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688 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 BTNL1/6-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 intestinal tissue. The functionality of the constructs to activate gamma-delta T cells and mediate killing of tumor cells was assessed using live cell imaging in vitro as well as a murine B-cell lymphoma model 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 BTNL1/6-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 BTNL1/6-Fc-CD19scFv led to anti-tumor effects in A20 tumor bearing BALB/c mice. Phenotyping from BTNL1/6-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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689 ATRC-101 DRIVES POTENT SINGLE-AGENT ACTIVITY IN MOUSE SYNGENEIC TUMOR MODELS VIA A NOVEL CELLULAR MECHANISM OF ACTION

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Background We have previously demonstrated adaptive antibody responses targeting public tumor antigens in cancer patients. ATRC-101, a clinical stage, engineered version of an antibody identified in such a patient, displays robust single-agent activity in syngeneic tumor models requiring Fc receptors (FcRs) expressed by innate immune cells and the presence of CD8+ T cells. The novel target of ATRC-101 was found to be a tumor-restricted ribonucleoprotein (RNP) complex, and because RNP complexes drive T cell responses in infectious and autoimmune disease via innate immune cells, we further characterized the mechanism-of-action of ATRC-101. Here we describe changes in immune cell populations in a tumor model proximal to treatment initiation with ATRC-101.
Methods Mice bearing EMT6 tumors received ATRC-101 beginning on day 7 post-tumor inoculation. Tissues were harvested between days 7 and 14 and analyzed by flow cytometry and immunohistochemistry. Transcriptome analysis was performed using RNA sequencing on whole tumors taken on days 7, 9, and 12.
Results The earliest significant changes induced by ATRC-101, relative to vehicle, were noted just 24 hours after dosing: increased numbers of cDC1 cells in blood, and decreased numbers of cDC2 cells in blood and M-MDSCs in tumor. A significant increase of CD8+ T cells was observed in blood 48 hours after dosing and in tumor 96 hours after dosing. Increased numbers of NK cells were also observed in blood and tumor at this later time. Multiplex analysis of circulating cytokines demonstrated a very early increase in myeloid chemo-attractants, such as MCP1 and MIP1a.Whole exome sequencing of tumor samples showed that ATRC-101 dosing drives a significant increase, relative to vehicle, in the expression of interferon-stimulated genes. Co-culturing experiments demonstrated that induced, bone marrow-derived dendritic cells are activated by ATRC-101 and its target in a dose-dependent fashion.
Conclusions Dosing with ATRC-101 in the EMT6 syngeneic tumor model, in which ATRC-101 displays notable single-agent activity, leads to changes in immune cell composition in the blood and tumor, with the earliest changes observed in myeloid or myeloid-derived cell populations, and to the early appearance of myeloid chemo-attractants. We believe these data indicate that ATRC-101 acts proximally on the myeloid cell populations in the tumor, leading to a remodeling of the tumor environment and an adaptive immune response that