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. Fluororescent 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
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 of 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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