CD117 antibody conditioning and multiplex base editing enable rapid and robust fetal hemoglobin reactivation in a rhesus autologous transplantation model

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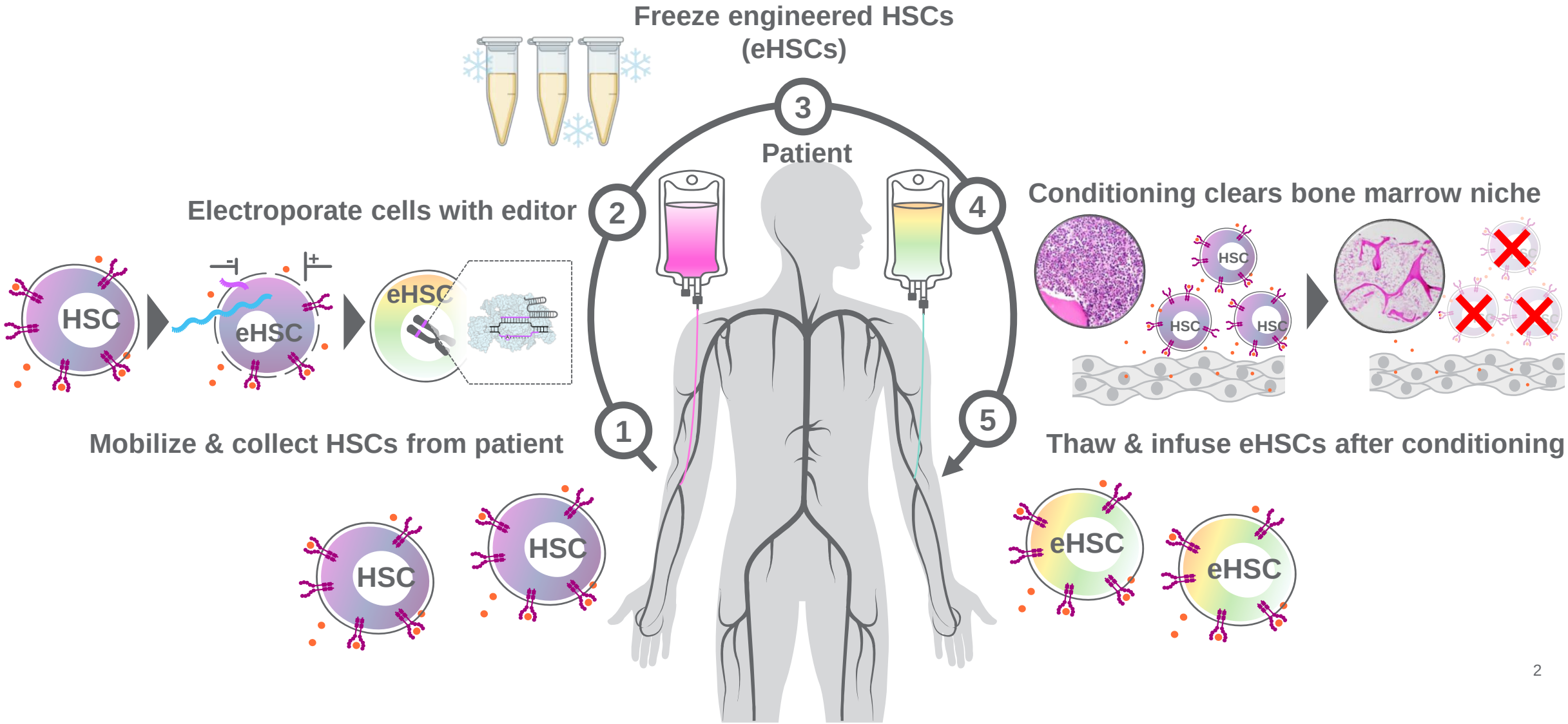
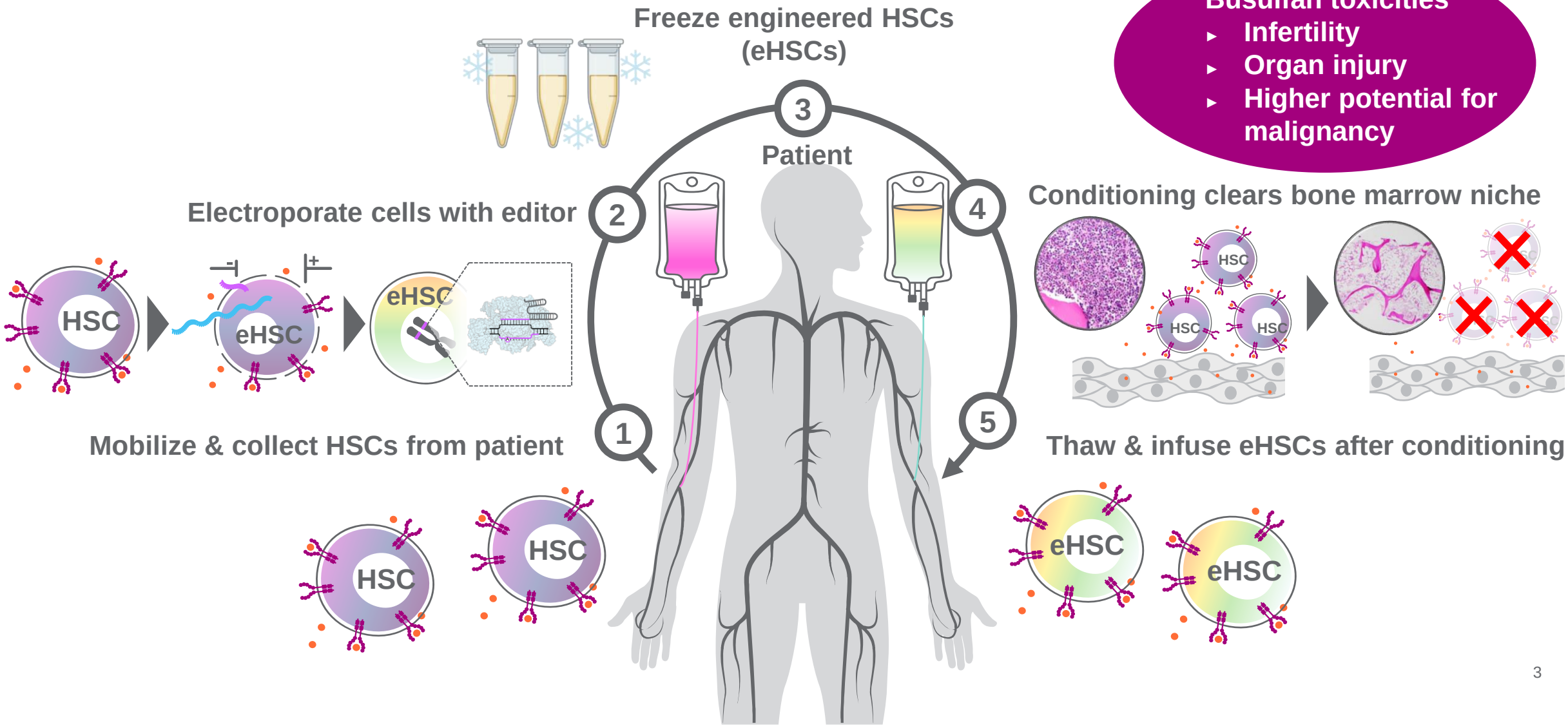
Curative gene therapies for sickle cell disease need myeloablative genotoxic conditioning with busulfan prior to autologous HSCT

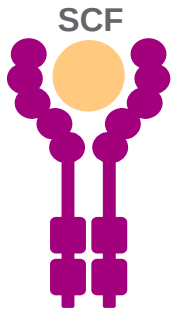


Toxicities associated with busulfan conditioning may exclude many SCD patients from receiving transformative gene therapies

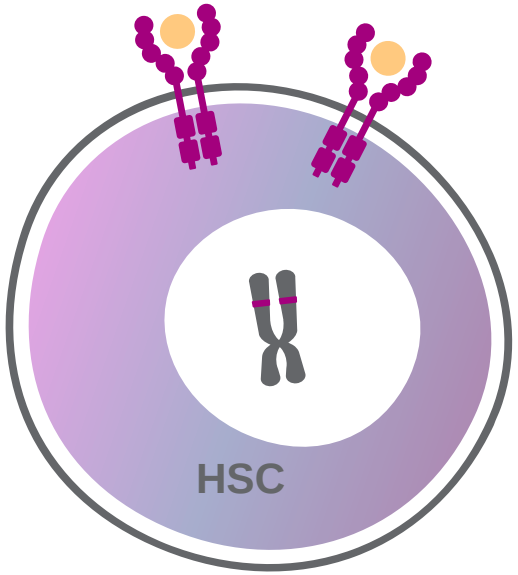
Busulfan toxicities
- Infertility
- Organ injury
- Higher potential for malignancy



Epitope engineering via base editing enables eHSCs to selectively ESCAPE mAb binding

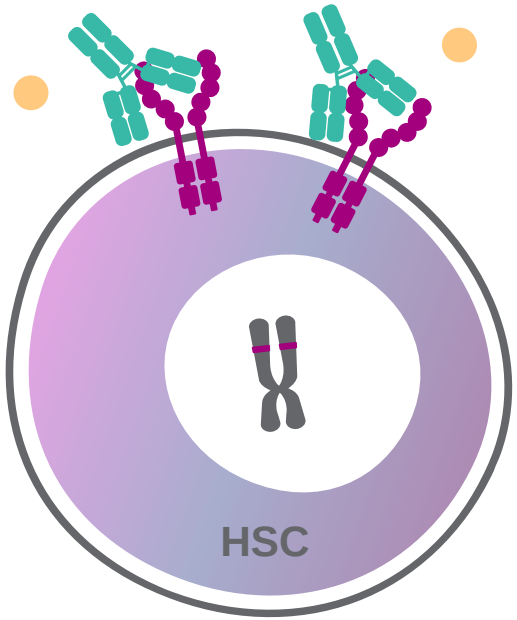


High expression in the long-term and short-term HSCs make CD117 an attractive target for immunologic conditioning



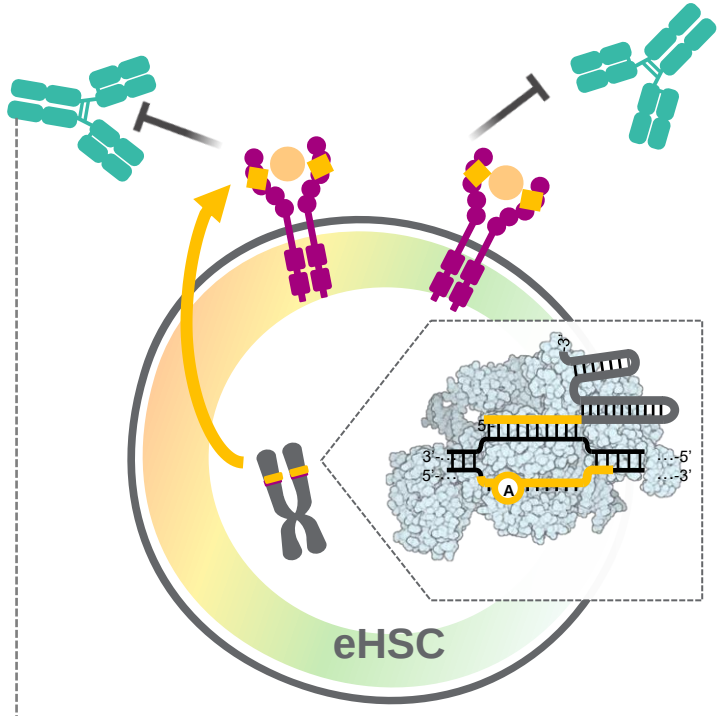
Unedited CD34 cell
No BEAM-103
Normal signaling

Cell Survives



Unedited CD34 cell
BEAM-103 blocks SCF
BEAM-103 blocked signaling

Cell Dies



Edited CD34 cell (BEAM-104)
Escapes BEAM-103
Normal signaling

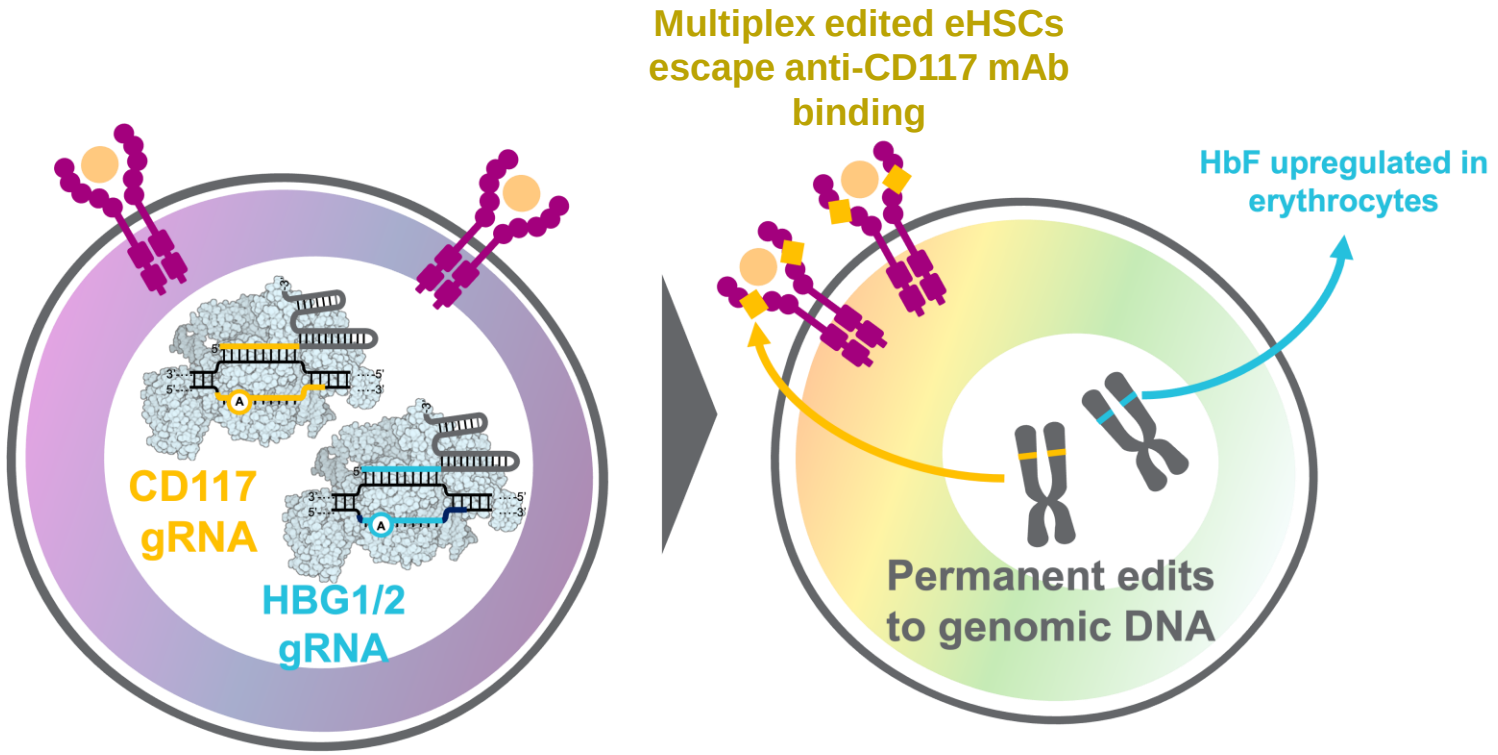
Cell survives
HBG1/2 editing leads to HBG induction

BEAM-104 = Multiplex edited eHSC
BEAM-103 = Anti-CD117 mAb

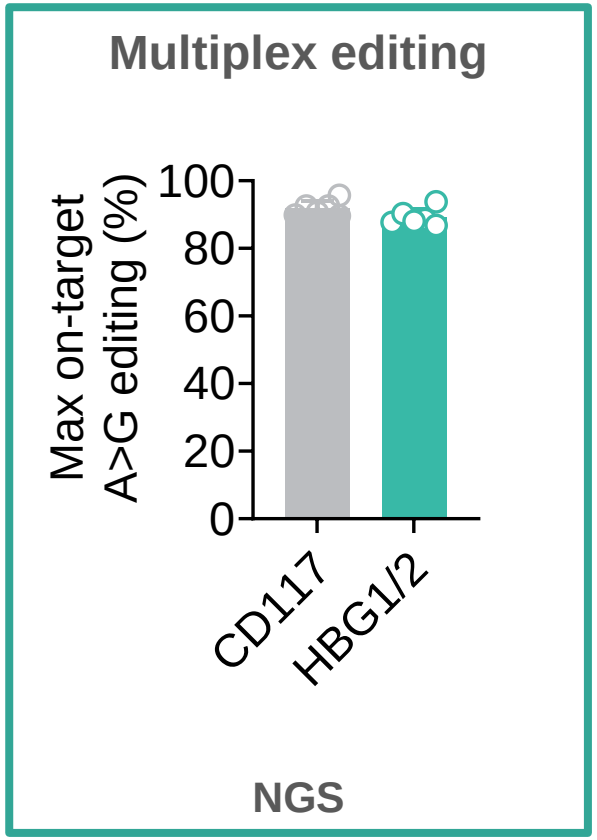
ESCAPE: Engineered Stem Cell Antibody Paired Evasion

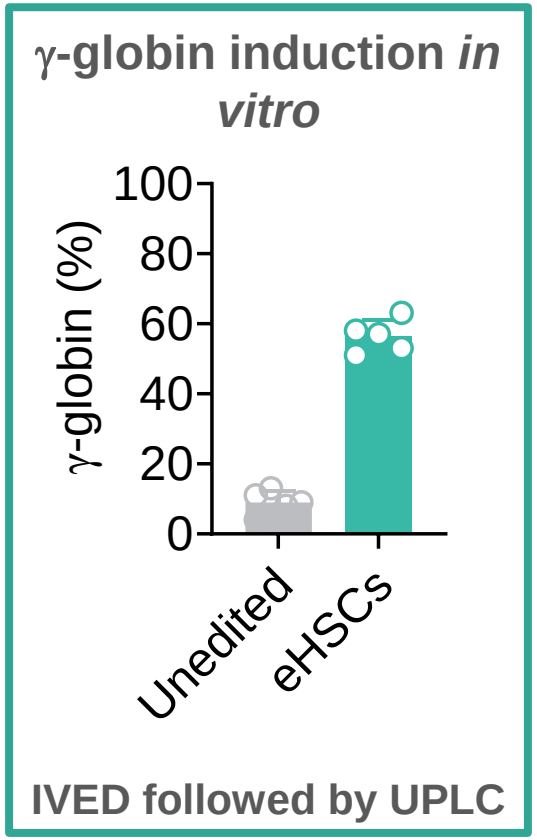
ESCAPE epitope engineering is predicated on a few key attributes

- High efficiency and balanced multiplex editing of both *CD117* and *HBG1/2*



Multiplex editing and γ-globin induction achieved



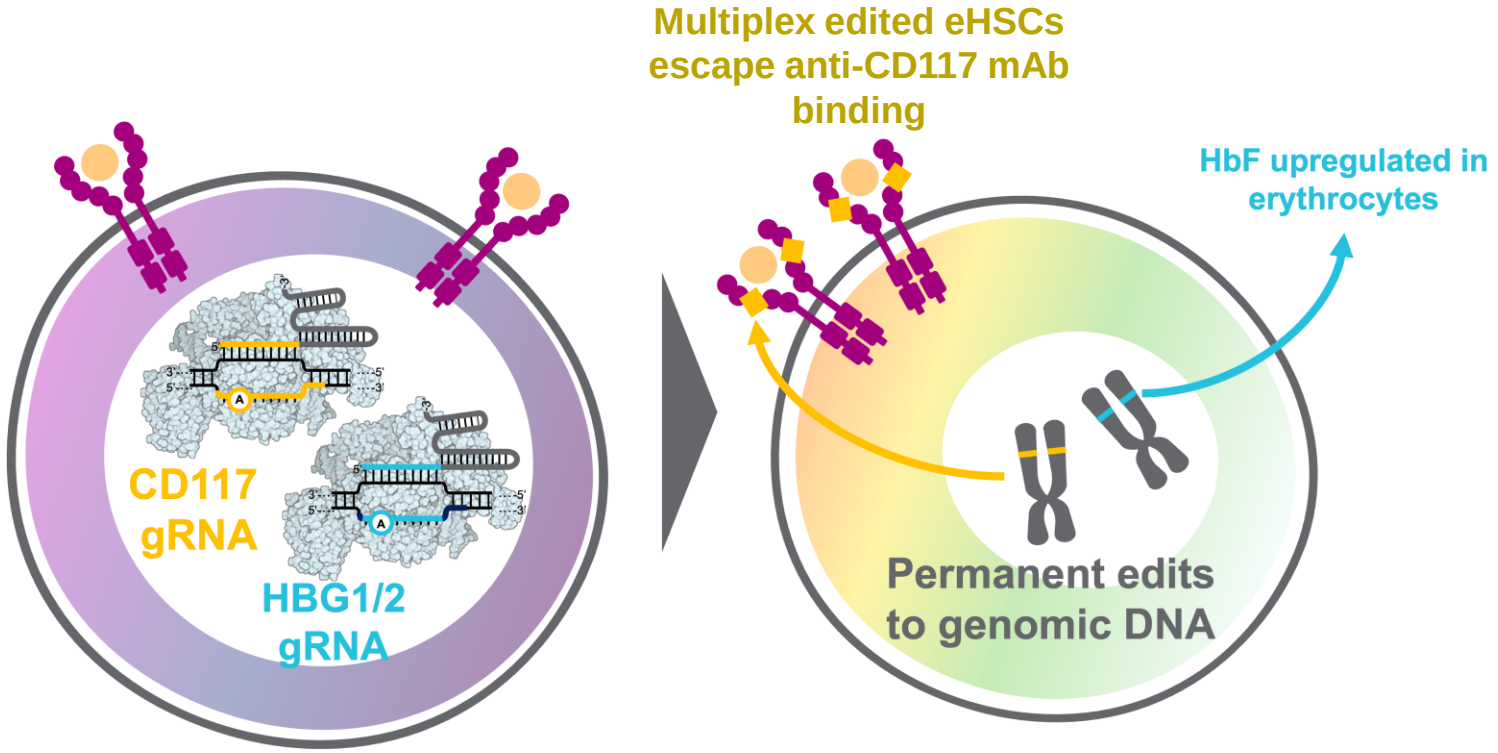


- >90% bulk CD117 and HBG1/2 editing
- Comparable to single-plex editing rates for each target site
- Single clonal analysis showed majority (>90%) of the clones harbored CD117 edit
- No CD117 only edited cells were identified
- *In vitro* differentiated (IVED) multiplex edited erythroid cells yielded >50% γ-globin

- Multiplex editing led to similar editing outcomes as single-plex editing for each target site
- >50% γ-globin by *in vitro* differentiated erythroid cells

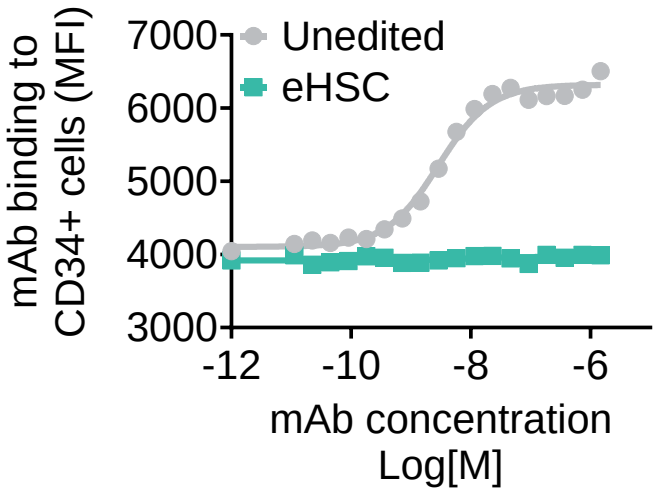
ESCAPE epitope engineering is predicated on a few key attributes

- High efficiency and balanced multiplex editing of both *CD117* and *HBG1/2*
- Engineered epitope should abrogate binding of anti-WT CD117 mAb



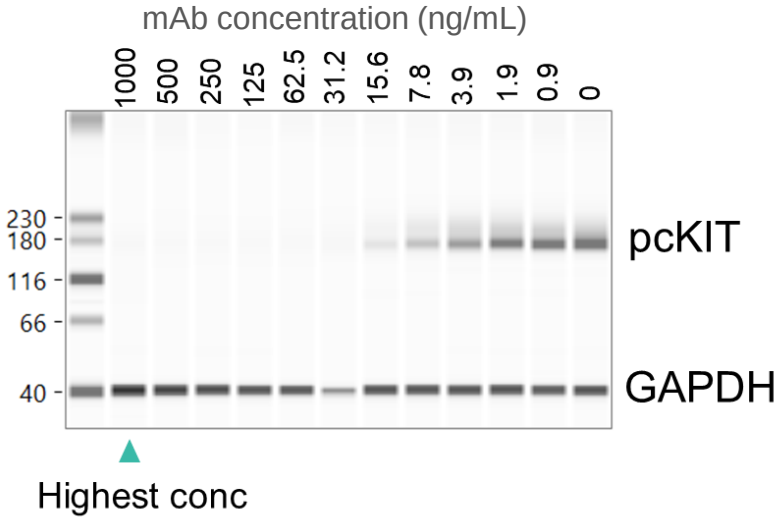
Anti-CD117 mAb selectively bound with high affinity and depleted WT CD117 expressing HSCs

Anti-CD117 mAb bound to unedited HSPCs but not to eHSCs



Flow cytometry of CD34s

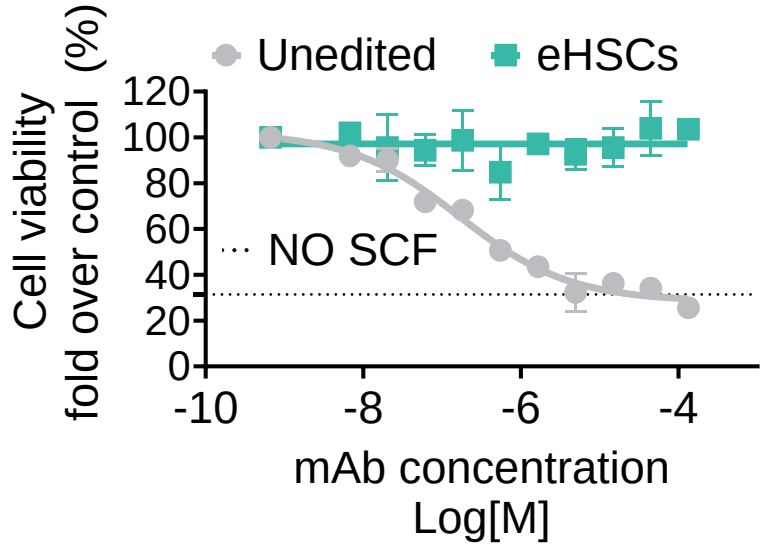
Anti-CD117 mAb abrogated CD117 phosphorylation



cKIT, CD117
pcKIT, phosphorylated CD117 (cKIT)
GAPDH, glyceraldehyde 3-phosphate dehydrogenase, used as loading control

Western blot

Anti-CD117 mAb selectively depleted unedited cells while eHSCs escaped depletion

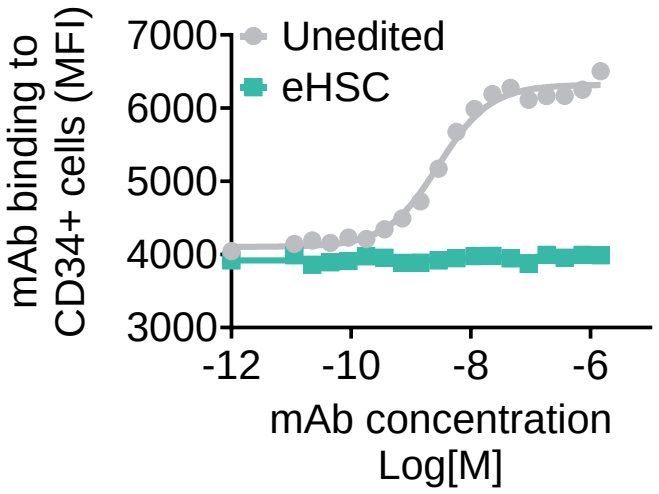


***In vitro* treatment of CD34s**

- Anti-CD117 mAb showed selective binding to WT CD117 and no binding to multiple edited eHSCs
- mAb binding led to complete abrogation of WT CD117 signaling
- Multiplex edited eHSCs are protected from mAb mediated depletion *in vitro*

Anti-CD117 mab selectively bound with high affinity and depleted WT CD117 expressing HSCs

Anti-CD117 mAb bound to unedited HSPCs but not to eHSCs



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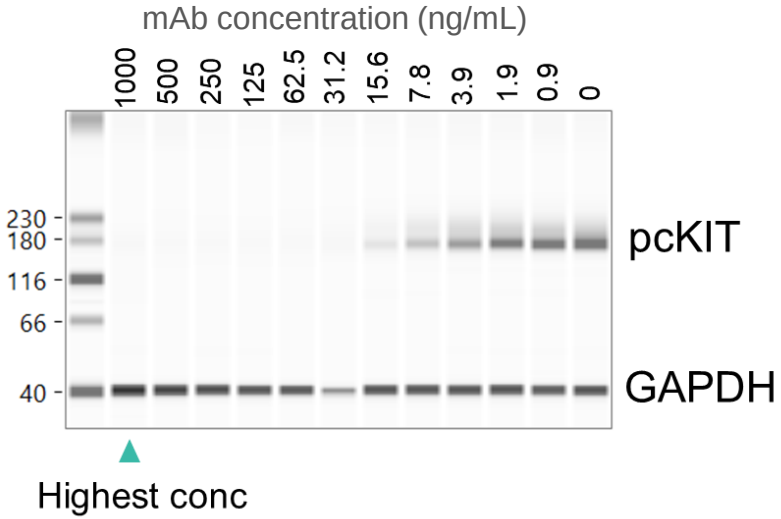

cKIT, CD117
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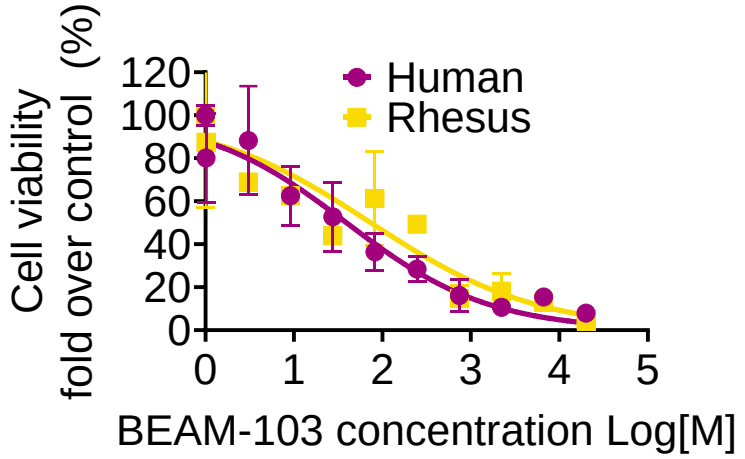
Western blot

Anti-CD117 mAb similarly depleted WT human and Rhesus HSPCs

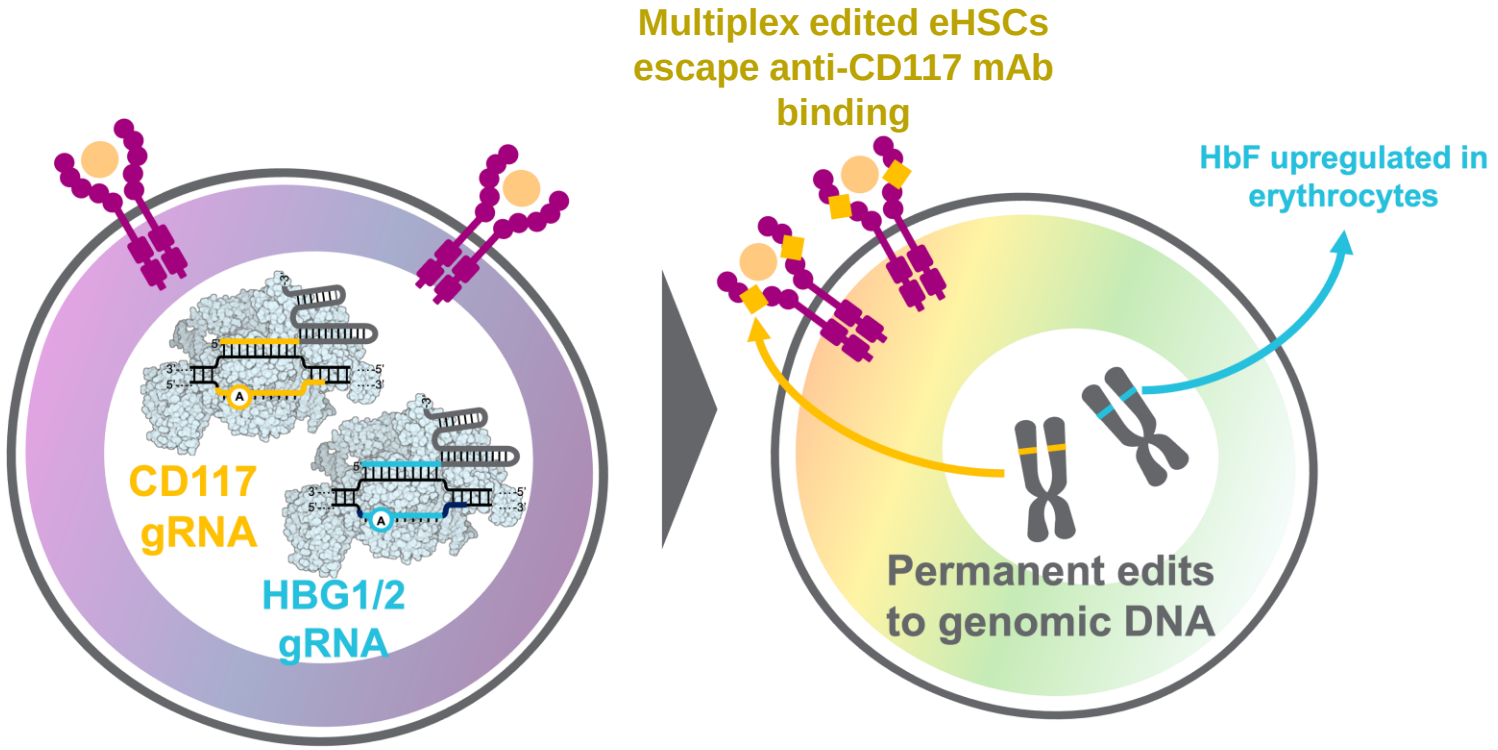


***In vitro* treatment of CD34s**

- Anti-CD117 mAb is cross-reactive to and led to depletion of Rhesus HSPCs *in vitro*

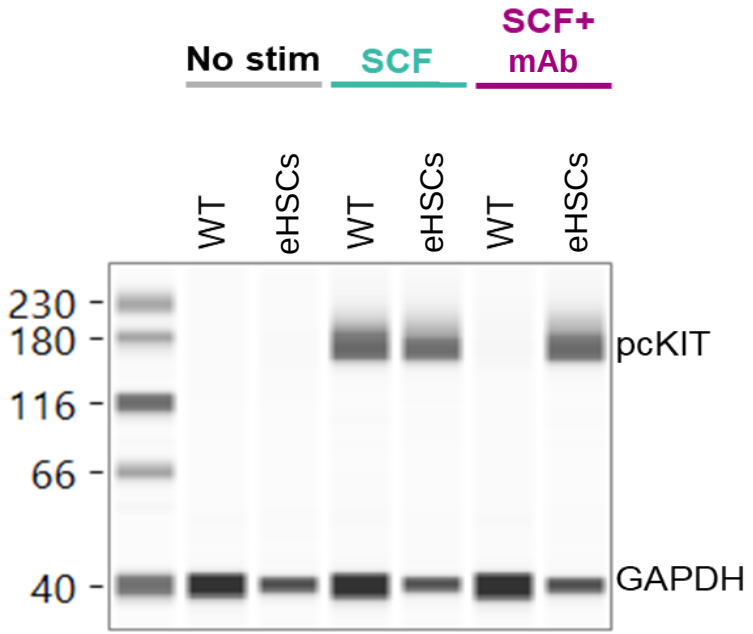
ESCAPE epitope engineering is predicated on a few key attributes

- High efficiency and balanced multiplex editing of both *CD117* and *HBG1/2*
- Engineered epitope should abrogate binding of anti-WT CD117 mAb
- Engineered CD117 epitope must preserve WT CD117 function



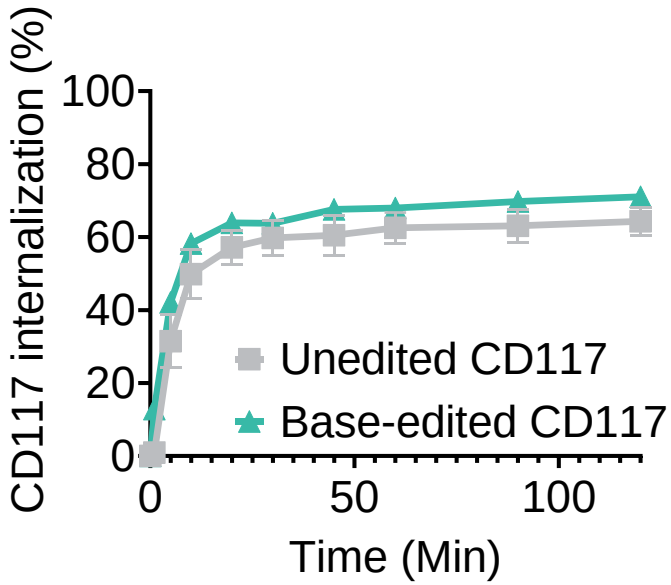
Base-edited CD117 variant retained comparable receptor binding and function to wild-type

Phosphorylation of WT and base-edited CD117 variant



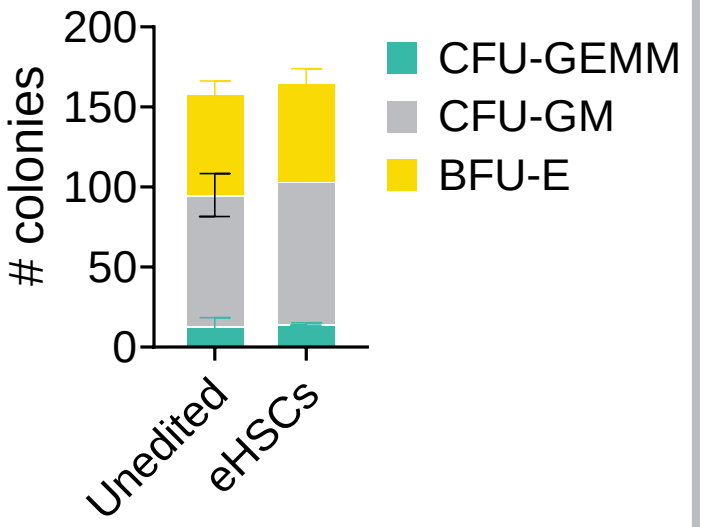
Western blot

Normal internalization of base-edited CD117 variant



Flow cytometry

Normal CFU output by eHSCs

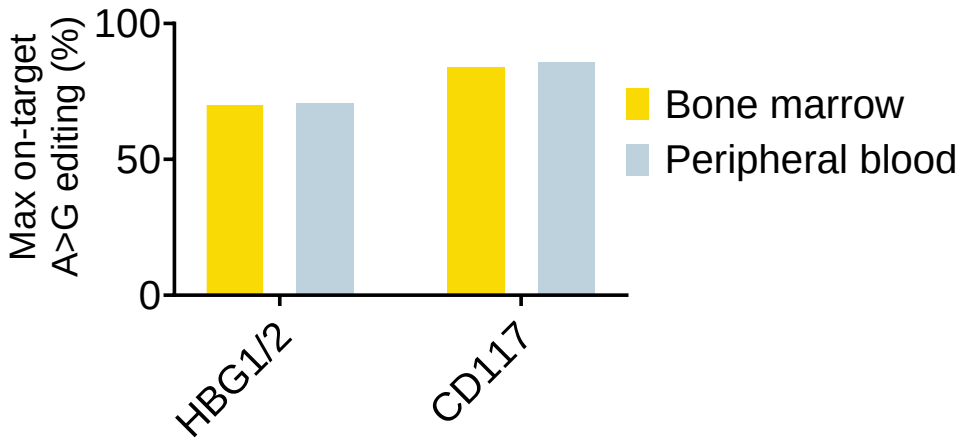


Colony forming unit (CFU) assay

- Base-edited CD117 retained normal ligand binding, phosphorylation and internalization properties
- Anti-CD117 mAb blocked phosphorylation of WT CD117 but not of base-edited CD117
- Multiplex edited eHSCs retained normal *in vitro* differentiation properties

Base-edited eHSCs produced durable engraftment and multi-lineage reconstitution in a traditional autologous transplant model with busulfan conditioning

Stable editing 1-year post-transplant



- Balanced multiplex editing rates within bone marrow and peripheral blood

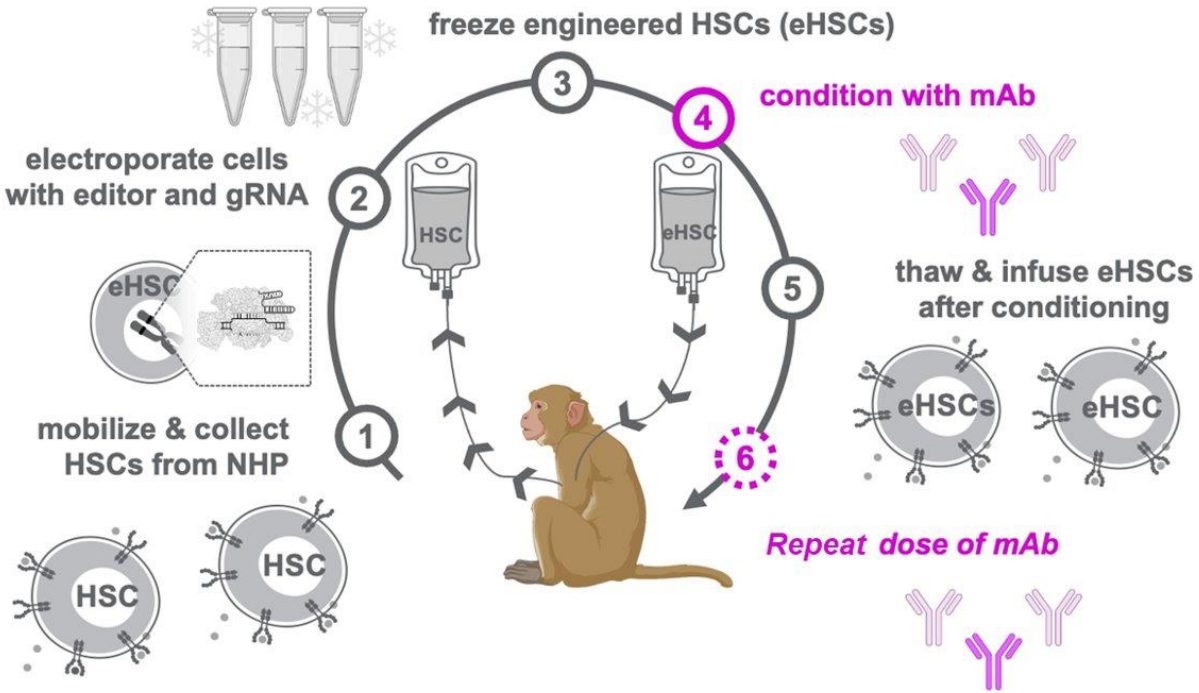
Multiplex edited eHSCs showed normal long-term engraftment

	Baseline (Pre-conditioning) (x10^3 cells/μL)	Counts 1-year post-transplant (x10^3 cells/μL)
WBC	7.18	7.2
ANC	4.25	2.97
Lymph	1.78	3.94
Mono	0.96	0.24
PLT	329	209
Hb	12.3	15.3
HCT	39.7	45.4
Retic	83.3	102.86

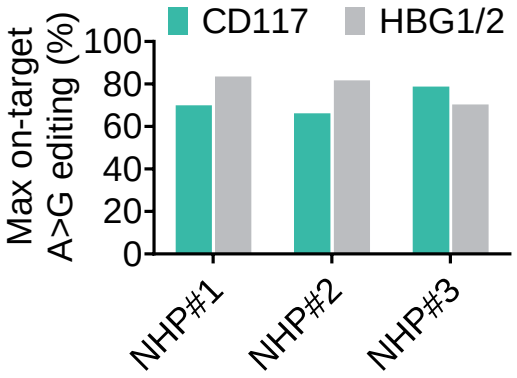
- Evidence of long-term engraftment by eHSCs
- Editing stability at both target sites

NHP autologous transplant model for our ESCAPE conditioning approach

Multiplex base-editing and erythroid differentiation of Rhesus CD34+ cells

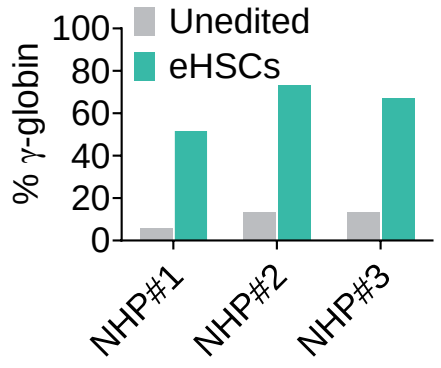


Multiplex editing in Rhesus HSPCs



Next generation sequencing

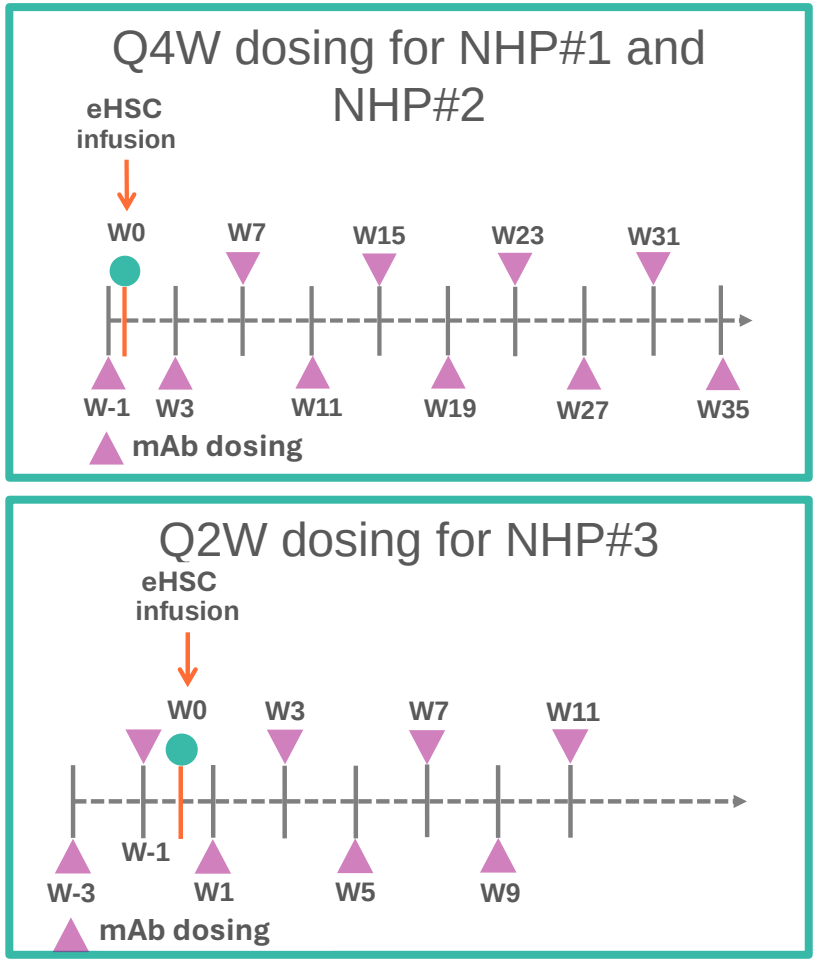
Potential for therapeutic levels of HBF upon multiplex editing

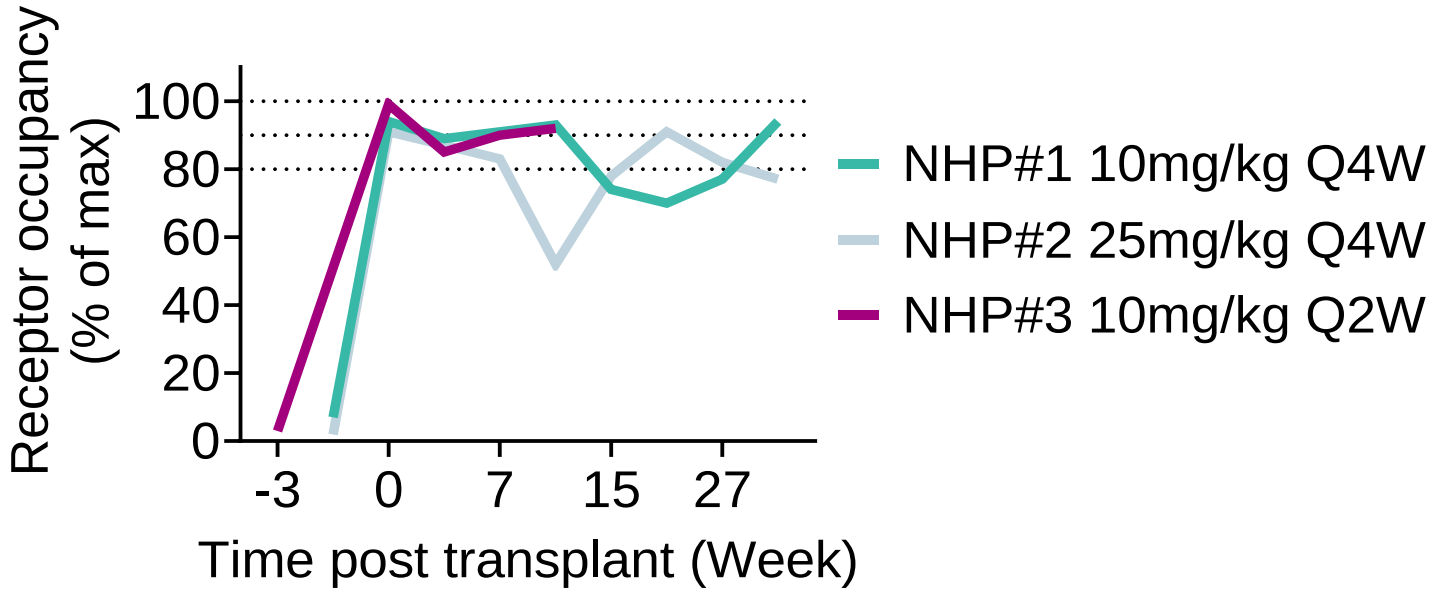


In vitro erythroid differentiation

Infusion product was manufactured with priority for maximizing total CD34+ cell dose for transplant

Receptor occupancy was maintained on target expressing cells via repeat dosing of anti-CD117 mAb





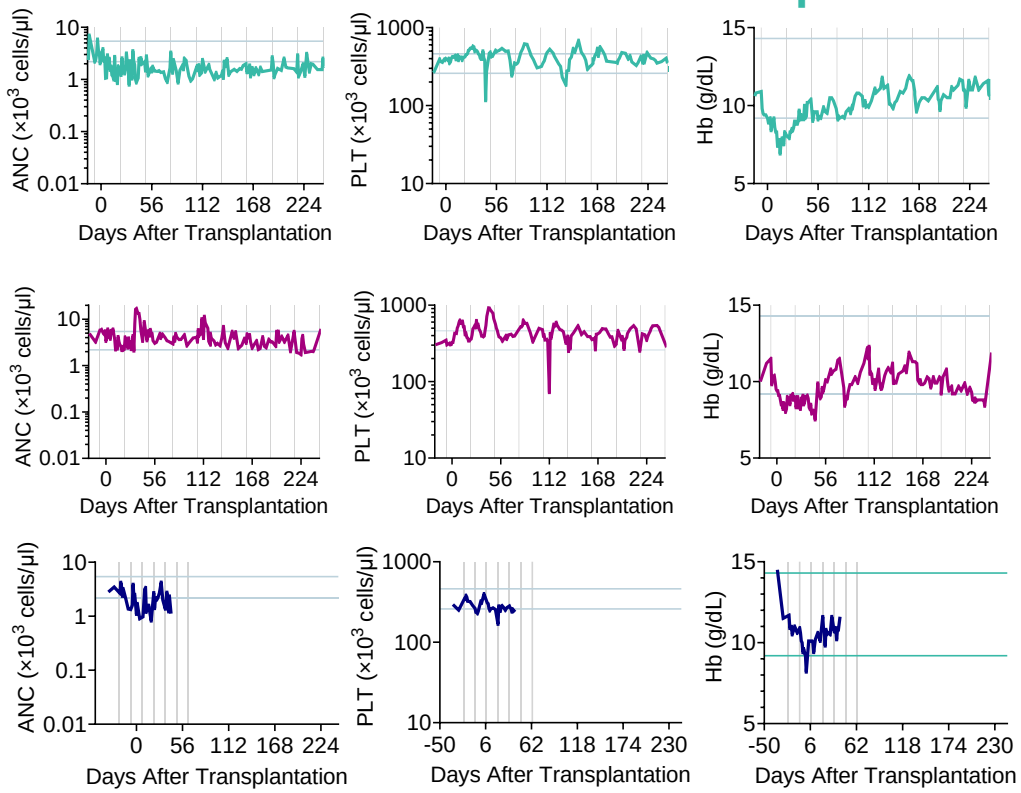
Repeat dosing of anti-CD117 mAb was able to maintain ~80-90% receptor occupancy

mAb dosing was well tolerated with no need for transfusions/antibiotic support

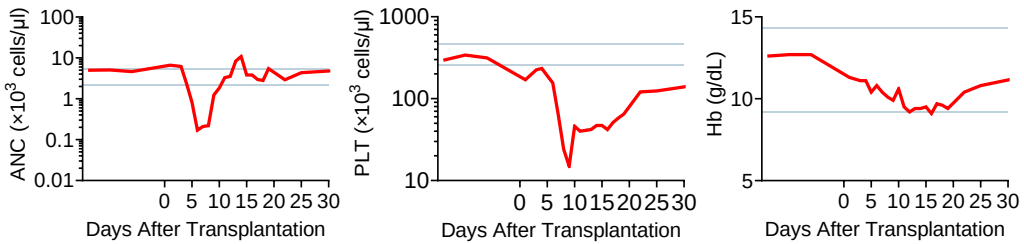
- In contrast with Busulfan conditioning, mAb administration led to only minor dips in neutrophil counts
- Although platelet counts dropped after each mAb dose, levels recovered quickly
- Minor drops in hemoglobin upon mAb dosing recovered post-transplant
- **The ESCAPE transplant strategy presents sharp contrast with Busulfan conditioning as the animals remained healthy without the need for transfusion/ antibiotics or additional supportive care**

Repeat dosing of anti-CD117 mAb was well tolerated

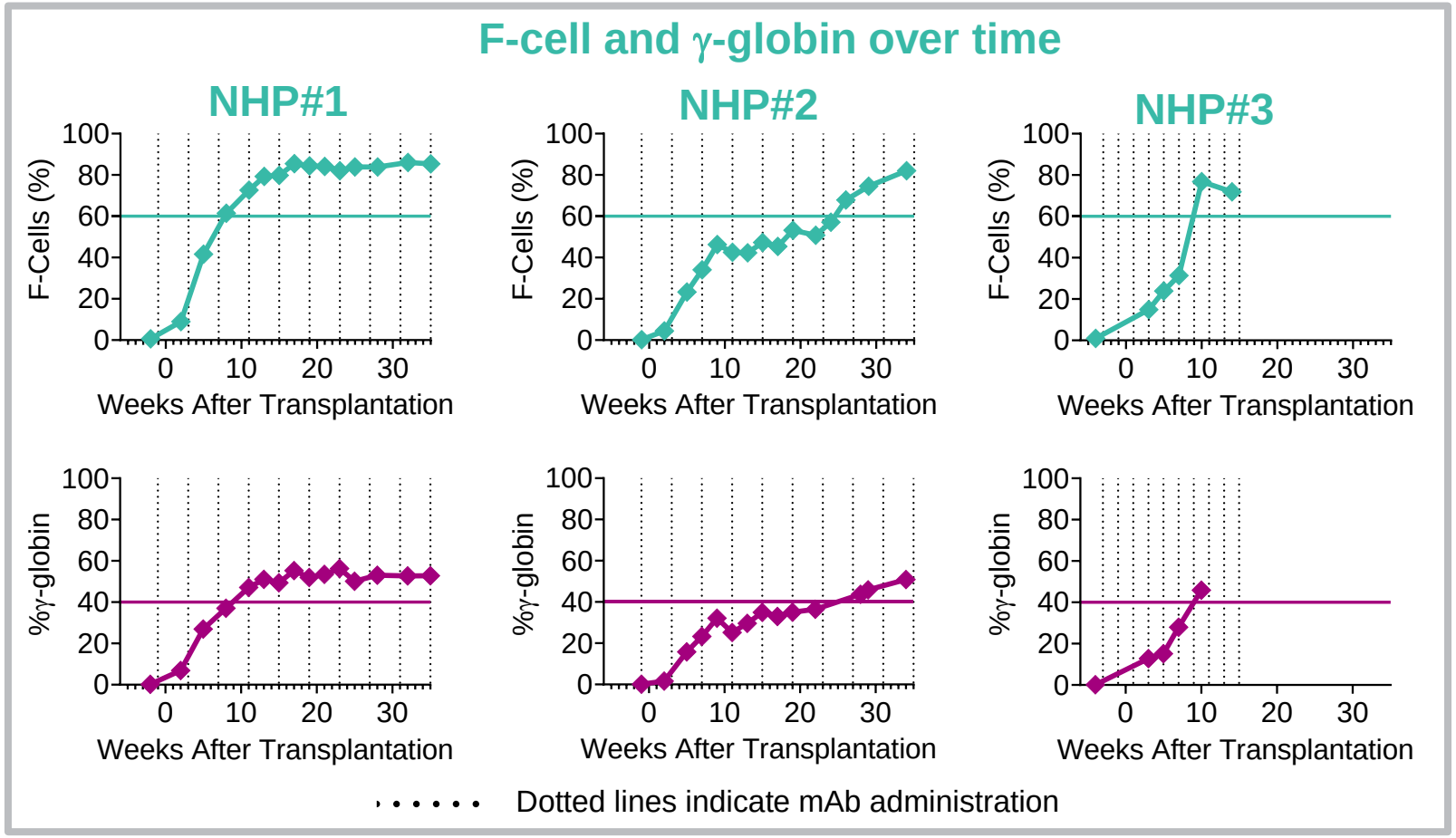
BEAM-103 conditioned transplants



Historical busulfan conditioned transplant



mAb dosing led to rapid turnover of unedited erythroid cells and early induction of therapeutic γ-globin levels



- Rapid and complete replacement of erythroid cells by edited cells
- F-cell levels reached ~60% as early as 8-weeks post-transplant
- Earliest time to achieve ~40% γ-globin was ~8 weeks post-transplant

BEAM-104 = Multiplex edited eHSC
BEAM-103 = Anti-CD117 mAb

Rapid reactivation of fetal hemoglobin post-transplant shows promise of early therapeutic benefit in SCD patients

Summary

- Busulfan-associated toxicity continues to be a major obstacle to expanding the use of autologous HSCT-based gene therapies for SCD
- The ESCAPE strategy can potentially address this unmet need by enabling HSC-targeted non-genotoxic naked anti-CD117 mAb conditioning
- The CD117 base-edit showed normal receptor function *in vitro,* and the multiplex edited eHSCs produced durable engraftment and multi-lineage reconstitution in an autologous transplant model with Busulfan conditioning
- Here we present non-human primate data demonstrating proof-of-concept for ESCAPE non-genotoxic conditioning, potentially removing the requirement for toxic, myeloablative conditioning for autologous HSCT
  - We observed rapid and complete replacement of host erythroid cells by edited cells leading to early induction of therapeutically relevant levels of fetal hemoglobin (60% F-cells and 40% γ-globin as early as 8-weeks post-transplant), providing potential early therapeutic benefit in SCD patients
  - The ESCAPE transplant strategy presents a sharp contrast to busulfan-based conditioning as the animals remained healthy without the need of transfusion, antibiotics or additional supportive care

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