ESCAPE-2: Paired HSC CD117-epitope engineering and direct editing of sickle allele through base editing for antibody-mediated autologous HSC therapy conditioning for potential treatment of sickle cell anemia

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Introduction

Introduction of the naturally-occurring Hb G-Makassar variation in the human betaglobin gene (*HBB*) through base editing to eliminate the polymerizing sickle protein, HbS, the major molecular driver for sickle cell anemia, represents a promising new paradigm for the potential treatment of individuals with this disease. While several *ex vivo* delivery of gene editing technologies for sickle cell anemia are advancing in the clinic, several challenges remain for this potentially transformative cell therapy, namely the co-requirement of genotoxic myeloablative conditioning prior to autologous hematopoietic stem cell transplant (HSCT). Aiming to address this, we have developed a strategy whereby a monoclonal antibody (mAb) that binds to CD117, a critical receptor on HSPCs essential for viability, is coupled with multiplexed engineered HSCs (eHSCs). Our eHSCs are designed to both evade mAb binding and harbor the Makassar therapeutic edit. Our **E**ngineered **S**tem **C**ell **A**ntibody **P**aired **E**vasion (**ESCAPE**) strategy is designed to provide a non-genotoxic alternative to current conditioning regimens.

Through an extensive antibody and base-edited-antigen screen, we identified a high affinity antagonist mAb that interacts with the ligand binding domain of CD117, blocking normal SCF ligand binding to the WT receptor, but allows for our engineered CD117 epitope to evade binding and cytotoxicity upon mAb treatment *in vitro*. Subsequent Fc receptor silencing modifications were included in our mAb to reduce any mast cell degranulation that could be the result of antibody-based binding to Fc-receptor on mast cells. Furthermore, we demonstrated *in vivo* efficacy of our mAb to deplete unedited, wildtype HSPCs. We also achieved highly efficient editing to both install the CD117 edit, but also Makassar edit with a single base editor.

Altogether, our ESCAPE-2 strategy is compatible with the installation of the therapeutic Makassar edit that, when combined with a mAb as a conditioning agent, represents a promising potential alternative to busulfan-based myeloablative regimens in an autologous HSCT setting for the treatment of sickle cell anemia.

Genome Editing with Adenine Base Editors (ABE)

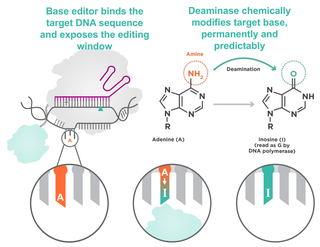


Figure 1. ABE is a fusion protein comprising an evolved TadA* deaminase (teal) connected to a CRISPR-Cas enzyme (grey). The base editor binds to a target sequence that is complementary to the guide-RNA (magenta) and exposes a stretch of single-stranded DNA. The deaminase converts the target adenine into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

Makassar β-globin Variant Direct Editing Strategy

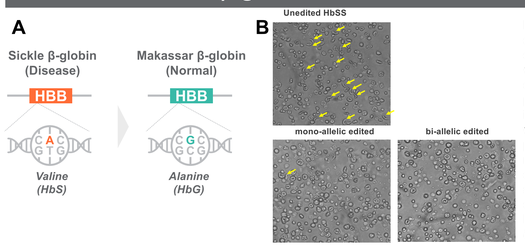


Figure 2. (A) ABE variants were designed to recognize and edit the opposite stranded adenine residue of the valine codon. This results in the conversion of valine to alanine and the production of a naturally occurring variant in human genetics, Hb G-Makassar, which presents with normal hematological parameters and red blood cell morphology (3-5). **(B)** We previously demonstrated that even with mono-allelically Makassar editing *in vitro* erythroid differentiated cells derived from HbSS CD34s exhibited reduced sickling *in vitro* upon exposure to hypoxia.

Auto-HSCT of Edited HSCs Requires Myeloablative Conditioning

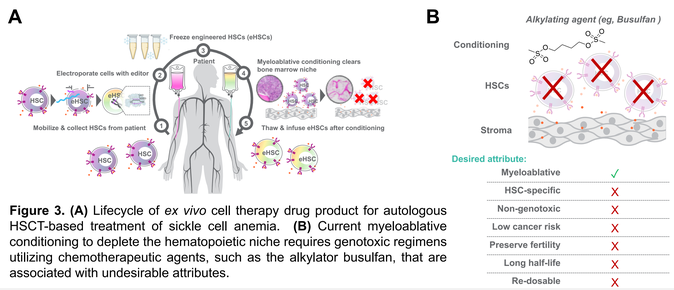


Figure 3. (A) Lifecycle of *ex vivo* cell therapy drug product for autologous HSCT-based treatment of sickle cell anemia. **(B)** Current myeloablative conditioning to deplete the hematopoietic niche requires genotoxic regimens utilizing chemotherapeutic agents, such as the alkylator busulfan, that are associated with undesirable attributes.

ESCAPE Strategy for Non-Genotoxic Conditioning

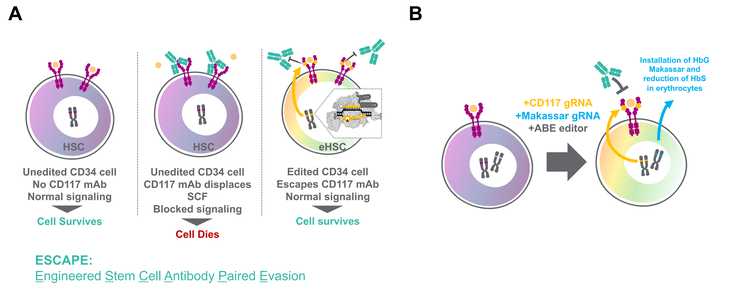
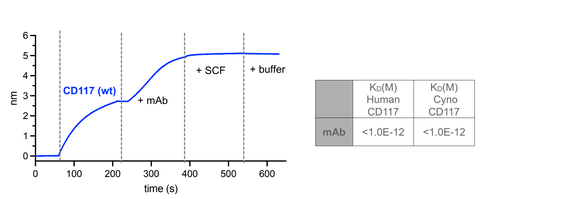


Figure 4. (A) ESCAPE strategy concept **(B)** ESCAPE-2 product whereby both a CD117 and therapeutic edit are introduced that allow for evasion from detection by mAb and harboring a therapeutic edit (Makassar installation) for the treatment of sickle cell anemia

Identification of an Effective mAb:edit Pair for ESCAPE-2



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

Figure 5. Biolayer Interferometry (BLI) demonstrating high affinity (pM) of lead mAb for WT CD117 protein that blocks SCF ligand binding.

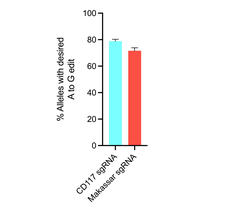


Figure 6. Highly efficient CD117 and Makassar editing (n=3) can be achieved with single base editor in mPB CD34⁺ discovered from base editor guide screening effort.

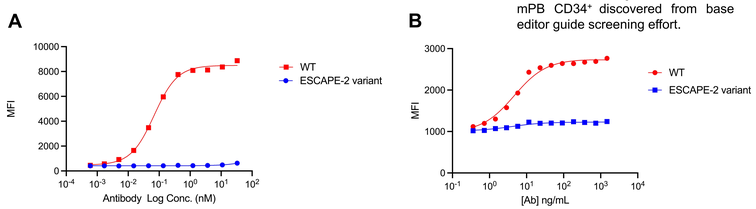


Figure 7. mAb cell binding as measured on **(A)** CD117 expressing M07e cell lines and **(B)** mobilized peripheral blood human CD34⁺s demonstrating high affinity binding of mAb for WT but not cells harboring CD117 ESCAPE variant.

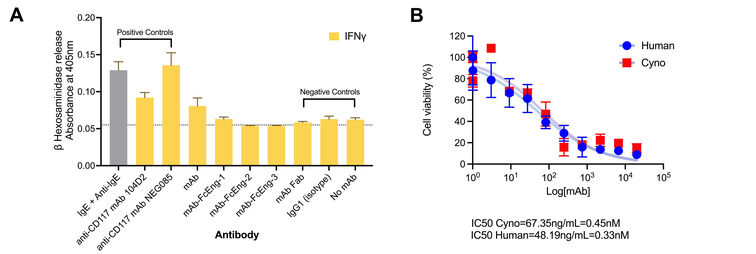


Figure 8. Fc engineering of mAb **(A)** reduced mast cell activation in primary mast cell model and is highly potent in reducing the viability of **(B)** hCD34+ HSPCs and cyno CD34⁺s *in vitro*.

ESCAPE-2 CD117 Variant Escapes Depletion by mAb

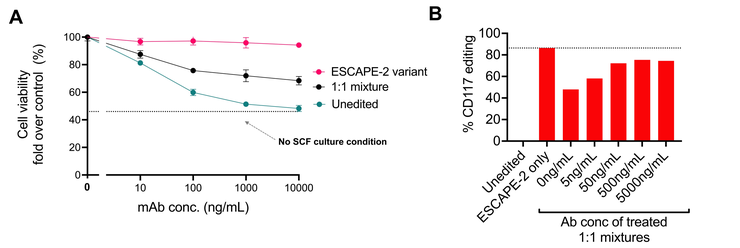


Figure 9. Fc Engineered mAb **(A)** selectively depletes unedited hCD34 HSPCs *in vitro* and **(B)** enriches for cells edited for the ESCAPE-2 CD117 variant

ESCAPE-2 CD117 Variant Exhibits WT CD117 Function

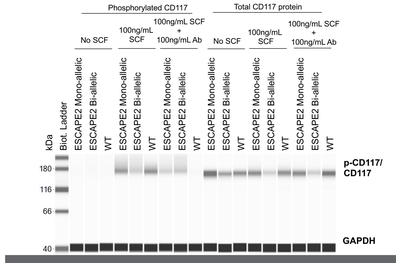


Figure 10. Both bi and mono-allelically M07e cells harboring the ESCAPE-2 CD117 variant retain CD117 phosphorylation upon stimulation with SCF, even in the presence of mAb. Phosphorylation of WT CD117 is blocked upon exposure of mAb.

In vivo Depletion of HSPCs with mAb

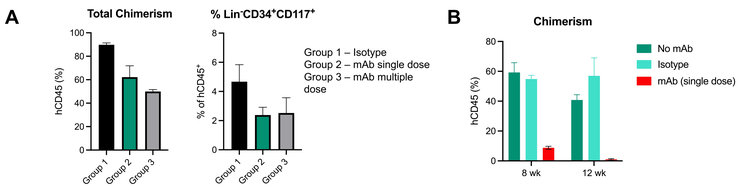


Figure 11. *In vivo* depletion of unedited HSPCs 2 weeks post-mAb dosing (4-week post-humanization) reflected in **(A)** total chimerism and Lin⁻CD34⁺CD117⁺ HSPC frequencies of animals (n = 3) dosed with mAb. **(B)** mAb single dosed mice at later time points exhibited sustained abrogation of long-term engraftment at 8 and 12 weeks post-mAb dosing.

Conclusions

Our Hb G-Makassar-compatible ESCAPE strategy demonstrated not only highly efficient editing of CD117 of eHSCs but also highly favorable mAb properties *in vitro*. We demonstrate that primary human HSPCs harboring our engineered epitope could effectively evade depletion by blocking of the CD117 ligand (SCF) binding by a highly specific and potent mAb *in vitro*. Early *in vitro* biological assessment of receptor function suggest that our engineered CD117 epitope is compatible with normal function. Furthermore, antibody engineering further minimized potential mast cell activation, potentially improving the safety of the mAb. *In vivo* studies that were conducted also suggest our mAb is effective in depleting non-edited HSPCs. *In vivo* studies including eHSCs are underway.

With this non-genotoxic conditioning approach, the direct editing of the causative sickle cell mutation to the naturally occurring and asymptomatic Hb G-Makassar is a promising new potential treatment paradigm for autologous HSCT for patients with sickle cell anemia.

References and Disclosures

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