Inducible MyD88/CD40 enhances proliferation and survival of PRAME-specific TCR-engineered T cells and increases anti-tumor effects 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), a rimiducid (AP1903)-dependent costimulatory molecule that enhances DC activation and T cell proliferation and survival.
- PRAME (PRReferentially 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.
- Herein, we investigate the feasibility and potential benefits of a "GoPRAME" TCR that incorporates iMC costimulation.

Technology

- "Costimulation on demand" is novel Bellicum technology.
- T cell activation and proliferation is TCR- and iMC-dependent.
- Maximal tumor-directed cytoxicity, as well as T cell persistence in vivo, requires synergistic signals from a tumor-specific TCR and rimiducid-activated iMC.

Methods

Retrovirus constructs: The α and β chains of a TCR recognizing the HLA A*0201-restricted peptide SUHLHFLG from PRAME was generated via gBlock synthesis (Integrated DNA Technologies). It was cloned either into an SFG retroviral backbone (SFG-PRAME) or in-frame with iMC (SFG-GoPRAME). Retroviral supernatants were generated by transient transfection of 293T cells using RD114 envelope and PegPam (gag/pol) plasmids.

T cell transduction: PBMCs from healthy donors were activated with αCD3 and αCD28 antibodies and transduced with retrovirus on RetroNectin-coated plates. Four days later, T cells were analyzed for transduction efficiency by flow cytometry using αCD3, αCD2 (the variable domain segment of the PRAME TCR β chain), αCD8, αCD4 and αCD19 antibodies.

Coculture and trans-well assays: T cells transduced with vectors expressing the PRAME TCR, GoPRAME TCR or iMC were cultured either with peptide (10 μg/ml)-pulsed T2 cells or U266 myeloma tumor cells that were GFP positive. T cells were activated with 10 nM rimiducid where indicated. Cytokine production was assessed at 48 hours using ELISAs or transduced with retrovirus on Retronectin-coated plates. Four days later, T cells were analyzed for transduction efficiency by flow cytometry using αCD3, αCD2 (the variable domain segment of the PRAME TCR β chain), αCD8, αCD4 and αCD19 antibodies.

Summary

- Rimiducid-driven activation of iMC provides potent costimulatory signals in transduced T cells, which synergize with signals from an exogenous PRAME-specific TCR, 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, possibly contributing to improved cytoxicity.

References