Translational assessment of triple combination with Tislelizumab (anti-PD-1), LBL-007 (anti-LAG-3) and Surzebicilimab (anti-TIM-3) highlights its strong anti-tumor activity and clinical potential in solid tumors such as HNSCC

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Background: Therapeutics targeting PD-(L)1 have demonstrated impressive clinical activity in several type of cancers. However, only a small proportion of patients develop long-term response and resistance frequently occurs. LAG-3 and TIM-3 are inhibitory immune checkpoints frequently upregulated and co-expressed with PD-1 on tumor-infiltrating T cells, contributing to T cell dysfunction. Thus, we hypothesized that simultaneously targeting PD-1/LAG-3/TIM-3 would further restore T cell response and the triple combination of Tislelizumab (Tisle, anti-PD-1), LBL-007 (anti-LAG-3) and Surzebiclimab (Surze, anti-TIM-3) would provide greater clinical benefit.

Methods: To evaluate the rationale and the anti-tumor activity of triple combination, LAG-3 and TIM-3 expression were analyzed in *in vitro* T cells, syngeneic mouse models and cancer patients with anti-PD-(L)1 therapy. The effects of dual blockade of PD-1/LAG-3, PD-1/TIM-3 and the triple blockade of PD-1/LAG-3/TIM-3 on T cell function were evaluated in *in vitro* activated PBMCs and the anti-tumor efficacy were evaluated in syngeneic mouse models. Relevant gene expression and immune signatures were ranked across 31 solid tumor types in TCGA.

Results: Both LAG-3 and TIM-3 expression on T cells were upregulated by anti-PD-1 treatment in syngeneic mouse tumors, similar trend was observed in cancer patients with anti-PD-(L)1 therapy. Dual combination of Tisle/LBL-007 or Tisle/Surze enhanced IFNγ production in *in vitro* activated human T cells. The dual blockade of PD-1/LAG-3 or PD-1/TIM-3 significantly enhanced tumor growth inhibition compared with anti-PD-1 monotherapy in syngeneic mouse models. The triple combination of Tisle/LBL-007/Surze further enhanced IFNγ production in *in vitro* activated T cells. In a mouse MC38 colon carcinoma model, the triple blockade of PD-1/LAG-3/TIM-3 demonstrated enhanced anti-tumor activity compared with either dual combination, evidenced by trend of increased tumor growth inhibition and higher tumor-free incidence rate. Finally, the responsiveness of PD-1/LAG-3/TIM-3 triple combination was predicted using 5 signatures, including early effector T cell signature, inflamed signature, LAG-3, TIM-3 and PD-L1 expression. The *in silico* analyses indicated strong potential in clinical utility such as squamous cell carcinoma of the head and neck (HNSCC).

Conclusions: The concurrent blockade of PD-1/LAG-3/TIM-3 represents a promising strategy to enhance T cell function and anti-tumor activity. The results demonstrated the therapeutic potential of the triple combination. A Ph2 study evaluating Tisle in combination with LBL-007 and/or Surze in first-line treatment of recurrent or metastatic HNSCC (NCT05909904) is recruiting.