Multiplex Deletion of Myeloid Antigens CD33 and CLL-1 by CRISPR/Cas9 in Human Hematopoietic Stem Cells Highlights the Potential of Next-Generation Transplantation for AML Treatment


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INTRODUCTION

► Acute myeloid leukemia (AML) is a heterogeneous disease characterized by aberrant cell division. It is the most common form of adult acute leukemia.

► Although hematopoietic cell transplantation (HCT) is the standard of care for patients with high-risk AML, post-HCT relapse occurs in 40% of these patients, highlighting the need for novel therapeutic approaches such as immunotherapy.

► Cluster of differentiation (CD)33 and CD11b are highly expressed in AML, patient-derived hematopoietic stem cells (HSCs), suggesting high mutational load and a potential therapeutic target. Editing these genes may reduce clonality and improve chances of patient remission. Targeting these antigens, however, can lead to cytokine release syndrome in normal hematopoietic cells.

► Demonstrating both CD33 and CD11b from hematopoietic progenitor cells prior to HCT reduces these antigens to AML cells in the event that relapse occurs post-HCT, thereby enabling the potential for subsequent immunotherapy without risk of on-target, off-tumor toxicities.

OBJECTIVE

► Demonstrate that multiplex (MP) deletion of CD33 and CLL-1 from CD34+ human hematopoietic stem and progenitor cells (HSPCs) does not impact HSC function.

► Demonstrate that cells derived for CD33 and CLL-1 are protected from targeted immunotherapies.

RESULTS

Fig. 1. CD33 and CLL-1 are highly expressed in AML patient-derived blasts and LSCs

- Percentage of CD33 and CLL-1-positive cells for flow cytometry in AML. (A) blasts (n=7) and (B) LSCs (n=7). CD33 and CLL-1 density on the cell surface quantified by flow cytometry in (C) blasts (n=7) and (D) LSCs (n=7).

Fig. 2. MPX-edited HSPCs for CD33 and CLL-1 retain high viability and normal distribution of hematopoietic progenitor subpopulations in vitro

- Multiplex progenitor blast CD34+CD38+ cells were isolated from autologous peripheral blood mononuclear cells (PBMCs) and used for multiplex editing using CRISPR/Cas9. The viability of MPX-edited HSPCs was evaluated by counting colony-forming units (CFUs) and by the flow cytometric analysis of CD3+CD19+ and CD14+CD33+ cells from MPX-edited HSPCs compared with non-edited control HSPCs.

- CD33 and CLL-1 expression levels were determined by flow cytometry in MPX-edited HSPCs and non-edited control HSPCs.

Fig. 3. CD33 and CLL-1 MPX-edited HSPCs maintain differentiation in vitro

- Treatment of MPX-edited HSPCs with interleukin-3 (IL-3) and granulocyte colony-stimulating factor (G-CSF) induces differentiation into granulocytes and monocytes.

Fig. 4. In vitro myeloid differentiated cells derived from CD33 and CLL-1 MPX-edited hHSPCs maintain function

- (A) Progeny frequency of CTR or MPX-edited hHSPCs. (B) Production of GM-CSF and TNF-α in response to CD33 or CLL-1 CAR-T killing.

Fig. 5. CD33 and CLL-1 MPX-edited hHSPCs maintain long-term engraftment and multilineage differentiation in vivo

- (A) Percentage of CD33 and CLL-1-positive cells by flow cytometry in AML. (B) blasts (n=7) and (C) LSCs (n=7). CD33 and CLL-1 density on the cell surface quantified by flow cytometry in (D) blasts (n=7) and (E) LSCs (n=7).

Fig. 6. Protection of MPX-edited cells from CAR-Ts targeting CD33 and CLL-1

- HLA-B matched cells were either edited for CD33 or CLL-1, or MPX-edited for both targets and knock-out (KO) cells were purified by FACs sorting prior to in vitro cytokine assay with CTR, CD33 + CAR-T, CLL-1 + CAR-T, and CD33 + CLL-1 + CAR-T-pretreated with CD11b and CD33 CAR-T cells. The percentage of live cells was plotted. CTR, Control anti-sense, CAR-T, chimeric antigen receptor T-