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

Degang Song, Ph.D.[1], Michael H. Swartz[1], Steven G. Biesecker[1], Fernando Carvajal Borda[1], Rutul R. Shah[1], Peter Emtage, Ph.D.[2], William G. Wierda, M.D., PhD[3], Laurence J. N. Cooper, M.D., Ph.D.[4], Tim Chan, Ph.D.[1]

[1]Intrexon Corporation, Germantown MD; [2]Formerly at Intrexon Corporation, Germantown, MD; [3]Department of Leukemia, The University of Texas MD Anderson Cancer Center, Houston, TX, [4]ZIOPHARM Oncology, Boston, MA

Abstract

Relapsed acute myeloid leukemia (AML) is an aggressive disease with very poor outcomes. Redirection 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 cells, 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 cell lines. CD33 CAR-T cells were also activated to produce IFNγ, TNF, and IL-2 cytokines in response to CD33$^+$ target cells. Furthermore, adoptive transfer of CD33 CAR-T in immunocompromised (NSG) mice bearing established CD33$^+$(CD19^{neg}) AML (MOLM-13) tumors resulted in reduction of tumor burden and improvement of overall survival, compared to control mice receiving CD19 CAR-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 unmet 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

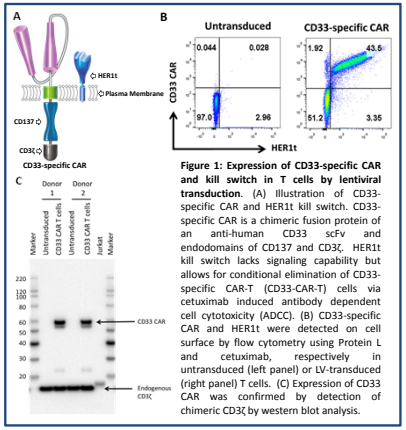

Figure 1: Expression of CD33-specific CAR and kill switch in T cells by lentiviral transduction. (A) Illustration of CD33-specific CAR and HER1t kill switch. CD33-specific CAR is a chimeric fusion protein of an anti-human CD33 scFv and endodomains of CD137 and CD3ζ. HER1t kill switch lacks signaling capability but allows for conditional elimination of CD33-specific CAR-T (CD33-CAR-T) cells via cetuximab induced antibody dependent cell cytotoxicity (ADCC). (B) CD33-specific CAR and HER1t were detected on cell surface by flow cytometry using Protein L and cetuximab, respectively in untransduced (left panel) or LV-transduced (right panel) T cells. (C) Expression of CD33 CAR was confirmed by detection of chimeric CD3ζ by western blot analysis.

Specific *in vitro* Cytotoxicity of CD33-CAR-T Cells

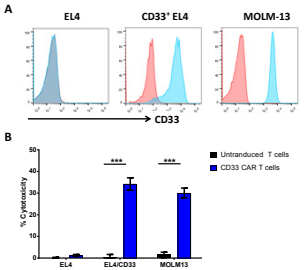

Figure 2: Redirected specificity of CD33-CAR-T cells to specifically lyse CD33$^+$ target cells *in vitro* using a co-culture assay with europium-labelled target cells (CD33^{neg} EL4, CD33$^+$ EL4 and CD33$^+$ MOLM-13). (A) CD33 surface expression levels in target cell lines as measured by flow cytometry. The red line represents staining with isotype control antibody and blue line represents staining with CD33-specific antibody. (B) Cytotoxicity of untransduced (control) T cell or CD33-CAR-T cells was performed at a 10:1 effector-to-target (E:T) ratio. *** p<0.001; Student's T-test. Shown is the mean ± standard deviation (SD).

Specific Cytokine Induction with CD33-CAR-T Cells

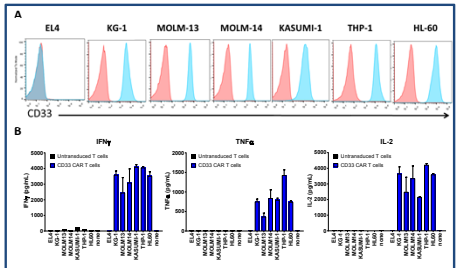

Figure 3: Cytokine expression by CD33-CAR-T cells in response to CD33$^+$ target cells. (A) CD33 surface expression levels in AML tumor cell lines as measured by flow cytometry. Red line represents the isotype and blue line represents anti-CD33 stained cells. (B) Supernatants were harvested after a 24 hr co-culture of tumor cells with untransduced (control) T cells or CD33-CAR-T cells. A multiplex cytokine analysis was performed to measure IFNγ, TNF and IL-2 levels in conditioned media. The effector:target (E:T) ratio was 5:1. Shown is the mean ± SD.

Conditional Elimination of HER1t Expressing CD33-CAR-T cells

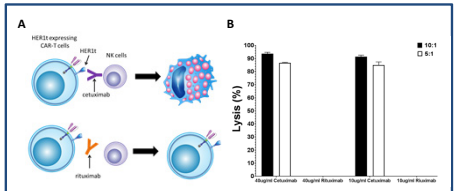

Figure 4: Ability of cetuximab to eliminate CD33-CAR-T cells using propagated allogeneic CD16$^+$ NK cells as effector cells in ADCC assay. (A) Schematic for mechanism of action. (B) NK cells were co-cultured with fluorescent (PKH26)-labelled CD33-CAR-T cells at two E:T ratios in the presence of cetuximab or rituximab (control). 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.

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

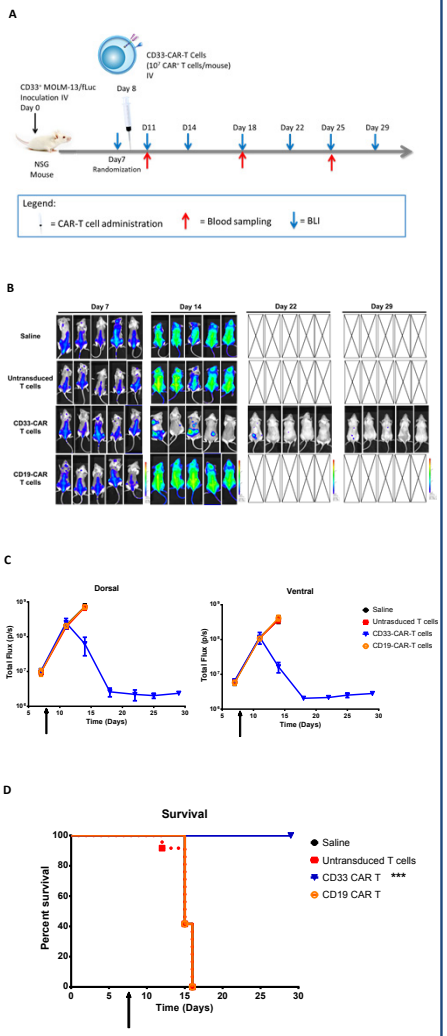

Figure 5: Efficacy of CD33-CAR-T cells in an AML mouse model. (A) Immunocompromised (NSG) mice were injected (Day 0) with CD33$^+$CD19^{neg} MOLM-13/fLUC AML tumor cell line, and engraftment (tumor flux) was confirmed by IVIS bioluminescence imaging (BLI). Tumor presence was confirmed on Day 7 prior to randomization. On Day 8, mice with established tumor burden were treated with CD33-CAR-T cells (10^7 CAR$^+$ T cells/mouse) or control treatments (untransduced T cells, CD19-specific CAR$^+$ T cells (CD19-CAR-T cells), or saline) from same donor T cells. Tumor burden was serially monitored by BLI and weekly blood draws were performed for flow cytometry analysis. (B) Images (dorsal view) from the same five representative mice from each group as shown 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 CD19-CAR-T cells; N=11 to 12 mice/group.)

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

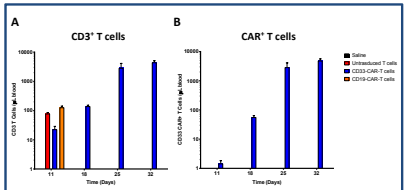

Figure 6: Persistence of CAR-T cells in mice analyzed by flow cytometry of peripheral blood samples. (A) Quantification of human CD3$^+$ T cells detected per µL of whole blood. (B) Quantification of the CAR-T cells detected per µL of whole blood. Shown is the mean ± SEM. n = 4 to 16 mice/group.

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

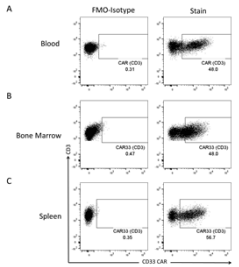

Figure 7: Presence of CD33-CAR-T cells in tissues of treated NSG mice. Flow cytometric analysis was performed on Day 18 (10 days after CD33-CAR-T cell IV injection) on (A) blood, (B) bone marrow, and (C) spleen recovered from tumor-bearing, CD33-CAR-Ttreated mice to detect the presence of the genetically modified T cells. Data from one representative mouse with the populations gated based on FSC/SSC/CD45/CD3 co-expression with the CAR expression gates determined using the fluorescence minus one (FMO-Isotype) gate.

Summary

- CD33-specific CAR and HER1t 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 and cytotoxicity in response to CD33$^+$ target cells.
- HER1t 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.

Conflict of Interest Disclosure:

DS, MHS, SGB, FB, RRS and TC are employees of Intrexon. PE has no financial disclosures. WGW is an employee of MD Anderson Cancer Center and has obtained research funding from Abbvie, Novartis, Acerta, Gilead and Genentech. LJNC is an employee of ZIOPHARM Oncology and has equity ownership in ZIOPHARM Oncology, Intrexon, Targazyme Inc., Immatics; Patents and Royalties from ZIOPHARM Oncology, Intrexon, City of Hope, Sangamo Biosciences; has received an honoraria from Miltenyi Biotec; and is a Visiting Scientist at MD Anderson Cancer Center.