Dual-switch TCR: A two-ligand system to control PRAME TCR-modified T cell proliferation and death using inducible MyD88/CD40 and caspase-9

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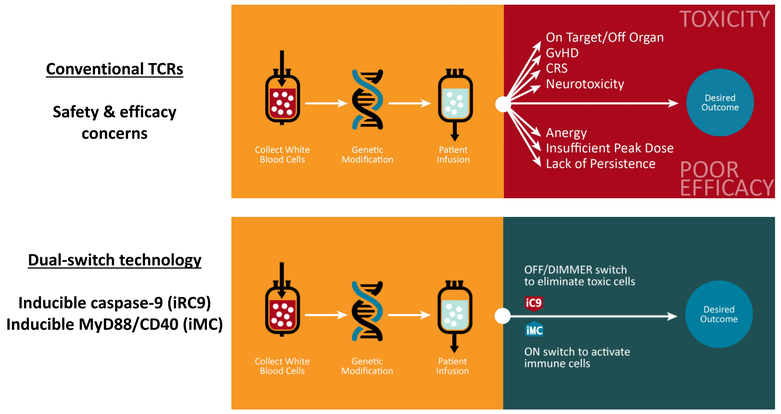
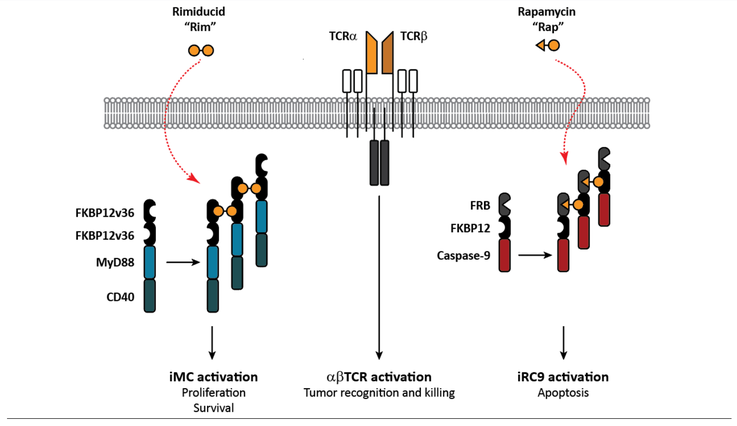
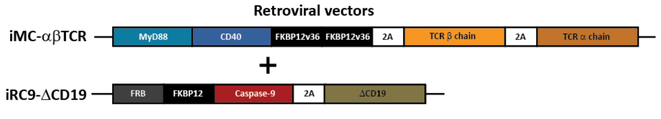
Background

T cells engineered to express the α and β chains of antigen-specific T cell receptors (TCRs) have shown promise as a cancer immunotherapy treatment; however, durable responses have been limited by poor persistence of gene-modified T cells. Additionally, severe toxicities, including patient deaths, have occurred upon infusion of large numbers of TCR-modified T cells. To enhance T cell persistence while providing a safeguard against life-threatening toxicity, we developed a dual-switch αβ TCR platform that uses a rapamycin (Rap)-induced caspase-9 (iRC9) together with a rimiducid (Rim)-controlled activation switch, inducible MyD88/CD40 (iMC).



Technology

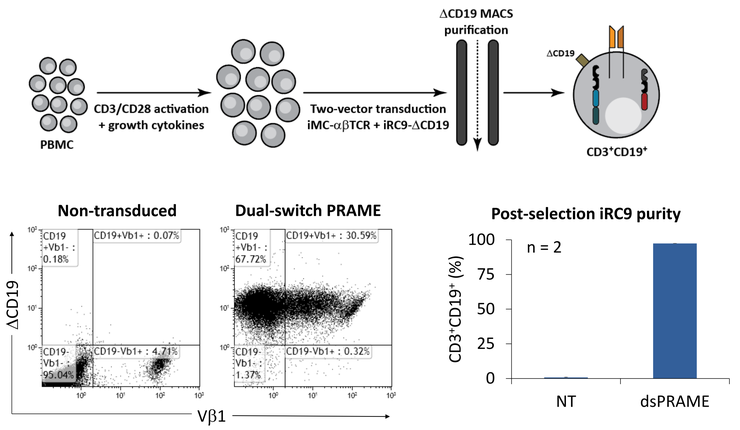




- Dual-switch technology based on two orthogonal ligands to enable tumor-specific TCRs
- High affinity αβ TCR recognizing HLA-A2-restricted PRAME epitope (SLLQHLIGL)
- Inducible MyD88/CD40 (iMC) is activated by the *homodimer* rimiducid (Rim) to provide costimulation which enhances T cell proliferation and survival
- Inducible Caspase-9 (iRC9) is activated by the *heterodimer* rapamycin (Rap) to induce rapid apoptosis of gene-modified T cells

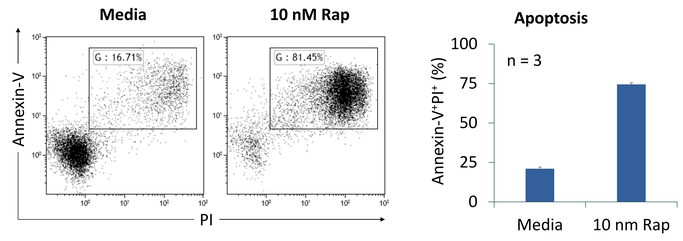
Results

Generation of dual-switch αβ TCR T cells



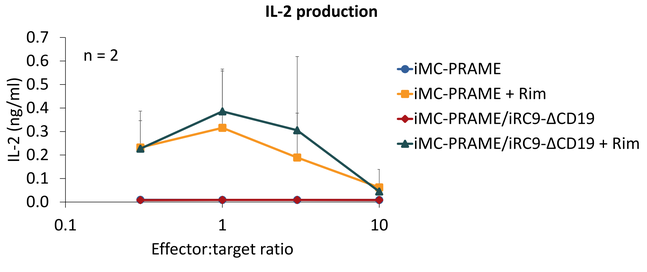
Generation of dual-switch αβ TCRs using a two-vector and selection protocol. PBMCs are stimulated with anti-CD3 and anti-CD28 antibodies followed by retroviral transduction with iMC-PRAME and iRC9-ΔCD19 vectors. After stable integration, T cells are purified for CD19 expression using magnetic separation and then expanded to yield dual-switch αβ TCRs.

iRC9 induces apoptosis of dual-switch αβ TCR T cells



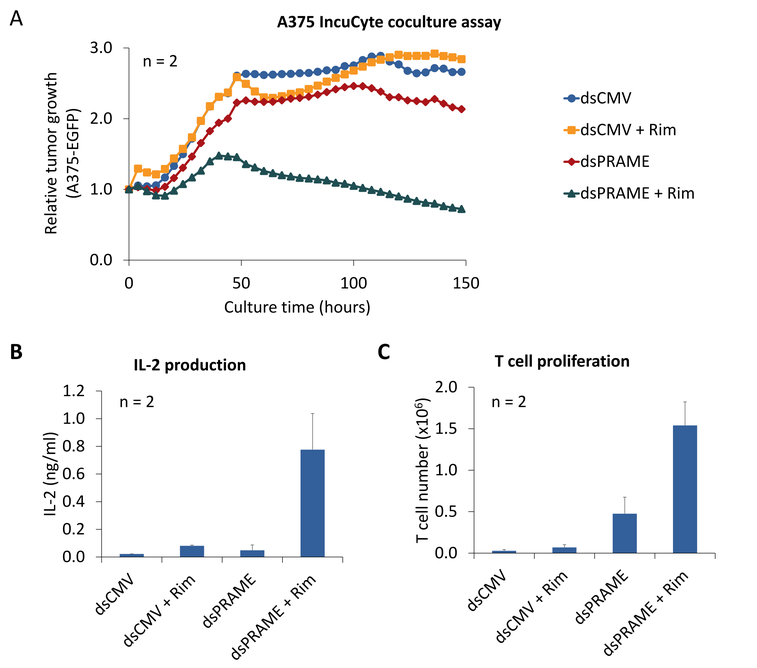
CD19-selected T cells transduced with iMC-PRAME and iRC9-ΔCD19 were cultured in media alone or with 10 nM rapamycin (Rap) for 6 hours and then analyzed for cell death by flow cytometry using Annexin-V and propidium iodide (PI).

iRC9 expression does not inhibit iMC costimulation



Signals from the PRAME TCR synergize with rimiducid-driven iMC costimulation in the presence or absence of iRC9 for the secretion of IL-2 following coculture with HLA-A2⁺ PRAME⁺ U266 myeloma cells at various effector to target ratios.

Dual-switch αβ TCRs retain iMC-dependent anti-tumor activity



(A) IncuCyte fluorescent live cell imaging was performed for coculture assays with dual-switch (ds)-modified T cells targeting PRAME or cytomegalovirus (CMV; recognizing the HLA-A2-restricted pp65 NLVPMVATV epitope) against the PRAME⁺ HLA-A2⁺ A375-GFP melanoma cell line. dsTCR-modified T cells were cultured in the presence or absence of 10 nM rimiducid (rim) **(B)** IL-2 production was measured 48 hours after coculture initiation. **(C)** After 7 days, cells were harvested and analyzed by flow cytometry and enumerated to determine transgenic T cell proliferation of PRAME and CMV-specific dsTCR T cells.

Summary

- Transduction with iMC-PRAME and iRC9-ΔCD19 yields acceptable αβ TCR expression and can be highly enriched for the iRC9 safety switch by magnetic selection
- iRC9 can be activated through heterodimerization by rapamycin to induce apoptosis of gene-modified T cells
- Co-expression of the iRC9 suicide gene does not impair rimiducid-induced MyD88/CD40 costimulation of dual-switch αβ TCR-modified T cells
- iMC activation with TCR engagement induces IL-2 production, enhanced T cell proliferation and greater anti-tumor activity through user-controlled costimulation
- This iMC-enhanced iRC9-incorporating TCR is the first reported prototype of a dual switch αβ TCR-engineered T cell therapy to increase efficacy, durability and safety of adoptive T cell therapies.