MATCH4 molecule was constructed containing bivalent low-affinity MSLN binding domains, a CD3 binding domain, and a serum albumin-binding domain for half-life extension. This molecule was tested in a cytotoxicity assay using human PBMCs co-cultured with H226 or MeT-5A cells, which express high or low levels of MSLN, respectively. The MeT-5A line, derived from mesothelial cells in the pleural fluid of non-cancerous individuals, represents normal MSLN-expressing cells. Soluble MSLN was added to determine effects on cytotoxicity. In vivo xenograft mouse studies were conducted using a tumor cell/PBMC co-implantation model, followed by regular dosing with molecules of interest.

**Results** Here we report the design and the promising preclinical activity of the MATCH4 molecule in vitro and in vivo. We demonstrate that the low-affinity bivalent MSLN T cell engager has increased in vitro potency in T cell activation and tumor cell killing, as compared to a high-affinity monovalent counterpart on high MSLN expressing cells. We also demonstrate that the activity on low MSLN expressing cells is reduced for the low-affinity bivalent compared to the high affinity monovalent molecule. Because soluble MSLN is shed from cancer cells into cancer patient serum, we also demonstrate that up to 500 ng/mL of soluble MSLN does not interfere with the cytotoxic activity of the low affinity bivalent T cell engager, compared to the effects of soluble MSLN on a high affinity monovalent T cell engager. Importantly, we demonstrate in vivo that the low-affinity bivalent molecule significantly inhibits tumor growth in a dose-dependent manner.

**Conclusions** Collectively, these data demonstrate anti-tumor efficacy by this novel multispecific low affinity bivalent T cell engager. These data indicate the potential of this molecule to increase the therapeutic window by reducing safety concerns on normal tissue where MSLN expression is low, and yet increase cytotoxicity to MSLN-expressing cancer cells.

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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.

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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).

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687 ENHANCEMENT OF ANTI-TUMOR IMMUNITY BY ICT01: A NOVEL G9D2 T CELL-ACTIVATING ANTIBODY TARGETING BUTYROPHILIN-3A (BTN3A)

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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 g9d2 T-cells by sensing intracellular accumulation of phosphorytogens.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 cyto-kines secretion (IFNγ, TNFα). In co-culture experiments, ~20% of target occupancy on tumor cells is sufficient for maximal g9d2 T-cell degranulation (e.g. CD107α/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)