Poster #133

CRISPR/Cas9 gene-edited allogeneic CAR-T cells targeting CD33 show high preclinical efficacy against AML without long-term hematopoietic toxicity

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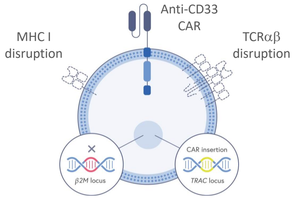
Abstract

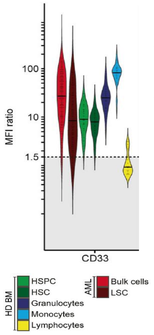
CD33 is the most consistently expressed antigen in AML, with high levels and homogeneous expression observed in malignant AML cells from most patients, including those with relapsed disease. Normal myelomonocytic cell lineages and a percentage of hematopoietic progenitors also express CD33, and the extreme myeloablation caused by the CD33-targeted antibody-drug conjugate (ADC) gemtuzumab ozogamicin reinforced concerns about targeting this antigen with more potent agents such as T-cell engaging bispecific antibodies and CAR-T cells. We have shown previously that allogeneic CRISPR/Cas9 gene-edited CAR-T cells targeting CD33 with TRAC disruption to reduce GvHD and B2M disruption to reduce allogeneic host rejection could eliminate tumors in xenograft models of AML. Given that off-target activity of the toxin could contribute to the myeloablation seen with CD33-targeted ADCs, we created *in vitro* and *in vivo* models to examine reconstitution of the myeloid compartment following treatment of CD33-targeted allogeneic CAR-T cells. Although co-culture of CD34+ stem cells *in vitro* with our CD33-targeted allogeneic CAR-T cells did significantly deplete the cell population, colonies still formed after removal of the CAR-T cells as the presumably CD33-negative stem/progenitor cells expanded and differentiated. A similar phenomenon was observed *in vivo* with CD34 humanized mice bearing an AML tumor (THP-1 cells) and treated with the CD33-targeted allogeneic CAR-T cells. The CAR-T cells completely eradicated the THP-1 tumor but did not lead to long-term myelosuppression or B cell aplasia. Thus, allogeneic CRISPR/Cas9 multiplex gene-edited CD33-targeted CAR-T cell therapy may be both efficacious and tolerable in AML.

Figure 1: CRISPR-edited allogeneic CAR-T cells for CD33⁺ malignancies



CRISPR-edited allogeneic CAR-T cells are produced by targeted disruption of the *TRAC* and *B2M* loci with concomitant insertion of an anti-CD33 CAR cassette into the *TRAC* locus by homology-directed repair. TCRαβ disruption is intended to prevent GvHD, while *B2M* KO to eliminate MHC I expression is intended to diminish host rejection.

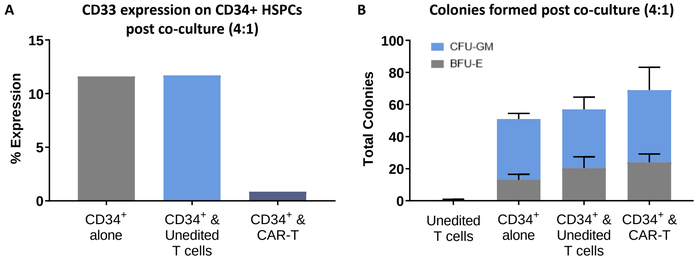
Figure 2: CD33 expression on AML blasts versus normal cells



Haubner, *et al*. quantified surface CD33 expression by flow cytometry in 356 AML patient samples and 34 healthy donor (HD) samples. Cells were classified as CD33⁺ if the median fluorescent intensity (MFI) ratio exceeded 1.5 (dotted line). Both AML bulk cells and leukemic stem cells (LSC) showed high rates of CD33 positivity at initial diagnosis (n=302) and relapse (n=54). HD bone marrow (BM) derived hematopoietic stem cells (CD34⁺ HSC), progenitor cells (CD34⁺CD38⁺ HSPC), granulocytes, and monocytes were CD33⁺ whereas lymphocytes were CD33⁻. These data suggest that CD33 CAR-T use poses a risk of long-term aplasia of monocytes and myeloid cells.

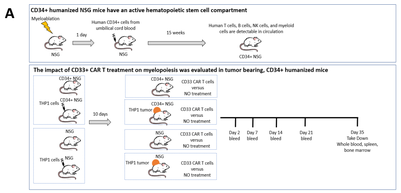
Haubner, S., et al. *Leukemia*, vol. 33, no. 1, 2018, pp. 64–74., https://doi.org/10.1038/s41375-018-0180-3.

Figure 3: Anti-CD33 CAR-T cells target CD33⁺ cell populations without affecting human HSPC function *in vitro*

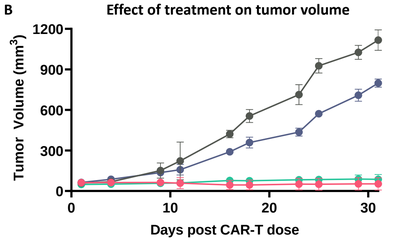


(A) 48-hour co-culture of CD34⁺ cells with anti-CD33 CAR-T cells reduces CD33⁺ populations. **(B)** Anti-CD33 CAR-T cells do not affect multi-lineage HSPC colony formation from granulocyte-macrophage progenitors (CFU-GM) or erythroid progenitors (BFU-E).

Figure 4: Anti-CD33 CAR-T cells exhibit specific and potent cell killing of AML cells in CD34⁺ humanized NSG mice



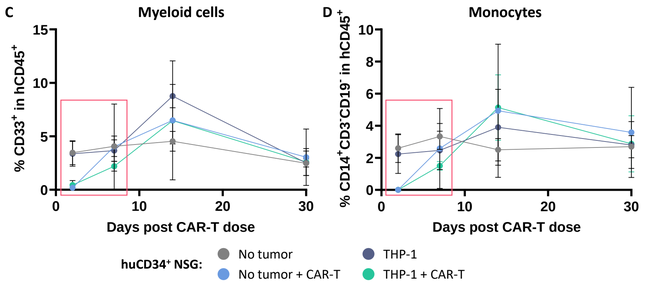
(A) NSG mice with engrafted human CD34⁺ cells were used to evaluate the effects of anti-CD33 CAR-T cells on normal and malignant cells.



(B) Anti-CD33 CAR-T cells show potent anti-tumor activity in both NSG and huCD34⁺ NSG mice transplanted with THP-1 tumor cells (AML cells).

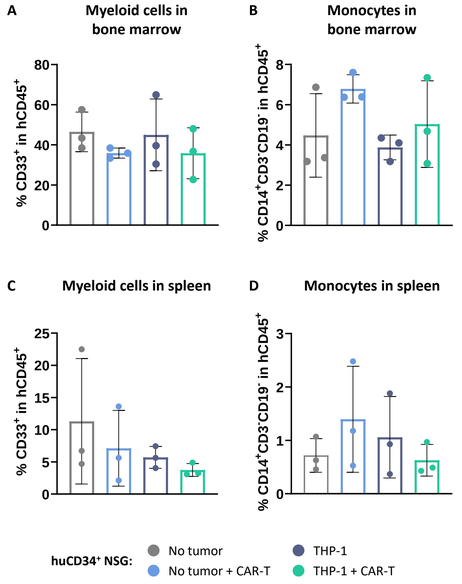
NSG:
- THP-1
- THP-1 + CAR-T

huCD34⁺ NSG:
- THP-1
- THP-1 + CAR-T



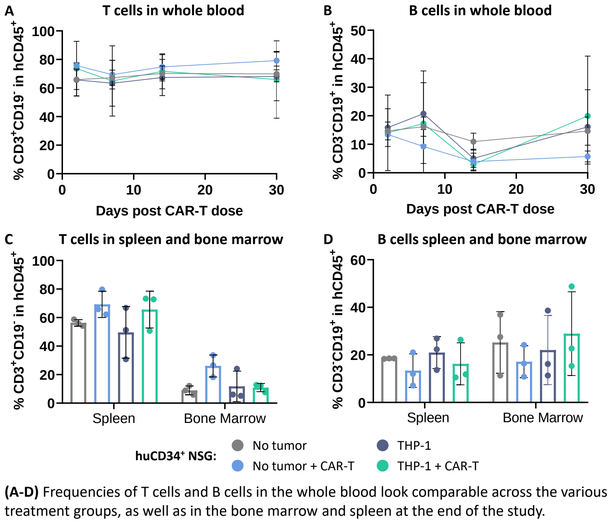
Anti-CD33 CAR-T treatment causes a transient drop in myeloid cells and monocytes that starts to rebound by Day 7 and has fully recovered by Day 14, as seen in the frequencies of circulating myeloid cells **(C)** and monocytes **(D)** in the whole blood of various treatment groups.

Figure 5: Anti-CD33 CAR-T cells did not cause long term depletion of healthy myeloid and monocytes



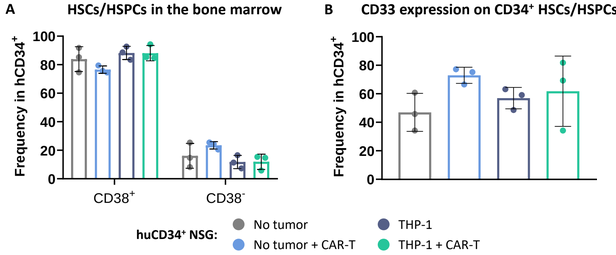
(A-D) Frequencies of myeloid cells and monocytes at the end of the study in the bone marrow and spleen look comparable across the various treatment groups.

Figure 6: Anti-CD33 CAR-T cells had no impact on B and T cell populations



(A-D) Frequencies of T cells and B cells in the whole blood look comparable across the various treatment groups, as well as in the bone marrow and spleen at the end of the study.

Figure 7: Anti-CD33 CAR-T cells did not affect human HSC/HSPC-enriched population frequencies



(A) Anti-CD33 CAR-T cells had no impact on phenotypically defined HSPC populations in the bone marrow at the end of the study. **(B)** The CD33⁺ fraction of CD34⁺ cells is unaffected by anti-CD33 CAR-T treatment at the end of the study.

Conclusions

CRISPR-edited allogeneic anti-CD33 CAR-T cells show high preclinical efficacy against AML with minimal long term hemato-lymphoid toxicity

These anti-CD33 CAR-T cells:

- Retained high potency against AML in a CD34-humanized NSG mouse model
- Transiently reduced human myeloid populations, which subsequently fully recovered
- Had no effect on HSPC cell function using *in vitro* colony assays
- Had no effect on multilineage cell production or on phenotypically defined HSPC populations *in vivo*