MyD88/CD40-Enhanced CD19-Specific CAR-T Cells Maintain Therapeutic Efficacy Following Resolution of Cytokine-Related Toxicity Using Inducible Caspase-9

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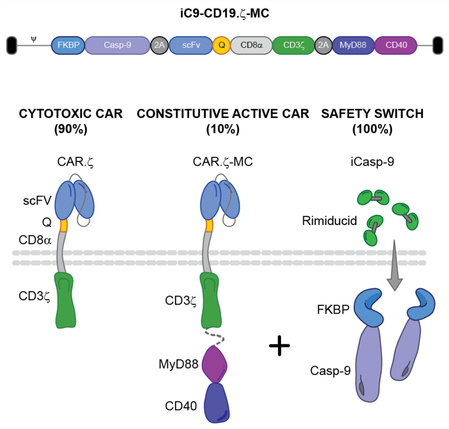
INTRODUCTION

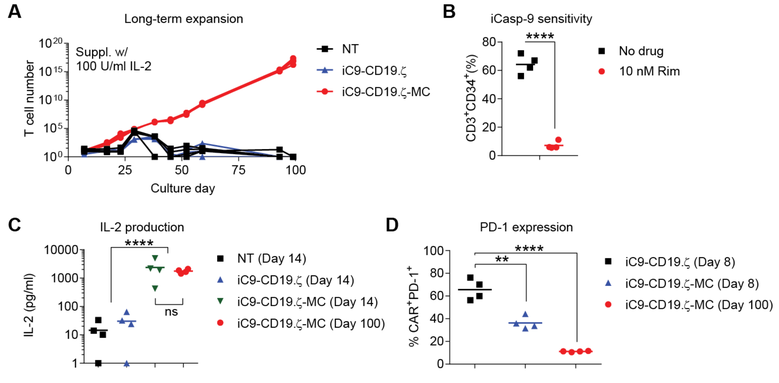
The efficacy of chimeric antigen receptor-modified T cells (CAR-T) targeting CD19⁺ leukemias and lymphomas is dependent on their *in vivo* expansion following adoptive transfer. Additional genetic augmentations to improve CAR-T expansion may improve therapeutic efficacy but risks increasing CAR-T toxicity. Here, we demonstrate that a highly active CD19-specific CAR-T cell, constitutively expressing a MyD88/CD40 (MC) costimulatory fusion protein and the inducible caspase-9 (iC9) safety switch, is effective at eliminating tumors but induces acute cytokine-related toxicity in animal models. Toxicity, however, can be fully resolved by rimiducid administration to induce partial CAR-T apoptosis, preserving long-term anti-tumor effects.

CIDeCAR TECHNOLOGY



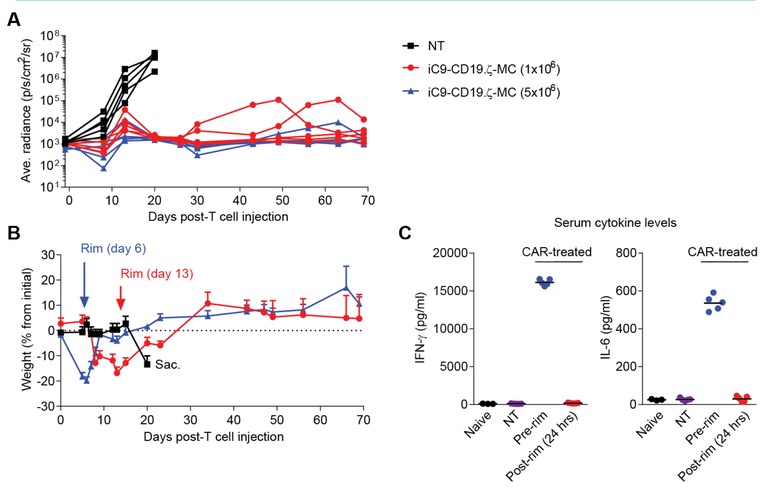
- Retroviral vector encoding a first-generation CAR targeting CD19 (FMC63 scFv), the anti-CD34 epitope (Q), CD8 stalk and transmembrane region and the CD3ζ domain were cloned in-frame with the iC9 safety switch and the MyD88/CD40 chimeric costimulatory molecule (MC).
- Inefficient 2A polypeptide cleavage results in two CAR species, a first generation CD19.ζ (~90%) and a third-generation CD19.ζ.MC (~10%) receptor. All T cells express iC9 (100%)
- iCasp-9 is activated by rimiducid (rim) dimerization, leading to T cell apoptosis

Robust proliferation with constitutive MC signaling



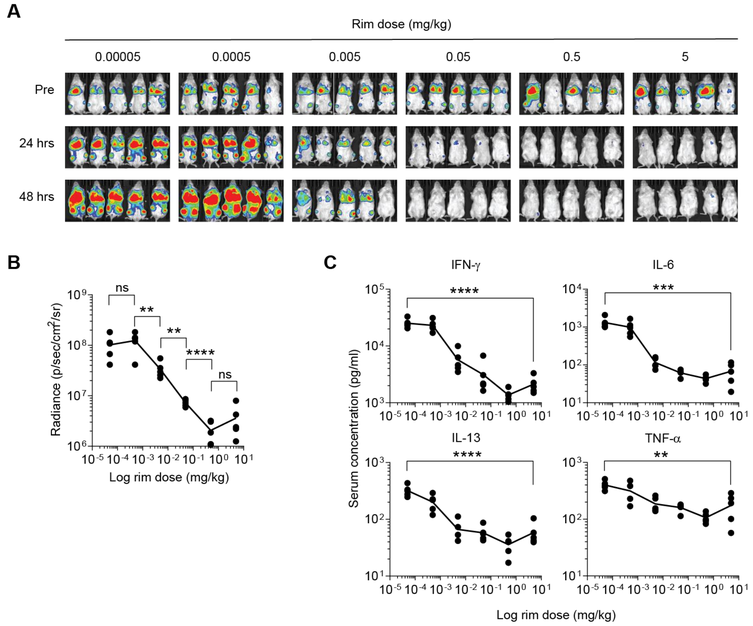
A) Non-transduced (NT), iC9-CD19.ζ and iC19-CD19.ζ-MC transduced T cells were expanded in IL-2 (100 U/ml)-supplemented growth media for 100 days. Cells were enumerated weekly and calculated for fold-expansion. **B)** Day 100 iC19-CD19.ζ-MC-modified T cells remained sensitive to iC9-mediated apoptosis when exposed to rim (10 nM). **C)** Short-term (day 14) and long-term (day 100) expanded iC19-CD19.ζ-MC-modified produced IL-2 in response to CD19 antigen stimulation in Daudi co-culture assays. **D)** iC19-CD19.ζ-MC-modified T cells downregulated PD-1 despite long-term expansion. ** p=<0.002, *** p<0.0002, **** p<0.0001

CAR efficacy after cytokine toxicity



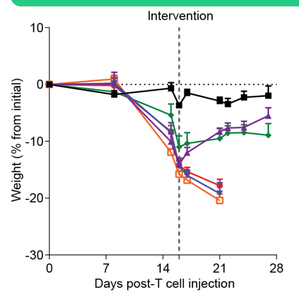
A) NSG mice (n=5 per group) were engrafted with Raji-luc tumor cells and treated with non-transduced (NT) or iC9-CD19.ζ-MC CAR-modified T cells on day 3. Tumor growth was measured by IVIS imaging and calculated by whole-body BLI . **B)** Mouse weight was measured to assess CAR-T-related cytokine toxicity. After ~20% weight loss, mice were treated with rim to eliminate CAR-T cells. **C)** Cytokine levels were assessed in naïve mice, mice receiving NT T cells, and CAR-T cells before and after (24 hrs) rim administration.

Titration of CAR-T activity using iCasp-9



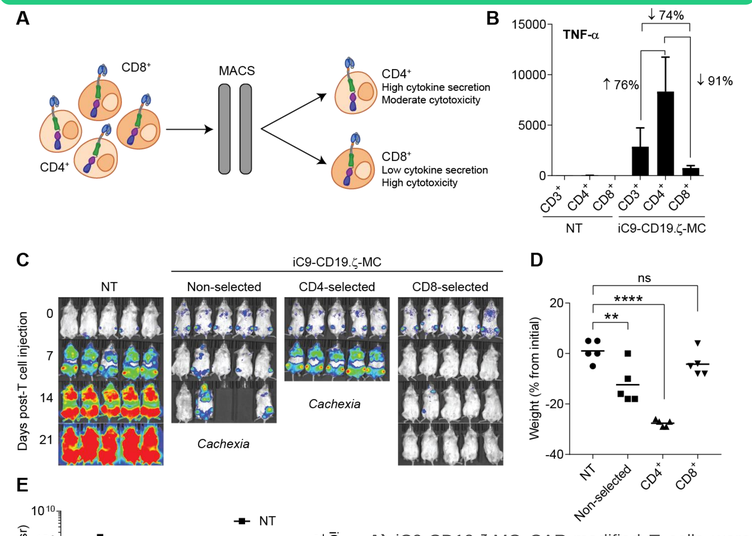
A and B) NSG mice (n=5 per group) were engrafted with CD19⁺ Raji lymphoma cells and treated with 5x10⁶ iC9-CD19.ζ-MC/luciferase-transduced T cells at day 3. After 6 days, mice were treated i.p. with log dilutions of rimiducid (0.00005 - 5 mg/kg). BLI of CAR-T cells was assessed prior to rimiducid treatment and at 24 and 48 hours post-injection. **C)** Serum cytokine levels were measured for IFN-γ, IL-6, IL-13 and TNF-α from each group before rimiducid and 24 hours post-injection. ** p=<0.002, *** p<0.0002, **** p<0.0001

TNF-α blockade resolves toxicity



5x10⁶ iC9-CD19.ζ-MC-modified T cells were injected into Daudi-bearing NSG mice and then treated with 0.5 mg/kg rim after >10% weight loss or with i.p. injections with 100 mg twice per week with an isotype antibody (control) or with neutralizing antibodies against human TNF-α, IL-6 and IFN-γ. Weight recovery was monitored until day 28.

CD8 selection enhances anti-tumor activity



A) iC9-CD19.ζ-MC CAR-modified T cells were purified into CD4⁺ and CD8⁺ fractions using MACS selection. B) TNF-α secretion from non-selected (CD3⁺), CD4⁺ or CD8⁺ CAR-T cells was measured following 48 hour exposure to CD19⁺ Daudi tumor cells C) NSG mice bearing Daudi-FFluc tumors were injected with 5x10⁶ NT, non-selected, CD4⁺ or CD8⁺ CAR-modified T cells and anti-tumor activity was measured by bioluminescent imaging. **D)** Toxicity from the different CAR-T therapies was assess by measuring weight. **E)** Subsequently, Daudi-Ffluc-bearing NSG mice were treated with a dose titration of CD8-selected iC9-CD19.ζ-MC CAR-modified T cells which showed anti-tumor efficacy to 50 days without the requirement for rimiducid or neutralizing antibody treatment. ** p=<0.002, **** p<0.0001

SUMMARY

- Constitutive MyD88/CD40 signaling due to incomplete 2A "cleavage" elicits robust CAR-T proliferation and anti-tumor activity
- Increased CAR-T activity is associated with CAR-produced cytokine-related toxicity in NSG mice
- CAR-T activity can be fully controlled by rimiducid titration to induce "partial" elimination of CAR-T cells and reduce cytokine levels
- Isolation of CD8⁺ CAR-T cells reduces cytokine related toxicities while preserving anti-tumor efficacy