CBL-B Inhibition Showed Differentiated Effects in a Mixed Lymphocyte Reaction Versus Other Immuno-oncology Targeted Approaches

#1354

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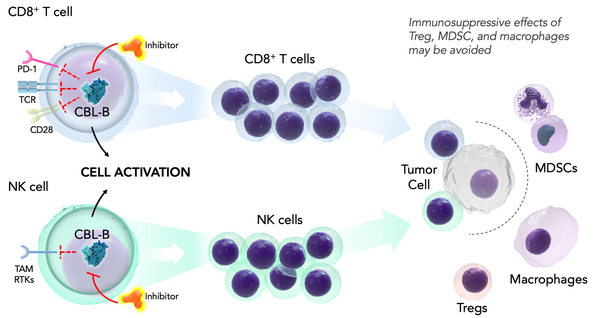
Introduction

- 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-Oncogene 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.
- 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.
- 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*.

CBL-B Inhibition Enhances Anti-tumor Immunity Through Several Key Biological Mechanisms



1 Lowers threshold for effector cell activation

2 Drives effector cell proliferation and inflammation

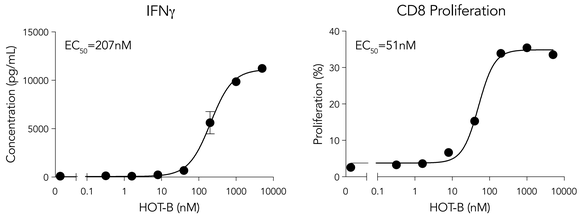
3 Reduces susceptibility to suppression

Method

- Two HotSpot allosteric CBL-B inhibitors, HOT-A and HOT-B, were used interchangeably through the studies
- Checkpoint inhibitors (CPIs) tested in MLR assay: aPD1: anti-human PD1 (Opdivo, BMS); aCTLA4: anti-human CTLA4 (Ipilimumab, MCE, Cat#HY-P9901); aLAG3: anti-human LAG3 Recombinant Antibody (TSR-033, Creative Biolabs, Cat#TAB-0367CL); aTIM3: anti-human TIM3 Recombinant Antibody scFv Fragment (mAb15, Creative Biolabs, Cat#TAB-666CT); aTIGIT: anti-human TIGIT Recombinant Antibody (clone HuTIG1-IgG1.AA, Creative Biolabs, Cat#HPAB-0669YY)

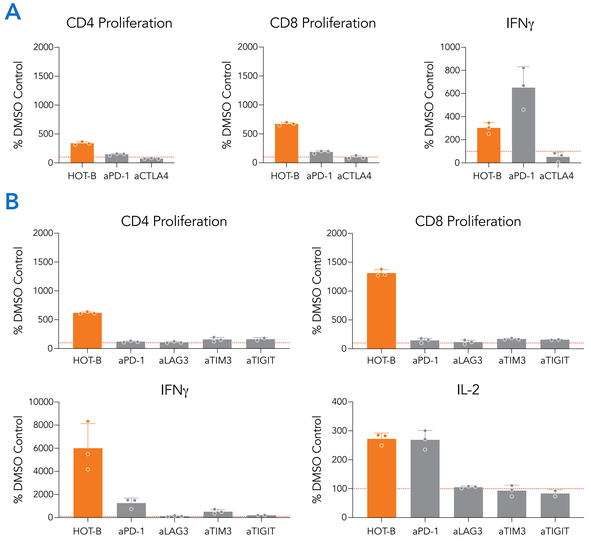
Results

Figure 1. CBL-B Inhibition Alone Dose-dependently Promoted CD8 T Cell Proliferation and IFNγ Production



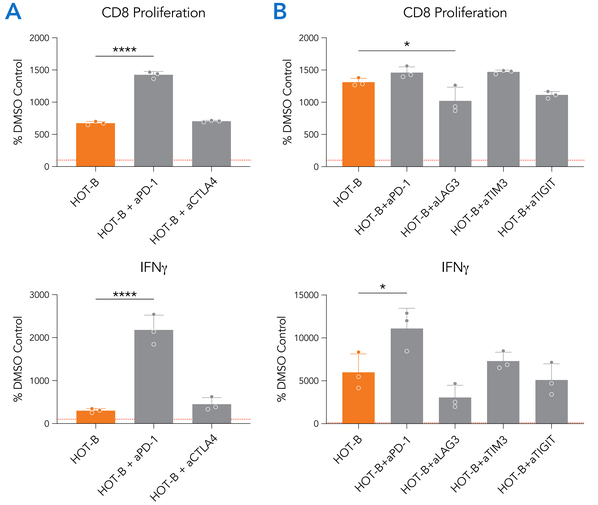
Human primary T cells (CellTraceViolet labeled, CTV) and *in vitro* differentiated allogeneic immature dendritic cells (imDCs) were cocultured for 4 days in the presence of varying concentrations of HOT-B. The percentage of CD8 T cells in proliferation was characterized by flow cytometry. IFNγ secretion at day 4 was measured by ELISA.

Figure 2. CBL-B Inhibition was Unique in MLR Assay Compared to Other CPIs



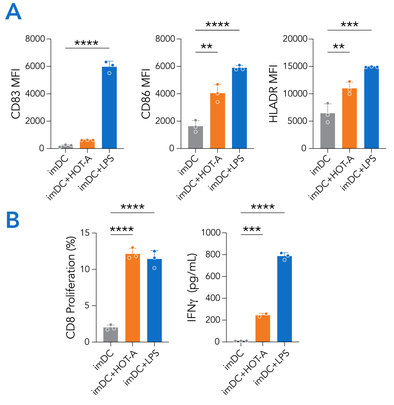
Human primary T cells (CTV labeled) and *in vitro* differentiated allogeneic immature dendritic cells were cocultured for 4 days in the presence of (A) DMSO control, HOT-B (1μM), aPD1 (10μg/ml) or aCTLA4 (10μg/ml); or (B) DMSO control, HOT-B (1μM), aPD1 (10μg/ml) , aLAG3 (10μg/ml), aTIM3 (10μg/ml), or aTIGIT (10μg/ml). The percentage of T cells with CTV dilution was characterized by flow cytometry. IL-2 (24hr) and IFNγ (96hr) secretion were measured from supernatant by ELISA.

Figure 3. Combination of CBL-B Inhibition and Anti-PD1 Showed Synergistic Activity in the MLR Assay



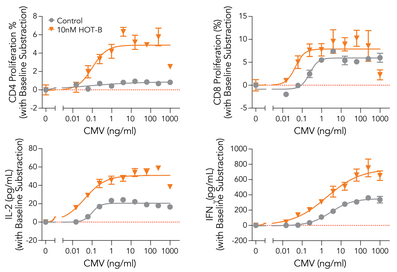
Human T cells and imDCs were cocultured for 4 days in the presence of (A) DMSO control, HOT-B (1μM), or HOT-B (1μM) + aPD1 (10μg/ml) or aCTLA4 (10μg/ml) combination; or (B) DMSO control, HOT-B (1μM), or HOT-B (1μM) + aPD1 (10μg/ml) , aLAG3 (10μg/ml), aTIM3 (10μg/ml), or aTIGIT (10μg/ml) combination. The percentage of T cells with CTV dilution was characterized by flow cytometry. IFNγ (96hr) secretion was measured from supernatant by ELISA.

Figure 4. CBL-B Inhibition Promoted Dendritic Cell Maturation



Human monocytes were differentiated into imDC (50ng/mL GM-CSF + 25ng/mL IL-4) in the presence of DMSO (imDC) or 1μM HOT-A (imDC+HOT-A) for 6 days. At day 6, imDC were stimulated with LPS (1μg/mL) for 24 hours to generate mature DC (imDC+LPS). Human primary T cells and *imDC, imDC+HOT-A, or imDC+LPS* were cocultured for 5 days. (A) CD83, CD86 and HLA-DR MFI on imDC, imDC+HOT-A, and imDC+LPS were analyzed by flow cytometry. (B) The percentage of CD8 T cell proliferation was characterized by flow cytometry. IFNγ secretion at day 5 was measured by ELISA.

Figure 5. CBL-B Inhibition Increased the Sensitivity to Antigen Mediated T Cell Activation



Human PBMCs were stimulated with various doses of CMV peptides pool for 4 days. The percentage of T cell proliferation was characterized by flow cytometry. IL-2 (24hr) and IFNγ (96hr) secretion were measured from supernatant by ELISA.

Figure 6. Mixed Lymphocyte Reaction (MLR) Assay: Potentially Predictive Correlate of Clinical Activity

Mechanism	Monotherapy		Combination with anti-PD-1	
	MLR	Clinical POC	MLR	Clinical POC
PD-(L)1 inhibitor	⊕	⊕	N/A	N/A
CTLA4 inhibitor	⊖	◐	⊕	⊕
LAG3 inhibitor	◐	⊖	⊕	⊕
TIGIT inhibitor	⊖	⊖	⊕	⊕
CD73 inhibitor	⊕	◐	⊕	⊕
4-1BB agonist	⊖	⊖	⊖	⊖
OX40 agonist	⊖	⊖	⊖	⊖
CBL-B inhibitor	⊕	TBD	⊕	TBD

⊕ Positive Data ◐ Mixed Data ⊖ Negative Data

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

- CBL-Bi had robust single agent effects on both cytokine release and T cell proliferation in human MLR assay.
- CBL-Bi plus anti-PD1 showed substantial combination effects.
- Antibodies directed against CTLA4, LAG3, TIGIT and TIM3 had no effect on either endpoint in this assay format.
- CBL-Bi promoted immature dendritic cell activation.
- CBL-Bi increased the sensitivity to antigen-specific T cell activation in a CMV challenge assay.

References

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