

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

Degang Song, Ph.D.¹, Michael H. Swartz¹, Steven G. Biesecker¹, Fernando Carvajal Borda¹, Rutul R. Shah¹, Peter Emtage, Ph.D.², William G. Wierda, M.D., PhD³, Laurence J. N. Cooper, M.D., Ph.D.⁴, Tim Chan, Ph.D.¹

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

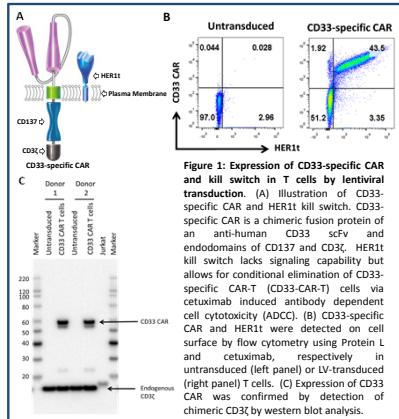
Abstract

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 IFN γ , 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.

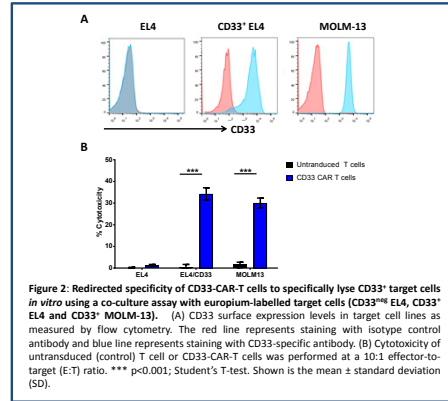
Background

- 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.

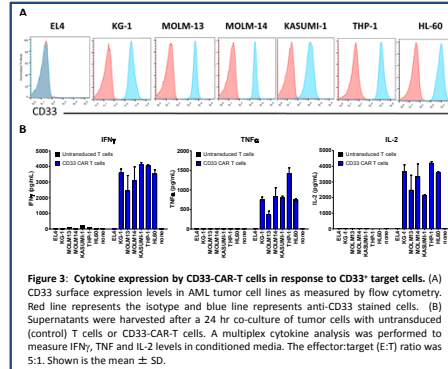
Co-expression of CAR and Kill Switch in T cells



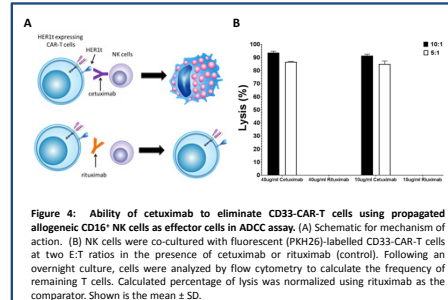
Specific *in vitro* Cytotoxicity of CD33-CAR-T Cells



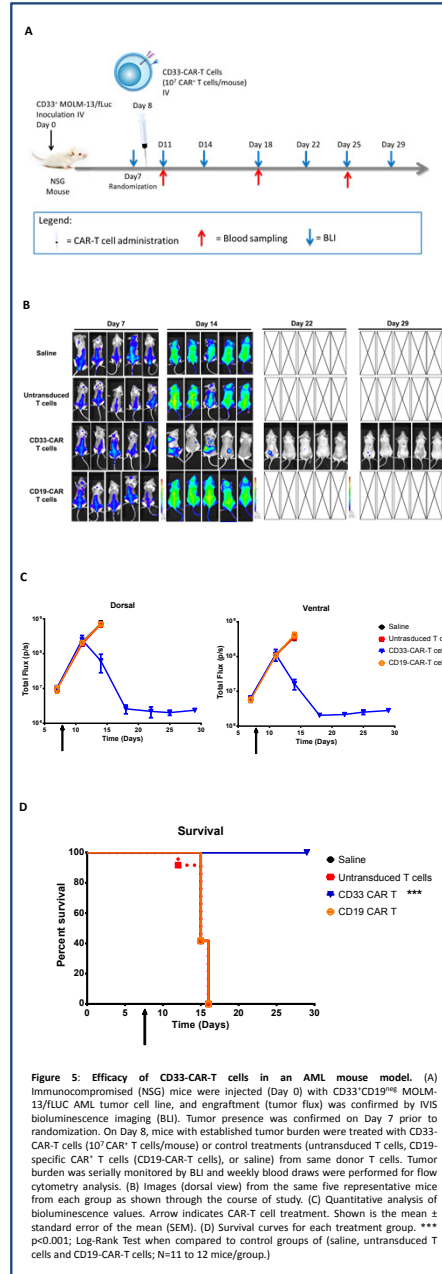
Specific Cytokine Induction with CD33-CAR-T Cells



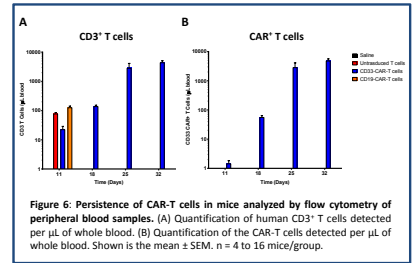
Conditional Elimination of HER11 Expressing CD33-CAR-T cells



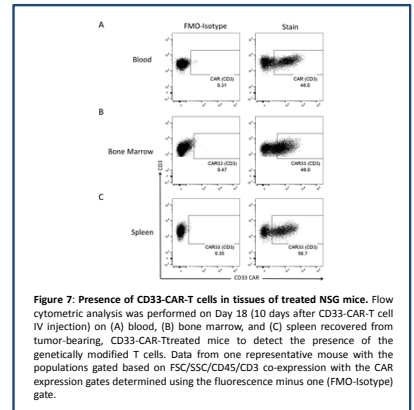
CD33-CAR-T Cells Eliminated AML Tumor & Improved Survival in an *in vivo* Mouse Model



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 HER11 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.
- HER11 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:

Dr. MHS, SGB, RL, RBS 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, Astra, Gilead and Genentech. LNC is an employee of ZIOPHARM Oncology and has equity ownership in ZIOPHARM Oncology, Intrexon, Targameer Inc., Immunatic, Patents and Royalties from ZIOPHARM Oncology, Intrexon, City of Hope, Sangamo Biosciences; has received an honoraria from Milteny Biotec; and is a Visiting Scientist at MD Anderson Cancer Center.