Autologous T Cells Modified to Co-express CD33-Specific Chimeric Antigen Receptor and a Kill Switch for Treatment of CD33⁺ Acute Myeloid Leukemia

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Abstract

Relapsed/refractory acute myeloid leukemia (AML) is an aggressive malignancy with poor outcomes underscoring the need to implement new therapies. Adoptive transfer of genetically modified T cells with specificity redirected through a chimeric antigen receptor (CAR) has resulted in clinical responses, particularly for B-cell malignancies. CD33 is an attractive target for immunotherapy of AML as this transmembrane protein is expressed on majority of AML blasts. On target, but off tumor effects are anticipated as this target is also expressed on normal myeloid cells and on a subset of activated T and NK cells. Therefore, we generated a lentiviral vector co-expressing a CD33-specific CAR with a safety (kill) switch, HER1t, for the generation of CD33-specific CAR^+HER1t^+ T (CD33-CAR-T) cells. HER1t improves the safety profile by providing a mechanism for selective *in vivo* depletion of these genetically modified T cells upon addition of the clinically-available monoclonal antibody, cetuximab. Co-expression of CD33-specific CAR and HER1t on transduced T cells was confirmed by flow cytometry and western blot analyses. The redirected specificity was demonstrated by the specific killing of $CD33^+$ tumor cells as well as significant release of cytokines IFNγ, TNFα, IP-10, IL-13, IL-18, and LIF upon co-culture with AML tumor cells. The ability to eliminate AML *in vivo* was determined in immunocompromised (NSG) mice bearing MOLM-13 ($CD33^+CD19^{neg}$) tumor. A single administration of human CD33-CAR-T cells resulted in a significant reduction in tumor burden and improvement in overall survival with an overall median survival time of 46 days compared to 15 days for control treated mice (saline only, untransduced (CAR^{neg}) T cells, or CD19-specific CAR^+ T(CD19-CAR-T) cells). Assessment from the plasma of CD33-CAR-T treated mice revealed production of cytokines consistent with observations from *in vitro* activation of genetically modified T cells. CD33-CAR-T cells were effectively eliminated in the presence of cetuximab both *in vitro* in an antibody dependent cell cytotoxicity assay and in an *in vivo* adoptive T cell transfer model in immunocompromised NOD/SCID mice. In preparation for a clinical trial, we manufactured CD33-CAR-T cells from an individual with relapsed AML. As with T cells from healthy volunteers, transduced T cells from an AML patient co-expressed CAR and HER1t and were effective at specifically eliminating autologous $CD33^+$ tumor as well as producing cytokines in response to CAR signaling. These pre-clinical data provide a strong rationale to evaluate CD33-CAR-T therapy in a clinical trial for treatment of AML. The FDA has approved an investigational new drug application to initiate phase 1 clinical trial at MD Anderson Cancer Center for treatment of $CD33^+$ relapsed/refractory AML with autologous CD33-CAR-T cells.

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 CD33-specific CAR and Kill Switch in T cells

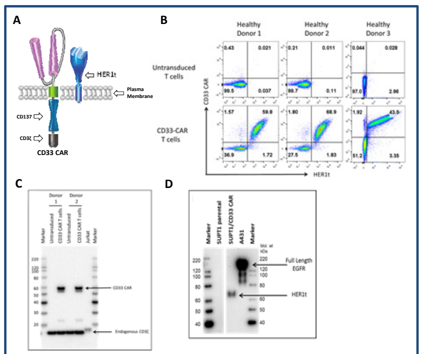


Figure 1: Expression of CD33-specific CAR and kill switch in T cells by lentiviral transduction. (A) Illustration of CD33-specific CAR and HER1t kill switch. CD33-specific CAR is a chimeric fusion protein of an anti-human CD33 scFv and endodomains of CD137 and CD3ζ. HER1t kill switch lacks signaling capability but allows for conditional elimination of CD33-specific CAR-T (CD33-CAR-T) cells via cetuximab mediated ADCC ~~activity~~. (B) CD33-specific CAR and HER1t were detected on cell surface by flow cytometry using CD33-Fc protein and cetuximab, respectively in untransduced (top row) or LV-transduced (bottom row) T cells. Three representative donors are shown (C) Expression of CD33-specific CAR by western blot analysis. (D) Expression of HER1t by co-immunoprecipitation with anti-EGFR antibody followed by western blotting with a different EGFR-specific antibody.

Specific *in vitro* Cytotoxicity and Cytokine Induction by CD33-CAR-T cells

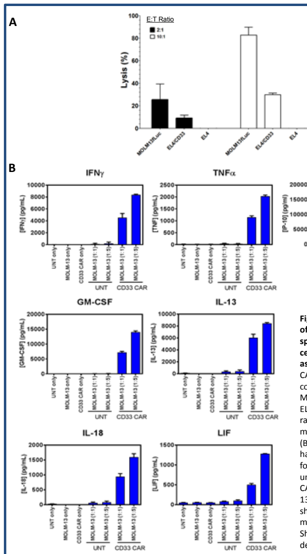


Figure 2: Redirected specificity of CD33-CAR-T cells to specifically lyse $CD33^+$ target cells *in vitro* using a co-culture assay. (A) Cytotoxicity of CD33-CAR-T cells was performed by co-culturing target cells ($CD33^+$ MOLM-13, $CD33^+$ EL4, $CD33^{neg}$ EL4) at effector-to-target (E:T) ratios shown. Shown is the mean ± standard deviation (SD). (B) Culture supernatants were harvested at 24 hour time point following co-cultures of untransduced (UNT) and CD33-CAR-T cells with $CD33^+$ MOLM-13 tumor cells at E:T ratios shown and subjected to a multiplex cytokine analysis. Shown is the mean ± standard deviation (SD) from a donor.

In vitro and *in vivo* Elimination of HER1t Expressing CD33-CAR-T cells

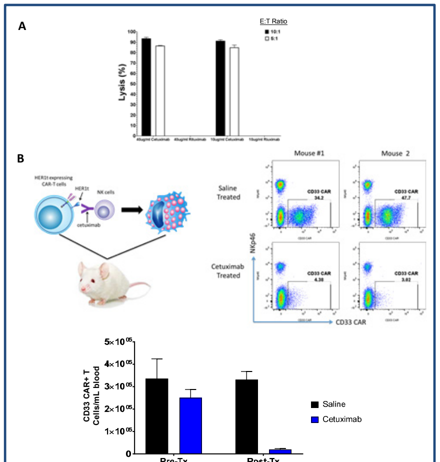


Figure 3: Ability of cetuximab to eliminate CD33-CAR-T cells expressing HER1t. (A) In vitro, allogeneic NK cells were co-cultured with fluorescent (PKH26)-labelled CD33-CAR-T cells at two E:T ratios in the presence of cetuximab or rituximab (control). Following an overnight culture, cells were analyzed 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. (B) In vivo assessment was performed in NOD/SCID mice lacking T and B cells but maintaining NK cell presence. Murine CTLL-2 cells were LV transduced to express CD33-CAR-T and kill switch and adoptively transferred into NOD/SCID mice. After verifying engraftment into the mice, mice were randomized and treated with saline only or cetuximab (1mg; IP). Flow assessment was performed 7 days after the antibody treatment. Representative flow data from 2 mice is shown in top right portion. Absolute cell counts of CTLL-2/CD33-CAR-T cells from flow cytometric analysis was performed. Shown is the mean ± SEM; n=4-5 mice/group.

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

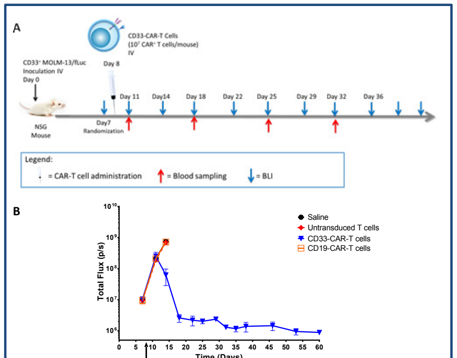
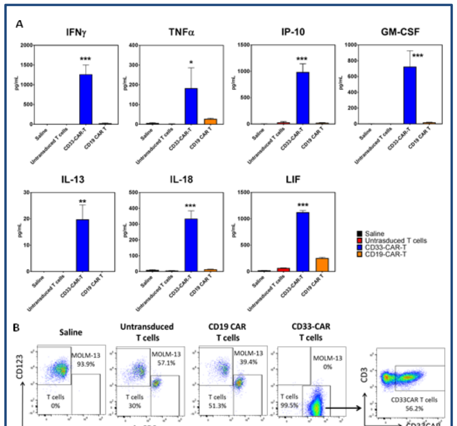


Figure 4: In vivo efficacy of CD33-CAR-T cells in AML Tumor model. (A) Study design. Immunocompromised (NSG) mice were injected (Day 0) with $CD33^+CD19^{neg}$ MOLM-13/fLUC AML tumor cell line, and engraftment (tumor flux) was confirmed by IVIS bioluminescence imaging (BLI). Tumor presence was confirmed 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; (10^7 CAR^+ T cells/mouse), 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) Quantitative analysis of bioluminescence values. Arrow indicates CAR-T cell treatment. Mice in control treatment groups were sacrificed by Day 18 due to tumor burden. Shown is the mean ± standard error of the mean (SEM).

CD33-CAR-T Cells Increase Cytokine Expression and Persist *in vivo*



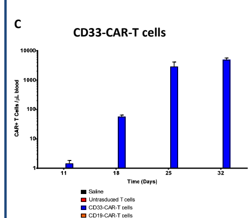


Figure 5: Cytokine expression and CAR T cell persistence. (A) Analyses of human cytokines and growth factors from the plasma of CAR-T cell administered NSG mice on study Day 11 (3 days after CAR-T cell infusion). Shown is the mean ± SEM of several analytes; N=11-15 mice/group. * p<0.05, ** p<0.01, *** p<0.001; one way analysis of variance (ANOVA) with Tukey's multiple comparison testing of saline, untransduced T cells and CD19-CAR-T cells. (B) Persistence of CAR-T cells in tumor-bearing mice. Blood samples were ~~taken~~ from mice treated with saline only, untransduced T cells or CD19-CAR-T cells on Day 16, as mice became moribund. Blood samples were obtained from remaining mice treated with CD33-CAR-T cells on Day 18. Data shown is gated on FSC/SSC/human $CD45^+$ cells. (C) Persistence of CAR-T cells in mice analyzed by flow cytometry of peripheral blood samples. Shown is the mean ± SEM. n = 4 to 16 mice/group.

In vitro Assessment of CD33-CAR-T cells From a Donor with AML

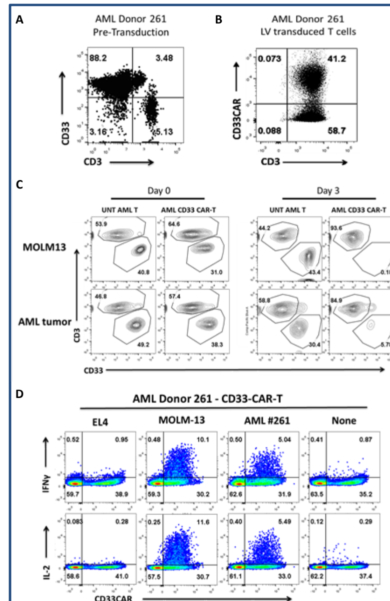


Figure 6: Anti-tumor activity of CD33-CAR-T from a donor with AML. (A) PBMC fraction from an AML donor was obtained and phenotypically characterized for CD33 and CD3 expression. (B) The AML donor's T cells were transduced with the LV-CD33-CAR construct and CAR expression is shown on Day 14 of the culture. (C) Co-culture of the AML donor CD33-CAR-T cells with either MOLM-13 or the autologous AML donor tumor cells is shown on Day 0 (start o f culture) and at Day 3. All cells are gated on FSC/SSC/viable cells. (D) Intracellular cytokine staining was performed following co-culture of CD33-CAR-T cells with designated tumor cells after an 18 hr time period. T cells were cell surface stained then fixed and permeabilized followed by staining for IFNγ and IL-2 cytokines. Cells are gated based upon FSC/SSC/viable/$CD3^+$ T cells. AML 261 cells refers to the patient's autologous $CD33^+$ tumor cells.

Summary

- CD33-specific CAR and 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 both *in vitro* and *in vivo*.
- CD33-CAR-T cells elicited cytokine response, persisted in mice and eliminated AML tumor that significantly improved survival.
- A Phase I clinical trial for treatment of relapsed/refractory AML has been initiated at MD Anderson Cancer Center (NCT03126864).

Conflict of Interest Disclosure:

Disclosures: Song: *Intrexon Corporation:* Employment. **Tian:** *Intrexon Corporation:* Employment. **Carvajal-Borda:** *Intrexon Corporation:* Employment. **Plummer:** *Intrexon Corporation:* Employment. **Shah:** *Intrexon Corporation:* Employment. **Wierda:** *Emergent:* Consultancy, Honoraria, Research Funding; *Merck:* Consultancy, Honoraria; *Karyopharm:* Research Funding; *Gilead:* Consultancy, Honoraria, Research Funding; *GSK/Novartis:* Consultancy, Honoraria, Research Funding; *Genentech/Roche:* Consultancy, Honoraria, Research Funding; *Celgene:* Consultancy, Honoraria; *AbbVie:* Consultancy, Honoraria, Research Funding; *Kite:* Research Funding; *Genzyme:* Consultancy, Honoraria; *The University of Texas MD Anderson Cancer Center:* Employment; *Pharmacyclics:* Consultancy, Honoraria, Research Funding; *Janssen:* Research Funding; *Sanofi:* Consultancy, Honoraria; *Acerta:* Research Funding; *Juno:* Research Funding. **Cooper:** *Organovo Holdings:* Equity Ownership; *Sangamo Biosciences:* Patents & Royalties; *Miltenyi Biotec:* Honoraria; *Ampliphi Biosciences:* Equity Ownership; *Argos Therapeutics:* Equity Ownership; *Intrexon Corporation:* Equity Ownership, Patents & Royalties; *Procter & Gamble:* Equity Ownership; *Targazyme, Inc.:* Equity Ownership; *Ferring:* Consultancy; *Immatics:* Equity Ownership, Patents & Royalties, Research Funding; *Ziopharm:* Employment, Equity Ownership, Research Funding. **Chan:** *Intrexon Corporation:* Employment.