Background gdT-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 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 g9d2 T-cells by sensing intracellular accumulation of phosphoantigens.\(^3\) ImCheck Therapeutics is developing ICT01, a humanized anti-BTN3A (IgG1, Fc-silenced), g9d2 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 g9d2 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 g9d2 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 g9d2 T-cells as shown by concentration dependent increase in cell surface CD69 and CD25 and cytokines secretion (IFN\(_\gamma\), TNF\(_\alpha\)). In co-culture experiments, ~20% of target occupancy on tumor cells is sufficient for maximal g9d2 T-cell degranulation (e.g. CD107a/b expression). ICT01-activated g9d2 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 pri-mates, ICT01 exposure and target engagement was dose-dependent, with all tested doses producing a specific g9d2 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 g9d2 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-cote d’Azur)
IN VIVO EXPANSION OF GAMMA DELTA T CELLS BY A CD19-TARGETED BUTYROPHILIN HETERODIMER LEADS TO ELIMINATION OF PERIPHERAL B CELLS

Suresh De Silva*, George Fromm, Louis Gonzalez, Arpita Patel, Kyung Yoon, Zachery Opheim, Robert Farmer, Dean Chamberlain, Taylor Schreiber. Shattuck Labs, Durham, NC, USA

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 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 BTN1L/6-Fc-CD19scFv bound to Vg4d4 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 cell populations 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.