high concentrations of long-acting, pegylated IL-10 have also shown anti-tumor activity. Here we investigated IL-10 and IL-10 receptor-alpha (IL-10RA) expression profiles in normal and tumor tissues as well as the immunological effects of modulating the IL-10 pathway via antibody-mediated blockade of IL-10RA.

**Methods** IL-10 and IL-10RA mRNA are expressed by several tumors, including renal, lung, breast, and colon cancers. Fluorescent in-situ hybridization revealed that the majority of IL-10RA was expressed by CD3-negative tumor-infiltrating cells, localized in close proximity to T cells in the tumor microenvironment (TME). Immunohistochemistry studies confirmed expression of IL-10RA in the TME, while no expression was detected in healthy tissues. Furthermore, dissociated tumor cells produced biologically active levels of IL-10 in culture.

**Results** Monoclonal antibodies (mAbs) against IL-10RA prevented IL-10 signaling and enhanced release of IL-12 by monocyte-derived dendritic cells activated with suboptimal LPS concentrations. The effect of IL-10RA blockade was greater than that observed with IL-10 neutralizing mAbs. In mixed lymphocyte reactions and superantigen-driven T-cell activation, IL-10RA blockade enhanced IL-2 secretion by T lymphocytes. Consistent with earlier observations in mouse models, the effect of IL-10RA blockade was nonredundant with blockade of the PD-1/PD-L1 axis, resulting in enhanced IL-2 and interferon-gamma secretion by T cells when both pathways were inhibited. Blockade of IL-10RA during CD3-redirected in vitro killing of tumor cells by PBMC induced IL-12 release as well as upregulation of CD86 and HLA-DR by CD3-negative cells. In vitro dissociated tumor cells, IL-10RA blockade induced release of IL-2, interferon-gamma and other proinflammatory cytokines; additional PD-1/PD-L1 axis blockade further enhanced cytokine release.

**Conclusions** In summary, antibody-mediated IL-10RA blockade can potentiate immune activation in the dissociated tumor cells and may be a valuable addition to cancer immunotherapies, including redirected T-cell killing and checkpoint blockade.

**REFERENCES**


http://dx.doi.org/10.1136/jitc-2020-SITC2020.0683
A HIGHLY SELECTIVE AND POTENT HPK1 INHIBITOR ENHANCES IMMUNE CELL ACTIVATION AND INDUCES ROBUST TUMOR GROWTH INHIBITION IN A MURINE SYNGENEIC TUMOR MODEL
1David Ciccone, 2Vad Lazeri, 2Ian Linney, 3Michael Briggs, 4Samantha Carreiro, 5Joshua McElwee, 6Ian Waddell, 7Chris Hill, 8Christine Loh, 9Peter Tummino, 10Alan Collis, 11Neelu Kaila, 12X Charlene Liao, 13An Song, 14Immuno-Onc Therapeutics, Palo Alto, CA, USA; 1University of Texas Health Science Center, Houston, TX, USA; 2University of Texas Southwestern, Dallas, TX, USA

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 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 pho-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.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0684

PRECLINICAL CHARACTERIZATION OF A NOVEL THERAPEUTIC ANTIBODY TARGETING LILRB2
1Maria Jose Costa, 2Ryan Stafford, 3Qi Li, 4Jing-Tyan Ma, 5Kris McCutcheon, 6Xiaoye Liu, 7Heyu Chen, 8Kyu Hong, 9Tao Huang, 10Ningyan Zhang, 11Zhiqiang An, 12Cheng Cheng Zhang, 13Charlene Liao, 14An Song, 15Immuno-Onc Therapeutics, Palo Alto, CA, USA; 1University of Texas Health Science Center, Houston, TX, USA; 2University of Texas Southwestern, Dallas, TX, USA

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.1–4 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.5–8 LILRB2/ILT4 has several ligands (classical and non-classical MHCI-I, ANGPTL2/S, SEMA4A and CD1) and most of these are known to contribute to immune suppression in the tumor microenvironment.9–14 Thus, LILRB2/ILT4 is a promising target to overcome protumor myeloid cell activity. IO-108 is a fully human IgG4 therapeutic candidate that binds LILRB2/ILT4 with high affinity and specificity, thereby blocking its ligand interactions.

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