Inducible MyD88/CD40 (iMC) Costimulation Provides Ligand-Dependent Tumor Eradication By CD123-Specific Chimeric Antigen Receptor T Cells

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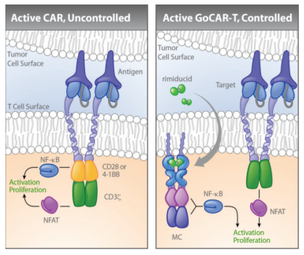
Background

- Promising clinical results with CD19-specific chimeric antigen receptor (CAR)-directed T cells for the treatment of B cell leukemia and lymphoma suggest that CARs may be effective in other hematological malignancies, such as acute myeloid leukemia (AML).
- CD123/IL-3Rα is an attractive CAR-T cell target due to its high expression on both AML blasts and leukemic stem cells (AML-LSCs). However, the antigen is also expressed at lower levels on normal stem cell progenitors presenting a major toxicity concern should CD123-specific CAR-T cells show long-term persistence.
- Here, we describe a CAR platform, "GoCAR-T", which uses a proliferation-deficient, first generation, CD123-specific CAR together with a ligand (rimiducid (Rim))-dependent costimulatory switch (inducible MyD88/CD40 (iMC)) to provide physician-controlled eradication of CD123⁺ tumor cells and regulate long-term CAR-T cell engraftment.

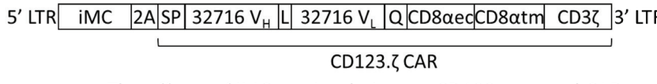
Technology

- Moves the costimulatory activation signal from the CAR to a rimiducid-controllable element
- Costimulation "on demand" is novel Bellicum technology
- Cell proliferation and activation is rimiducid-dependent
- Full activation and tumor cell killing requires second target-specific CD3ζ signal



Methods

Retrovirus and transduction: T cells were activated with anti-CD3/28 antibodies and subsequently transduced with a bicistronic retrovirus encoding tandem Rim-binding domains (FKBP12v36), cloned in-frame with MyD88 and CD40 cytoplasmic signaling molecules, and first generation CAR targeting CD123 (SFG-iMC-CD123.ζ).



Coculture assay: The effects of iMC costimulation on CD123-targeted CARs were assessed in coculture assays with CD123⁺, EGFP/*luciferase* (EGFP/*luc*)-modified leukemic cell lines (KG1, THP-1 and MOLM-13) with and without Rim using the IncuCyte live cell imaging system. IL-2 production was examined by ELISA from coculture supernatants.

Animal experiments: *In vivo* efficacy of iMC-CD123.ζ-modified T cells was assessed using an immune-deficient NSG tumor xenograft model. One million EGFP/*luc*-expressing CD123⁺ THP-1 tumor cells were injected i.v. into the animals, followed by a single i.v. injection on day 7 with varying non-transduced or iMC-CD123.ζ-modified T cells. Groups receiving CAR-T cells subsequently received i.p. injections of Rim (1 mg/kg) or vehicle only on days 0 and 15 post-T cell injection. Animals were evaluated for THP-1-EGFP/*luc* tumor burden and weight change on a weekly basis using IVIS bioluminescent imaging (BLI) and for T cell persistence by flow cytometry and qPCR at day 30 post-T cell injection.

Results

CD123 GoCAR-T cells mediate rimiducid-dependent killing of CD123⁺ tumor cells.

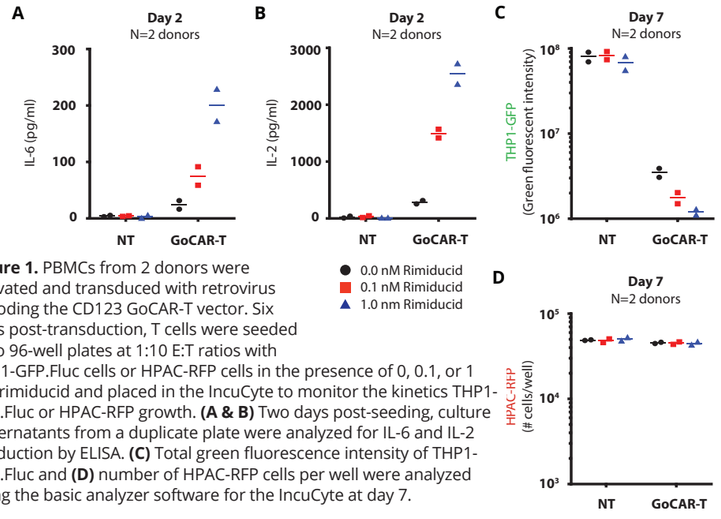


Figure 1. PBMCs from 2 donors were activated and transduced with retrovirus encoding the CD123 GoCAR-T vector. Six days post-transduction, T cells were seeded onto 96-well plates at 1:10 E:T ratios with THP1-GFP.Fluc cells or HPAC-RFP cells in the presence of 0, 0.1, or 1 nM rimiducid and placed in the IncuCyte to monitor the kinetics THP1-GFP.Fluc or HPAC-RFP growth. **(A & B)** Two days post-seeding, culture supernatants from a duplicate plate were analyzed for IL-6 and IL-2 production by ELISA. **(C)** Total green fluorescence intensity of THP1-GFP.Fluc and **(D)** number of HPAC-RFP cells per well were analyzed using the basic analyzer software for the IncuCyte at day 7.

Rimiducid-dependent proliferation of GoCAR-T cells following CD123⁺ tumor cell killing.

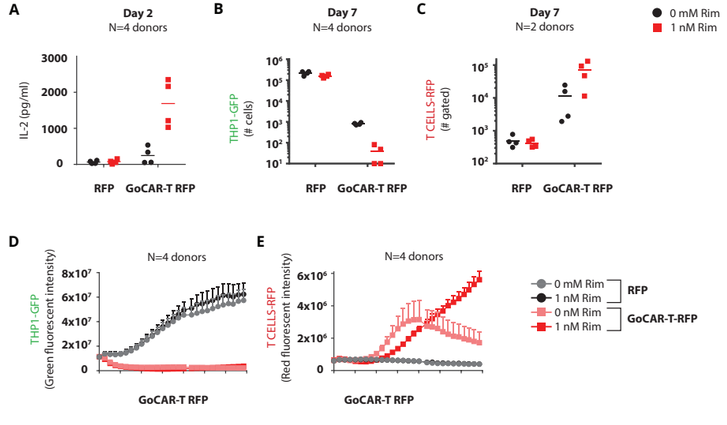


Figure 2. PBMCs from 4 donors were activated and co-transduced with retroviruses encoding the CD123 GoCAR-T and RFP vectors. Ten days post-transduction, T cells were seeded onto 96-well plates at 1:1 E:T ratios with THP1-GFP.Fluc cells in the presence of 0 or 1 nM rimiducid and placed in the IncuCyte to monitor the kinetics THP1-GFP.Fluc and T cell-RFP growth. **(A)** Two days post-seeding, culture supernatants from a duplicate plate were analyzed for IL-2 production by ELISA. **(B)** On day 7, cells were analyzed for the number of THP1-GFP.Fluc and **(C)** T cell-RFP remained in the coculture by flow cytometry. **(D)** Time course monitor of THP1-GFP.Fluc green fluorescence and **(E)** T cell-RFP red fluorescence analyzed using the IncuCyte for a total of 7 days.

iMC costimulation is required for killing of tumor cells by CD123 GoCAR-T cells *in vivo*.

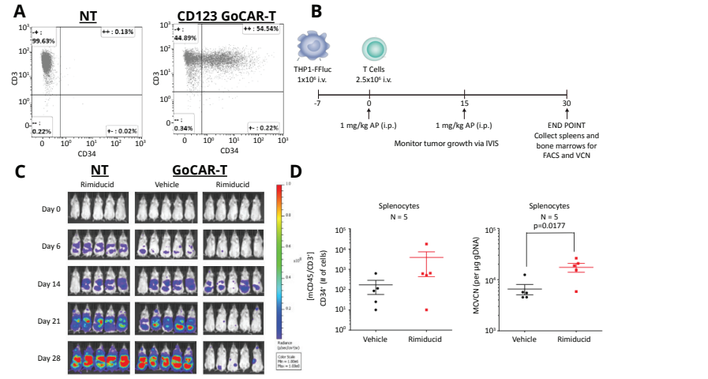


Figure 3. (A) PBMCs were activated and transduced with retrovirus encoding the CD123 GoCAR-T vector. Twelve days after transduction, CAR expression was determined using anti-Q-bend10 antibody before injection into mice. **(B)** NSG mice were engrafted with 1x10⁶ THP1-GFP.Fluc cells i.v. for 7 days followed by infusion of 2.5x10⁶ non-transduced (NT) or CD123 GoCAR-T cells i.v. Rimiducid or placebo were given i.p. on days 0 and 15 after T cell infusion at 1 mg/kg. **(C)** THP1-GFP.Fluc growth was measured using IVIS bioluminescence. **(D, E)** On day 30, mice were sacrificed and spleens were analyzed for the presence of CAR-T cells by flow cytometry and vector copy number assay.

CD123 GoCAR-T demonstrates dose-dependent killing of THP1 tumors *in vivo*.

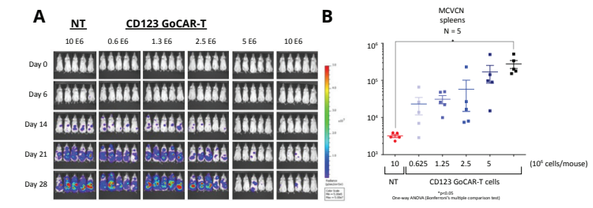


Figure 4. (A) NSG mice were engrafted with 1x10⁶ THP1-GFP.Fluc cells i.v. for 7 days followed by treatment with 1x10⁶ NT T cells or various doses of CD123 GoCAR-T cells i.v. Rimiducid was given i.p. on days 0 and 15 after T cell infusion at 1 mg/kg. **(B)** On day 29, mice were sacrificed and spleens were analyzed for the presence of CAR-T cells by vector copy number assay.

Summary

- GoCAR-T, a platform comprising a ligand-dependent activation switch and a proliferation-deficient first generation CAR, efficiently eradicates CD123⁺ leukemic cells when costimulation is provided by systemic rimiducid administration.
- Infrequent iMC costimulation results in reduction of CAR-T levels, providing a user-controlled system for managing persistence and safety of CD123-specific CAR-T cells.