National Heart Lung and Blood Institute

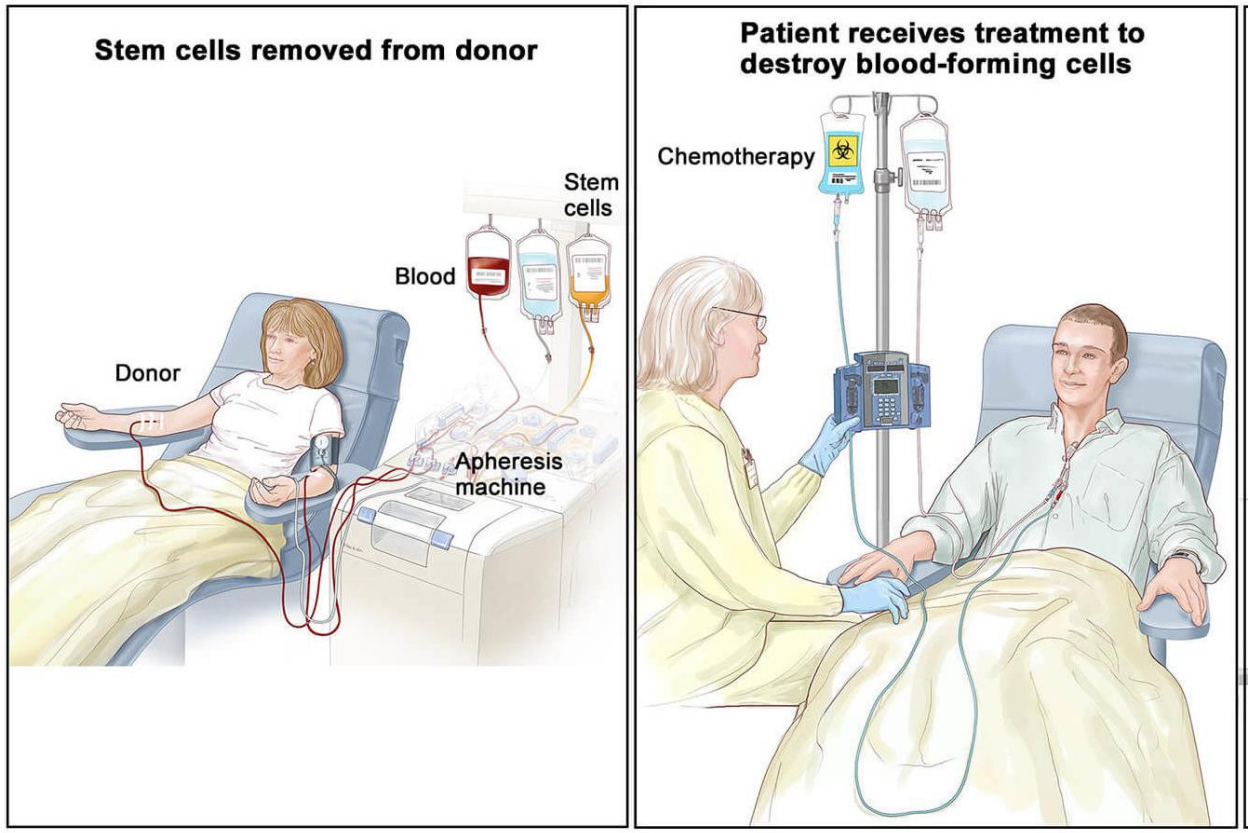
ASGCT 26TH ANNUAL MEETING Los Angeles

Efficient Engraftment of Genome Edited/Modified CD34+ HSPCs in CD45 Antibody-Drug Conjugate (ADC) Conditioned Non-Human Primates

Selami Demirci, PhD

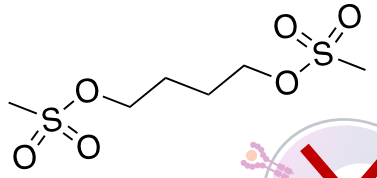
May 19, 2023

Hematopoietic stem cell transplantation



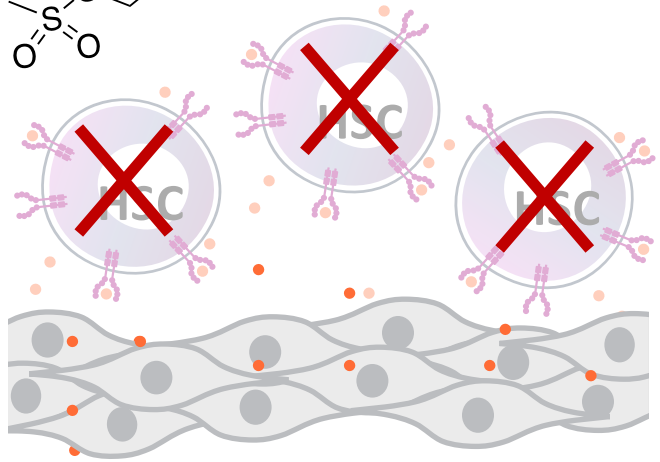
Alkylating agent (i.e., Busulfan)

Conditioning



HSCs

Stroma

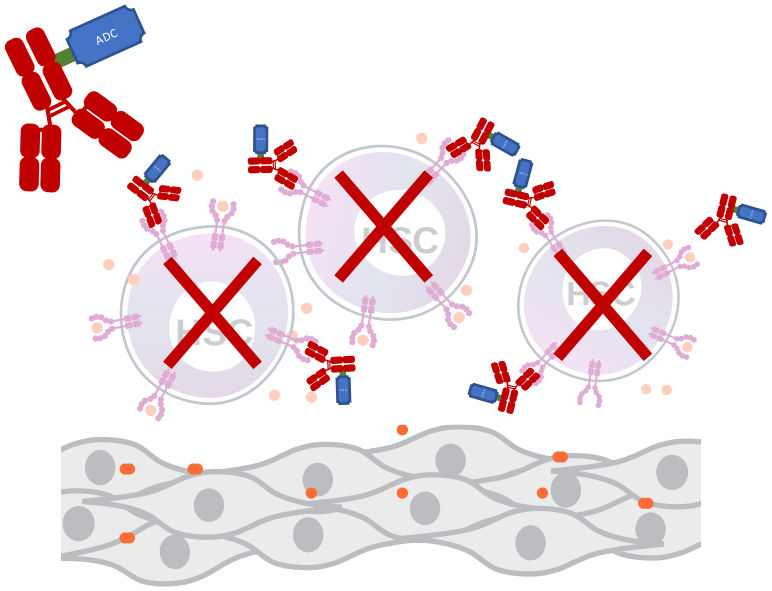


Desired attribute:

Myeloablative	✓
Specificity	None
Non-genotoxic	X
Low cancer risk	X
Preserve fertility	X

Alternative conditioning regimens

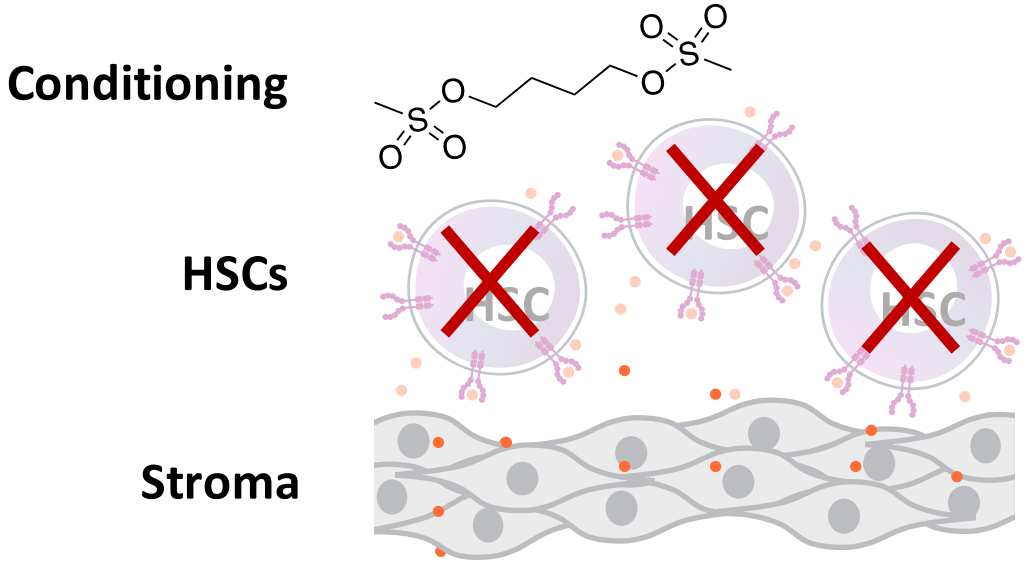
Antibody drug conjugate (i.e. CD117 or CD45)



Desired attribute:

Myeloablative	✓
Specificity	High
Non-genotoxic	✓
Low cancer risk	✓
Preserve fertility	✓

Alkylating agent (i.e., Busulfan)



Desired attribute:

Myeloablative	✓
Specificity	None
Non-genotoxic	X
Low cancer risk	X
Preserve fertility	X

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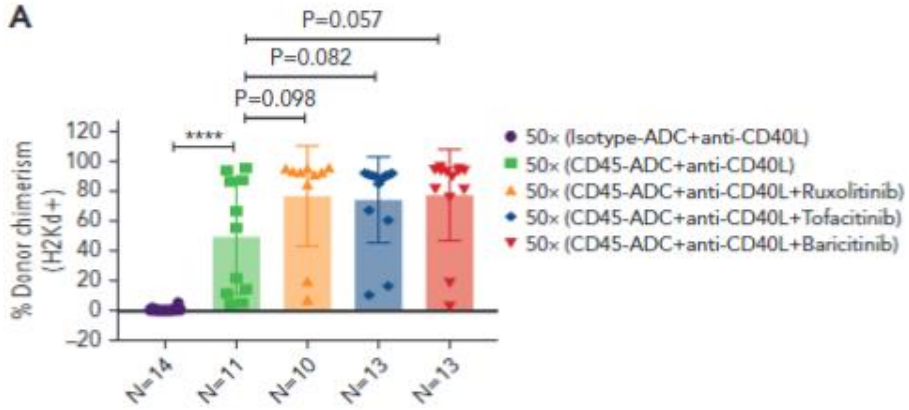
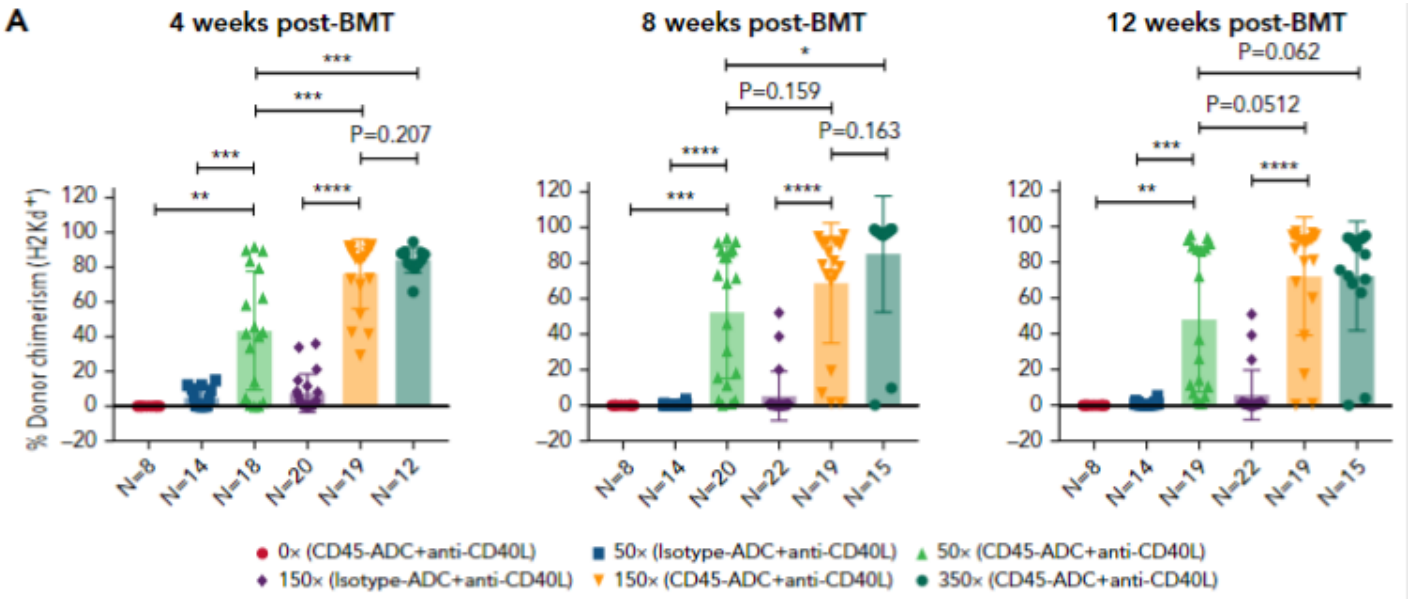
Regular Article

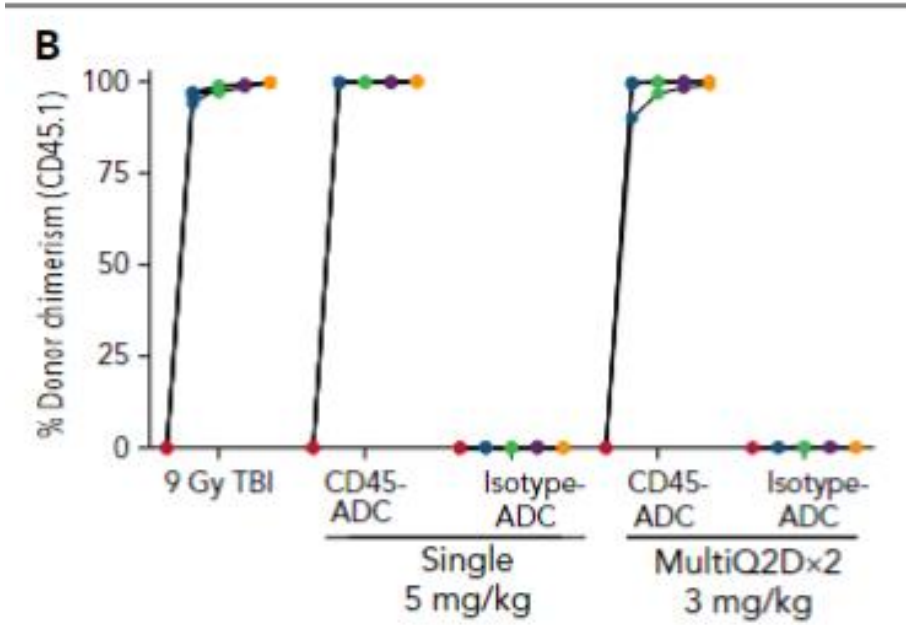
TRANSPLANTATION

A CD45-targeted antibody-drug conjugate successfully conditions for allogeneic hematopoietic stem cell transplantation in mice

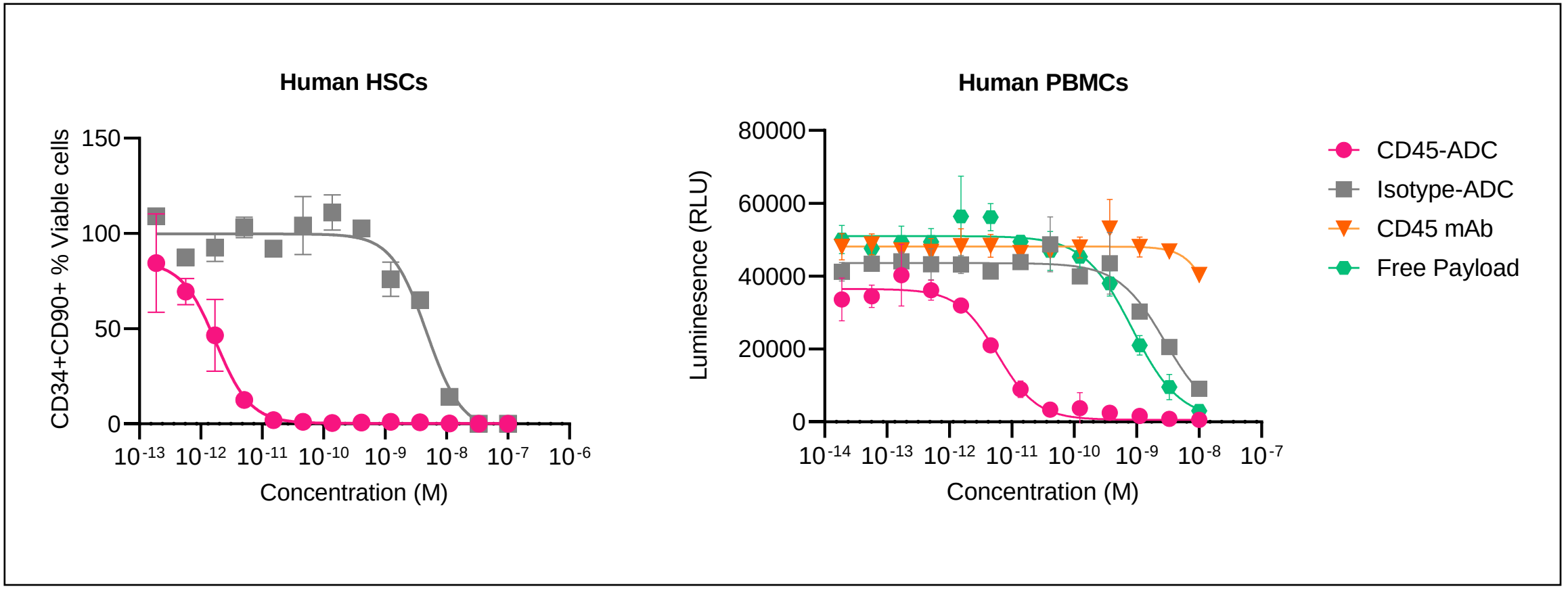
Asim Saha,[1] Sharon Hyzy,[2] Tahirih Lamothe,[2] Katelyn Hammond,[2] Nicholas Clark,[2] Leanne Lanieri,[2] Prashant Bhattarai,[2] Rahul Palchaudhuri,[2] Geoffrey O. Gillard,[2] Jennifer Proctor,[2] Megan J. Riddle,[1] Angela Panoskaltsis-Mortari,[1] Margaret L. MacMillan,[1] John E. Wagner,[1] Hans-Peter Kiem,[3] Lisa M. Olson,[2] and Bruce R. Blazar[1]





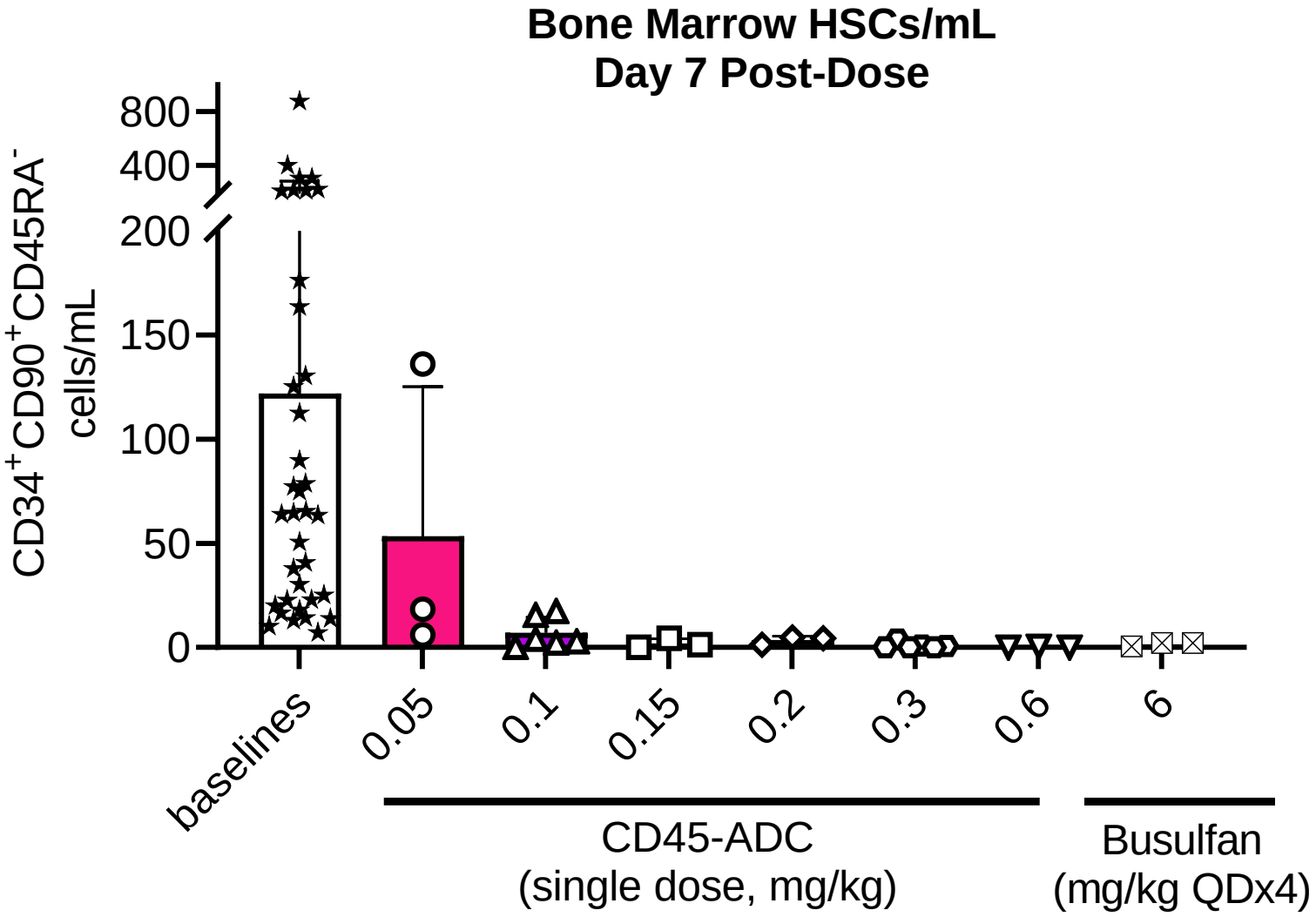


CD45-ADC kills HSCs and immune cells *ex vivo*

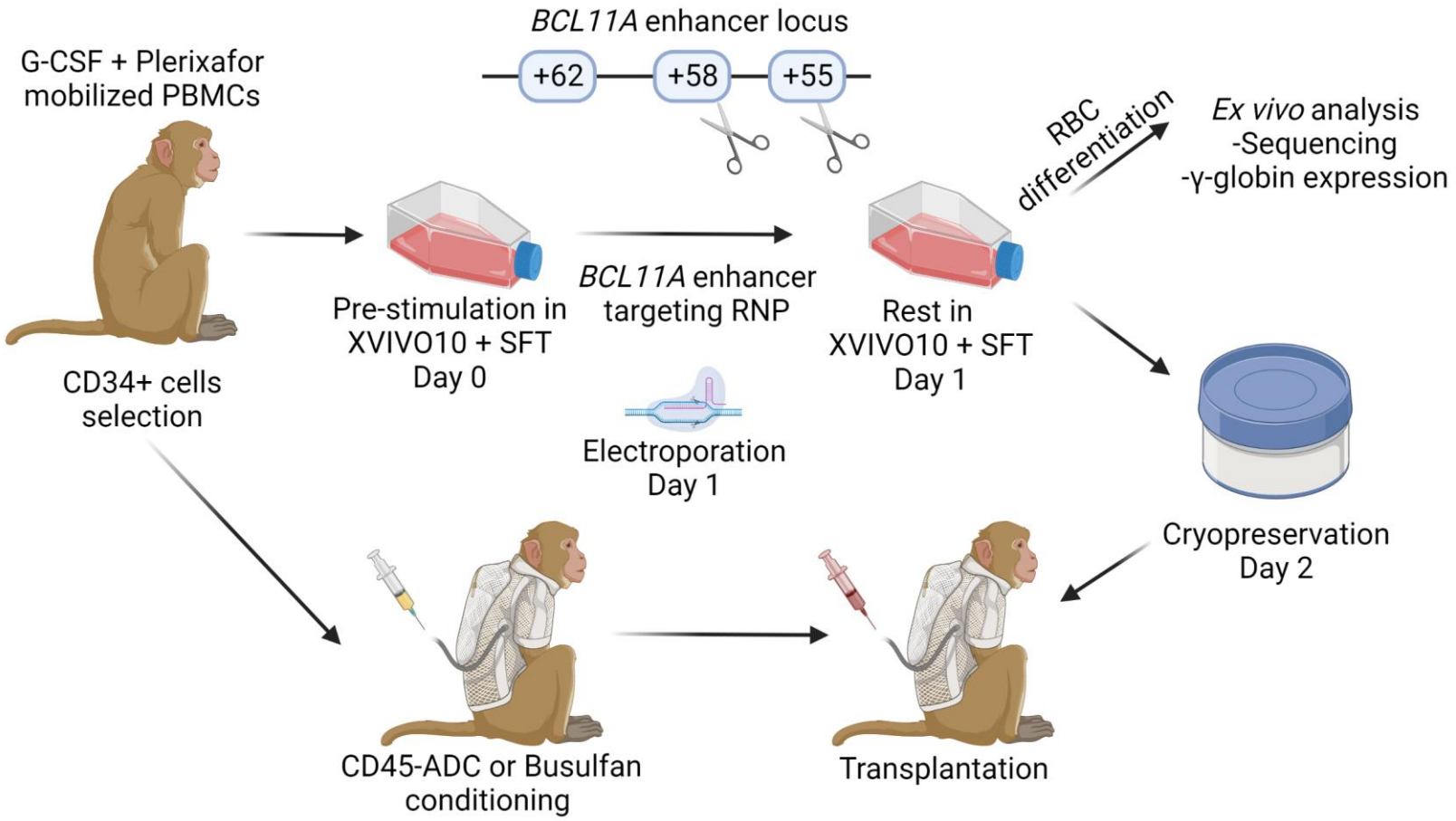


HSCs: Hematopoietic stem cells, *PBMCs*: Peripheral blood mononuclear cells

CD45-ADC kills HSCs in cynomolgus macaques



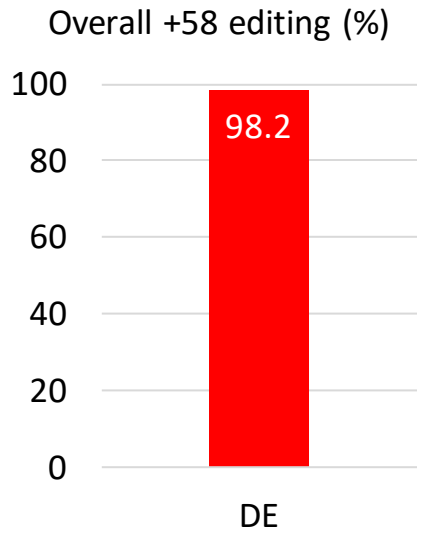
Experimental design for edited HSPC transplantation

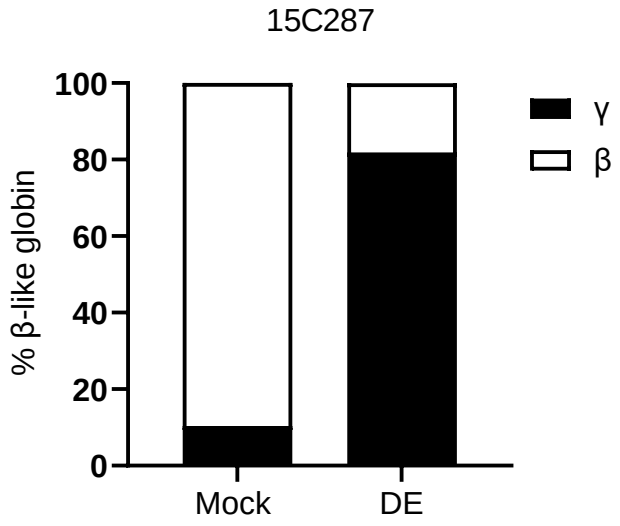


Demirci *et al.*, JCI, 2020
Jing *et al.*, Blood, 2021

G-CSF: Granulocyte colony stimulating factor*; PBMCs:* Peripheral blood mononuclear cells*; SFT:* Stem cell factor-thrombopoietin-fms-like tyrosine kinase *3; RBC:* red blood cell*; RNP:* ribonucleoprotein

Partial engraftment in 0.2mg/kg CD45-ADC conditioning



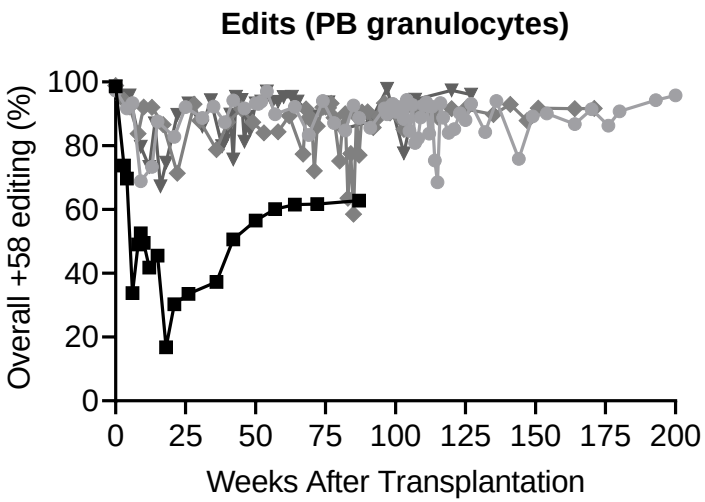


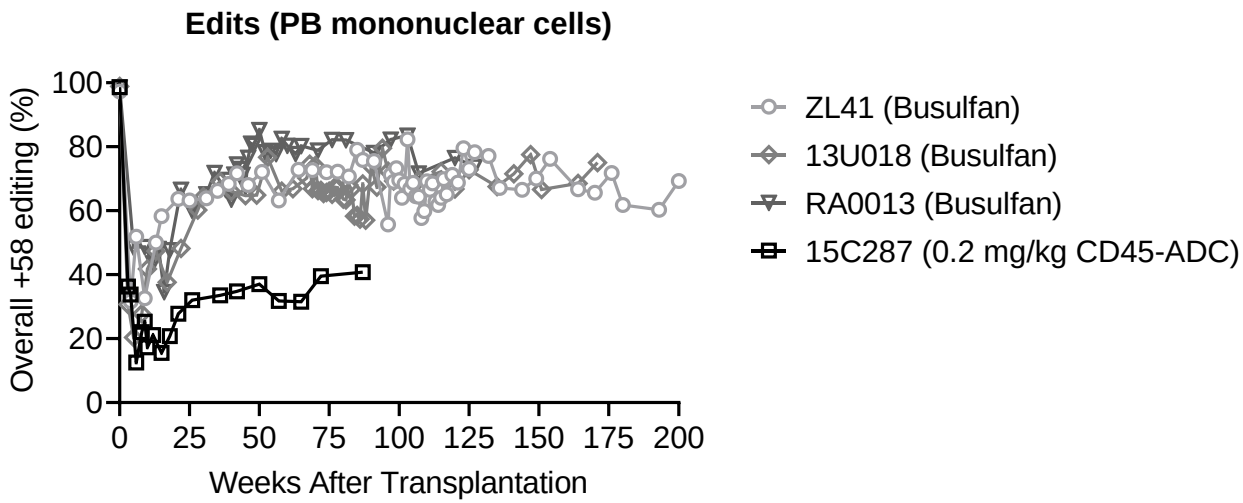
Mock: Non-electroporated control cells, *DE*: Dual edited cells

Cells collected:
2.76×10^7

Transplanted viable cells:
1.56×10^7
(2.57×10^6 cells/kg)

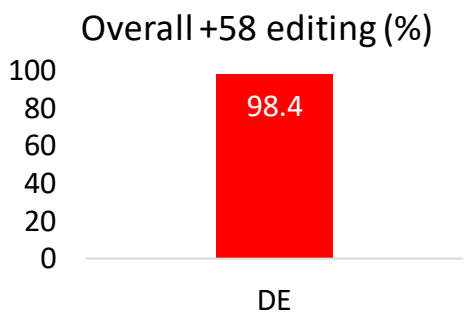
Viability:
90%

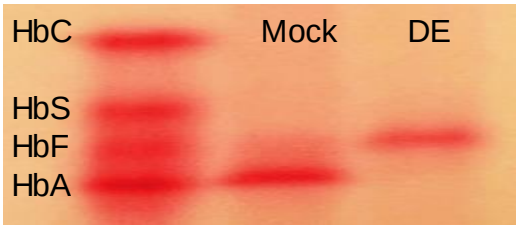


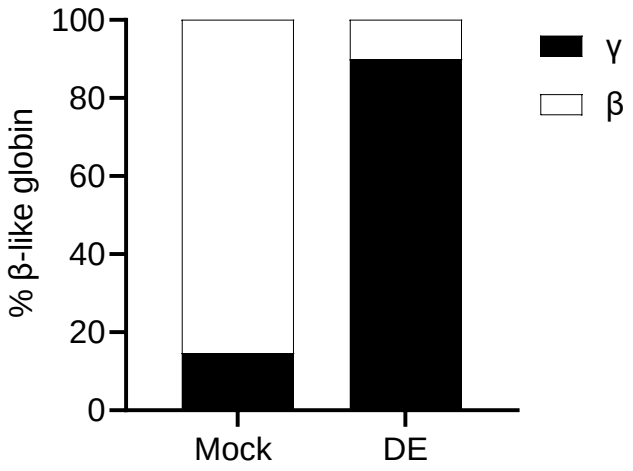


Higher dose (0.3 mg/kg) CD45-ADC conditioning

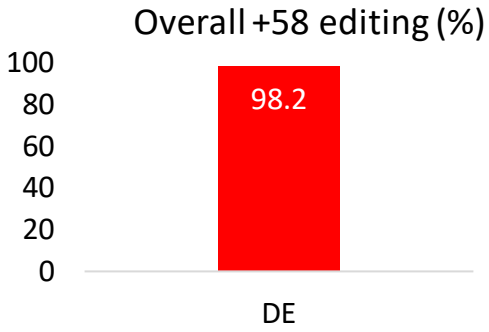
13U013

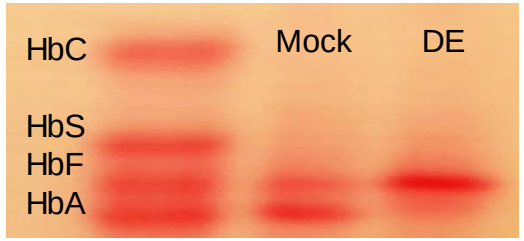


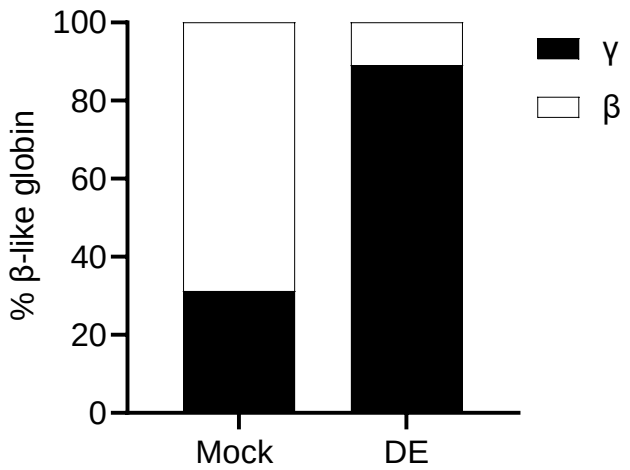




13U072

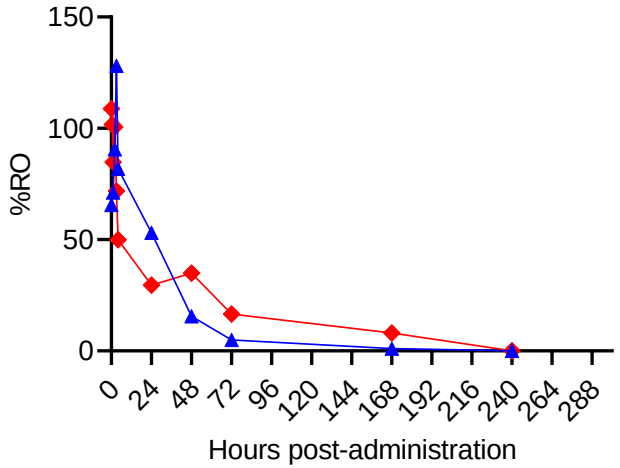


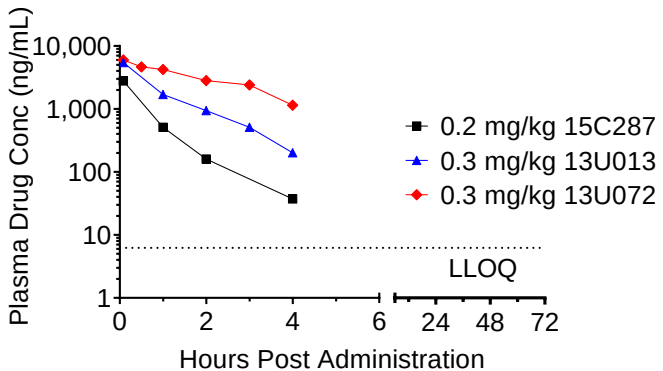




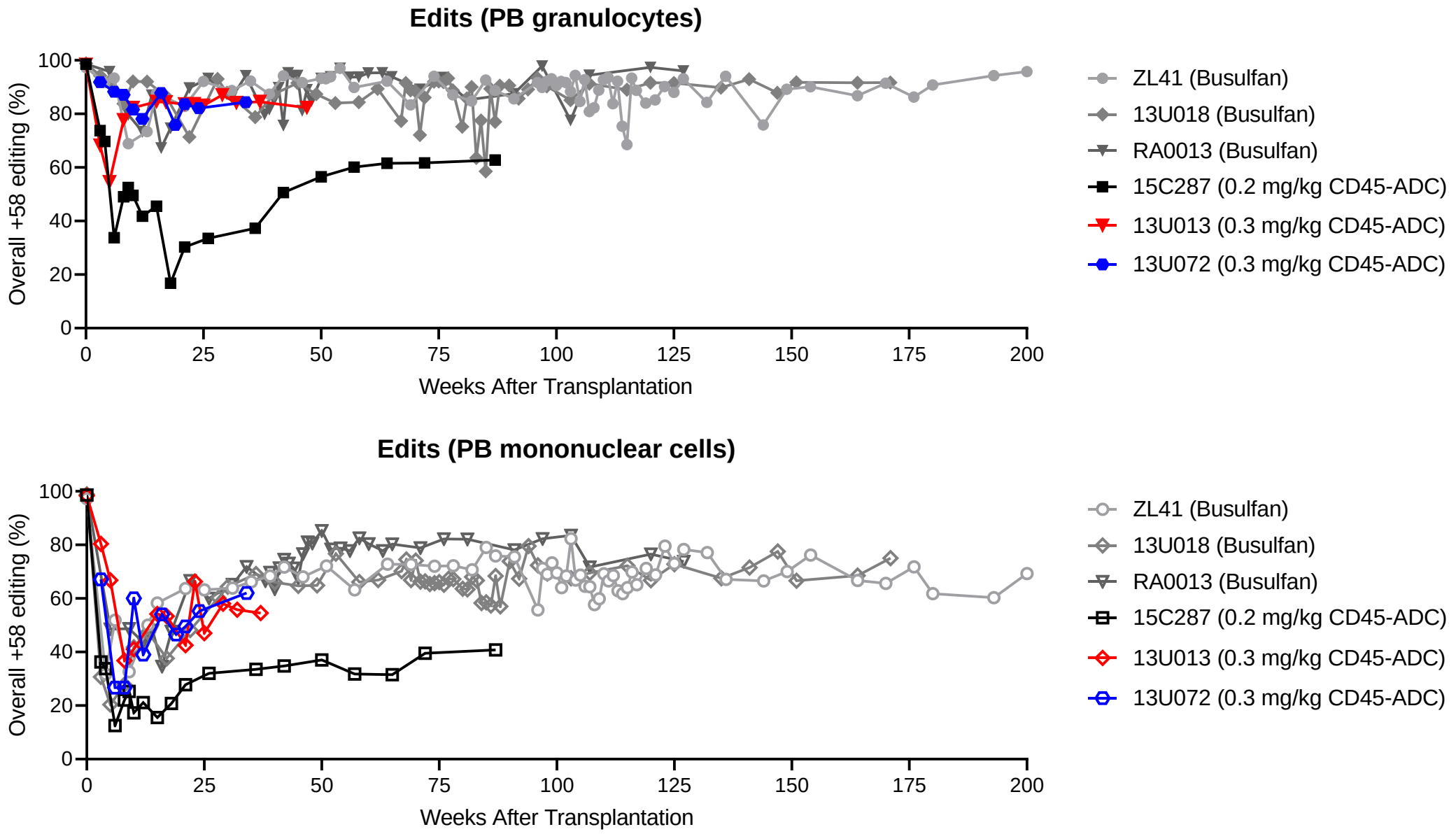
	Infusion	Infusion Day post-ADC	Cell dose
13U013	0.3mg/kg - IV Bolus	Day 10	4.07×10^6 cells/kg
13U072	0.3mg/kg - IV Bolus	Day 10	1.81×10^6 cells/kg

Total PB CD45+ cells

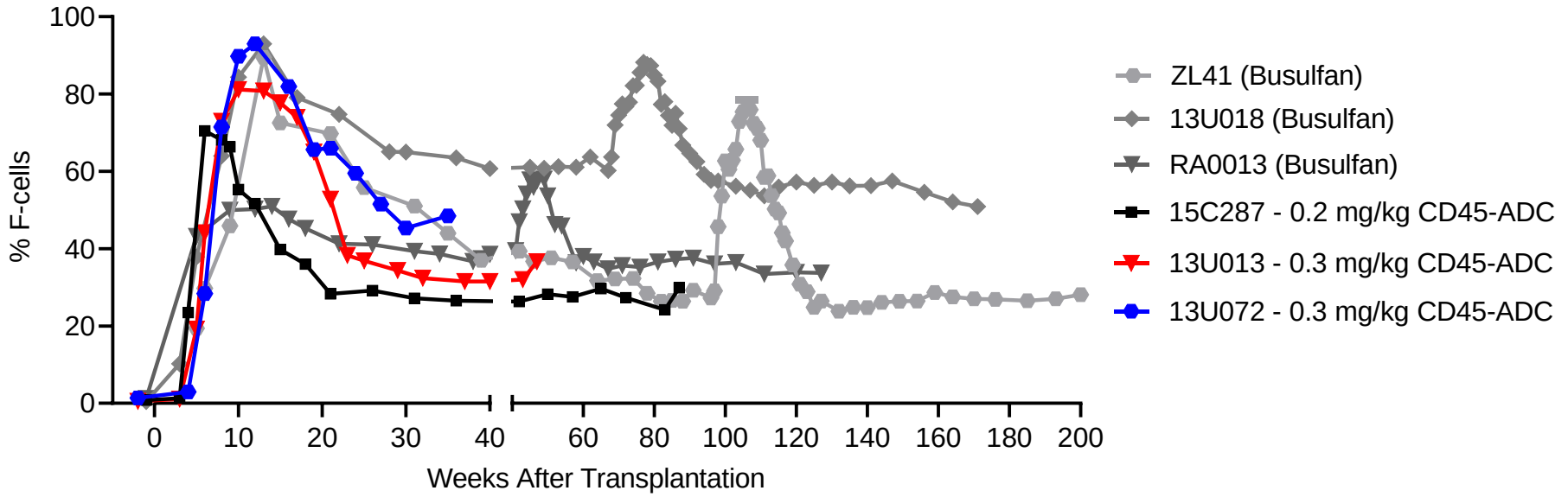


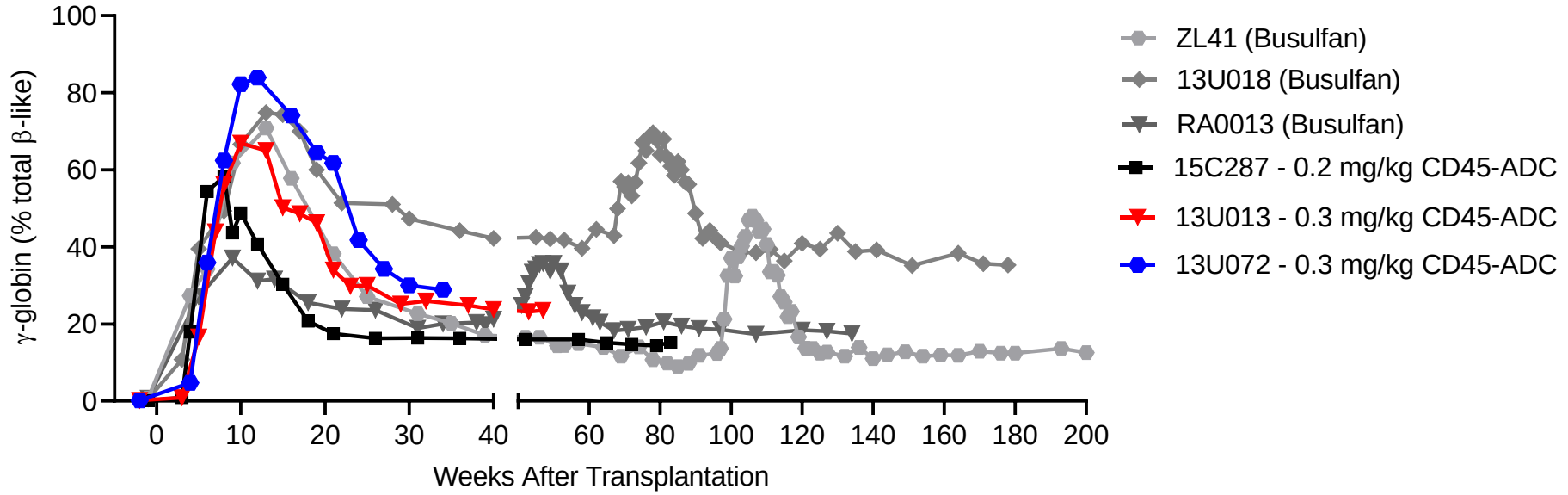


Robust engraftment in 0.3 mg/kg CD45-ADC conditioning

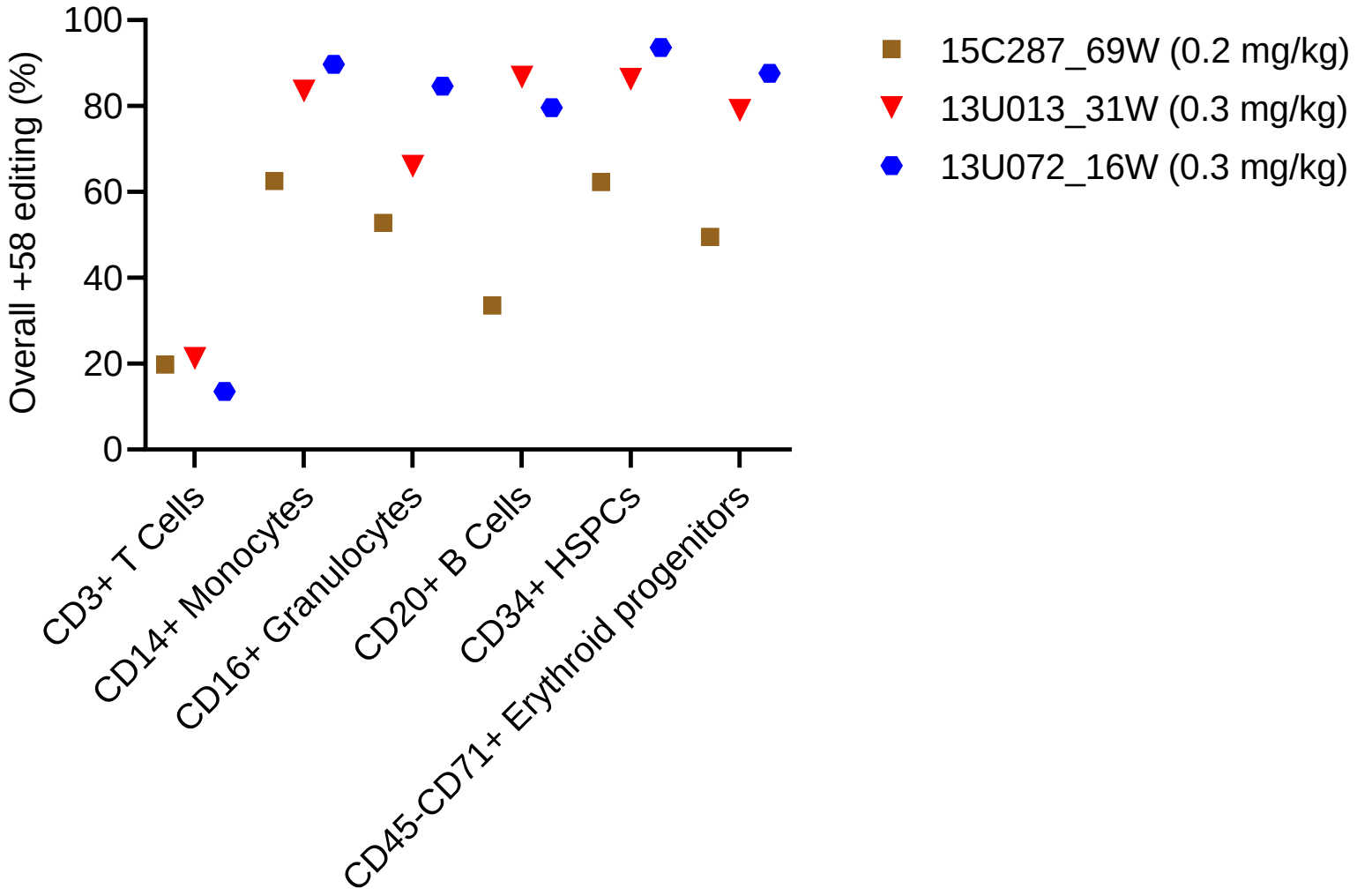


Robust HbF induction in 0.3 mg/kg CD45-ADC conditioning

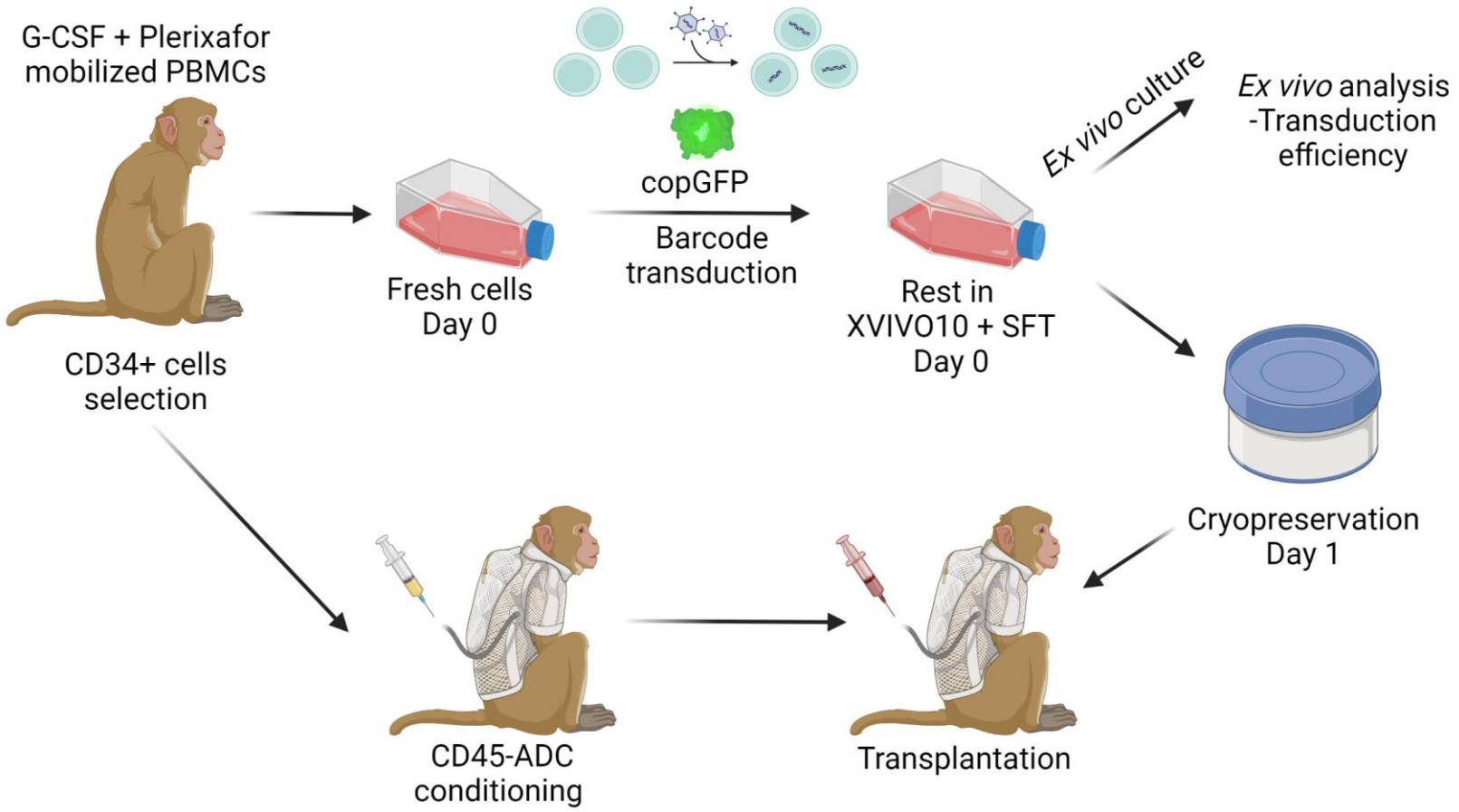




Indel frequencies in BM cell subsets



Barcoded HSPC transplantation experimental design



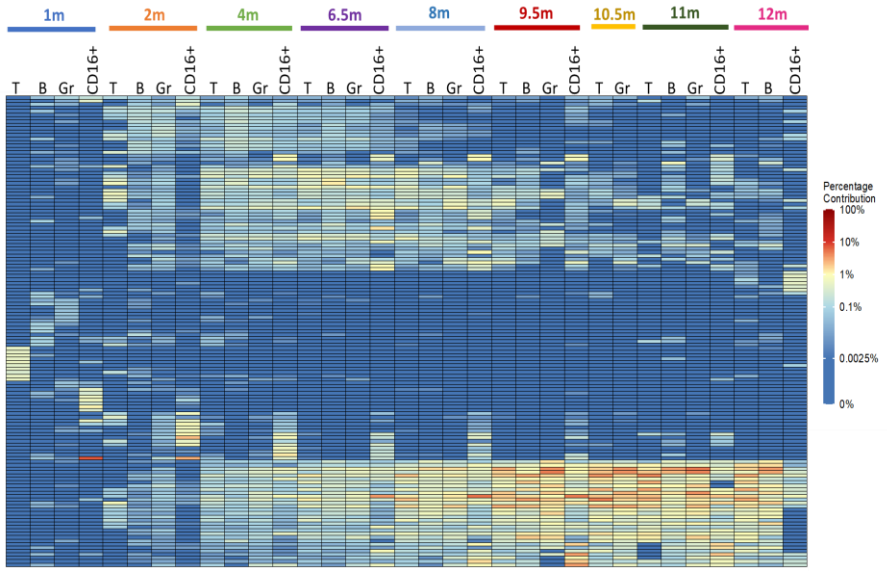
Espinoza *et al., Blood*, 2017

G-CSF: Granulocyte colony stimulating factor*; PBMCs:* Peripheral blood mononuclear cells*; SFT:* Stem cell factor-fms-like tyrosine kinase *3*-thrombopoietin

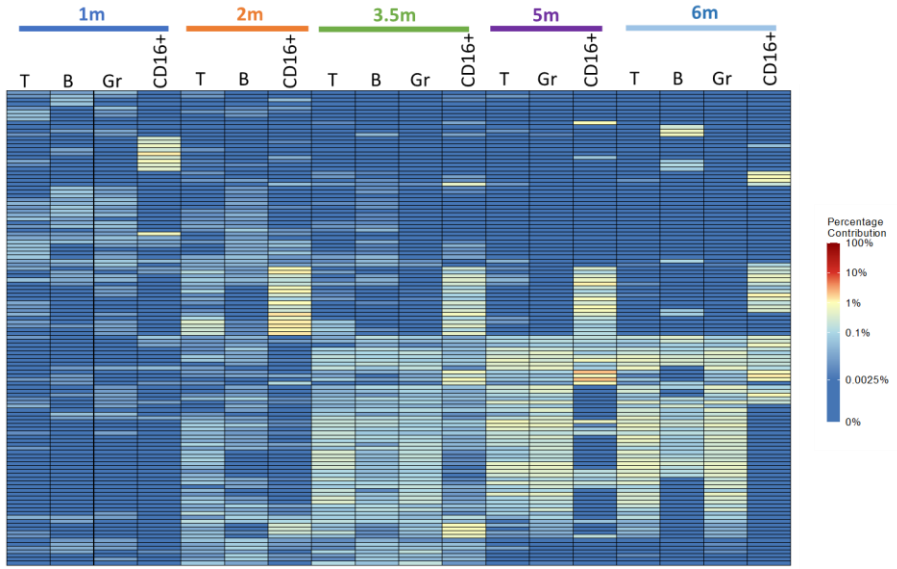
CD45-ADC conditioning supported polyclonal engraftment

Barcode heatmaps(Top 10 Clones)

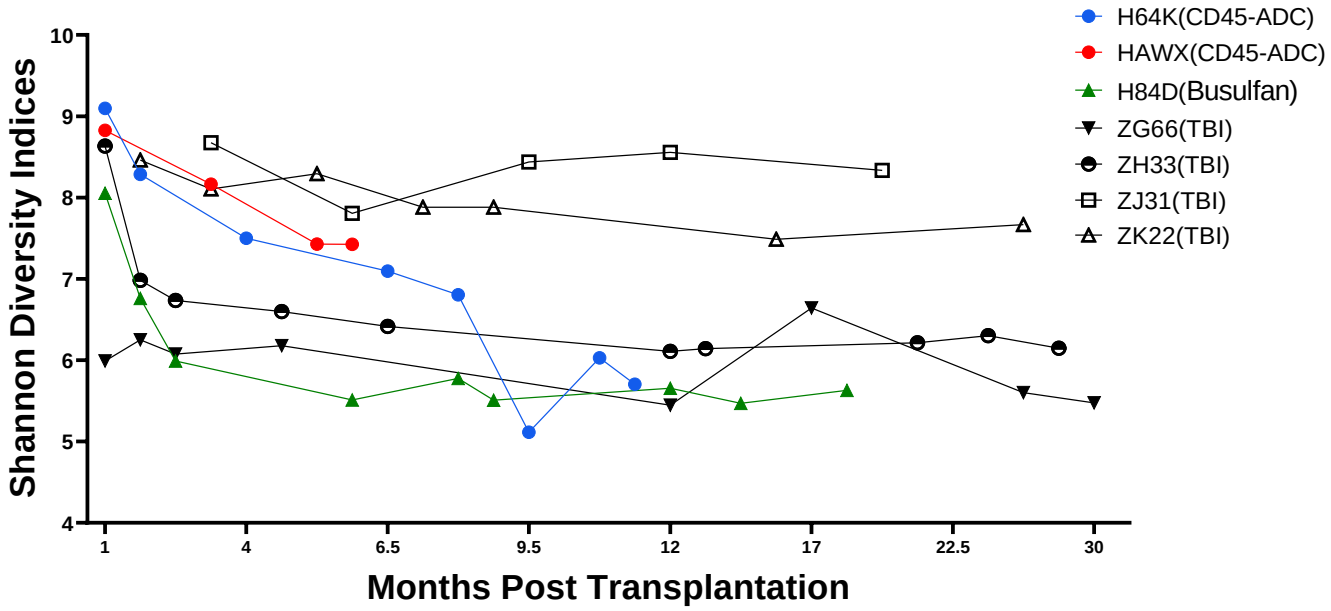
H64K



HAWX



Shannon diversity indices of PB Gr compared across CD45-ADC, Busulfan, and TBI conditioned macaques



Conclusion

- 0.2 mg/kg CD45-ADC conditioning regimen provided partial engraftment of *BCL11A* enhancer edited HSPCs and moderate HbF induction.
- 0.3 mg/kg CD45-ADC conditioning regimen provided robust engraftment of *BCL11A* enhancer edited HSPCs and significant HbF induction.
- The CD45-ADC conditioning was able to support polyclonal HSPC engraftment, and maintain the clones for several months.
- No off-target cytotoxicity nor hematological perturbations were noted to date.

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Dr. Cynthia E. Dunbar

Dr. Chuanfeng Wu
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Dr. Daniel Bauer

Dr. Jing Zeng
Anh Nguyen

magenta THERAPEUTICS

Dr. Lisa Olson
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Dr. Pradeep Dagur
Dr. Maria Lopez-Ocasio
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Dr. Duck-Yeon Lee

selami.demirci@nih.gov