# NKG2D Chimeric Antigen Receptor-Expressing Lymphocytes Target Acute Myeloid Leukemia Cells

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## Introduction & Objectives

Acute myeloid leukemia (AML) is a hematological malignancy with a very low overall survival. Among the new treatment modalities, chimeric antigen receptor (CAR) therapy is showing promising results in other hematological malignancies. Since AML exhibits high heterogeneity and does not have specific differential antigens of the hematopoietic stem cell, using NKG2D-CAR cells could be an appropriate therapeutic strategy against AML. NKG2D receptor has a wide range of specific tumor cell ligands (MICA, MICB, ULBP-1, ULBP-2 and ULBP-3) which are expressed in more than 80% of all tumors. For this reason, the objective of this work was to evaluate the anti-tumor activity of activated and expanded natural killer cells (NKAE) and T cells expressing an NKG2D CAR.

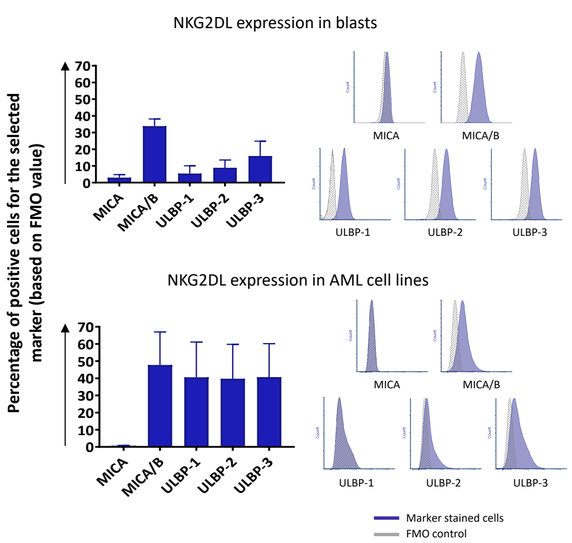
## Patients & Methods

T cells and NK cells were isolated from the healthy donor peripheral blood mononuclear cells (PBMCs) ring (n=5) by immunomagnetic depletion. NKAE cells were obtained by co-culture with subletally irradiated CSTX002 cells. The purified NKAEs and T cells were transduced with an NKG2D CAR with 4-1BB and CD3z signaling domains. The viral supernatant was produced by transient transfection of HEK293T cells with the vector genome plasmid and lentiviral packaging helper plasmids. NKAE cells were transduced 10 days after co-culture with CSTX002 in RPMI-1640 medium with Il-2 at 100 IU/mL and 10% AB serum. T cells were incubated in XVIVO-15 medium with IL-2 at 250 IU/mL and anti-CD3/anti-CD28 antibodies. T cells were transduced 24 hours after activation.

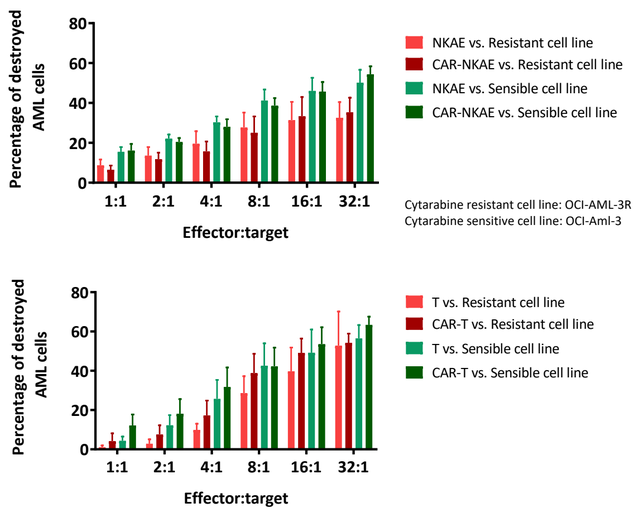
The efficiency of transduction was evaluated by flow cytometry detecting NKG2D expression. Also, the immunoprofiling of surface molecules, as well as the expression of NKG2D ligands and PD-1 ligands in tumor cells (AML cell lines -n=5- and primary blasts -n=3-) were analyzed by flow cytometry. The cytotoxicity of untransduced NKAE, CAR-NKAE cells, untransduced T cells and CAR-T cells was evaluated by 4 hour europium release assay. Also cytotoxicity after 24 hour exposition was evaluated by flow cytometry of $PI^+$ and annexin $V^+$ cells. Toxicity on healthy tissue (healthy lung cells -NL-20- and PBMCs from third party) was analyzed in the same way by 4 hour Eur-TDA release assay.

## Results

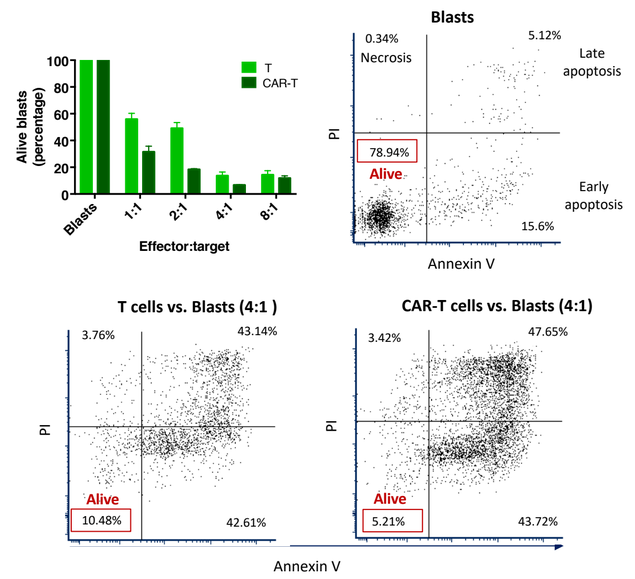
### 1. NKG2D ligands are expressed in AML



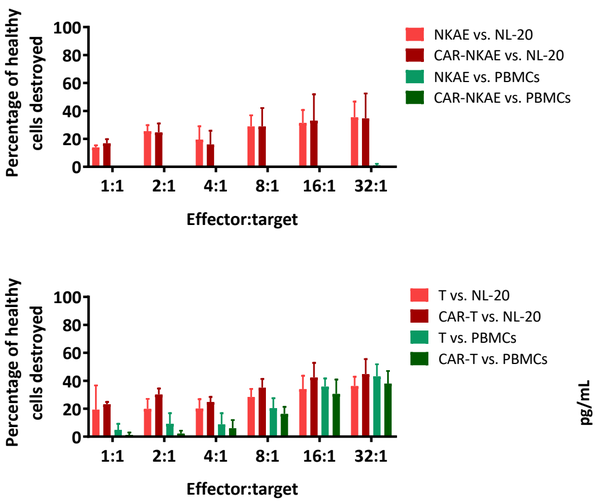

### 2. CAR-NKAE cells and CAR-T cells destroy AML cells



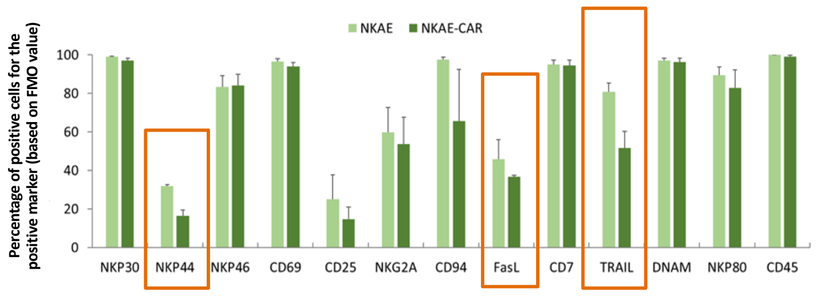

### 3. CAR-T cells near completely destroyed AML blast after 24 hours




### 4. CAR-NKAE cells exhibited better toxicity profile than CAR-T cells



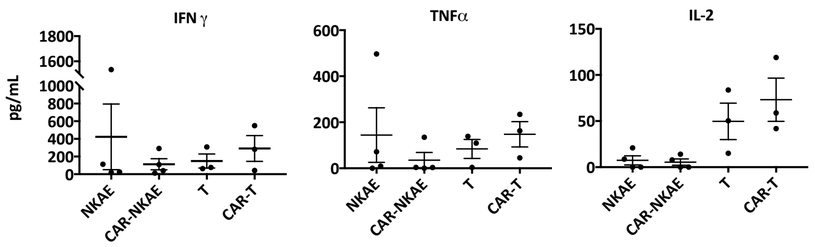

### 5. CAR-NKAE cells exhibited an exhausted immunophenotype



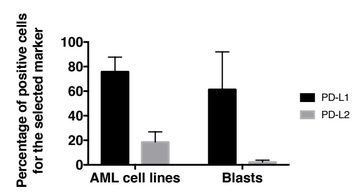

### 6. CAR-T cells exhibited central memory and effector memory phenotype ($CD45RA^-$ $CD45RO^+$)

| | LT | LT-CAR |
|---|---|---|
| CD45RA+ | 24.7% | 12.7% |
| CD45RO+ | 9.8% | 60.1% |
| CD8+ | 51.1% | 50.6% |
| CD4+ | 31.4% | 33.5% |
| CD25+ | 21% | 19.4% |
| CD127+ | 35% | 20.5% |
| CD62L+ | 48.7% | 42.3% |

### 7. CAR-NKAE cells and CAR-T cells release IFN-γ, TNF-α and Il-2




### 8. PD-L1 expression could constitute a resistance mechanism




## Conclusions

- We have demonstrated that AML cells could be target with an NKG2D-CAR.
- Primary NKAE cells and T cells can be transduced with an NKG2D-CAR at low MOI to enhance their antileukemic activity.
- NKG2D-CAR-T cells exhibited a highly activated phenotype and were more effective than CAR-NKAE cells.
- Moreover, CAR-T cells were able to near completely destroy AML blasts.
- Although further studies are needed, these results show the potential of NKG2D-CAR T and NK cell therapy in AML.

## Acknowledgements

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**Conflict of interest Disclosures**:

DJPJ hold patents in the area of CAR T cell therapy. DAL declares an equity interest, advisory role, and intellectual property licensing to CytoSen Therapeutics and Kiadis Pharma, and advisory role with Caribou BioSciences and Courier Biosciences.

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