Paired HSC Epitope Engineering of CD117 (cKIT) for Antibody-Mediated Autologous Hematopoietic Stem Cell Therapy Conditioning for The Potential Treatment of Hemoglobinopathies

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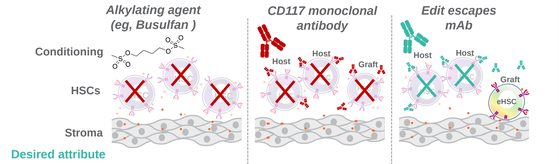
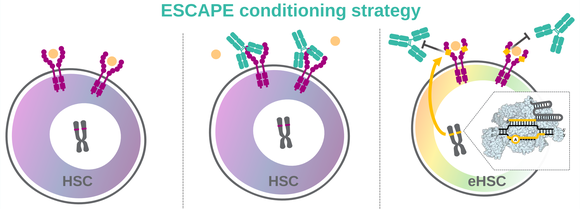
Introduction

- Busulfan and other chemotherapeutic agents are currently used for myeloablative conditioning prior to autologous hematopoietic stem cell transplant (HSCT).
- Busulfan is genotoxic with undesired morbidities (e.g. does not preserve fertility) and is non-specific and not re-dosable.
- To overcome this unmet need, we are developing Engineered Stem Cell Antibody Paired Evasion (ESCAPE), a non-genotoxic conditioning approach where we pair an engineered HSC (eHSC) expressing a modified CD117 epitope with a wild type (WT) CD117-targeted mAb for selectively depleting WT, disease harboring stem cells
- The ESCAPE1 therapeutic edit causes upregulation of HbF, via *HBG1/2* promoter base editing



Desired attribute	Alkylating agent (eg, Busulfan)	CD117 monoclonal antibody	Edit escapes mAb
HSC-specific	X	✓	✓
Non-genotoxic	X	✓	✓
Low cancer risk	X	✓	✓
Preserve fertility	X	✓	✓
Long half-life	X	X	✓
Re-dosable	?	X	✓

ESCAPE conditioning strategy

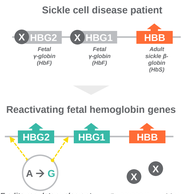


Unedited CD34 cell
No CD117 mAb
Normal signaling
Cell Survives

Unedited CD34 cell
CD117 mAb displaces SCF
Blocked signaling
Cell Dies

Edited CD34 cell
Escapes CD117 mAb
Normal signaling
Cell survives

Therapeutic edit for ESCAPE1



Key attributes of ESCAPE strategy

High efficiency multiplex editing of both CD117 and HBG1/2

Engineered CD117 epitope must preserve WT CD117 function

Engineered epitope should abrogate binding of anti-WT CD117 mAbs

Successful high efficiency multiplex editing in human HSPCs

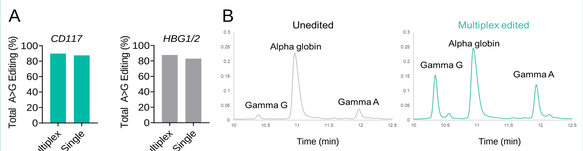


Figure 1. (A) NGS results showing total on-target A>G editing was comparable between single- and multiplex edited CD34+ cells **(B)** Multiplex base editing of *CD117* target with *HBG1/2* site results in >55% γ-globin induction.

ESCAPE1 editing preserves WT CD117 receptor function *in vitro*

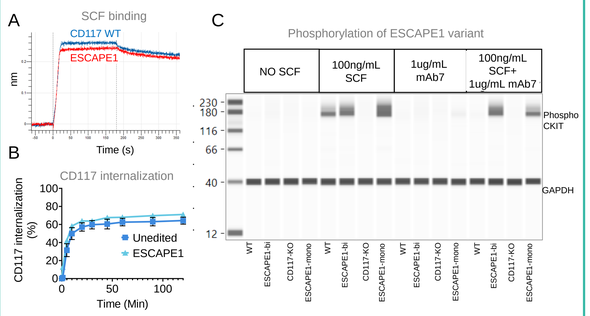


Figure 2. (A) Biolayer Interferometry (BLI) demonstrating comparable binding of wild type and ESCAPE1-edited CD117 proteins to SCF **(B)** Unedited and ESCAPE1-edited CD117 showed similar internalization upon SCF stimulation as measured by surface availability of CD117 **(C)** Unedited and ESCAPE1 edited CD117 exhibit competent induction of phosphorylation when stimulated by SCF. Phosphorylation of unedited protein could be blocked by SCF blocking mAb.

ESCAPE1 HSPCs retain normal myeloid and erythroid differentiation *in vitro*

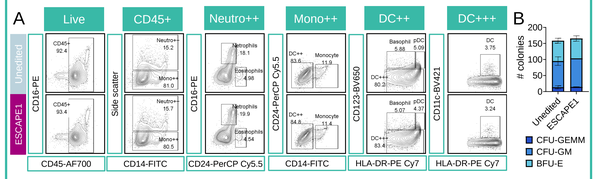


Figure 3. (A) Flow cytometry of different myeloid cells generated via *in vitro* differentiation. Unedited and ESCAPE1 HSPCs show equivalent myeloid differentiation potential to generate neutrophils, monocytes, Eosinophils, Basophils, Dendritic cells, and pDCs *in vitro.* **(B)** CFU assays demonstrate that ESCAPE1 HSPCs had similar colony forming efficiency and comparable outputs of CFU-GEMM (myeloid+ erythroid), CFU-GM (myeloid), and BFU-E (erythroid) colonies.

CD117 epitope engineering prevents anti-CD117 mAb binding

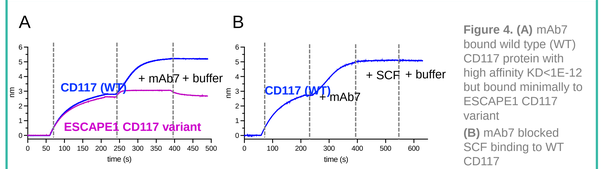


Figure 4. (A) mAb7 bound wild type (WT) CD117 protein with high affinity KD<1E-12 but bound minimally to ESCAPE1 CD117 variant
(B) mAb7 blocked SCF binding to WT CD117

ESCAPE1 edited HSPCs evade anti-CD117 mAb mediated cell depletion *in vitro*

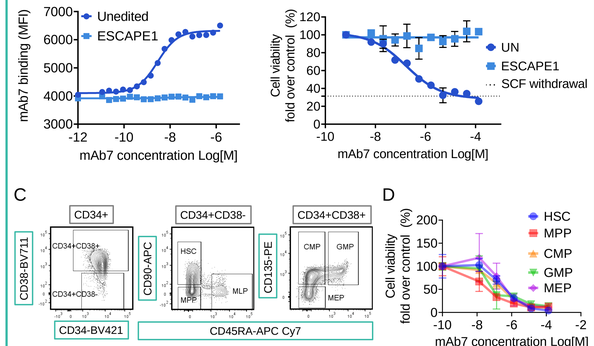


Figure 5. (A) mAb7 bound with high affinity to unedited CD34+ HSPCs but did not recognize ESCAPE1 edited HSPCs **(B)** mAb7 selectively depleted unedited HSPCs while ESCAPE1 HSPCs retained viability **(C)** Gating and sorting strategy for LT-HSC, MPP, CMP, GMP, and MEP subpopulations based on cell surface marker expression **(D)** LT-HSC and other progenitor subpopulations exhibited similar sensitivity to mAb7 treatment *in vitro.*

mAb7 treatment induced HSPC apoptosis *in vitro*

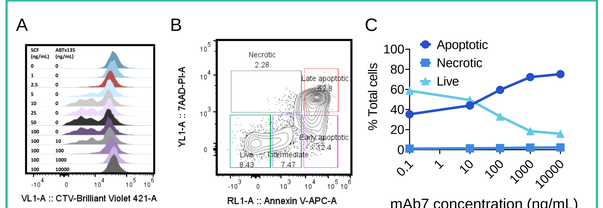
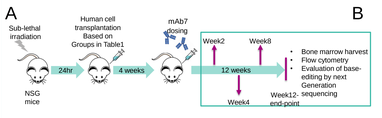


Figure 6. (A) mAb7 treatment mimicked SCF withdrawal and led to proliferation arrest in CD34+ HSPCs as measured by Cell trace violet dye dilution **(B)** Flow cytometry contour plot showing gating strategy to identify live and apoptotic cells **(C)** mAb7 treatment led to dose-dependent apoptosis in unedited HSPCs.

Anti-CD117 mAb selectively depleted unedited HSPCs *in vivo*



Group	Cells (1e6/mouse)	mAb treatment
A	Unedited : unedited	None
B	Unedited : unedited	Isotype
C	Unedited : unedited	mAb7
D	Unedited : multiplex edited	Isotype
E	Unedited : multiplex edited	mAb7

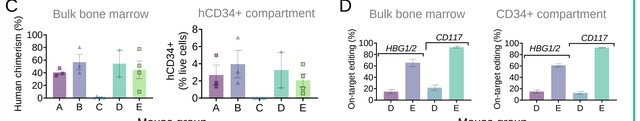
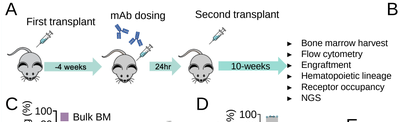


Figure 7. (A) *In vivo* study design **(B)** Transplant and dosing groups **(C)** Marked reduction in human chimerism at 12 weeks post mAb dosing in both bulk bone marrow (*Left*) and human CD34+ HSPC compartment *(Right).* **(D)** Marked enrichment of *CD117* and *HBG1/2*-editing within the bulk bone marrow (*Left*) and sorted CD34+ cells (*Right*) of the mAb7 treatment group compared with the isotype control group.

Engraftment of ESCAPE1 edited HSPCs post anti-CD117 mAb-based conditioning



Group	First transplant ("Host")	Second transplant ("Graft")	mAb treatment
1	Unedited	CD117/HBG1/2-Multiplex	Isotype
2	Unedited	CD117/HBG1/2-Multiplex	mAb7

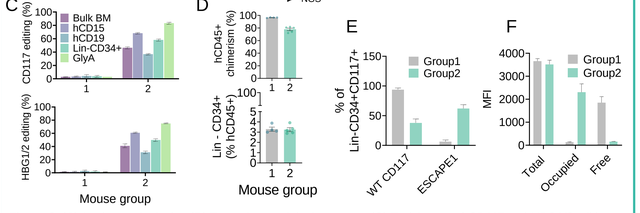


Figure 8. (A) *In vivo* study design **(B)** Transplant and dosing groups **(C)** Enrichment of *CD117* and *HBG1/2* editing in group 2 mice indicating engraftment of multiplex edited cells in mice treated with mAb7. Isotype control group showed absence of marrow editing **(D)** High human chimerism in mAb7 treated group that received a second transplant of multiplex edited cells, in bulk bone marrow (*Top*) and human Lin-CD34+ (*Bottom*) compartment **(E)** Depletion of HPSCs expressing WT-CD117 and enrichment of ESCAPE1 HSPCs in bone marrow **(F)** Receptor occupancy assay showing occupied receptors on unedited human HSPCs in mouse bone marrow, post mAb treatment.

Conclusions

- Non-Genotoxic conditioning continues to be a major unmet need in HSCT-based gene therapy for hemoglobinopathies.
- We have developed a base-edited antigen:antibody pair that enables edited cells to ESCAPE binding of a mAb that can bind and deplete unedited (WT) HSPCs both *in vitro* and *in vivo.*
- We achieved high level of multiplex editing for ESCAPE1 eHSCs.
- Our ESCAPE1 *CD117* edit preserves WT CD117 receptor biology.
- Our anti-CD117 mAb, mAb7, selectively depletes unedited HSPCs while sparing ESCAPE HSPCs both *in vitro* and *in vivo.*
- We demonstrate proof of concept that mAb7 can potentially be used as a conditioning agent that leads to engraftment of multiplex edited HSPCs.
- Collectively, our ESCAPE strategy present a promising new paradigm for autologous stem cell therapies in treatment of hemoglobinopathies including sickle cell disease.

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