CAR-targeted IL-2 Drives Selective CAR-T cell Expansion and Improves Anti-tumor Efficacy

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Background

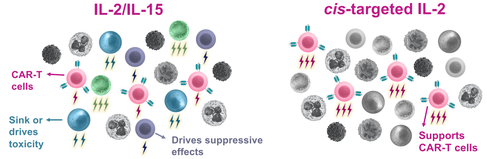
Chimeric antigen receptor T cell (CAR-T) therapies have enabled the treatment of some hematological malignancies and have demonstrated promising preliminary results in solid tumors. Recent studies have shown that in vivo expansion and persistence of CAR-Ts are correlated with improved therapeutic outcomes in patients[1,2]. Administration of IL-2 enhances CAR-T engraftment, persistence, and functionality in preclinical models[3-4]. However, the clinical potential of IL-2R stimulation with cell therapies is limited using current molecules due to severe toxicity of high-dose IL-2 and the inadequate selectivity of existing engineered IL-2 and IL-15 variants, which expand multiple endogenous cells in addition to CAR-T cells, including regulatory T cells and NK cells[5-7]. To address this challenge, we have applied our *cis*-targeting technology to develop CAR-T selective IL-2 fusion molecules that specifically target CAR-T cells, while exhibiting minimal activity on CAR-negative cells.

Cis-targeting of CAR-T cells

Figure 1: Engraftment and Expansion of CAR-T cells Correlates with Clinical Outcome

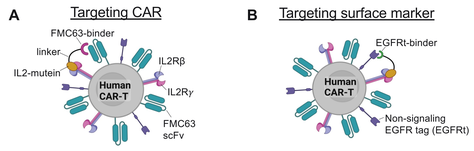
Survival in CAR-T treated patients is correlated with CAR-T expansion and persistence in patients[1,2]. Cytokines that promote CAR expansion and functionality may provide superior activity and durability.

Figure 2: *cis*-Targeted IL-2 Selective for CAR-T cells over Endogenous IL-2R-expressing Cells



Untargeted IL-2/IL-15-based therapies activate both CAR-T cells and endogenous IL-2R-expressing cells which limits cytokine benefit to CAR-T cells by acting as a sink, driving toxicity, or opposing effects (left). *Cis*-targeted IL-2 selectively supports CAR-T cells without activating endogenous cells (right).

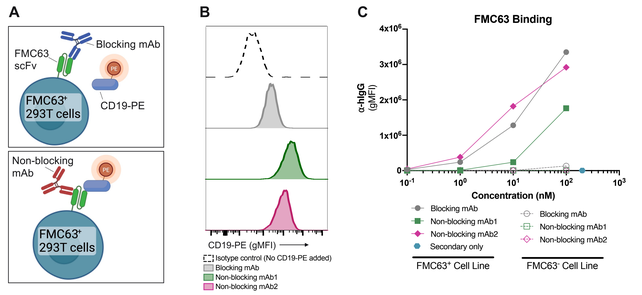
Figure 3: CAR-T Selective IL-2 Molecular Designs



Cis-targeting was used to design molecules with CAR-T cell restricted IL-2 signaling. Cis-targeted IL-2 fusions are comprised of a targeting antibody and an attenuated IL-2 mutein. Targeting is achieved either via binding to CAR directly (A) or to a marker co-expressed on the surface of CAR-Ts (e.g. EGFRt, B)

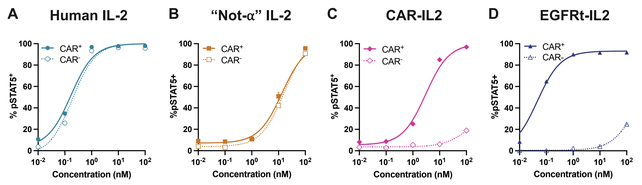
Results

Figure 4: FMC63-binding mAb is Non-blocking Allowing for CAR-T Interaction with CD19



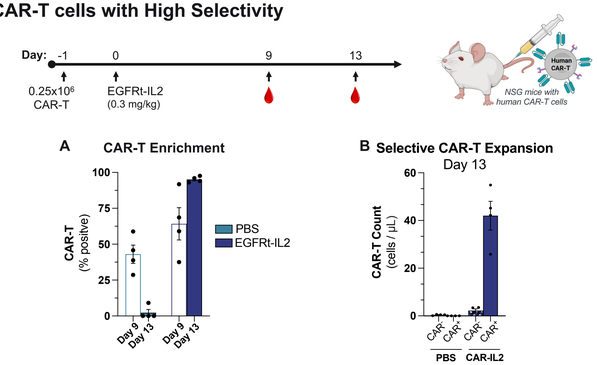
Generated mAbs were incubated with FMC63-scFv$^+$ 293T cells and CD19 protein conjugated to PE to determine the blocking or non-blocking status of the mAbs (A-B) . FMC63-binders were incubated with FMC63$^+$ 293T cells and stained with α-hIgG-APC (C). Non-blocking mAb2 was chosen for CAR-IL2 as it displayed substantially better binding to FMC63 than non-blocking mAb1.

Figure 5: CAR-IL2 and EGFRt-IL2 Fusion Molecules are Highly Selective for CAR T cells



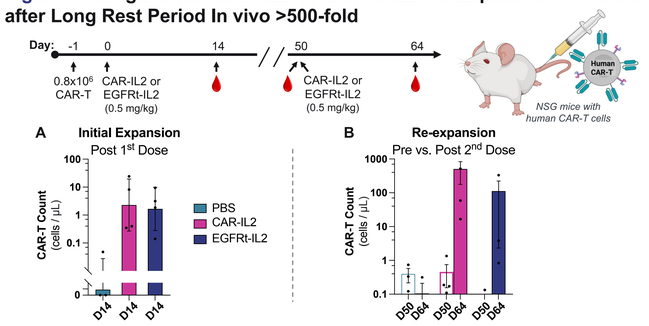
Induction of pSTAT5 signaling by non-selective molecules, human IL-2 cytokine (A) and "not-alpha" IL-2 (B), versus targeted IL2 fusion molecules (C-D) in human CAR-T cells. *Cis*-targeting of CAR T cells directed by non-blocking FMC63 binder (C) or an EGFRt molecule binder (D) exhibits high selectivity (>100 fold) for CAR$^+$ T cells over CAR$^-$ T cells.

Figure 6: A Single Low Dose of EGFRt-IL2 Expands Low Frequency CAR-T cells with High Selectivity



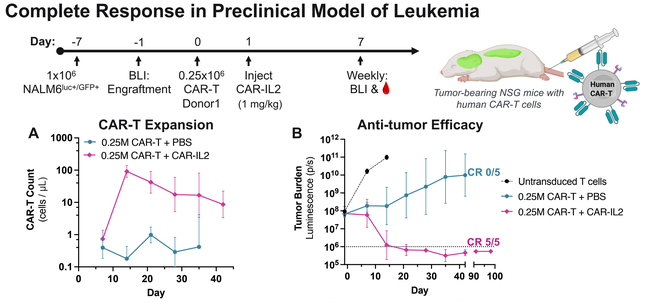
Peripheral blood was collected from inoculated mice on indicated days (relative to molecule injection) and analyzed by flow cytometry. A single dose of EGFRt-IL2 molecule resulted in substantial enrichment of CAR T cells to >95% of CD3$^+$ T cells (A) and selective expansion of CAR$^+$ cells over CAR$^-$ cells (B) .

Figure 7: Single Dose CAR-IL2 or EGFRt-IL2 Re-Expand CAR-T cells after Long Rest Period In vivo >500-fold



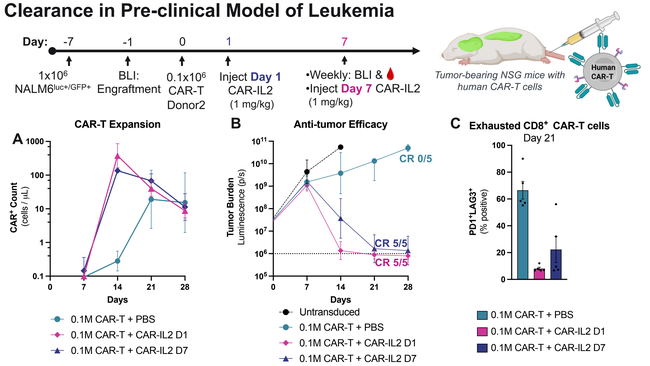
CAR-T cell expansion was examined in peripheral blood post 1st dose of targeted IL-2 molecules (A). Mice were re-treated 50 days after initial dosing. Re-expansion of CAR T cells was examined 2 weeks after 2nd dose (D64) and compared to pre-dose (D50) counts (B).

Figure 8: CAR-IL2 Converts Non-curative Dose of CAR-T cells to Complete Response in Preclinical Model of Leukemia



Substantial expansion of a low number of CAR-T cells by CAR-IL2 (A) conferred complete regression of tumors with no recurrence over 100 days (B). CAR-IL2 substantially augmented the efficacy of a non-curative CAR-T cell dose enabling 5/5 complete responses (CRs) in NALM6 model. Dashed line indicates baseline luminescence.

Figure 9: Single Delayed CAR-IL2 Dose Results in Complete Tumor Clearance in Pre-clinical Model of Leukemia



A single dose of CAR-IL2 was administered on D1 or D7. CAR-IL2 induced substantial expansion of CAR-Ts (A) and tumor regression (B) regardless of being administered on D1 or D7. Fewer exhausted CAR-T cells were detected in mice that received CAR-IL2 as indicated by the percent of PD-1$^+$LAG3$^+$ CD8+ CAR-T cells on day 21 (C). Dashed line indicates baseline luminescence.

Conclusions

- **Cis-targeting of IL-2 to CAR-T cells enables selective activation of CAR-T cells without activating endogenous IL-2R-expressing cells.**
  - Targeting can be achieved either via binding to CAR directly or to a marker co-expressed on the surface of CAR-T cells.
- **Cis-targeted CAR-IL2 molecules selectively activate CAR-T$^+$ cells over CAR-T$^-$ cells in vitro and in vivo.**
  - CAR-IL2 construct targeting FMC63 scFv selectively activate CAR-T cells by >100-fold without interfering with CD19 binding.
  - CAR-T cells can be selectively expanded in vivo to in non-tumor bearing NSG mice.
  - CAR-T cells can be re-expanded by >500-fold following a 2nd dose of CAR-IL2 after a long rest period in vivo.
- **CAR-IL2 augments the anti-tumor efficacy of CAR-T therapy in a preclinical model of leukemia.**
  - A single dose of CAR-IL2 converts a suboptimal dose of CAR-T cells into a curative therapy by substantially expanding the low number of transferred CAR-T cells.
  - Delaying a single dose of CAR-IL2 until seven days after CAR-T cell transfer still induces complete responses in leukemia model.

Methods

- Human T cells were isolated from the peripheral blood of healthy donors and transduced with FMC63-scFv 41BB CD3ζ (CD19 CAR) construct. Transduction efficiency was directed to ~50% allowing for an even mix of CAR-T$^+$ and CAR-T$^-$ cells for use in assays.
- Human phospho-STAT5 (pSTAT5) assays were performed by incubating FMC63 CAR-T cells with the indicated concentrations of cytokine molecules for 25 minutes. Cells were placed on ice, surface stained, washed, and then fixed using 4% PFA. Cells were then permeabilized with methanol, stained intracellularly, and analyzed by flow cytometry.
- In vivo studies with non-tumor bearing mice were performed by injecting the indicated number of human FMC63 CAR-T cells into NSG mice (Jackson Laboratory). The IL-2 fusion molecules were administered at the indicated dosing schedule. Peripheral blood was collected from the retro-orbital sinus and stained for flow cytometry in the presence of cell-counting beads.
- Tumor efficacy studies were performed by injecting 1x10^6 NALM-6$^{luc+/gfp+}$ cells intravenously (IV) via tail vein into NSG mice. After six days, tumor engraftment was analyzed by bioluminescence imaging (BLI) and mice were randomized into treatment groups. The designated number of FMC63 CAR-T cells were injected IV and the indicated treatments were administered one day later using the dosing schedule as described. Tumor volume was assessed by BLI and peripheral blood was collected, weekly.

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