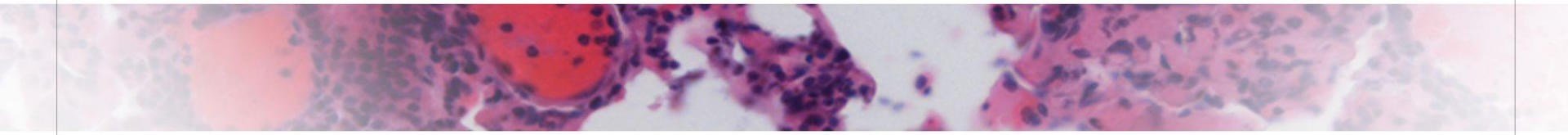
American Society of Hematology
Helping hematologists conquer blood diseases worldwide



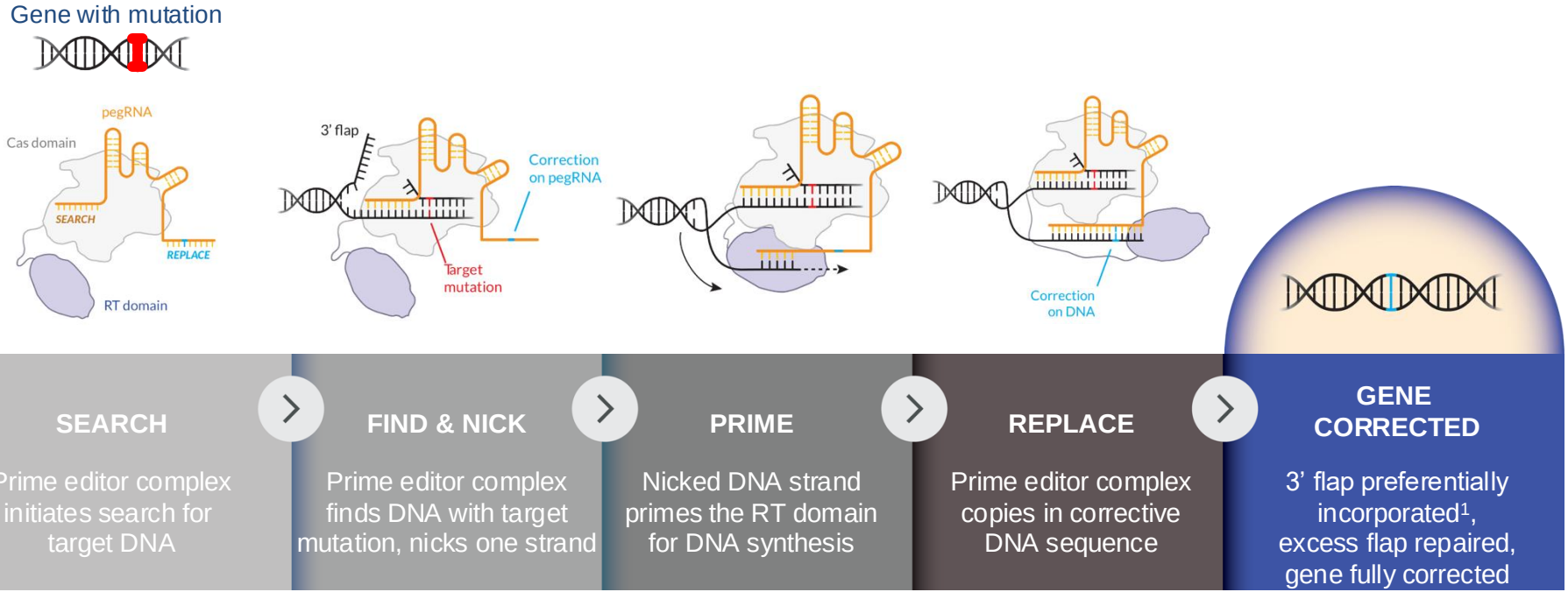
Prime Editing enables precise and efficient single amino acid substitutions to shield CD34+ hematopoietic stem cells from anti-CD117 antibody-based conditioning

prime medicine

CIMEIO

Prime Editing is programmable for both search and replace

Prime Editing can directly write new genetic information into a targeted DNA site without requiring a DSB



pegRNA = Prime Editing guide RNA; RT = reverse transcriptase; Cas = CRISPR associated protein; DSB = Double-stranded break

Preparative ‘Conditioning’ in HSCT is required to create space for engraftment of transplanted CD34+ cells

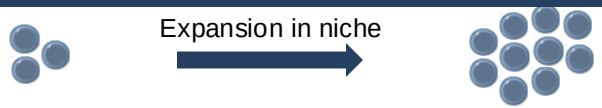
prime medicine

Myeloid Conditioning is required for successful engraftment of incoming CD34+cells

- Clearance of bone marrow niche space
- Deplete resident hematopoietic stem cells (HSC)

1. ABLATION OF BLOOD CELLS:
Kills dividing cells & create space in bone marrow

2. ENGRAFTMENT: Incoming CD34+ cells (allogeneic or gene edited autologous) have space to engraft & repopulate bone marrow



3. LONG TERM BLOOD RECONSTITUTION with grafted cells is impacted by initial conditioning

Non-selective Conditioning has unintended toxicity to patients

Chemotherapy and radiation may carry risks to patients:

- Infertility
- Risk of complications due to protracted pancytopenia
- Off -target organ toxicity (e.g. mucositis, pulmonary toxicity)

Selective antibody-based conditioning targets hematopoietic cells and may improve patient access and/or outcomes from HSCT

- Reduced off-target organ toxicity
- Reduced risk of graft failure / need for re-transplant: shielding edit plus CD117 Ab Tx post HSCT to increase chimerism
- Reduced Cost: Reduced length-of-stay + supportive care

Ab = Antibody; Tx = treatment; HSCT = Hematopoietic stem cell transplantation

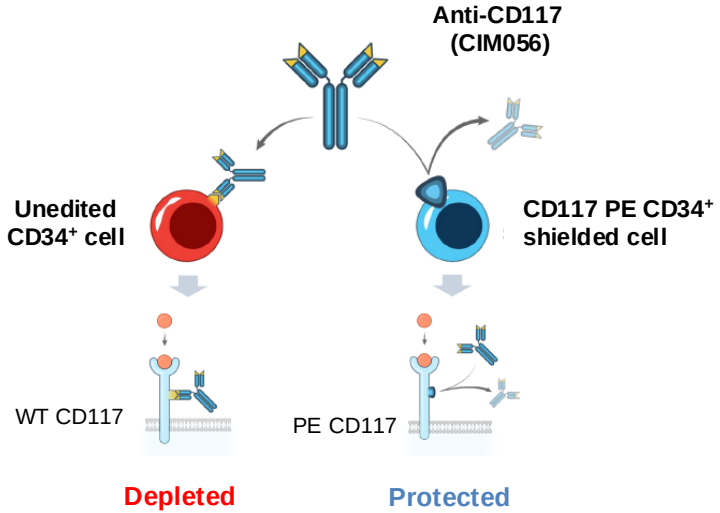
Cimeio CD117 antibody (CIM056) for reduced toxicity of conditioning and cell shielding of HSC

CD117 is a receptor that is critical for the survival and proliferation of hematopoietic stem cells (HSC)

Prime Editor designed to precisely install CD117 shielding mutation in CD34+ cells

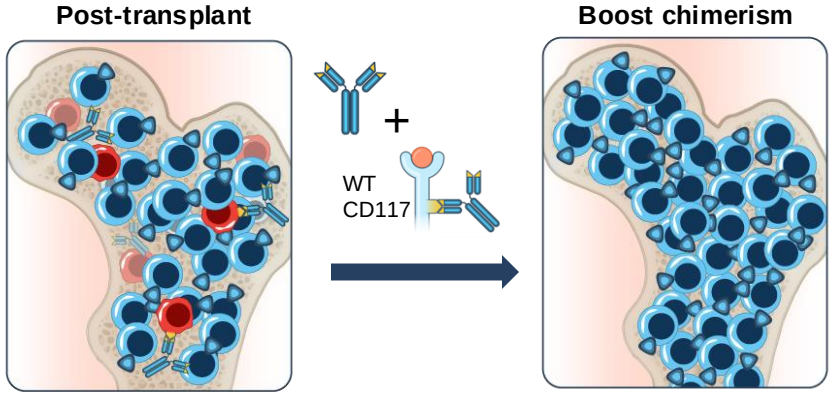
Cimeio CD117 antibody engineered to deplete HSCs that do not contain shielding mutation

Selective depletion of unedited cells



Shielding uses an antibody to boost proportion of edited HSCs by depleting unedited cells to increase effective editing

Targeted conditioning of WT HSCs

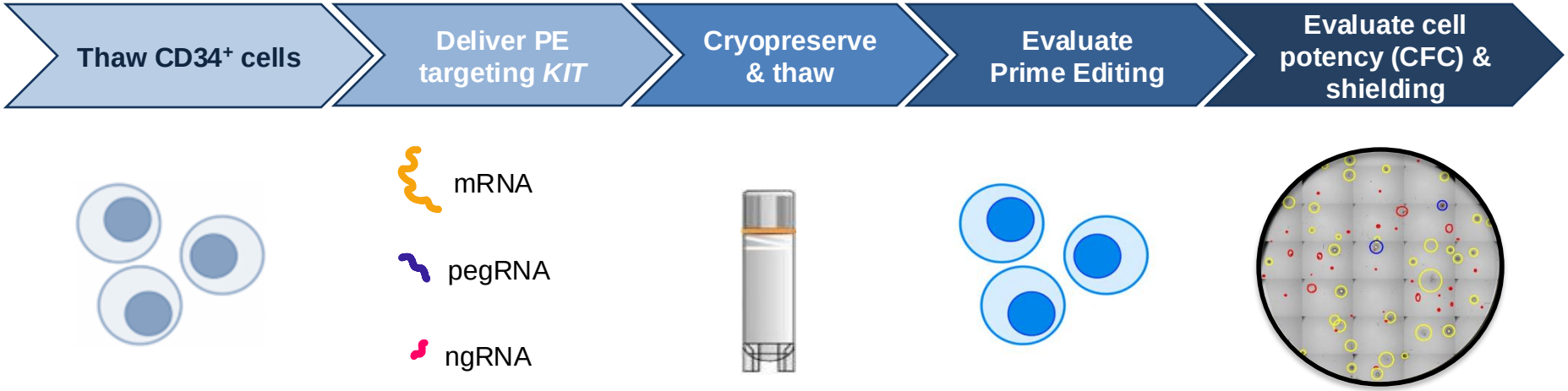


- Anti-CD117 to **selectively deplete WT HSC** prior to HSCT
- Shielding protects Prime Edited (PE) cells from Ab depletion
- Anti-CD117 post HSCT to **selectively deplete unedited cells** in marrow creating space for expansion of PE HSC to **boost chimerism**

PE = Prime Editor or Prime Edited; WT = wild type; HSCT = Hematopoietic stem cell transplantation

Research and Development workstream for shielding Prime Editors in CD34+ cells ex vivo

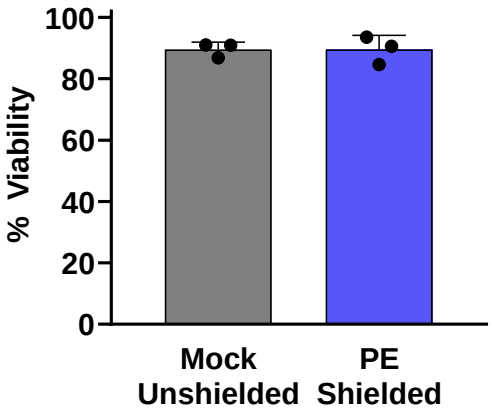
Assessment of cell health, function, editing efficiency, & cell shielding



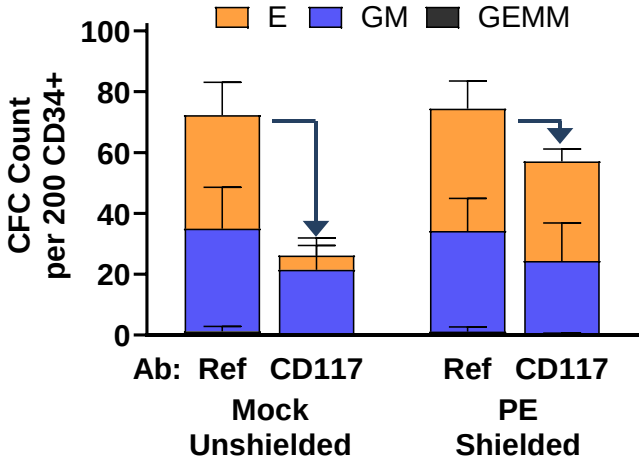
PE = Prime Editor or Prime Edited; mRNA = messenger ribonucleic acid; pegRNA = Prime Editor guide RNA; ngRNA = nicking guide RNA; CFC = colony forming cell

Prime Editor shields CD34+ cells from CD117 Ab with no impact on cell viability or potency ex vivo

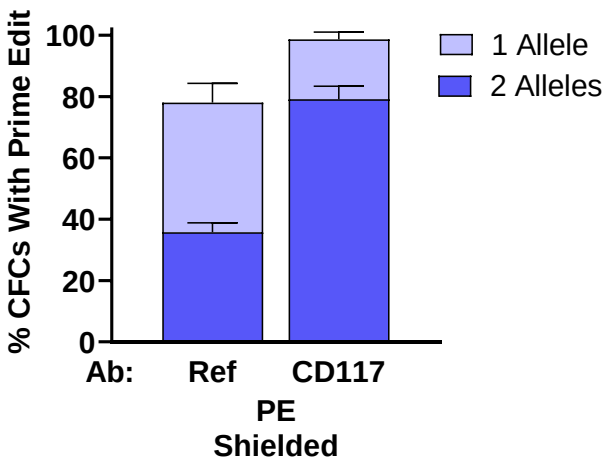
PE does not impact cell viability



CD117 Ab depletes unedited cells, PE cell potency maintained



CD117 Ab enriches PE cells with biallelic edits



- 78 ± 6 % of PE-treated CD34+ cell clones carry a shielding edit; no unintended edits detected (n=3 donors)
- Approximately half of PE-treated CD34+ cell clones with a shielding edit have biallelic editing
- Shielded cells are enriched in the presence of CIM056 (CD117 Ab)

PE = Prime Editor or Prime Edited; Ab = Antibody; Ref = Reference non-CD117 antibody; E = Erythroid; GM = Granulocyte/Macrophage; GEMM = Multilineage

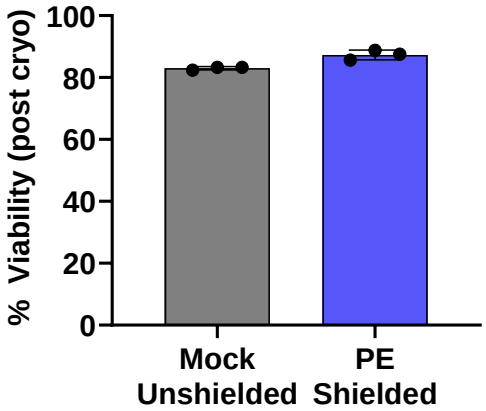
Clinical scale production of >70% Prime Edited CD34+ cells with no impact on cell viability or multipotency

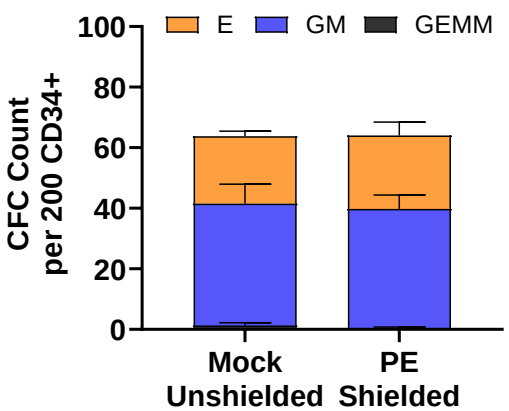
Half of PE CD34+ cell clones have biallelic shielding edit at *KIT* (CD117) gene

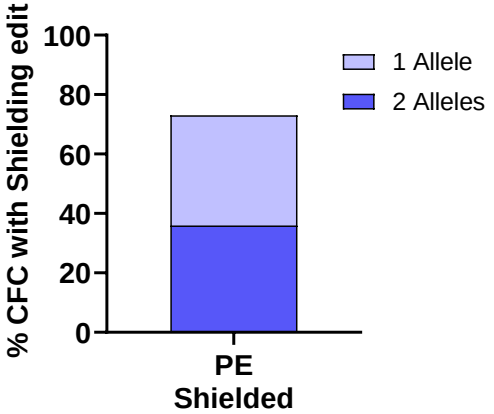
Clinical Scale Process does not impact cell viability

PE CD34+ cell potency maintained

73% of CD34+ cell clones carry Prime Edit; 50% of which have biallelic edits
No unintended edits detected

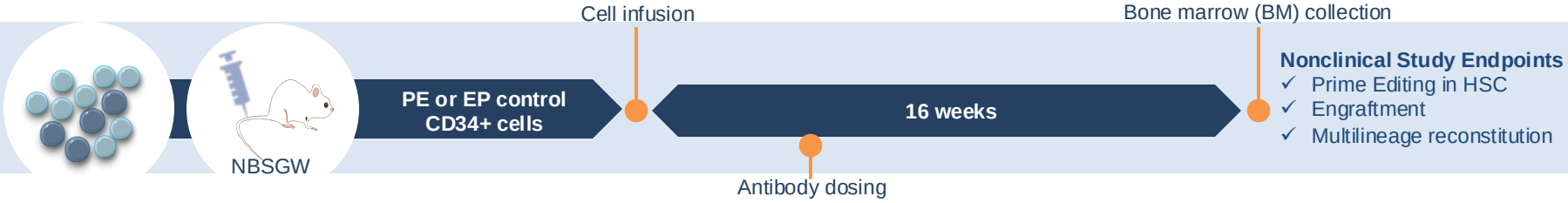




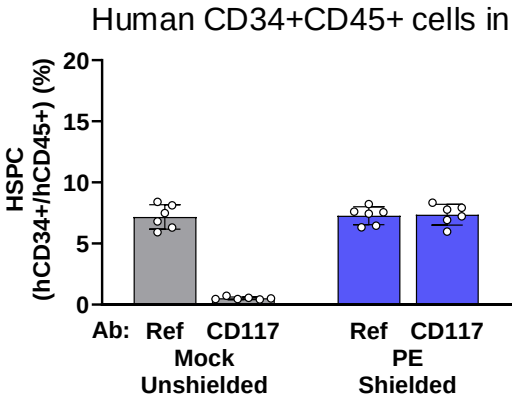


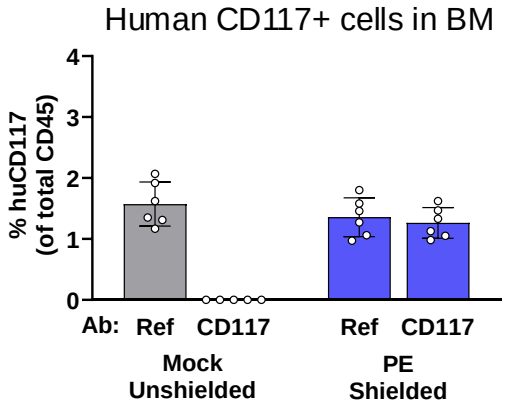
PE: Prime Editor or Prime Edited; ; E = Erythroid; GM = Granulocyte/Macrophage; GEMM = Multilineage

Prime Edited hematopoietic stem progenitor cells (HSPC) are shielded from CD117 Ab mediated depletion in vivo



Bone marrow engrafted PE HSPC are shielded vs. unedited HSC are depleted by Ab treatment





PE = Prime Edited; Ab = Antibody; HSPC = Hematopoietic stem and progenitor cells; Ref = Reference non-CD117 antibody

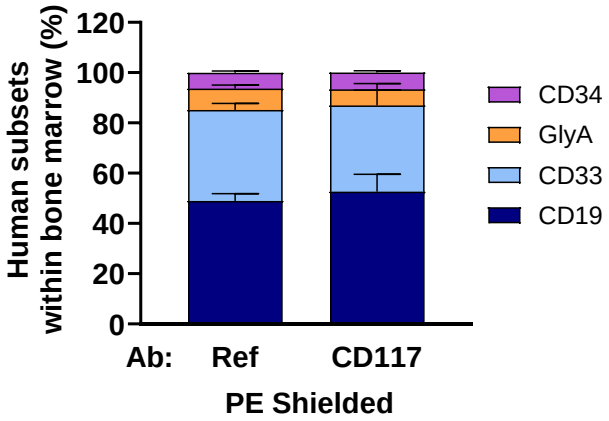
In vivo shielding boosts PE CD34+ cells in bone marrow to 100% with 80% of cells carrying biallelic edits

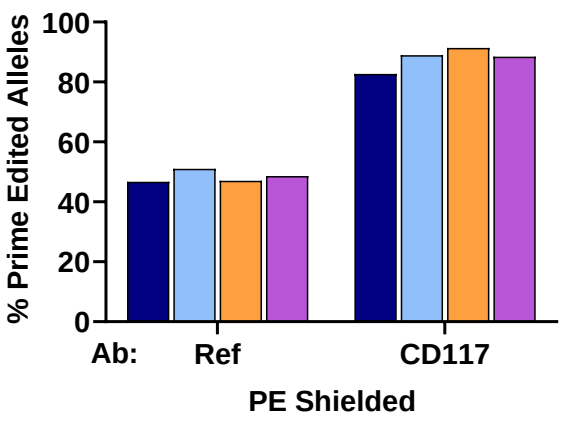
% of CD34+ cell clones with biallelic edit increases to 80% after CD117 Ab Tx

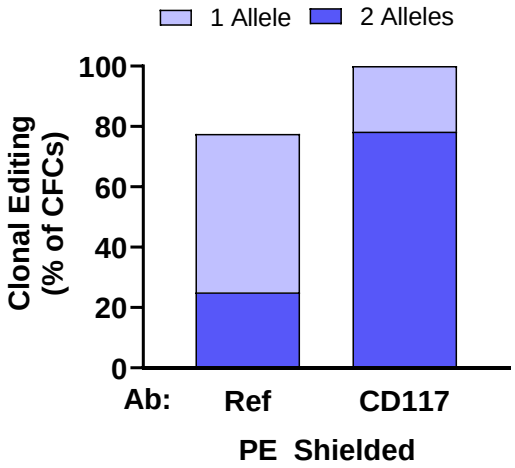
Comparable Bone Marrow reconstitution in both antibody treatment groups

Shielded HSCs are enriched and maintain ability to repopulate erythroid, myeloid, & lymphoid lineages

100% of BM engrafted HSC carry Prime Edit after in vivo selection





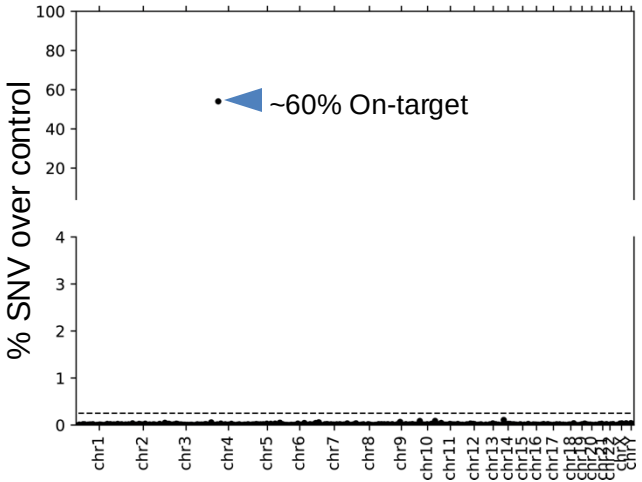


Ab = Antibody; Tx = Treatment; PE = Prime Editor or Prime Edited; HSC = hematopoietic stem cell; CFC = colony forming cell; Ref = Reference non-CD117 antibody

No unintended edits, off-target editing, or chromosomal rearrangements detected in PE CD34+ cells

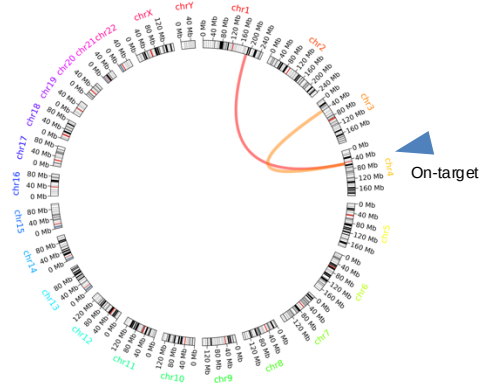
Three orthogonal methods used to determine presence of Off Target Editing

No detectable off-Target edits

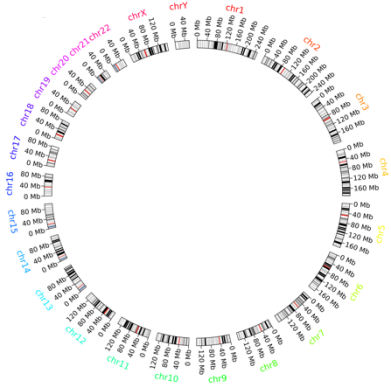


No Chromosomal Rearrangements Detectable

Positive Control
Shielding gRNAs + RNF2 + spCas9

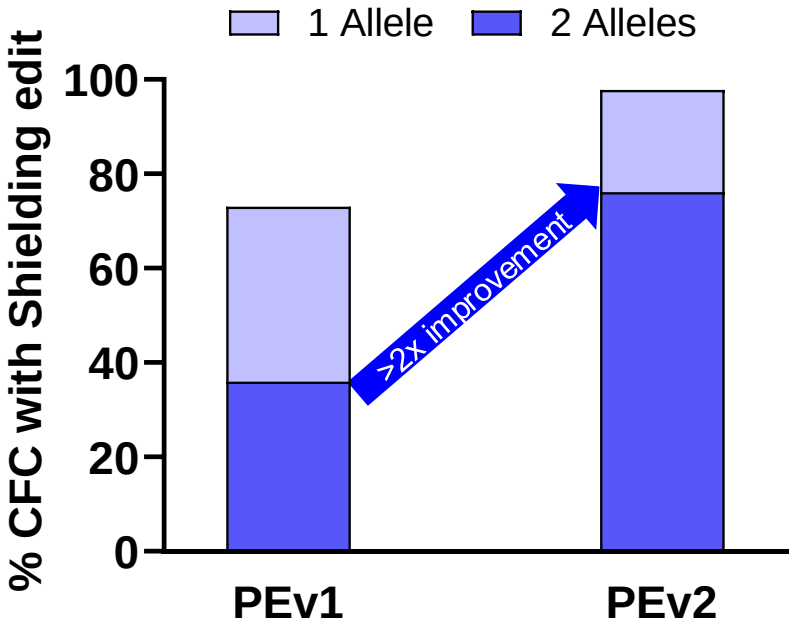


Test Article
Shielded PE CD34+ cells



SNV = Single Nucleotide variation; gRNA = guide RNA; PE = Prime Editor or Prime Edited; RNF2 = Ring Finger Protein 2

Optimized PEv2 Prime Editor increases PE efficiency to 100% with 80% of cells carrying biallelic shielding edits without CD117 Ab treatment

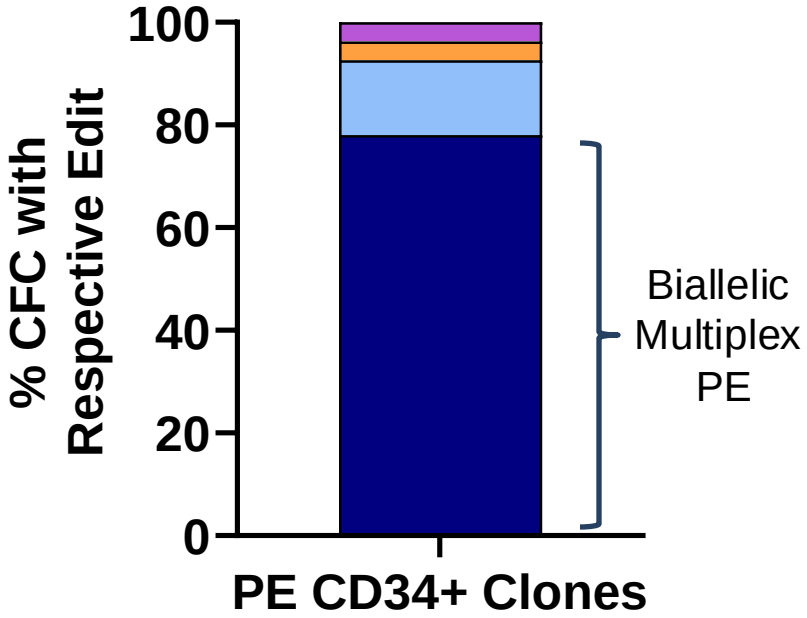


*PEv1 shown in preceding studies

PEv1 = Prime Editor version 1; PEv2 = Prime Editor version 2

~80% biallelic Multiplex Prime Editing efficiency with i) disease correcting Prime Editor and ii) shielding Prime Editor v2

CD117 Shielding Edit and a second Prime Edit at a clinically relevant target site after single-step electroporation



Single Edit Outcomes:

Therapeutic Edit

Shield

Multiplex Outcomes:

Monoallelic Shield + Therapeutic Edit

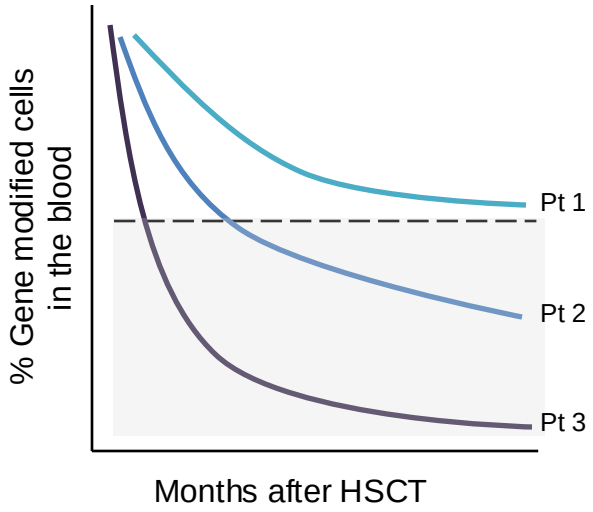
Biallelic Shield + Therapeutic Edit

PE Prime Editor or Prime Edited; PEv2 = Prime Editor version 2

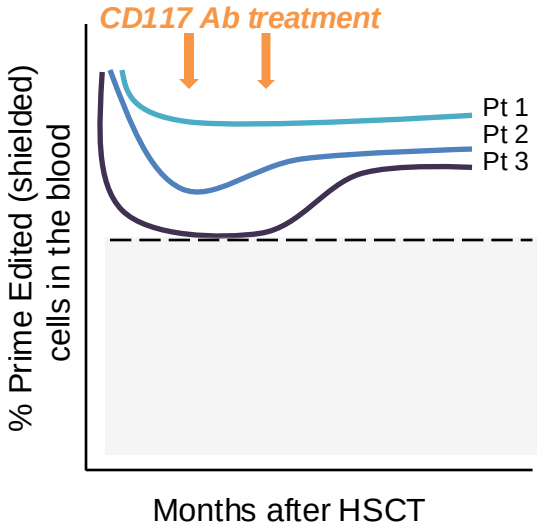
Shielding has potential to provide a measure of control to physician over HSCT outcome following engraftment

ILLUSTRATION: Shielding potential to increase engraftment in patients post PE CD34+ cell infusion

No Shielding = No Opportunity to increase chimerism post HSCT



Shielding Prime Edit + CD117 Ab = Opportunity to increase chimerism post HSCT



Threshold for clinical benefit

No/minimal clinical benefit

Ab = Antibody; HSCT = Hematopoietic stem cell transplantation

Summary

- **Clinical scale production of Prime Edited CD34+ cells** with a Prime Editor showed no observable impact on cell viability, or multipotency, with >70% cells carrying the shielding edit
- **In vivo, shielding with anti-CD117 Antibody boosts Prime Edited CD34+ cells to 100%** with ~80% of cells carrying biallelic edits
- **No off-target editing**, chromosomal rearrangements, or unintended edits were observed in shielded CD34+ cells
- **Optimized Prime Editor Increased PE efficiency to 100%** with 80% of cells carrying biallelic edits without anti-CD117 antibody treatment
- **~80% biallelic Multiplex Prime Editing efficiency** was observed with i) disease correcting Prime Editor multiplexed with ii) shielding Prime Editor