TCGA. IO-108 binds to LILRB2 with high affinity and specificity and blocks LILRB2/ILT4 ligand binding and activation. IO-108 enhanced the production of multiple proinflammatory cytokines in LPS- and anti-CD3-stimulated PBMC cultures from healthy donors and potentiated DC maturation/activation in response to LPS. Moreover, IO-108 polarized primary CD14+ cells isolated from solid tumor patient PBMC and ovarian cancer-associated ascites towards a proinflammatory phenotype and attenuated their suppressive effect on autologous T-cell proliferation and production of tumoricidal cytokines.

Conclusions The preclinical characterization of IO-108, a novel LILRB2/ILT4 antagonistic antibody, demonstrates its ability to polarize tumor-associated myeloid cells towards a proinflammatory phenotype and suggests potential therapeutic benefit in tumors unresponsive to immune checkpoint blockade.

Acknowledgements We acknowledge the funding support from National Cancer Institute (1R01 CA248736 and 2P30 CA142543), the Welch Foundation (AU-0042-20030616) and the Cancer Prevention and Research Institute of Texas (RP150551 and RP190561).

Ethics Approval PBMCs were isolated from buffy coats of healthy donors (Interstate Blood Bank). Hematopoietic samples from cancer patients were obtained through the services of the Simmons Cancer Center’s Tissue Management Shared Resource with IRB approved protocol (STU 102010-051). All animal work was approved and conducted under the oversight of the UT Southwestern Institutional Animal Care and Use Committee (IACUC).

REFERENCES

Background gd' T-cells are innate-like lymphocytes described as potent killer of cancer cells whose infiltration into tumors is associated with a positive prognosis.1 2 This supports gd T-cells use in cancer immunotherapy. BTN3A, which belongs to the B7-subfamily of Ig proteins, is required for the recognition of malignant or infected cells by human g9d T-cells by sensing intracellular accumulation of phosphorytens.3 ImCheck Therapeutics is developing ICT01, a humanized anti-BTN3A (IgG1, Fc-silenced), g9d T-cell-activating antibody for the treatment of patients with solid or hematologic tumors.

Methods A complete IND-enabling program was conducted to characterize the preclinical activity and safety of ICT01. ICT01 effects on human and cynomolgus PBMCs were characterized in vitro using flow cytometry. ICT01-mediated killing activity of g9d T-cells was assessed using in vitro co-cultures with tumor and non-tumor cells. Immunocompromised mice bearing human tumors and adoptively transferred with human g9d T cells were used to assess ICT01 anti-tumor activity in vivo. The PK, PD and safety of intravenous ICT01 (0.1 to 100 mg/kg single- and repeated-dose) were evaluated in Cynomolgus monkeys.

Results ICT01 selectively binds to all three BTN3A isoforms with high affinity (<10nM). When assayed in human and cynomolgus PBMCs in vitro, ICT01 promoted a robust and specific activation of g9d T-cells as shown by concentration dependent increase in cell surface CD69 and CD25 and cytokines secretion (IFNγ, TNFα). In co-culture experiments, ~20% of target occupancy on tumor cells is sufficient for maximal g9d T-cell degranulation (e.g. CD107a/b expression). ICT01-activated g9d T-cells continuously and serially kill a wide range of tumor cells in multi-day co-culture conditions. In contrast, non-tumoral BTN3A-expressing B cells, HUVEC and fibroblasts were unaffected. In mouse AML and ovarian cancer models, repeated injections of ICT01 delayed tumor growth and significantly prolonged animal survival. In primates, ICT01 exposure and target engagement was dose-dependent, with all tested doses producing a specific g9d T cell activation and trafficking out of the circulation within 1 hour. ICT01 administration was well tolerated with no safety signals observed at doses up to 25 mg/kg/week based on clinical, laboratory, and anatomic pathology parameters.

Conclusions The combined in vitro and in vivo pharmacology data provide evidence that ICT01 is an attractive and novel therapeutic approach for enhancing the innate anti-tumor potential of g9d T-cells by activating BTN3A. Importantly, ICT01 did not affect healthy BTN3A-expressing cells, and NHP studies confirmed ICT01 safety with a wide therapeutic index. Therefore, ICT01 is being tested in the ongoing EVIC-TION trial (NCT04243499).

Ethics Approval Pseudonymized samples isolated from healthy volunteers’ whole blood by ImCheck Therapeutics under the agreement n° 7173 between ImCheck Therapeutic SAS and EFS PACA (Etablissement Français du Sang Provence-Alpes-Côte d’Azur)
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http://dx.doi.org/10.1136/jitc-2020-SITC2020.0688

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 Vγ9d2 T cell receptor (TCR), but does not activate the primary gamma-delta T cell population in mucosal tissues, comprising the Vγ4 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 BTN1L6-Fc-CD19scFv heterodimeric fusion proteins were purified and binding to CD19 or the respective gamma-delta TCR 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 demonstrated by binding to CD19 or the respective gamma-delta TCR.

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 chemoattractants (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-attracants, 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-attracants. 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