

IDENTIFICATION OF A NOVEL ALLOSTERIC ORAL CBL-B INHIBITOR THAT AUGMENTED T CELL RESPONSE AND ENHANCED NK CELL KILLING IN VITRO AND IN VIVO

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Background Immunotherapies aiming to boost anti-tumor cell responses in cancer patients has been proven successful by checkpoint inhibitors targeting PD1 or CTLA-4, but the majority of cancer patients do not garner durable benefit. Co-stimulation through the CD28 pathway is one potential approach to maximize the benefits of immunotherapies. The E3 ubiquitin ligase Cbl-b (casitas b-lineage lymphoma proto-oncogene b) has been established as a master negative regulator of T-cells and NK cells and plays an important role in immune suppression. Genetic ablation of Cbl-b or functional inactivation of its E3 ligase activity in mice resulted in CD8 T-cell-mediated rejection of primary tumors in several mouse models. Based on the overwhelming evidence supporting the role of Cbl-b in immune suppression, targeting Cbl-b with small molecule inhibitors is attractive for cancer immunotherapy.

Methods Cbl-b is activated by tyrosine kinases and undergoes a large conformational change from closed inactive form to open active form. Historically, it had been difficult to identify inhibitors of Cbl-b. Through the utilization of our proprietary SpotFinder platform, a druggable phosphoregulatory pocket was identified in the inactive form of Cbl-b. Learnings from the platform allowed for the development of screening assays utilizing specifically designed protein constructs. Assays were developed to identify inhibitors that bind to the hotspot and lock Cbl-b in its inactive form.

Results Here we report on a member of our lead series of inhibitors, a low nanomolar potent inhibitor identified via application of our SpotFinder platform. This inhibitor binds to the inactive form of Cbl-b, its binding mode in the identified hotspot confirmed by co-crystal structures. It inhibits the phosphorylation of Cbl-b by kinases, inhibits the E3 ligase activity of Cbl-b, promotes cytokine release and enhances T cell proliferation well as NK cell activation and killing. In vivo, our CBL-B inhibitors efficaciously augmented the T cell response in anti-CD3 treated mice.

Conclusions We herein demonstrated the validation of our proprietary SpotFinder platform via the prediction and drugging of a regulatory hotspot on an important immune oncology target that has to date been very difficult to drug.

<http://dx.doi.org/10.1136/jitc-2021-SITC2021.864>