HEMATOPOIETIC STEM CELLS EXPRESSING ENGINEERED CD45 ENABLE A NEAR UNIVERSAL TARGETED THERAPY FOR HEMATOLOGIC DISEASES

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INTRODUCTION

- Current untargeted cytotoxic conditioning regimens for hematopoietic stem cell transplantation (HSCT) are directly or indirectly associated with transplant related morbidity and mortality.
- Antigen-specific cell depleting therapies have revolutionized clinical practice in hematology.
- The pan-hematopoietic marker CD45, a protein tyrosine phosphatase which is exclusively expressed on all nucleated hematopoietic cells, could enable targeted depletion of the entire hematopoietic system including HSCs.
- CD45 is critical for the function of immune cells (i.e, CD45 mutations can lead to severe combined immunodeficiency (SCID)).
- HSPCs engineered to be shielded from a CD45-targeted antibody-drug conjugate (ADC) could enable selective tumor ablation with preserved hematopoiesis.

AIM

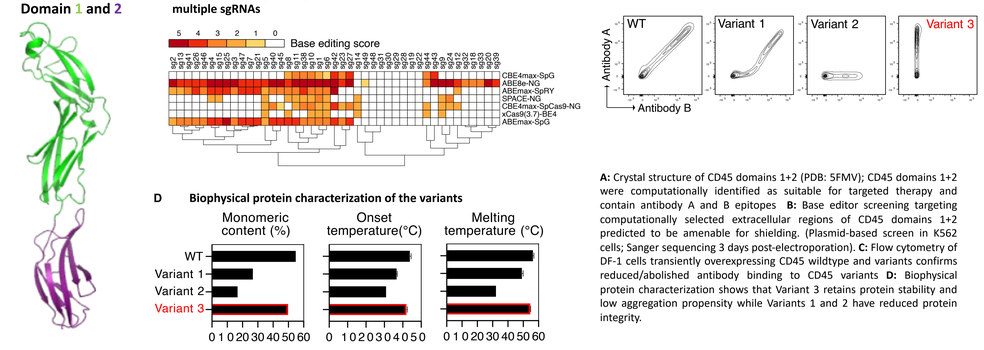
Identify and characterize CD45 variants that shield from a novel, concurrently developed, highly potent CD45-antibody drug conjugate (CIM053-ADC) while preserving CD45 function.

METHODS

- Biophysical characterisation of the leading variants (recombinant proteins).
- Base editing of HSPCs.
- In vitro colony forming assay of HSPCs.
- Internalisation of antibody B.
- In vitro CIM053-ADC mediated killing of tumor cells co-cultured with HSPCs.
- In vivo: Injection of base edited HSPCs into NBSGW mice and application of CIM053-ADC. Secondary transplants and NGS from mouse organs.
- In vivo: Injection of MOLM-14 tumor cells (AML cell line) into humanized mice and application of CIM053-ADC.

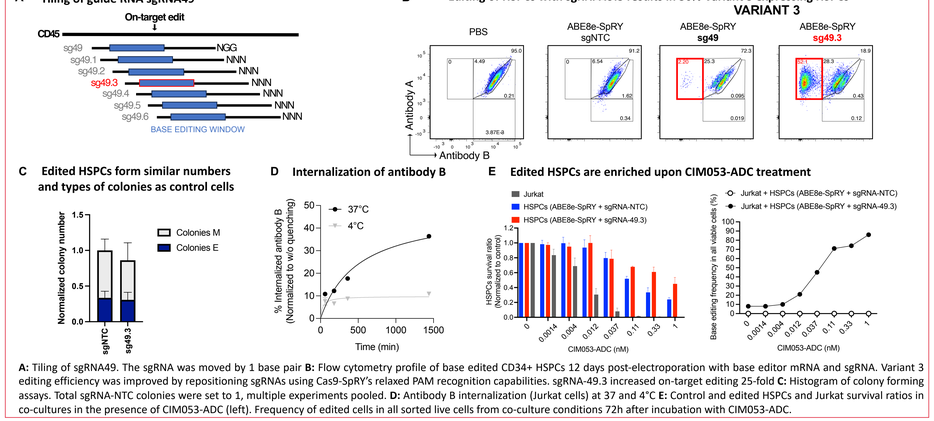
RESULTS

1. Identification of base editable CD45 extracellular domain regions to achieve shielding from targeted therapies



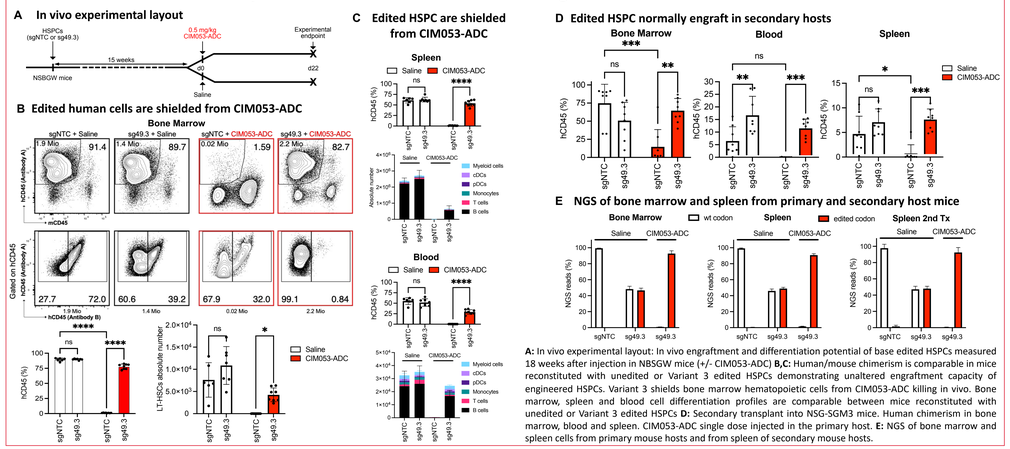
A: Crystal structure of CD45 domains 1+2 (PDB: 5FMV); CD45 domains 1+2 were computationally identified as suitable for targeted therapy and contain antibody A and B epitopes **B:** Base editor screening targeting computationally selected extracellular regions of CD45 domains 1+2 predicted to be amenable for shielding. (Plasmid-based screen in K562 cells; Sanger sequencing 3 days post-electroporation). **C:** Flow cytometry of DF-1 cells transiently overexpressing CD45 wildtype and variants confirms reduced/abolished antibody binding to CD45 variants **D:** Biophysical protein characterization shows that Variant 3 retains protein stability and low aggregation propensity while Variants 1 and 2 have reduced protein integrity.

2. Base editing in CD34+ hematopoietic stem and progenitor cells (HSPCs) in vitro



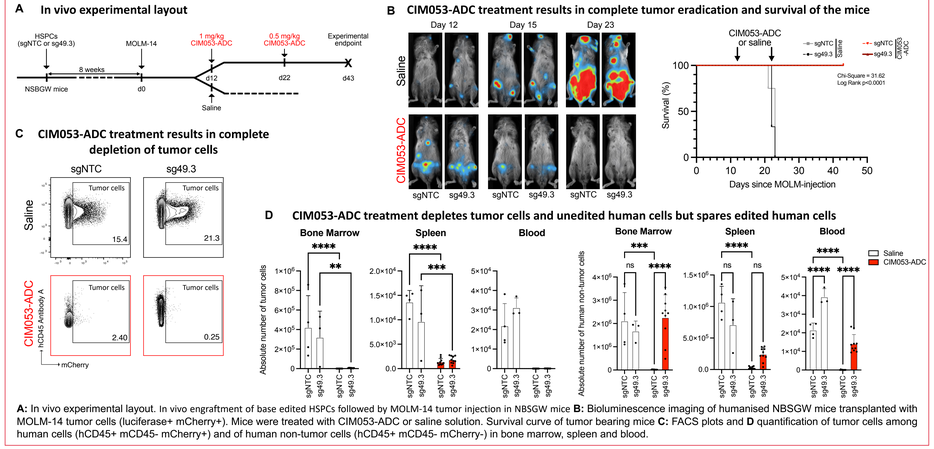
A: Tiling of sgRNA49. The sgRNA was moved by 1 base pair **B:** Flow cytometry profile of base edited CD34+ HSPCs 12 days post-electroporation with base editor mRNA and sgRNA. Variant 3 editing efficiency was improved by repositioning sgRNAs using Cas9-SpRY's relaxed PAM recognition capabilities. sgRNA-49.3 increased on-target editing 25-fold **C:** Histogram of colony forming assays. Total sgRNA-NTC colonies were set to 1, multiple experiments pooled. **D:** Antibody B internalization (Jurkat cells) at 37 and 4°C **E:** Control and edited HSPCs and Jurkat survival ratios in co-cultures in the presence of CIM053-ADC (left). Frequency of edited cells in all sorted live cells from co-culture conditions 72h after incubation with CIM053-ADC.

3. Variant 3 shields hematopoietic cells from CIM053-ADC killing in vivo



A: In vivo experimental layout: In vivo engraftment and differentiation potential of base edited HSPCs measured 18 weeks after injection in NBSGW mice (+/- CIM053-ADC) **B,C:** Human/mouse chimerism is comparable in mice reconstituted with unedited or Variant 3 edited HSPCs demonstrating unaltered engraftment capacity of engineered HSPCs. Variant 3 shields bone marrow hematopoietic cells from CIM053-ADC killing in vivo. Bone marrow, spleen and blood cell differentiation profiles are comparable between mice reconstituted with unedited or Variant 3 edited HSPCs **D:** Secondary transplant into NSG-SGM3 mice. Human chimerism in bone marrow, blood and spleen. CIM053-ADC single dose injected in the primary host. **E:** NGS of bone marrow and spleen cells from primary mouse hosts and from spleen of secondary mouse hosts.

4. In vivo CIM053-ADC mediated selective tumor eradication with preserved hematopoiesis



A: In vivo experimental layout. In vivo engraftment of base edited HSPCs followed by MOLM-14 tumor injection in NBSGW mice **B:** Bioluminescence imaging of humanised NBSGW mice transplanted with MOLM-14 tumor cells (luciferase+ mCherry+). Mice were treated with CIM053-ADC or saline solution. Survival curve of tumor bearing mice **C:** FACS plots and **D** quantification of tumor cells among human cells (hCD45+ mCD45- mCherry+) and of human non-tumor cells (hCD45+ mCD45- mCherry-) in bone marrow, spleen and blood.

CONCLUSIONS

- Identified CD45 variants with favorable biophysical properties.
- Generation of a novel, potent anti-CD45 antibody drug conjugate (CIM053-ADC) which depletes tumor cells and HSPCs. CD45 variant 3-expressing HSPCs are shielded from CIM053-ADC while maintaining intact protein properties.
- Edited HSPCs engraft, differentiate in vivo and are shielded from CIM053-ADC.
- Selective tumor and unedited human cell depletion in vivo with preservation of edited human hematopoietic cells.

REFERENCES

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