IN VIVO EXPANSION OF GAMMA DELTA T CELLS BY A CD19-TARGETED BUTYROPHILIN HETERODIMER LEADS TO ELIMINATION OF PERIPHERAL B CELLS

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Background A primary mechanism of cancer immunotherapy resistance involves downregulation of specific antigens or major histocompatibility complex based antigen presentation, which renders tumor cells invisible to alpha-beta T cells, but not gamma-delta T cells. Recently, a two-step model of gamma-delta T cell activation has emerged, wherein one butyrophilin (BTN, ie. BTN2A1) directly binds the gamma-delta TCR but is only activated if certain molecular patterns (eg. phosphoantigens) facilitate recruitment of a second BTN (ie. BTN3A1) into a complex to form a BTN2A1/3A1 heterodimer. The BTN2A1/3A1 complex specifically activates the predominant gamma-delta T cell population in the peripheral blood, comprising the Vg9d2 T cell receptor (TCR), but does not activate the primary gamma-delta T cell population in mucosal tissues, comprising the Vg4 TCR. The unique mechanism of action and specificity of gamma-delta TCR/BTN interactions suggests that therapeutic proteins comprising specific BTN heterodimers could be used to target specific gamma-delta T cell populations, with a lower risk of off-target activation common with CD3-directed T cell engagers.

Methods Human BTN2A1/3A1-Fc-CD19scFv and mouse BTN1L/6-Fc-CD19scFv heterodimeric fusion proteins were purified and binding to CD19 or the respective gamma-delta TCRs was assessed by ELISA, Octet and flow cytometry using gd T-cells isolated from human peripheral blood and mouse tumor models in vivo.

Results The CD19-targeting scFv domains of the BTN heterodimer fusion proteins bound to human and mouse CD19 with low nanomolar affinity. The BTN2A1/3A1-Fc-CD19scFv compound specifically bound to the Vg9d2 TCR on human gd T cells while the mouse BTN1L/6-Fc-CD19scFv bound to Vg7d4 TCR on mouse gd T cells. Both compounds were able to activate gd T cells in a co-culture assay resulting in degranulation and increased surface expression of CD107a and also increased apoptosis of CD19+ tumor cells. Intraperitoneal administration of the mouse BTN1L/6-Fc-CD19scFv led to anti-tumor effects in A20 tumor bearing BALB/c mice. Phenotyping from BTN1L/6-Fc-CD19scFv treated mice revealed profound and rapid expansion of the endogenous gamma-delta T cells in the circulation and tumor, with concomitant depletion of peripheral CD19+ B-cells, confirming the mechanism of action of the heterodimer as a gamma-delta T cell specific engager.

Conclusions These results provide proof of mechanism for in vivo manipulation of gamma-delta T cells using antigen-targeted butyrophilin heterodimeric fusion proteins for the treatment of cancer.

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includes CD8+ T cells driving tumor regression. Our data demonstrate that ATRC-101, bound to its target which is an RNP complex, can activate myeloid cells and are consistent with this activation occurring via FcR and Toll-like receptor (TLR) pathways.

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CD122-SELECTIVE IL-2 COMPLEXES TREAT OVARIAN CARCINOMAS, INDUCE TREG FRAGILITY AND PROMOTE T CELL STEM CELLS

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Background Ovarian cancer (OC) responds poorly to immunotherapies. Regulatory T cells (Treg) engage IL-2 by high-affinity CD25 for differentiation and function,1 and anti-tumor effector T cells (Teff) use intermediate affinity CD122. We studied IL-2 complexes (IL-2c) that selectively activate CD122 (Teff) over CD25 (Tregs).

Methods Orthotopic ID8agg-luc mouse OC burden was measured by in vivo imaging. Tumor, ascites and draining lymph nodes (TDLN) were analyzed by flow and tSNE. IL-2c was complexed using 1.5 μg/mouse IL-2 and 7.5 μg/mouse αIL-2 (clone JES6-5H4) before i.p. injection every other day x 4 starting at day 7. antiPD-L1 was given at 100ug/mouse every 3 days x 4 starting from Day 11. FIR mice were used to sort live Tregs.

Results IL-2c but not antiPD-L1 potently inhibits ID8agg (figure 1). IL-2c decreased ascites Treg functional markers (e.g., CD25, granzymeB) while upregulating the same markers on Teffs (figure 2). IL-2c inhibited Treg suppression in ascites while TDLN Tregs were unaffected (figure 3). tSNE showed great similarity of TDLN Tregs treated with isotype and IL-2c while ascites Tregs after IL-2c showed a fragile phenotype (e.g., increased PD-1, T-bet, and IFNgamma with maintained FoxP3 expression [figure 4]) which is known to contribute to better response to cancer immunotherapy.3 4 We observed a complete reduction of tumor bioluminescence with IL-2c alone (figure 5). A CD8+CXCR5+TCF-1+ T cell stem cell (TCSC) population reportedly improves immune checkpoint blockade efficacy.5 6 Since CD122 is regulated by TCF-1,7 we explored the effect of IL-2c on these TCSC. IL-2c significantly induced a CD8+CXCR5+TCF-1+ TCSC population in ID8agg tumors (figure 6), possibly through a positive feedback loop by further enhancing CD122 expression on TCF-1+ cells but not TCF-1− cells (figure 7). tSNE analysis of detailed immune phenotype of IL-2c induced TCSC revealed that these TCSC differed from those induced by antiPD-L1. In ID8agg, antiPD-L1-induced TCSC are mostly CXCR5+ and PD1+, consistent with previous reports in other cancers3 4 while IL-2c-induced TCSC were PD1− (figure 8), expressed CCR2 and CXCR3, and produced TNFalpha (figure 9).

Abstract 690 Figure 1 IL-2c but not aPD-L1 treats ID8agg Luciferase signal of ID8agg-luc tumors treated with isotype, ?PD-L1, or IL-2c measured by in vivo imaging. Arrows indicate treatments.

Abstract 690 Figure 2 IL-2c inhibits functional markers on tregs and promotes teff Expression of CD25 and granzymeB were measured by flow cytometry in indicated population from isotype or IL-2c treated ascites 3 weeks after final IL-2c dose.

Abstract 690 Figure 3 IL-2c reduces ascites Treg suppressive function Treg:CD4*CD25+ T cell ratio

Abstract 690 Figure 4 IL-2c induces Treg fragility in ascites but not TDLN tSNE analysis on CD45+CD3+CD8+FoxP3+ cells from ascites and TDLN of isotype and IL-2c treated ID8agg-luc challenged mice. Right, representative bar graphs of flow data.