Selective Support of CAR-T Cell Therapies By Cis-Targeted IL-2 or IL-21 Cytokines Results in Enhanced Anti-Tumor Activity

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INTRODUCTION

Approximately 50% of patients with acute lymphoid leukemia (ALL) or with aggressive lymphoma who receive anti-CD19 CART cells (CART-19) remain relapse-free at 1 year. In vivo behavior of CART-19 correlates with improved outcomes, spurring interest in the development of approaches to selectively control the in vivo expansion of infused CART cells. Administration of exogenous cytokines belonging to the IL-2 family is one such approach. However, the clinical potential of combining IL-2 family of cytokines with CART cells is hampered by the pleiotropic nature of the current molecules, which leads to severe toxicities and expansion of multiple endogenous cells (e.g. Tregs) in addition to CAR-T cells. While mutations have been introduced into existing engineered IL-2 variants to alter potency, these molecules are not entirely selective since endogenous cells are still stimulated. Other approaches using orthogonal cytokine/cytokine receptors are interesting but require further genetic modification of the T cells.

Cis-targeting of CAR-T Cells

Selective Stimulation of CAR-T cells

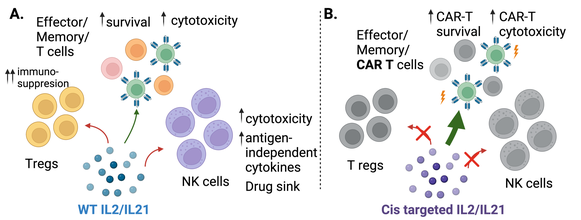


Figure 1. (A) IL-2 family cytokines cause severe toxicities and expansion of multiple endogenous cells (e.g. Tregs) in addition to CAR-T cells which hampers their clinical potential. (B) To address these challenges, we developed CAR-T specific IL-2 or IL-21 fusion molecules that selectively activate CART cells by recognizing an extracellular tag.

Cis-targeted Fusion Product Elements

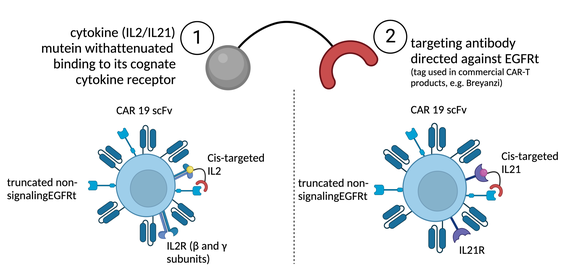


Figure 2. Cis-targeted cytokine fusions are comprised of (1) a targeting antibody directed against a tag expressed on the CAR-T surface (truncated non-signaling epidermal growth factor receptor [EGFRt] that is co-expressed with the CAR) and (2) a cytokine mutein with attenuated binding to its cognate cytokine receptor.

RESULTS

Cis-targeting Selectivity on CAR-T cells

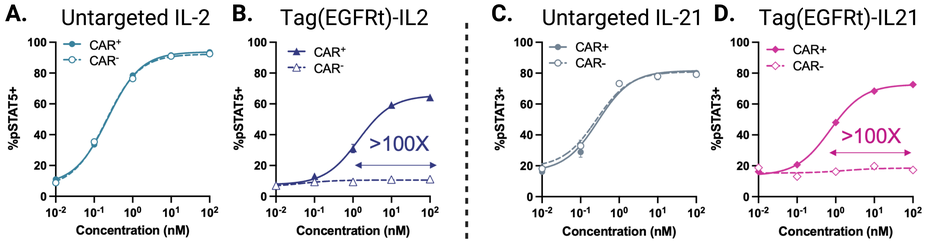
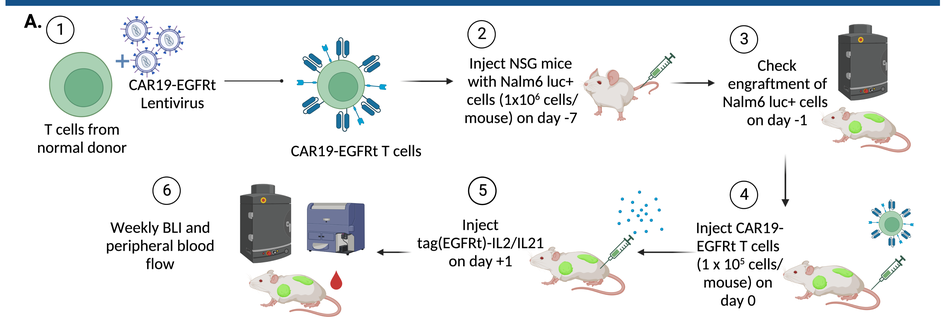


Figure 3. Induction of pSTAT5 signaling (A-B) or pSTAT3 signaling (C-D) in a mixture of T cells containing 50% human CAR-T and 50% non-CART cells. Non-selective WT cytokines IL-2 (A) or IL-21 (C) induce STAT signaling in both CAR+ and non-CART cells. *Cis*-targeted-IL2 (B) and *cis*-targeted-IL21 (D) fusion molecules, that are directed by an EGFRt molecule binder, exhibit high selectivity (>100 fold lower EC50) for CAR+ T cells over non-CAR T cells.

Outline of Preclinical In vivo Experiment



B. Treatment Groups

1. UTD
2. PBS
3. 0.1M CAR19-EGFRt + Tag-IL2 1mg/kg
4. 0.1M CAR19-EGFRt + Tag-IL21 0.3mg/kg
5. 0.1M CAR19-EGFRt + Tag-IL21 1mg/kg

C. Baseline CAR Expression

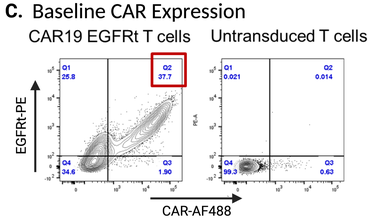


Figure 4. Using normal donor T cells, CAR19-EGFRt T cells were produced. On day -7, **1x10⁶** Nalm6 luc+ cells / mouse were injected. On day -1 (6 days later) we checked engraftment of Nalm6 luc+ cells via measuring bioluminescence. On day 0, **1x10⁵** CAR19-EGFRt T cells/mouse were injected. On day 1, *cis*-targeted cytokines were injected. Mice were imaged weekly to check for tumor burden and peripheral blood was used to analyze the phenotype of CAR T cells. (B) Treatment groups. (C) Baseline CAR19+ EGFRt+ %.

RESULTS

EGFRt-IL2 and EGFRt-IL21 Induced Substantial Tumor Regression

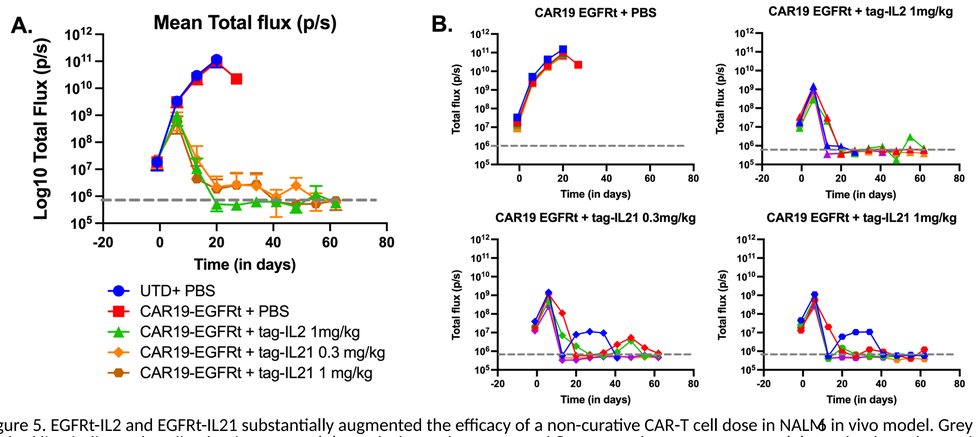


Figure 5. EGFRt-IL2 and EGFRt-IL21 substantially augmented the efficacy of a non-curative CAR-T cell dose in NALM6 in vivo model. Grey dashed line indicates baseline luminescence. (A) Graph shows the mean total flux per each treatment group. (B) Graphs show the total flux for each mouse per treatment group.

EGFRt-IL2 Induced Stronger Initial Expansion of CART19 In vivo than EGFRt-IL21

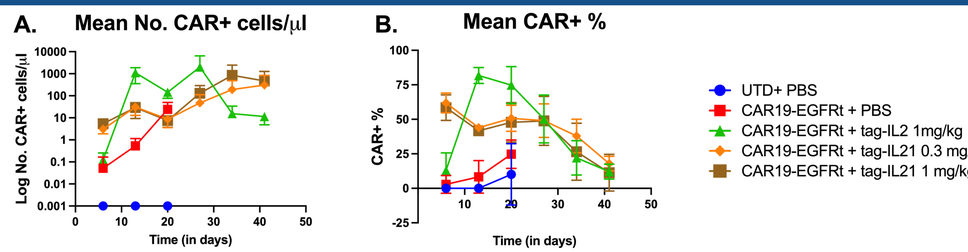


Figure 6. (A) EGFRt-IL2 induced a stronger initial expansion of CART19 in vivo than EGFRt-IL21. (B) Graph shows the mean CAR+ % of CD3+ T cells. Mice treated with EGFRt-IL2 showed a 2-fold increase in CAR+% compared to the injected CAR+ % (which is 50%).

METHODS

- Two *cis*-targeted fusion molecules were engineered (one EGFRt-IL2 fusion molecule and one EGFRt-IL21 fusion molecule).
- Fusion molecules were comprised of (1) a targeting antibody against an external non-functional EGFR tag (coexpressed with the CAR) and (2) a cytokine mutein with attenuated binding to its cognate cytokine receptor.
- In vivo activity was tested in a "stress test" model of B-cell ALL by engrafting 1x10⁶ NALM6 cells that expressed luciferase into NSG mice for six days, prior to IV injection with a low dose of human CART19 (0.1x10⁶).
- Cis-targeted cytokine fusions were administered intraperitoneally once, one day after CAR-T infusion.
- Anti-tumor activity was measured weekly by bioluminescence imaging (BLI) and analysis of peripheral blood was performed to examine the phenotype of the CAR-Ts.

CONCLUSION

- Cis-targeted IL-2 or IL-21 cytokine fusion molecules selectively augment CAR-Ts in vitro and enhance in vivo anti-tumor activity and survival.
- Temporal control of the cis-targeted cytokines directed by anti-tag antibodies represent a promising approach to enhance CART cell therapies.

ACKNOWLEDGMENTS

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