Base-Edited HSPCs Are Shielded from Targeted CD33 Therapy but Preserve CD33 Expression

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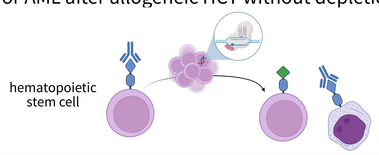
INTRODUCTION

- CD33 is expressed in >90% of patients with acute myeloid leukemia (AML)
- Due to higher expression on leukemic blasts compared to their healthy counterparts, CD33 is an attractive target and already used in clinical routine (antibody drug conjugate gemtuzumab ozogamicin)
- Additionally, there are clinical trials evaluating allogeneic hematopoietic cell transplantation (HCT) with CD33 knock-out cells to avoid hematotoxicity. However, longterm outcome of a CD33 deficient hematopoiesis remains unclear
- Our group and others recently demonstrated that single amino acid changes can protect hematopoietic stem and progenitor cells (HSPCs) from targeted therapies while maintaining their function[1,2,3]
- The adenine base editor ABE8e allows for targeted introduction of A > G changes

AIM

Identification and characterization of a base-editable CD33 variant, that maintains function while loosing binding to a therapeutic CD33 antibody. Thereby, we create a tumor-specific antigen allowing targeted therapy for AML after allogeneic HCT without depletion of HSPCs.

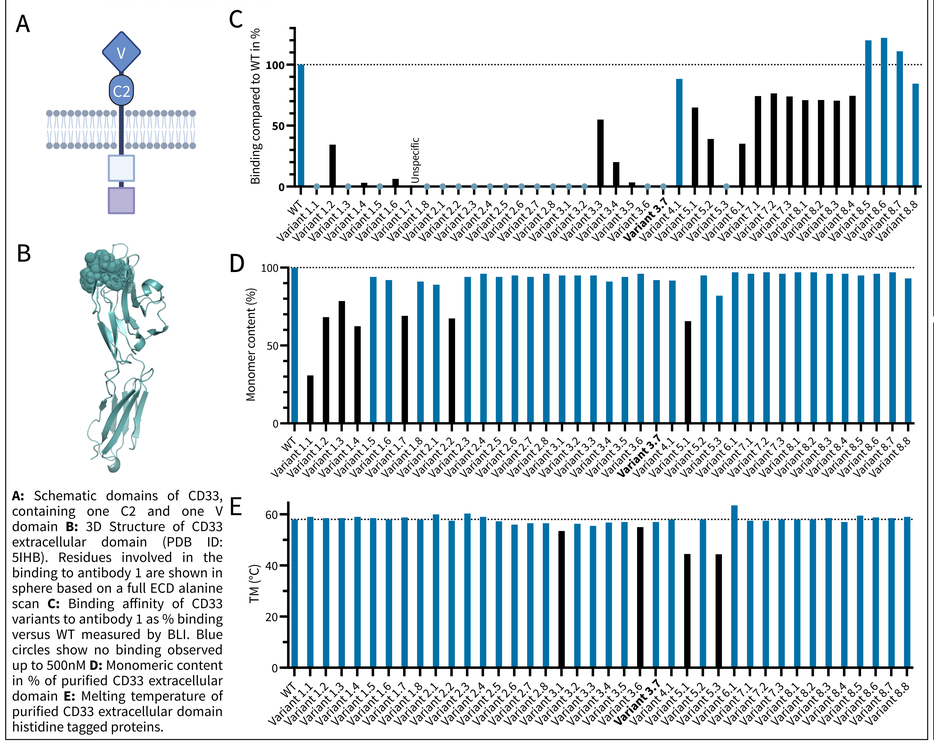


METHOD

- Alanine scan epitope mapping to identify single amino acid substitutions within the extracellular domain of CD33 maintaining protein structure
- Affinity screening by bio-layer interferometry (BLI) and biophysical characterization of variants by assessing melting temperature and monomer content
- Base editing screen using ABE8e_SpRY and 21 tiled sgRNAs
- Readout after editing by assessing binding to therapeutic CD33 antibody and CD33 control antibody via flow cytometry and Sanger sequencing of bulk cells
- NGS of sorted cell populations after base editing
- In vitro differentiation and colony forming assay of base-edited HSPCs

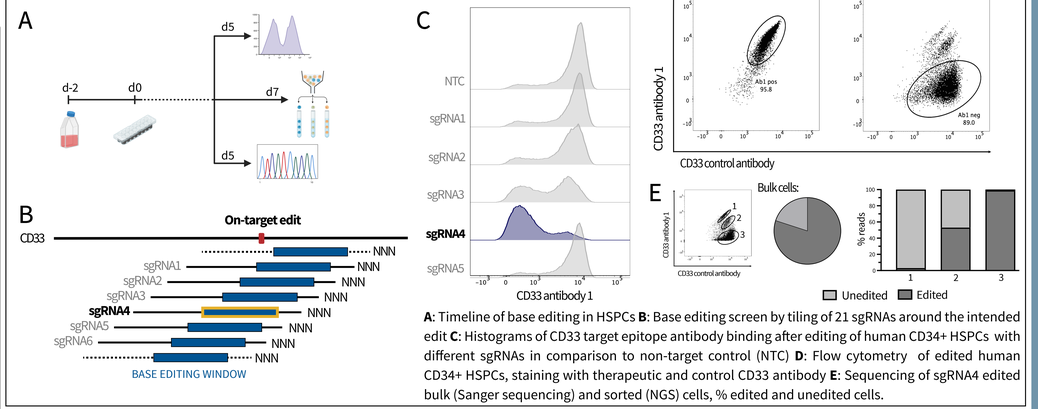
RESULTS

Figure 1: Biophysical characterization of CD33 protein variants harboring single amino acid substitutions



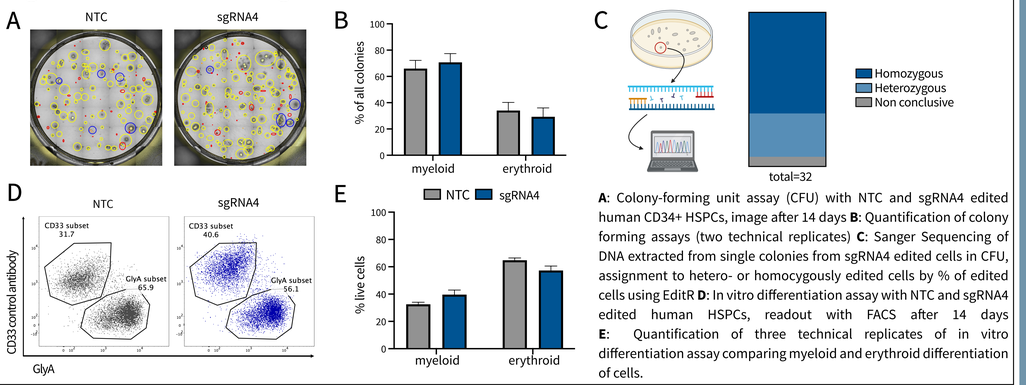
A: Schematic domains of CD33, containing one C2 and one V domain **B:** 3D Structure of CD33 extracellular domain (PDB ID: 5IHB). Residues involved in the binding to antibody 1 are shown in sphere based on a full ECD alanine scan **C:** Binding affinity of CD33 variants to antibody 1 as % binding versus WT measured by BLI. Blue circles show no binding observed up to 500nM **D:** Monomeric content in % of purified CD33 extracellular domain **E:** Melting temperature of purified CD33 extracellular domain histidine tagged proteins.

Figure 2: Base editing of human CD34+ HSPCs in vitro



A: Timeline of base editing in HSPCs **B**: Base editing screen by tiling of 21 sgRNAs around the intended edit **C**: Histograms of CD33 target epitope antibody binding after editing of human CD34+ HSPCs with different sgRNAs in comparison to non-target control (NTC) **D**: Flow cytometry of edited human CD34+ HSPCs, staining with therapeutic and control CD33 antibody **E**: Sequencing of sgRNA4 edited bulk (Sanger sequencing) and sorted (NGS) cells, % edited and unedited cells.

Figure 3: Differentiation of sgRNA4 edited HSPCs in vitro



A: Colony-forming unit assay (CFU) with NTC and sgRNA4 edited human CD34+ HSPCs, image after 14 days **B**: Quantification of colony forming assays (two technical replicates) **C**: Sanger Sequencing of DNA extracted from single colonies from sgRNA4 edited cells in CFU, assignment to hetero- or homocygously edited cells by % of edited cells using EditR **D**: In vitro differentiation assay with NTC and sgRNA4 edited human HSPCs, readout with FACS after 14 days **E**: Quantification of three technical replicates of in vitro differentiation assay comparing myeloid and erythroid differentiation of cells.

CONCLUSIONS/ OUTLOOK

- We identified a base-editable CD33 variant showing loss of antibody 1 binding while maintaining binding to a control CD33 antibody and unaltered biophysical properties compared to wildtype CD33
- HSPCs expressing this variant show a differentiation potential comparable to NTC edited HSPCs in CFU assay and in vitro differentiation assay
- Studies to further characterize the differentiation of edited cells in vivo as well as tumor models to investigate selective killing of tumor cells while preserving edited HSPCs are ongoing

REFERENCES

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