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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**A HIGHLY SELECTIVE AND POTENT HPK1 INHIBITOR ENHANCES IMMUNE CELL ACTIVATION AND INDUCES ROBUST TUMOR GROWTH INHIBITION IN A MURINE SYNGENEIC TUMOR MODEL**

**Background** HPK1, a member of the MAP4K family of protein serine/threonine kinases, is involved in regulating signal transduction cascades in cells of hematopoietic origin. Recent data from HPK1 knockout animals and kinase-inactive knock-in animals underscores the role of HPK1 in negatively regulating immune cell activation. This negative-feedback role of HPK1 combined with its restricted expression in cells of hematopoietic origin, make it a compelling drug target for enhancing anti-tumor immunity.

**Methods** A structure-based drug design approach was used to identify potent and selective inhibitors of HPK1. Biochemical assays, as well as primary human and mouse immune cell-based activation assays, were utilized for multiple iterations of structure-activity relationship (SAR) studies. In vivo efficacy, target engagement and pharmacodynamic data were generated using murine syngeneic tumor models.

**Results** A highly potent, HPK1 inhibitor was identified, that showed high selectivity against T cell-specific kinases and kinases in the MAP4K family. In vitro, HPK1 small molecule inhibition resulted in enhanced IL-2 production in primary mouse and human T cells, enhanced IL-6 and IgG production in primary human B cells, and enhanced mouse dendritic cell activation and antigen presentation capacity. Furthermore, HPK1 inhibition alleviated the immuno-suppressive effects of PGE2 on naïve human T cells and restored the proliferative capacity of exhausted human T cells. In vivo, HPK1 inhibition HPK1 inhibition abrogated T cell receptor-stimulated phospho-SLP-76, enhanced cytokine production, and mediated robust tumor growth inhibition in a murine syngeneic tumor model.

**Conclusions** Pharmacological blockade of HPK1 kinase activity represents a novel and potentially valuable immunomodulatory approach for anti-tumor immunity.

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**PRECLINICAL CHARACTERIZATION OF A NOVEL THERAPEUTIC ANTIBODY TARGETING LILRB2**

**Background** Myeloid-derived suppressor cells and tumor-associated macrophages inhibit anti-cancer immune responses systemically and in the tumor microenvironment, respectively, thereby limiting the efficacy of immune checkpoint blockers. However, the plasticity of myeloid cells may enable therapeutic intervention. The inhibitory receptor LILRB2/ILT4, which is expressed primarily in myeloid cells (monocytes, macrophages, dendritic cells and neutrophils), has emerged as a key immune checkpoint mediating the tolerogenic activity of myeloid cells associated with cancer.

**Methods** We used computational approaches to evaluate LILRB2/ILT4 expression in solid tumors from TCGA. IO-108 was discovered from a phage-displayed human single chain variable fragment antibody library. IO-108 contains the S228P mutation in the hinge region to prevent Fab-arm exchange. The binding affinity of IO-108 was measured using biolayer interferometry. The specificity of IO-108 was confirmed by two methods: 1) ELISA using recombinant LILR family members; 2) flow cytometry using cell lines engineered to express the extracellular domain of every LILR on the cell surface. Reporter and ligand binding assays were used to demonstrate LILRB2/ILT4 blocking activity of IO-108. Functional studies using primary immune cells from healthy donors and solid tumor cancer patients were performed to characterize IO-108 activity and mechanism of action. The in vivo efficacy of IO-108 is currently being evaluated in mouse models.

**Results** We found high LILRB2/ILT4 expression associated with macrophage infiltration in many solid tumor types from...
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).

REFERENCES
10. He J, Xu J, Xu Y et al. Overexpression of ANGPTL2 and LILRB2 as predictive and specific activation of g9d2 T-cells as shown by concentration dependent increase in cell surface CD69 and CD25 and cytokine secretion (IFNγ, TNFα). 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, HUVAC 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)