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Blood 142 (2023) 3422–3423

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

701.EXPERIMENTAL TRANSPLANTATION: BASIC AND TRANSLATIONAL

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

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Targeted therapy for AML remains challenging as there is broad overlap between targetable surface proteins on transformed cells and healthy hematopoietic stem and progenitor cells (HSPC). CD33 is expressed in about 90% of patients with AML but also on healthy myeloid cells with strong expression on progenitor cells and only weak expression on mature neutrophils. Given the higher expression on leukemic blasts compared to their healthy counterparts, CD33 is an attractive target in AML. However, targeting CD33 e.g. with the antibody-drug conjugate (ADC) gemtuzumab ozogamicin often leads to prolonged cytopenia due to suppression of normal myelopoiesis. Transplantation of genetically engineered CD33 knock-out allogenic HSPCs protects reconstituting hematopoietic cells from targeted immunotherapy administered for disease control after transplantation. Early clinical trials explore this option but the long-term effect of loss-of-function of CD33 remains unknown. We recently demonstrated that single amino acid substitutions engineered into CD45, CD117 or CD123 protect HSPCs from targeted therapies against these antigens while preserving their function. Here, we describe the identification of CD33 shielding amino acid substitutions that can be engineered by base editing and preserve CD33 expression on HSPCs.

We identified binding sites of two CD33 antibodies by alanine scanning of the entire extracellular domain. The most favorable amino acid substitutions at key residues were analyzed computationally and binding reduction was subsequently validated by flow cytometry. 23 individual amino acid substitutions at 3 positions were tested. Eleven retained similar expression, purity and biophysical properties as wildtype CD33. Those variants are shielded from antibody 1 even at high concentrations when assessed by biolayer interferometry. In addition, three variants are shielded from antibody 2 binding.

We used the ABE8e_SpRY base editor to engineer HSPCs and tiled 21 sgRNAs centered around the intended edit. Five days after electroporation, HSPCs were evaluated for editing efficiency (Sanger sequencing) and binding to antibody 1 (flow cytometry). sgRNA4 showed high editing efficiency of about 80% as well as loss of binding to antibody 1 (Figure A) while binding to the control antibody that binds a different epitope was maintained. Importantly, Sanger sequencing did not show off-target editing of the closely related SIGLEC22P pseudogene. Edited (sgRNA4) and non-edited control (NTC) HSPCs formed comparable numbers of myeloid and erythroid colonies (Figure B). Furthermore, in vitro differentiation showed no difference in frequency of CD14, CD15, CD19, CD33, CD41 and GlyA positive cells indicating that the edited HSPCs did not display major functional alterations.

In summary, CD33 variants that can be engineered into HSPCs with high editing efficiency by base editing protected from binding to antibody 1 but preserved CD33 expression. These edited HSPCs could be therapeutically useful, in particular for continued immunotherapy after allogenic HSC transplantation. Similar to HSPCs devoid of CD33 these engineered cells could enable tumor-selective immunotherapy using ADCs or CAR T cells but with preserved CD33 expression and function. Furthermore, since base editing is suitable for multiplexing, they may in the future be multiplexed to protect CD33 and other targets for combination immunotherapy.

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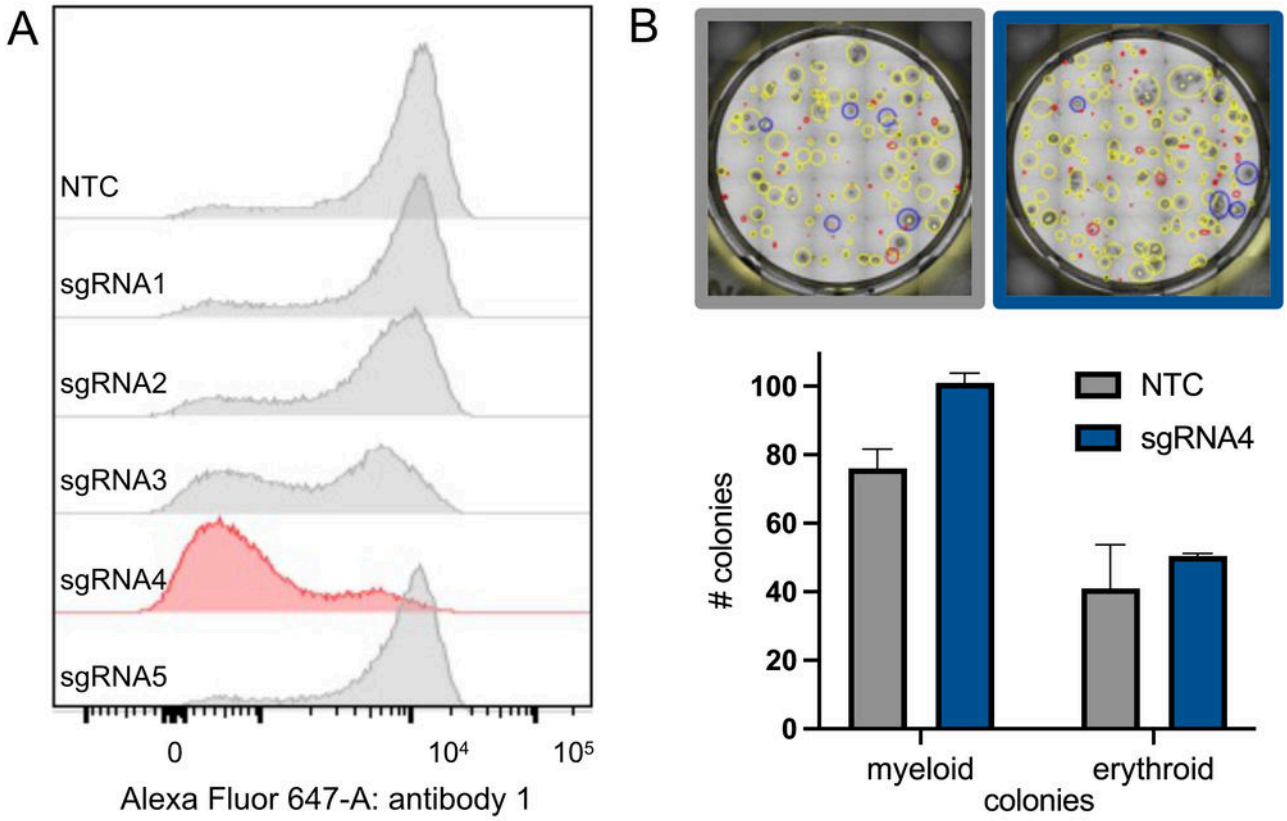

Figure 1

A: Flow cytometry histogram of base edited HSPCs stained with antibody 1. NTC: non-targeting control sgRNA; sgRNA1-5: tiled sgRNAs. **B**: Image and quantification of myeloid and erythroid colonies of colony forming assays

Figure 1

https://doi.org/10.1182/blood-2023-177706

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