GoTCR: Inducible MyD88/CD40 (iMC) enhances proliferation and survival of tumor-specific TCR-modified T cells and improves anti-tumor efficacy in myeloma

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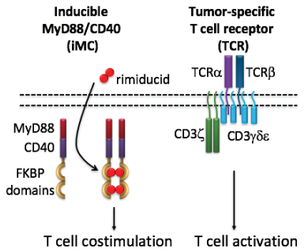
Background

- Cancer immunotherapy using T cells engineered to express tumor antigen-specific TCRs has shown promise in the clinic; however, durable responses have been limited by poor T cell expansion and persistence *in vivo*.
- In addition, downregulation of MHC class I on tumor cells diminishes T cell recognition, leading to reduced therapeutic efficacy.
- Inducible MyD88/CD40 (iMC) is a rimiducid (AP1903)-dependent costimulatory molecule that enhances DC activation[1] and T cell proliferation and survival.
- PRAME (PReferentially expressed Antigen in MElanoma) is a cancer testis (CT) antigen that is overexpressed in a number of cancers, including melanoma, sarcoma, AML, CML, neuroblastoma, breast, lung, head and neck cancers, but not in normal tissues.
- Bob1 (also known as OCA-B, OBF1 or POU2AF1) is a B cell-specific transcriptional co-activator that is highly expressed in CD19$^+$ B cells, ALL, CLL, MCL and multiple myeloma (MM).
- Herein, we investigate the feasibility and potential benefits of "GoPRAME" and "GoBob1" TCRs that incorporate iMC costimulation.

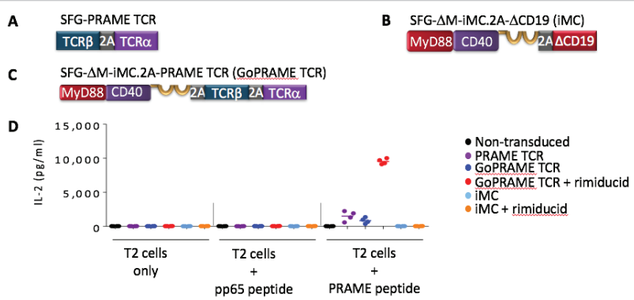
Technology

- "Costimulation on demand" via iMC is novel technology to better regulate potent T cell therapy.
- T cell activation and proliferation is TCR- and iMC-dependent.
- Maximal tumor-directed cytotoxicity, as well as T cell persistence *in vivo*, requires synergistic signals from a tumor-specific TCR and rimiducid-activated iMC



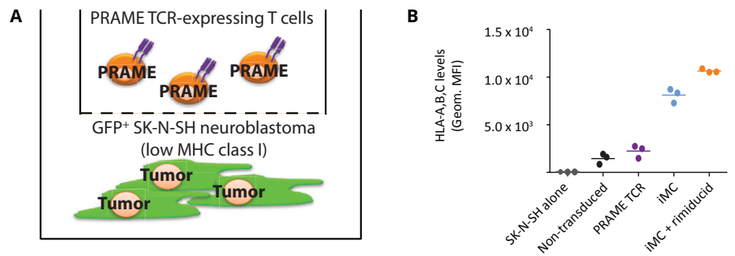
Results

Specific recognition of SLL peptide-pulsed APCs



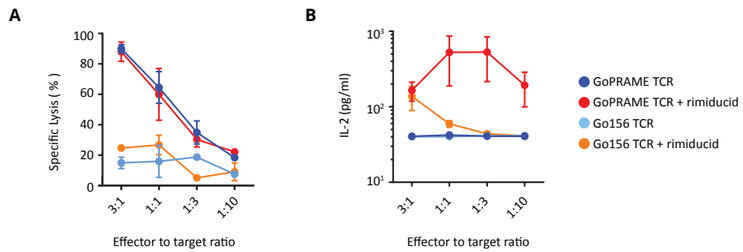
(A-C) Retroviral vectors expressing PRAME TCR[2], iMC/surface marker and GoPRAME TCR. **(D)** PRAME TCR recognition of SLL-peptide pulsed T2 cells synergizes with rimiducid-dependent iMC signals for maximal IL-2 secretion.

iMC and rimiducid upregulate MHC class I on tumors



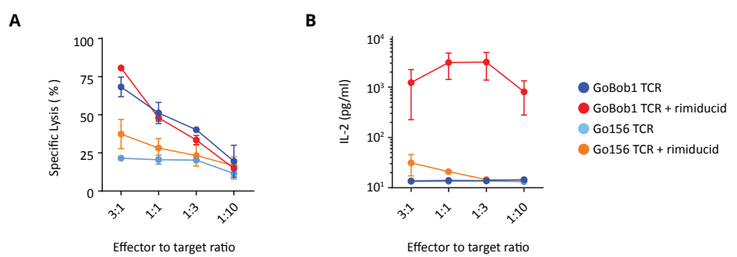
(A) Trans-well assay set-up. **(B)** Cytokines secreted by transduced T cells in the top well upregulate HLA class I on the surface of SK-N-SH neuroblastoma cells in an antigen-independent, but iMC- and rimiducid-dependent manner.

iMC costimulation enhances PRAME TCR function *in vitro*



(A) GoPRAME TCR-mediated cytotoxicity against HLA-A2$^+$PRAME+ U2OS osteosarcoma is rimiducid-independent **(B)** Signals from the PRAME TCR synergize with rimiducid-driven iMC costimulation, resulting in maximal IL-2 secretion. The Go156 TCR is a negative control TCR.

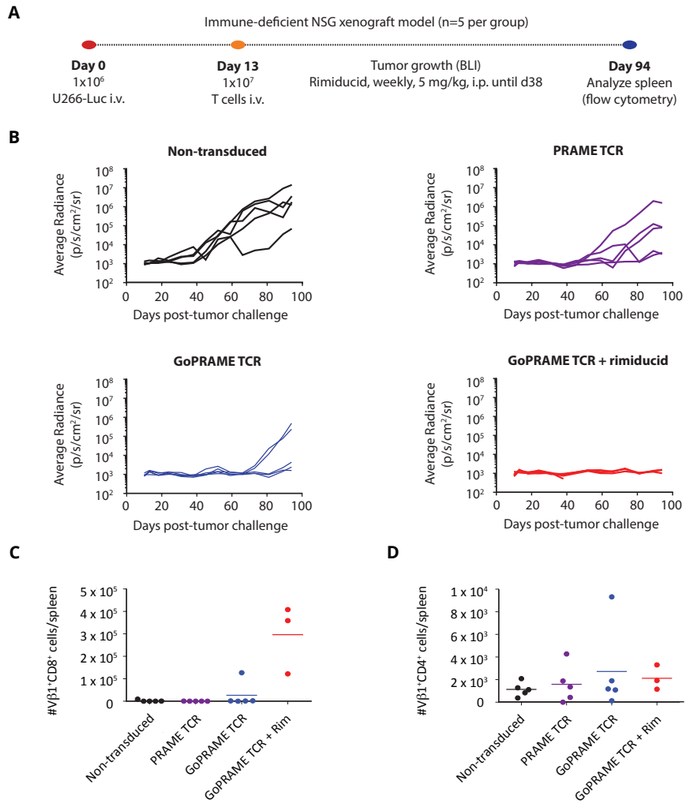
iMC costimulation enhances Bob1 TCR function *in vitro*



(A) GoBob-1 TCR-mediated cytotoxicity against HLA-B7$^+$Bob-1$^+$ U266 multiple myeloma is rimiducid-independent. **(B)** Signals from the Bob-1 TCR synergize with rimiducid-driven iMC costimulation, resulting in maximal IL-2 secretion. Go156 TCR is a negative control TCR.

References:
1. Narayanan P et al., A composite MyD88/CD40 switch synergistically activates mouse and human dendritic cells for enhanced antitumor efficacy. J Clin Invest. (2011) 121:1524.
2. Amir AL et al., PRAME-specific Allo-HLA-restricted T cells with potent antitumor reactivity useful for therapeutic T-cell receptor gene transfer. Clin Cancer Res (2011) 17:5615.

iMC costimulation enhances PRAME TCR efficacy *in vivo* by augmenting T cell proliferation and/or persistence



(A) NSG mice were engrafted with 1x10^6 luciferase-expressing U266 myeloma cells and treated with 1x10^7 non-transduced, PRAME TCR- or GoPRAME TCR-transduced T cells on day 13. Starting on day 14, five of the mice that received GoPRAME-transduced T cells received 5 mg/kg rimiducid i.p. weekly until day 38. **(B)** Tumor growth was measured by bioluminescence imaging. **(C,D)** Mice were sacrificed on day 94 and the spleens were analyzed for persistence of human T cells. iMC costimulation significantly increased the number of Vβ1$^+$CD8$^+$ T cells **(C)** but not the number of Vβ1$^+$CD4$^+$ T cells **(D)**.

Summary

- Rimiducid-driven iMC activation provides potent costimulatory signals in transduced T cells, synergizing with signals from exogenous PRAME- or Bob1-specific TCRs, leading to enhanced T cell proliferation/survival and improved anti-tumor efficacy both *in vitro* and *in vivo*.
- iMC activation upregulates HLA class I levels on tumor targets, which should lead to improved cytotoxicity via both engineered and endogenous T cells.