1371 NOVEL ORALLY ACTIVE AND HIGH TISSUE PENETRANT SMALL MOLECULES TARGETING SURFACE AND INTRACELLULAR PD-L1 SIGNALS FOR CANCER IMMUNOTHERAPY

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Background Currently approved PD-L1/PD-1 targeted immuno-oncology therapy involves intravenous administered monoclonal antibodies against PD-1 or PD-L1 that can effectively block the interaction between PD-1/PD-L1 proteins located on cell membrane surfaces. Small-molecule PD-L1 inhibitors have been demonstrated to have distinct mechanisms from and potential advantages over well-known mAbs by targeting both the surface and intracellular PD-L1. This study focuses on the discovery and identification of two novel orally active small-molecule inhibitors, CU-B103 and CU-B206.

Methods The ALPHA assay was used for PD-L1/PD-1 interaction evaluation. T cell activation was assessed through NFAT-PD-1 Jurkat T cell reporter assay and IFN-γ/IL-2 ELISA. Mechanisms of PD-L1 signaling blockade were studied using flow cytometry, confocal imaging, and western blotting with enzyme digestion. The anti-tumor effects were evaluated in 2D and 3D tumor models using co-cultures of PBMCs and A375 cells. Immune cell infiltration in the 3D model was examined by confocal microscopy. *In vivo* efficacy and tissue penetration were investigated in humanized PD-L1 mice bearing MC38-hPD-L1 tumors. MDCKII-WT, MDCKII-BCRP and Caco-2 monolayers were used in *in vitro* transporter studies.

Results CU-B103 and CU-B206 potently inhibited hPD-L1/hPD-1 protein-protein interaction (IC50 = 0.2 nM), and effectively induced Jurkat T cell activation and rescued IFN-γ/IL-2 secretion from human T cells when co-cultured with hPD-L1 overexpressing cells. Distinct from the anti-PD-L1 antibody atezolizumab, the compounds promoted PD-L1 internalization, intracellular retention and degradation, leading to a long-lasting loss of PD-L1 signal from the cell surface. They also altered the glycosylation pattern of PD-L1. In the 2D tumor-killing assay format, they demonstrated T cell cytolytic activity comparable to anti-PD-L1 antibody. In the 3D tumor spheroid model, both compounds exerted a greater cytotoxic effect vs the antibody, with greater increased immune cell infiltration and decreased tumor size. Consistent with the *in vitro* mechanistic studies, the orally dosed CU-B206 demonstrated *in vivo* target occupancy in mice bearing MC38 hPD-L1 tumor, at a level much greater than anticipated based on the corresponding *in vitro* assay. Additionally, CU-B206, at Ctrough/EC50 ratio of <0.2, exhibited comparable efficacy as approved mAbs. CU-B206 also showed excellent tumor and brain exposure in mice, consistent with its high passive permeability and insusceptibility to P-gp and BRCP efflux transporters.

Conclusions CU-B103 and CU-B206 are small-molecules with distinct mechanisms in inhibiting PD-L1, and with demonstrated corresponding *in vitro* and *in vivo* efficacy profiles. They exhibited favorable ADME/PK properties in preclinical studies, with acceptable safety profile, supporting further development as potential preclinical candidates.

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