PRECLINICAL MECHANISM OF ACTION AND ANTI-TUMOR ACTIVITY OF CB-668, A POTENT, SELECTIVE AND ORALLY BIOAVAILABLE SMALL-MOLECULE INHIBITOR OF THE IMMUNO-SUPPRESSIVE ENZYME INTERLEUKIN 4 (IL-4)-INDUCED GENE 1 (IL4I1)

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Background Tumors evade destruction by the immune system through multiple mechanisms including altering metabolism in the tumor microenvironment. Metabolic control of immune responses occurs through depletion of essential nutrients or accumulation of toxic metabolites that impair immune cell function and promote tumor growth. The secreted enzyme interleukin 4 (IL-4)-induced gene 1 (IL4I1) is an L-phenylalanine oxidase that catabolizes phenylalanine and produces phenyl-pyruvate and hydrogen peroxide. IL4I1 regulates several aspects of adaptive immunity in mice, including inhibition of cytotoxic T cells through its production of hydrogen peroxide (reviewed in1). In human tumors, IL4I1 expression is significantly elevated relative to normal tissues and is notably high in ovarian tumors and B cell lymphomas. Motivated by the hypothesis that IL4I1 is an immuno-metabolic enzyme that suppresses anti-tumor immunity, we discovered CB-668, the first known small-molecule inhibitor of IL4I1.

Methods IL4I1 enzymatic activity was measured using an HRP-coupled enzyme assay. RNA in-situ hybridization was carried out on the RNAscope platform. Syngeneic mouse tumor models were used to evaluate the anti-tumor activity of CB-668. The level of phenyl-pyruvate in tumor homogenates was measured by LC/MS.

Results Our clinical candidate, CB-668 is a potent and selective non-competitive inhibitor of IL4I1 (IC_{50} = 15 nM). CB-668 has favorable in vitro ADME properties and showed low clearance and high oral bioavailability in rodents. Twice-daily oral administration of CB-668 was well-tolerated in mice and resulted in single-agent anti-tumor activity in the syngeneic mouse tumor models B16-F10, A20, and EG7. Oral CB-668 administration reduced the levels of phenyl-pyruvate in the tumor, consistent with inhibition of IL4I1 enzymatic activity. Anti-tumor activity of CB-668 was immune cell-mediated since efficacy was abrogated in CD8-depleted mice, and CB-668 treatment caused increased expression of pro-inflammatory immune genes in the tumor. Moreover, CB-668 had no direct anti-proliferative activity on tumor cells grown in vitro (IC_{50} > 50 μM). CB-668 also favorably combined with anti-PD-L1 therapy to reduce tumor growth in the B16-F10 tumor model.