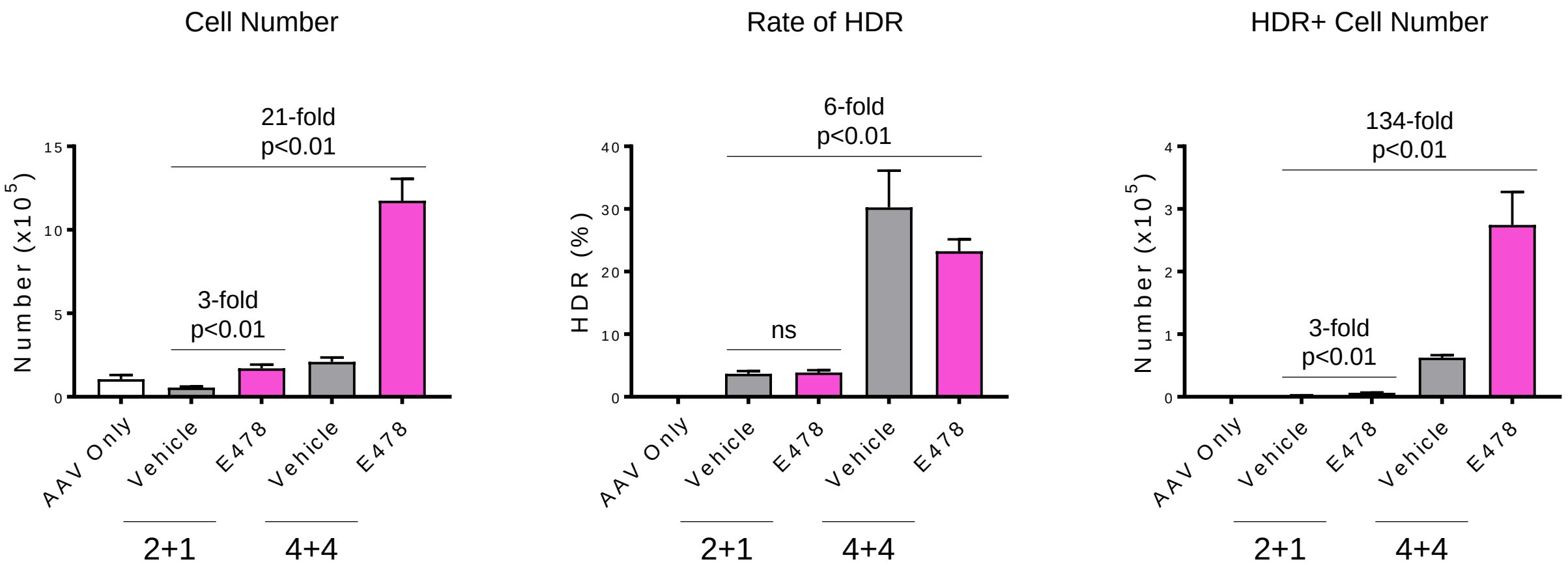


DMSO Vehicle: dashed line
E478: solid line

n=2 cell donors

Expansion with E478 Leads to Higher Rates of HDR and Numbers of HDR+ CD34+CD90+ Cells Compared to Conventional Approaches

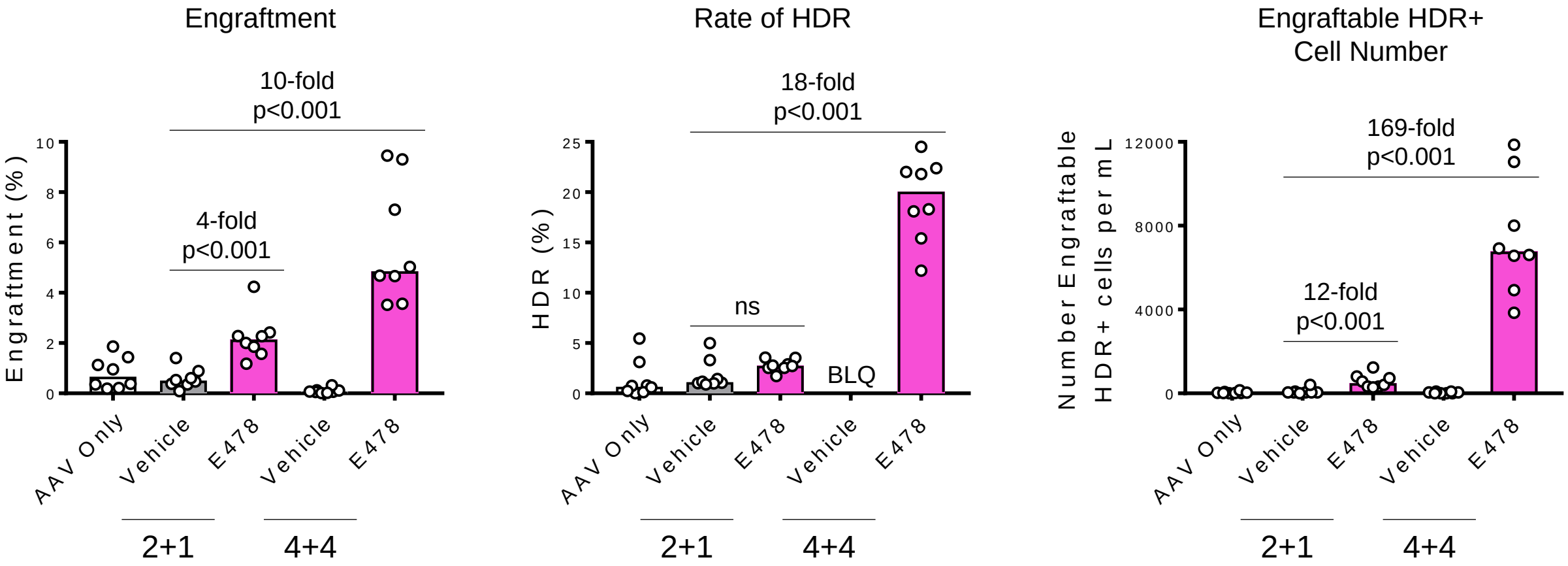
IN VITRO EXPANSION OF CD34+CD90+ CELLS



n=2

Expansion with E478 Leads to Higher Rates of HDR and Numbers of HDR+ NSG-Engrafting Cells Compared to Conventional Approaches

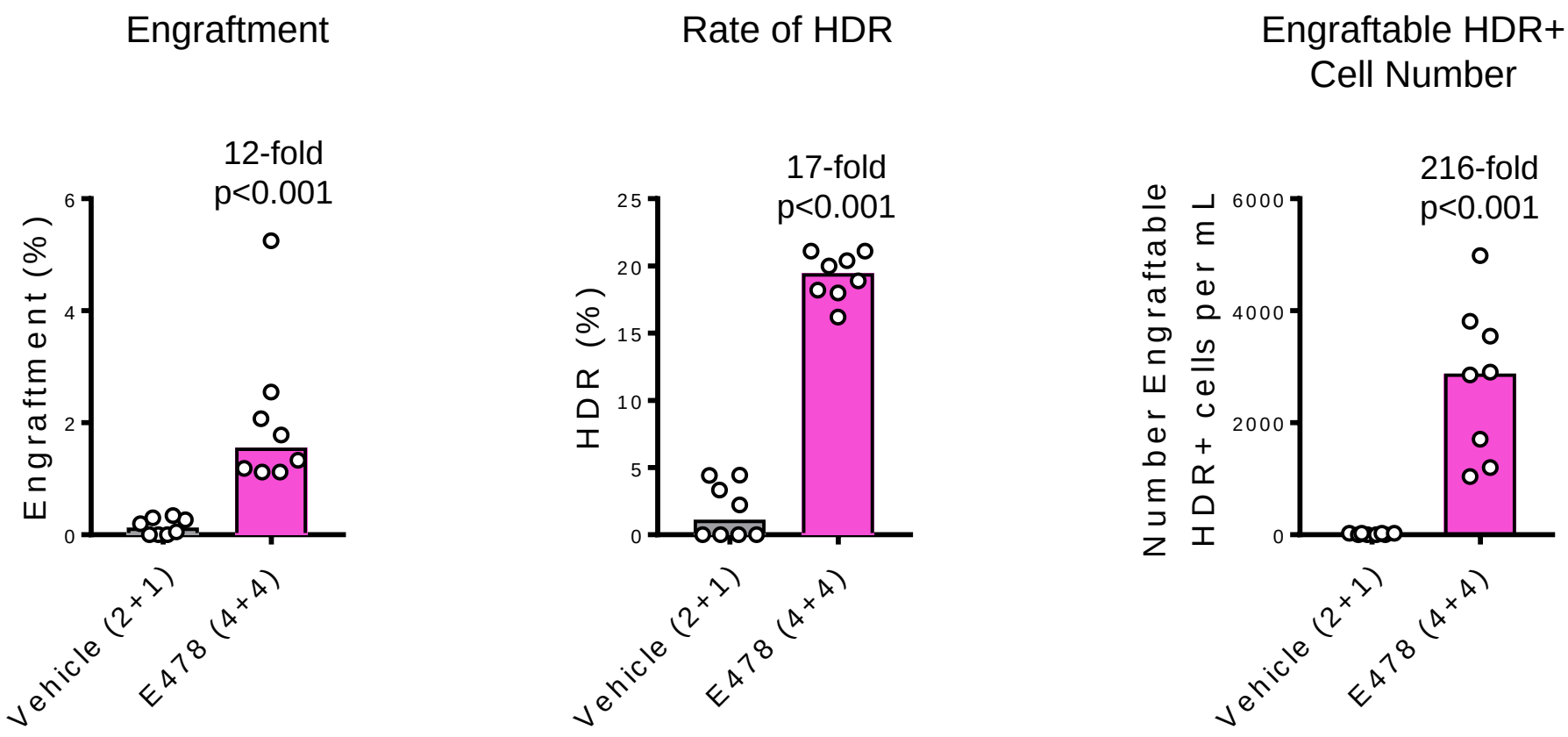
WEEK 16 NSG ENGRAFTMENT



n=8 mice

Expansion with E478 Leads to High Rates of HDR and Numbers of HDR+ NSG-Engrafting Cells in Secondary Recipients

WEEK 16 NSG ENGRAFTMENT



E478 Increases the Dose of Gene-Modified HSCs

- Increased CD34+ cell dose leads to improved engraftment and gene therapy outcomes
- AHR antagonism allows for robust expansion of HSCs by blocking differentiation
- MGTA-456 provides clinical validation for expansion of HSCs via AHR antagonism
- E478 is a novel, potent AHR antagonist capable of expanding all human HSC sources: mobilized blood, bone marrow, and cord blood
- Compared to conventional approaches, *ex vivo* expansion with E478:
  1. Results in significantly higher engraftment of lentiviral vector-transduced cells by 7-fold
  2. Results in higher engraftment of CRISPR-Cas9 knockout cells by 12-fold
  3. Enables 18-fold higher rates of HDR, a high dose of HDR+ HSCs, and a ~200-fold increase in the engraftment of HDR+ HSCs

Acknowledgments

Magenta R&D Team

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