AN ALLOSTERIC, ORALLY ADMINISTERED CBL-B INHIBITOR REMODELS THE TUMOR MICROENVIRONMENT AND ENHANCES IMMUNE-MEDIATED TUMOR GROWTH INHIBITION

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Background Casitas B-lineage lymphoma proto-oncogene b (CBL-B), an E3 ubiquitin-protein ligase, is a critical regulator of immunity. Genetic ablation or inactivation of CBL-B bypasses the requirement of a co-stimulatory signal for T cell activation in an antigen-dependent manner. CBL-B knockout mice spontaneously reject tumor growth, and the effect is largely dependent on CD8+ T cells. Therefore, CBL-B represents a novel target for cancer immunotherapy. Previously, Hotspot has disclosed a series of allosteric CBL-B inhibitors which exhibited potent in vitro and in vivo properties.

Methods Our CBL-B inhibitor (CBL-Bi) was evaluated in a set of syngeneic mouse tumor models. In addition to the measurement of tumor growth, tumor gene expression immune signatures were characterized by Nanostring analysis. Immunohistochemistry and flow-based immunophenotyping were used in profiling the tumor microenvironment.

Results CBL-Bi, as a single agent, demonstrated a spectrum of anti-tumor responses. It not only showed anti-tumor activity in immunologically hot tumor models, such as H22 and CT26, but also in immunologically cold tumor models, such as B16-F10. Responding tumors showed enhancement of antigen presentation, T cell cytotoxicity, and interferon and TNF pathway activation. Furthermore, an in vivo study using the CT26 model demonstrated that the anti-tumor effect was mediated by the immune system, as there was no tumor growth inhibition observed in immune deficient NCG mice. We further investigated the combinational effect of CBL-Bi with anti-PD1 in the CT26 syngeneic mouse tumor model. Additive or synergistic anti-tumor activity was observed. Tumor microenvironment profiling demonstrated significant enhancement of T cell tumor infiltration, and a shift of tumor associated macrophages to a pro-inflammatory status was observed in the combination group.

Conclusions Taken together, the pre-clinical in vivo data presented here demonstrate that inhibition of CBL-B induces immune mediated tumor growth inhibition; the anti-tumor effect is further enhanced when combined with anti-PD1. Thus, CBL-B inhibition has the potential to overcome the low-antigen and high immune suppressive tumor environment.