Engineered Stem Cell Antibody Paired Evasion 1 (ESCAPE-1): Paired HSC epitope engineering and upregulation of fetal hemoglobin for antibody-mediated autologous hematopoietic stem cell therapy conditioning for the potential treatment of hemoglobinopathies

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Introduction

A major hurdle to the successful application of autologous hematopoietic stem cell transplant (HSCT) gene therapy, a potential treatment for a variety of hemoglobinopathies including sickle cell disease (SCD), is risk of severe adverse effects associated with use of the chemotherapeutic drug busulfan for pre-transplant myeloablative conditioning. Aiming to overcome this hurdle, we are developing a non-genotoxic investigational therapy for hemoglobinopathies that combines multi-plex base edited engineered HSCs (eHSCs) with antibody-based conditioning.

Our engineered stem cell antibody paired evasion (ESCAPE) strategy (Figure 1) consists of a multiplex base-edited eHSC that includes, a therapeutic edit at the promoter region *of HBG1/2*, and a missense mutation in the extracellular domain of CD117 (cKIT), a receptor tyrosine kinase expressed by hematopoietic stem and progenitor cells (HSPCs) that regulates HSPC survival, proliferation, and differentiation.

Base edited CD117 epitope enables engineered HSCs to selectively ESCAPE antibody binding

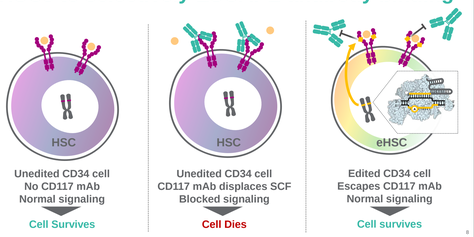


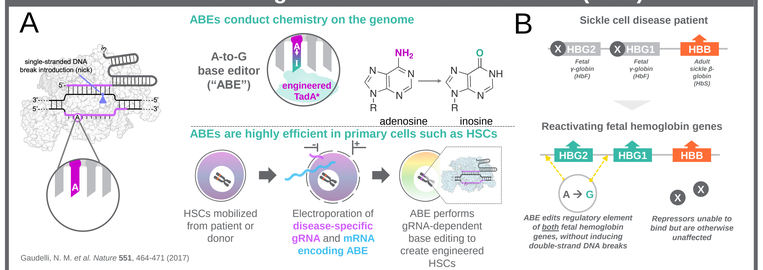
Figure 1. ESCAPE strategy

Here we report the use of multi-plex editing of CD34+ cells to generate eHSCs that contain mutations within the *HBG1/2* and *CD117* genes. While *HBG1/2* mutation leads to upregulation of fetal globin; *CD117* edit causes amino acid substitutions within *CD117* ectodomain, which in turn, lead to loss of binding by our conditioning monoclonal antibody (mAb). Our conditioning mAb is designed to orthogonally bind and eliminate endogenous, unedited HSCs, but not recognize, and thereby spare, our ESCAPE eHSCs with *CD117* mutations.

Our lead anti-CD117 mAb, mAb-7, bound with high affinity to unedited HSPCs but not to *CD117*-edited cells (Figure 4). mAb-7 binding to CD117 blocked SCF-CD117 interaction (Figure 4) thereby selectively suppressing survival of unedited HSPCs (Figure 5, 6). Treatment of HSPCs with mAb-7 *in vitro* resulted in >85% reduction in viability of unedited HSPCs, while *CD117*-edited cells remained unaffected (Figure 5, 6). Since CD117 signaling has been linked with mast cell degranulation, we evaluated effects of native and Fc-engineered versions of mAb-7 on *in vitro* culture-differentiated mast cells. The Fc-engineered versions of mAb-7 did not lead to any level of mast cell degranulation *in vitro* (Figure 6). We compared phosphorylation of our modified CD117 to wild type CD117 (Figure 7) *in vitro.* Our CD117 variant protein bound normally to SCF and underwent similar levels of phosphorylation (compared to WT protein) after SCF binding. We achieved ~80% bi-allelic *CD117* editing and near complete editing of the *HBG1/2* locus in HSPCs (Figure 8). In xenotransplantation studies, we observed that *CD117*-edited HSPCs were capable of long-term multi-lineage hematopoietic engraftment in immunocompromised mice (Figure 9). Importantly, mAb-7 treatment led to significant reduction of human chimerism as well as bone marrow CD34+ cell frequency in mice humanized with only unedited human CD34+ cells. Interestingly, in mice receiving edited:unedited mixture, mAb-7 led to enrichment of *CD117*-edited cells both in whole bone marrow and in the CD34+ cell compartment, as indicated by high editing levels in these compartments (Figure 10).

Together, our ESCAPE strategy may enable less toxic pre-transplant conditioning for autologous HSC-based SCD therapies, while minimizing treatment-related toxicities that arise from current busulfan-based conditioning.

Genome Editing with Adenine Base Editors (ABE)



Gaudelli, N. M. *et al. Nature* **551**, 464-471 (2017)

Figure 2. A. ABE is a fusion protein comprising an evolved TadA* deaminase (teal) connected to a CRISPR-Cas9 nickase (grey). The base editor binds to a target sequence that is complementary to the guide-RNA (magenta) and exposes a stretch of single-stranded DNA. The deaminase converts the target adenine into inosine (which is read as guanine by DNA polymerases) and the Cas enzyme nicks the opposite strand. Following DNA replication and repair, an A:T to G:C conversion is made permanent (1-2).

B. Naturally-occurring single nucleotide variation within the *HBG1/2* gene are known to result in the hereditary persistence of fetal hemoglobin (HPFH) and is an effective mechanism to ameliorate the effects of sickle cell disease and beta thalassemia (3). Using base editors, we can install mutations in the promoter region of the *HBG1/2* gene, resulting in high and persistent levels of fetal hemoglobin

ABE compatible CD117 antigen engineering and antibody screening

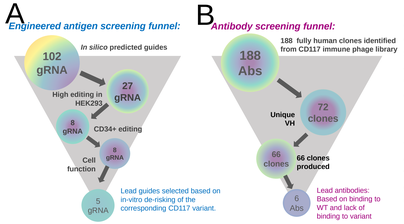
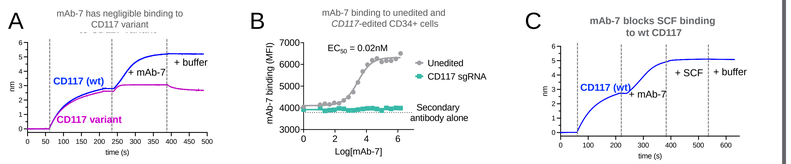


Figure 3. A. Engineered CD117-antigen screening. 102 guide RNAs capable of installing missense mutations were computationally identified. Among these, 27 gRNAs were selected based on highest editing efficiencies in HEK293 cells. 8 gRNAs were selected based on high editing efficiencies in CD34+ cells. CD117 mutations produced by these gRNAs were characterized and 5 were chosen based on retention of normal ligand binding and phosphorylation capability *in vitro*.

B. Antibody screening. 188 mAb clones were identified and of these 72 clones had unique heavy chain variable domains. 66 of these clones were screened for binding to wild type and variant CD117 proteins. 6 such antibodies were selected based on binding to wild type CD117 and lack of binding to variant CD117. One of the lead antibodies blocked CD117 binding to its natural ligand SCF and did not bind to one of the lead edits.

In vitro characterization of lead *CD117* edit and anti-CD117 mAb

Lead anti-CD117 antibody mAb-7 did not recognize the lead CD117 variant



	KD(M) Human CD117	KD(M) Cyno CD117
mAb-7	<1.0E-12	<1.0E-12

Figure 4. A. Biolayer Interferometry (BLI) showed binding of lead antibody mAb-7 to wild type (WT) CD117 (blue line) and lack of binding to lead CD117 variant (magenta line).

B. Flow cytometry showed binding of mAb-7 to unedited CD34+ cells (grey line) and lack of binding to CD34+ cells edited with *CD117* sgRNA.

C. BLI showed that mAb-7 blocked binding of stem cell factor (SCF) to wild type CD117.

mAb-7 treatment mimicked complete SCF withdrawal and enriched for *CD117*-edited cells *in vitro*

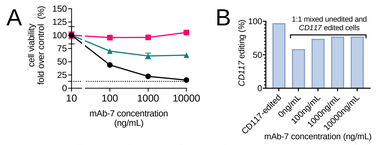


Figure 5. A. mAb-7 treatment depleted unedited cells (black line) to the level of complete SCF starvation (dotted line), while *CD117*-edited cells retained viability (magenta line). Cell viability levels for the mixed population maintained an intermediate value (green line).

B. Next generation sequencing showed enrichment of *CD117* editing upon mAb-7 treatment.

Fc-Engineering of mAb-7 generated highly potent mAb that did not produce mast cell degranulation *in vitro*

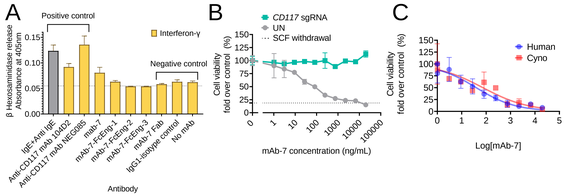


Figure 6. A. Fc-engineered mAb-7 reduced degranulation of mast cells generated via *in vitro* differentiation of CD34+ cells.

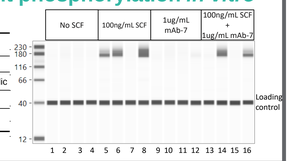
B. Fc-engineered mAb-7 selectively depleted unedited CD34+ cells (Grey line) *in vitro*, while *CD117*-edited cells retained viability.

C. Fc-engineered mAb-7 led to similar levels of cell depletion in human (blue line) and Cynomolgus (red line) CD34+ cells.

CD117 base-edited cells bound SCF and underwent phosphorylation *in vitro*

Figure 7. M07e cell lines expressing wild type CD117 underwent phosphorylation upon SCF stimulation, as did cells containing *CD117* mono-allelic and bi-allelic edits. mAb-7 inhibited phosphorylation of WT CD117. Bi-allelically edited cells underwent phosphorylation even in presence of mAb-7. mAb-7 did not completely inhibit CD117 phosphorylation in cells containing mono-allelic *CD117* edit. *CD117*-KO cells served as negative control.

Lane numbers	Cell type
1, 5, 9, 13	Wild type
2, 6, 10, 14	*CD117* sgRNA bi allelic
3, 7, 11, 15	*CD117*-KO
4, 8, 12, 16	*CD117* sgRNA monoallelic



Multiplex editing

Base editing enabled highly efficient multiplex editing of both *CD117* and *HBG1/2* in primary CD34+ HSPCs

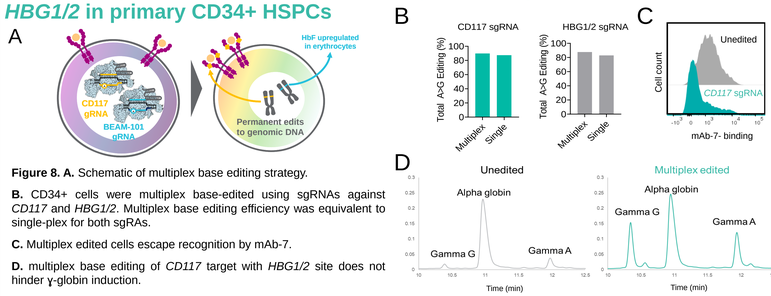


Figure 8. A. Schematic of multiplex base editing strategy.

B. CD34+ cells were multiplex base-edited using sgRNAs against *CD117* and *HBG1/2*. Multiplex base editing efficiency was equivalent to single-plex for both sgRAs.

C. Multiplex edited cells escape recognition by mAb-7.

D. multiplex base editing of *CD117* target with *HBG1/2* site does not hinder γ-globin induction.

In vivo characterization of lead *CD117* edit and anti-CD117 mAb

Base editing of *CD117* in CD34+ cells did not alter long-term engraftment and multi-lineage reconstitution in rodent model

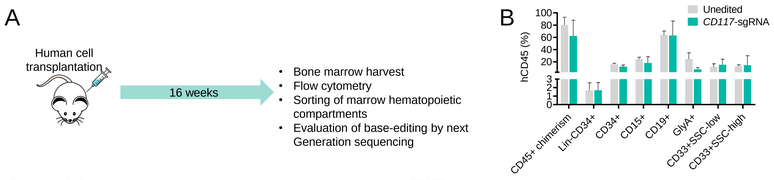
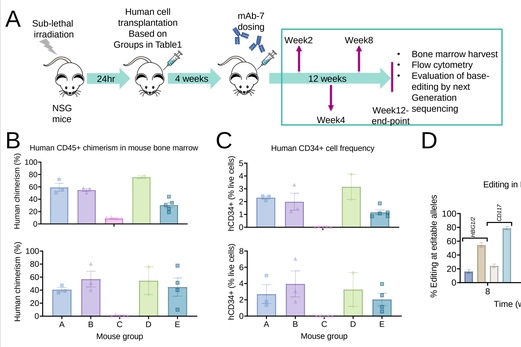


Figure 9. A. Schematic of *in vivo* long-term engraftment study. Unedited or *CD117*-edited CD34+ cells were transplanted into NBSGW mice. Mouse bone marrow was harvested and subjected to flow cytometry and NGS analyses 16 weeks post transplantation.

B. *CD117*-base edited cells produced stable long-term (16-week) engraftment in NBSGW mice. Unedited (grey bars) and *CD117*-base edited (teal bars) CD34+ cells produced similar levels of multi-lineage reconstitution.

C. NGS showing high *CD117* editing levels within different hematopoietic compartments.

mAb-7 selectively depleted unedited cells from the bone marrow of mice transplanted with hCD34+ cells



Group	Cells (1e6/mouse)	Ab treatment
A	Unedited : unedited	None
B	Unedited : unedited	Isotype
C	Unedited : unedited	mAb-7
D	Unedited : multiplex edited	Isotype
E	Unedited : multiplex edited	mAb-7

Figure 10. A. Schematic of *in vivo* study to evaluate mAb-7-mediated myeloablation. Irradiated NSG mice were first humanized with either unedited cells or a 1:1 mixture of unedited and multiplex-edited CD34+ cells. 4 weeks post-transplantation, mice were either left untreated, or treated with either isotype control antibody or mAb-7 according to mouse groups listed in right panel. Mouse bone marrow was harvested at indicated time points and were analyzed using flow cytometry. NGS was performed with bulk bone marrow and sorted CD34+ cells.

B. Significant reduction in human chimerism was observed at 8 weeks (top) and 12 weeks (bottom) post dosing. **C.** CD34+ cell population was also abrogated at 8 (top) and 12-week (bottom) time points.

D. Significant enrichment of *CD117* as well as *HBG1/2*-editing was observed withing the bulk bone marrow (left) and sorted CD34+ cells (right) of the mAb-7 treatment group compared with the isotype control group.

Conclusions

- We have developed Engineered Stem Cell Antibody Paired Evasion (ESCAPE) strategy, wherein a base-edited CD117 antigen and anti-CD117 mAb pair enabled edited cells to escape recognition by the mAb that selectively depletes unedited cells
- Anti-CD117 antibody mAb-7 binds with high affinity to wild-type CD117 but minimally to our base edited CD117 variant protein
- In *in vitro* assays, mAb-7 selectively depleted unedited primary HSPCs via blocking CD117-SCF interaction, while HSPCs containing CD117 base-edit evaded such depletion
- Edited CD117 showed comparable phosphorylation to the wild-type protein *in vitro*
- Fc engineered mAb-7 did not induce mast-cell degranulation *in vitro*
- Multiplexing CD117 sgRNA with therapeutic sgRNA HBG1/2 using a single ABE8 editor achieved >85% CD117 base editing in CD34+ cells which also contained the therapeutic edit
- Base editing of CD117 in HSPCs cells did not alter long-term engraftment and multi-lineage reconstitution in rodent model
- mAb-7 treatment selectively depleted unedited cells from the bone marrow of mice transplanted with human HSPCs
- Collectively, our ESCAPE strategy present a promising new paradigm for autologous stem cell therapies in treatment of hemoglobinopathies including sickle cell disease.

References and Disclosures

1. Gaudelli et al. Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage. Nature, 551:464–471 (2017)
2. Gaudelli, N.M., et al. Directed evolution of adenine base editors with increased activity and therapeutic application. Nat Biotechnol (2020).
3. Bauer et al, Reawakening fetal hemoglobin: prospects for new therapies for the β-globin disorders. Blood, 120(15), pp.2945-2953 (2012).

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