Engineering allogeneic 'off-the-shelf' CD19-directed CAR-iNKT cells without additional genetic manipulations for the treatment of hematological malignancies

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Background

- invariant Natural Killer T (iNKT) cells are a unique subset of T cells that naturally target and kill cancer cells[1]
- iNKT cells express an invariant T cell receptor (iTCR) that recognizes glycolipids presented in the context of the monomorphic, MHC-class I related molecule, CD1d
- Engineering a Chimeric Antigen Receptor (CAR) makes iNKT cells dual targeting, thereby enhancing cytotoxicity[2]
- iNKT cells promote anti-tumor activity by reprogramming the immunosuppressive tumor microenvironment to be immunostimulatory[3]
- iNKT cells can target cancers without the risk of graft-versus-host disease (GvHD), circumventing the need to delete or knock out the endogenous TCR for an allogeneic product[4,5]

Allogeneic Off-the shelf CAR-iNKT cells

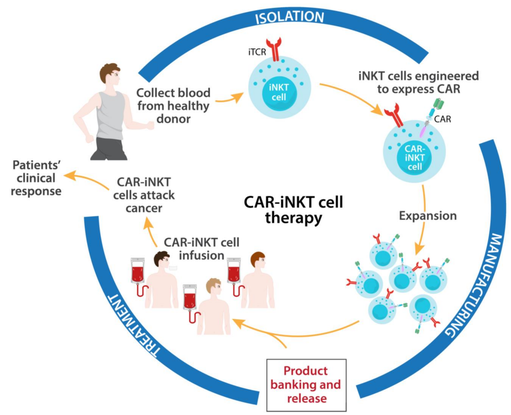
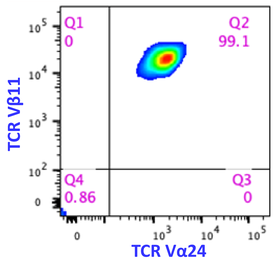


Fig 1. Schematic representation of CAR-iNKT cell manufacturing using lentiviral vector

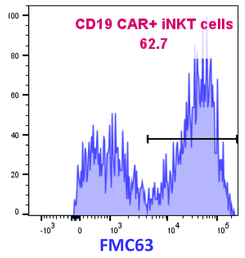
Methods

Briefly, peripheral blood-derived iNKT cells were isolated from healthy donors and engineered to express a CD19 CAR using a 3rd generation lentiviral vector. Cells were then expanded for 21 days. To demonstrate CAR19-dependent and independent anti-tumor activity, CAR19-iNKT cells **(ALA-101)** were compared *in vitro* against non-transduced (NT) iNKT cells in cytotoxicity assays and for cytokine secretion. Finally, the anti-tumor activity of cryopreserved CAR19-iNKT cells (ALA-101) were evaluated in an established aggressive NSG mice model of SEM-luc, a B cell lymphoblastic leukemia cell line expressing luciferase.

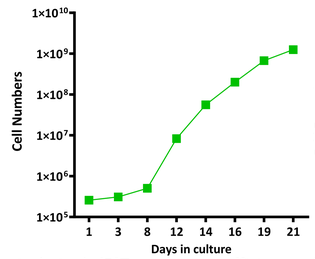
A. iNKT Cell Purity



B. Transduction Efficiency



C. Growth Curve



D. Fold Expansion

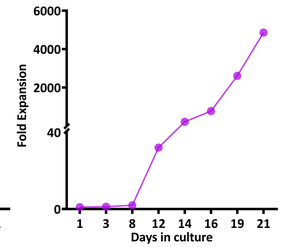
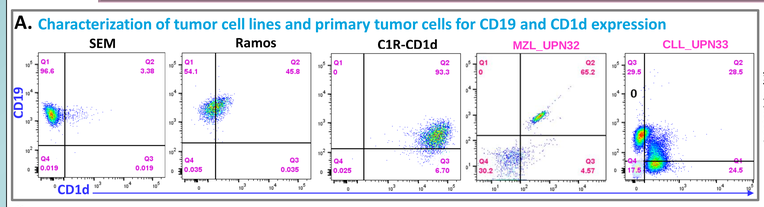


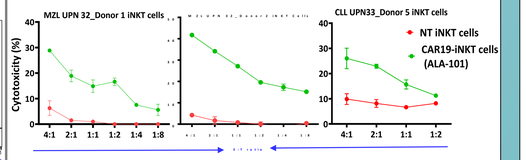
Fig 2. (A) iNKT purity after isolation (0.17% of CD3+ cells in PBMC to 99.1% after isolation), **(B)**Transduction efficiency of purified iNKT cells, CAR19-iNKT cell (ALA-101) research scale **(C)** Growth Curve & **(D)** Fold Expansion calculated as change from Day 1.

CAR19-iNKT cells (ALA-101) are cytotoxic to CD19+/CD1d+/- tumor cell lines and primary tumor cells

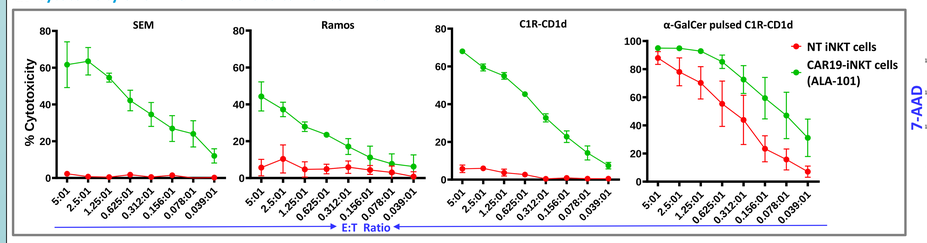
A. Characterization of tumor cell lines and primary tumor cells for CD19 and CD1d expression



B. I. Cytotoxicity to primary MZL and CLL cells



C. Cytotoxicity of CAR19-iNKT cells to tumor cell lines



II. Elimination of CD19+ cells in MZL sample by Donor 2 derived NT iNKT cells and ALA-101

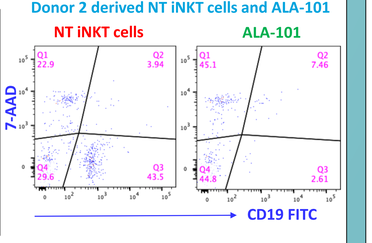
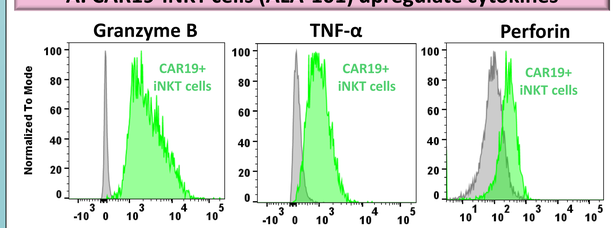


Fig 3. (A) Characterization of tumor cell lines & primary marginal zone B cell lymphoma (MZL) and chronic lymphocytic leukemia (CLL) cells. **(B)** Cytotoxicity of ALA-101 to **(I)** primary tumor cells, **(II)** FACS plots showing elimination of CD19+ cells **(C)** Cytotoxicity of ALA-101 (n=2) to tumor cell lines in 20 h co-culture.

A. CAR19-iNKT cells (ALA-101) upregulate cytokines



B. Antigen specific proliferation of CAR19-iNKT cells (ALA-101)

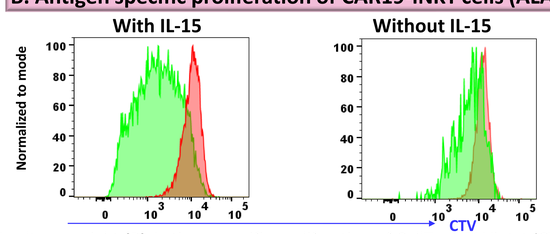
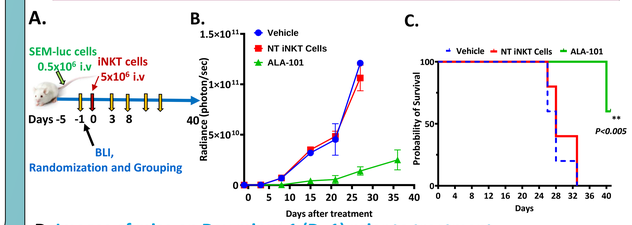
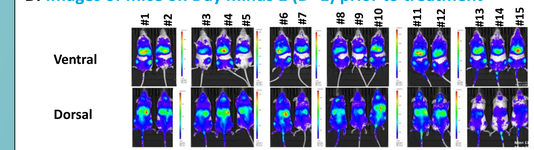


Fig 4. **(A)** Intracellular cytokine staining of CAR19-iNKT cells co-cultured with SEM cells overnight **(B)** Antigen specific proliferation of CellTrace™ Violet (CTV) labeled CAR19+ iNKT cells upon exposure to 3 rounds of irradiated tumor cell challenge with **SEM (CD19+)** or **K562 (CD19-)** cells every 24h for 3 days. Based on the dilution of CTV, proliferation of CAR19+ iNKT cells in the presence or absence of exogenous IL-15 was assessed on day 7 following the first stimulation.

ALA-101 Mediated Antitumor Activity & Survival Benefit in an Aggressive Disseminated NSG Mouse Model of Acute Lymphoblastic Leukemia (SEM-luc)



D. Images of mice on Day minus 1 (D -1) prior to treatment



E. Images of mice Day 8 (D8) post treatment

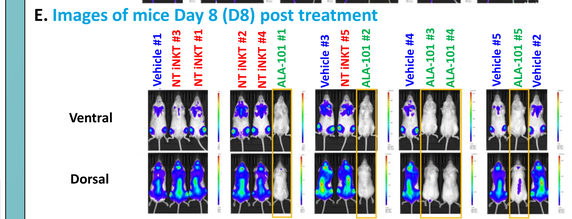


Fig 5. (A) NSG mice were injected i.v on D -5 with 0.5x10^6 SEM-luc tumor cells. On D -1 mice were imaged, & randomized. On D0, mice (n=5) were treated with 5x10^6 Non-transduced (NT) iNKT cells, or CAR19-iNKT cells (ALA-101), or vehicle. **(B)** Total flux of the tumor at different time points based on the quantification by bioluminescence imaging (BLI). **(C)** Survival curve. **(D)** Images of mice on D -1 and **(E)** D8 post treatment.

Summary & Conclusions

- iNKT cells can be isolated from PBMC with a high purity (≥ 99.1%) and can be efficiently transduced to express CD19 CAR
- CAR19-iNKT cells (ALA-101) can be expanded to ~ 5000- fold
- ALA-101 is cytotoxic to CD1d- (SEM), CD1d+ (Ramos) and CD1d+++ (C1R-CD1d) cell lines and primary MZL and CLL tumor cells
- ALA-101 displays excellent proliferative response after at least 3 rounds of serial killing of CD19+ tumor cells
- ALA-101 prolongs survival and mediates anti-tumor activity in an aggressive CD1d-negative ALL model of SEM-luc *in vivo*
- ALA-101 has the potential to treat CD19+ malignancies

Bibliography

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