Background The mixed lymphocyte reaction (MLR) mimics an immune reaction between T cells and antigen presenting cells and has been used to assess a variety of immune-oncology (I-O) agents as a potential predictor of clinical effects. Inhibition of the E3 ligase, Casitas B-Lineage Lymphoma Proto-Onco-gene B (CBL-B), is a novel I-O approach shown to lower the threshold for antigen-specific T cell activation, even in the absence of co-stimulatory signaling or in the presence of an immunosuppressive environment. Moreover, genetic ablation of CBL-B and functional inactivation of its E3 ligase activity in mice or primary human T cells enhanced immune-mediated anti-tumor effects. Previously, Hotspot has disclosed a series of allosteric CBL-B inhibitors (CBL-Bi) with potent in vitro effects on T cells and NK cells and immune-mediated anti-tumor effects in vivo.

Methods A human allogenic MLR assay was applied to compare the effects of CBL-Bi and antibodies directed against PD1, CTLA4, LAG3, TIGIT and TIM3 on cytokine release and T cell proliferation, followed by evaluation of CBL-Bi effects on dendritic cells maturation and antigen specific CMV response.

Results CBL-Bi significantly enhanced interferon-gamma production comparable to anti-PD1 treatment, with an additional profound effect on CD8 T cell proliferation. Antibodies directed against CTLA4, LAG3, TIGIT and TIM3 had no effect on either endpoint in this assay format. The effects of CBL-Bi extended to CD4 T cells and interleukin-2 (IL-2) production; anti-PD1 also showed similar effects on IL-2. CBL-Bi effects were concentration-dependent, suggesting potential to optimize the extent of desired immune enhancement. The combination of CBL-Bi plus anti-PD1 demonstrated additive or possibly synergistic effects on both cytokine release and T cell proliferation. Combination of CBL-Bi plus other I-O agents did not show any enhancement. Mechanistically, CBL-Bi promoted immature dendritic cell activation and increased the sensitivity to antigen-specific T cell activation in a CMV challenge assay. An integrated literature review further suggested that the MLR assay has been generally correlated with clinical effects of I-O agents as monotherapy and/or in combination with anti-PD1.

Conclusions In summary, CBL-Bi had robust single agent effects on both cytokine release and T cell proliferation in the human MLR assay. These effects were differentiated from a range of other I-O mechanisms and CBL-Bi plus anti-PD1 showed substantial combination effects. These data support the potential for CBL-Bi to drive anti-tumor immunity in patients as both monotherapy and in combination with anti-PD1 standard of care therapy.