Chimeric Antigen Receptor-Modified T cells for the Treatment of Acute Myeloid Leukemia Expressing CD33

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Abstract

Relapsed acute myeloid leukemia (AML) is an aggressive disease with very poor outcomes. Reduction of T-cell specificity via chimeric antigen receptor (CAR) has shown promising anti-tumor activity in clinical trials, particularly for B cell lineage malignancies. CD33 is a transmembrane protein expressed on normal and malignant myeloid-derived cells (as well as on subsets of activated T cells and NK cells). Since this protein is commonly expressed on AML, we sought to evaluate the efficacy of targeting AML with CD33-specific CAR T cells. We generated a lentiviral construct to co-express CD33-specific CAR and a kill switch based on a tag derived from the epidermal growth factor receptor. The latter allows for the conditional elimination of CAR-T cells in vivo. Following transduction of primary T cells, we confirmed CAR and kill switch co-expression by flow cytometry and western blot analyses. Elimination of genetically modified T cells was demonstrated using the clinically available antibody, cetuximab. CD33 CAR-T cells demonstrated specific cytotoxicity to CD33+ target cells. CD33+ T cells were also activated in co-culture using IFN-γ and IL-12 cytokines in response to CD33+ target cells. Furthermore, adoptive transfer of CD33-CAR T cells in immunocompromised (NSG) mice resulted in reduction of tumor burden and improvement of overall survival, compared to control mice receiving CD33+ T cells or no immunotherapy. Sampling of blood demonstrated the persistence of the CD33 CAR-T cells with no detection of AML (MOLM-13) tumor cells. These pre-clinical data demonstrate the effectiveness of CD33+ CAR-T cells in targeting CD33+ AML tumor cells and provide a rationale for future clinical evaluation in AML patients with urgent medical need.

Background

- Patients with AML often face treatment failures and high relapse rates. The overall survival following relapse is poor.
- Limited treatment options are currently available for patients with relapse/refractory AML.
- CD33 is a transmembrane glycoprotein commonly expressed on AML blast cells but also expressed on normal myeloid cells and on some activated T and NK cells.
- CD33 is an attractive target for immunotherapy.

Co-expression of CAR and Kill Switch in T cells

Conditional Elimination of HER1T Expressing CD33-CAR-T cells

Specific in vitro Cytotoxicity of CD33-CAR-T Cells

Specific Cytokine Induction with CD33-CAR-T Cells

Specific in vivo Cytotoxicity of CD33-CAR-T Cells

CD33-CAR-T Cells Eliminated AML Tumor & Improved Survival in an in vivo Mouse Model

CD33-CAR-T Cells Engrafted & Expanded in CD33+ AML Tumor-Bearing Mice

Presence of CD33-CAR-T Cells in AML Tumor-Bearing NSG Mice

Summary

- CD33-specific CAR and HER1 t kill switch were co-expressed in T cells using a lentiviral vector.
- CD33-CAR-T cells exhibited redirected specificity for CD33 in vitro as evident by cytokine release.
- CD33-CAR-T cells demonstrated specific cytotoxic activity in response to CD33+ target cells.
- HER1 t expressing CD33-CAR-T cells were conditionally eliminated by cetuximab-mediated ADCC.
- CD33-CAR-T cells eliminated AML and significantly improved survival in mice.
- Pre-clinical data support clinical evaluation of CD33-CAR-T for treatment of relapsed/refractory AML in human trials.

Graphical Abstract

- Figure 1: Expression of CD33-specific CAR and kill switch in T cells by lentiviral transduction. (A) Depiction of CD33-specific CAR and kill switch constructs. CD33-specific CAR is a chimeric fusion protein of human CD33 extracellular domain and humanFcγRIIIa transmembrane and intracellular domains. (B) Western blot analysis of lysates from engineered (right panel) and control (left panel) T cells. (C) Expression of CD33-specific CAR was confirmed by detection of chromatin GAD by western blot analysis.
- Figure 2: Redirected specificity of CD33-CAR-T cells to specifically lyse CD33+ target cells in vitro using co-culture with autologous target cells (CD33+ KASUMI-1) or CD33+ EL4 and CD33+ MOULM-13. (A) CD33 surface expression levels in target cell lines were measured by flow cytometry. Red square represents staining with human control antibody and blue line represents staining with CD33-specific antibody. (B) Cytotoxicity of unmodified (control) T cell or CD33-CAR T cells was performed at a 5:1 effector:target (E:T) ratio. *** p<0.001, Student’s T test. Shown is the mean ± standard deviation (SD).
- Figure 3: Cytokine expression by CD33-CAR-T cells in response to CD33+ target cells. (A) CD33 surface expression levels in AML tumor cell lines as measured by flow cytometry. Red line represents the isotype and blue line represents anti-CD33 stained cells. (B) Supernatants were harvested after a 24 h culture of tumor cells with untransduced (parent) T cells or CD33-CAR-T cells. A multiplex cytokine analysis was performed to measure IFN-γ, TNF-α and IL-12 levels in conditioned media. The effector target (E:T) ratio was 5:1. Shown is the mean ± SD.
- Figure 4: Ability to turn off CD33-specific CAR and kill switch in T cells using propagated allogeneic CD33+ target cells as effector cells and AML assay. (A) Schematic diagram of mechanism of action. (B) T cells were co-cultured with fluorescent PKH26 labeled CD33+HL60 cells at two E:T ratios in the presence of unmodified control cultures. Following an overnight culture, cells were analyzed by flow cytometry to calculate the frequency of remaining T cells. Calculated percentage of lysis was normalized using rituximab as the comparator. Shown is the mean ± SD.
- Figure 5: Efficacy of CD33-CAR T cells in an AML reseeding model. (A) Immunocompromised (NSG) mice were reseeded (Day 18) with CD33-CAR+ or control (LV) AML tumor cell lines, and engraftment (tumor load) was confirmed by bioluminescence imaging (BLI). Tumor presence was confirmed on Day 7 prior to reseeding. On Day 8, mice with established tumor burden were treated with CD33-CAR-T cells (5×106 CAR+ T cells) or control treatments (unmodified T cells; CD33- specific CAR-T cells; or saline) from same donor T cells. Tumor burden was monitored by BLI and weekly blood drawn was performed for flow cytometry analysis. (B) Images (oral view) from the same five representative mice from each group drawn through the course of study. (C) Quantitative analysis of bioluminescence values. Arrow indicates CAR T cell treatment. Shown is the mean ± standard error of the mean (SEM). (D) Survival curves for each treatment group. *** p<0.001, log-rank Test when compared to control groups of saline, untransduced T cells and CD33-CAR T cells (n=10 to 12 per group).
- Figure 6: Presence of CAR-T cells in mice analyzed by flow cytometry of peripheral blood samples. (A) Quantification of human CD3+ T cells detected per µL of whole blood. (B) Quantification of the CAR-T cells detected per µL of whole blood. Shown is the mean ± SEM in n = 4 to 16 mice/group.