Abstract 4041

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

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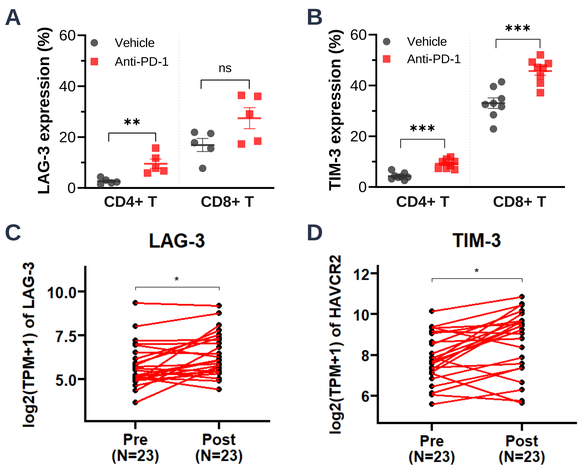
Introduction

- Co-expression of multiple immune checkpoint proteins is a hallmark of T cell exhaustion. The immune checkpoints LAG-3 and TIM-3 are often co-expressed with PD-1 on tumor-infiltrating T cells and contribute to tumor-related T cell dysfunction.
- Therapeutics targeting PD-(L)1 have demonstrated impressive clinical activity in several cancer types. However, only a small proportion of patients develop long-term response and resistance is common.
- Both LAG-3 and TIM-3 were upregulated in tumor infiltrating T cells following anti-PD-1 therapies. Furthermore, LAG-3 was increased in tumor-infiltrating T cells after co-blockade of PD-1/TIM-3 in preclinical models, suggesting the potential of triple blockade of PD-1/LAG-3/TIM-3.
- Therefore, we hypothesized that simultaneous targeting of PD-1/LAG-3/TIM-3 would further restore T-cell response and that the triple combination of tislelizumab (anti-PD-1), LBL-007 (anti-LAG-3) and surzebiclimab (anti-TIM-3) would improve clinical benefit.
  - Tislelizumab is an anti-PD-1 humanized IgG4 mAb that blocks the PD-1/PD-L1 immune checkpoint. Tislelizumab was designed to minimize FcγR binding on macrophages, reducing ADCP mediated killing of T cells. Tislelizumab has been approved for multiple indications in China, US, and Europe.
  - LBL-007 is an investigational, anti-LAG-3 fully human IgG4 mAb that potently blocks LAG-3-ligand interaction.
  - Surzebiclimab is an investigational, anti-TIM-3 humanized IgG1 mAb with high specificity and affinity.

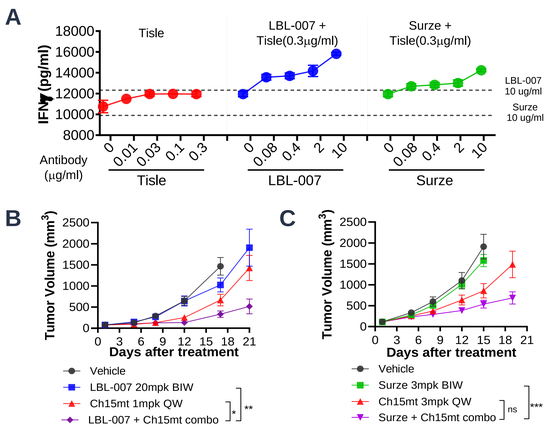
The expression of LAG-3 and TIM-3 expression was upregulated after anti-PD-1 treatment



(A) LAG-3 and **(B)** TIM-3 surface expression on tumor-infiltrated CD4+ T and CD8+ T cells after treatment with anti-mouse PD-1 antibody (Ch15mt) were assessed by flow cytometry in CT26 and A20 syngeneic models, respectively.

(C) LAG-3 and **(D)** HAVCR2 (gene encoding TIM-3) expression were analyzed by RNA sequencing in non-responding patients with various tumor types, including HNSCC, melanoma and others, after anti-PD-1 therapy (dataset from Yang et al. 2021).

Dual blockade of PD-1/LAG-3 or PD-1/TIM-3 demonstrated enhanced T-cell activation and anti-tumor activity

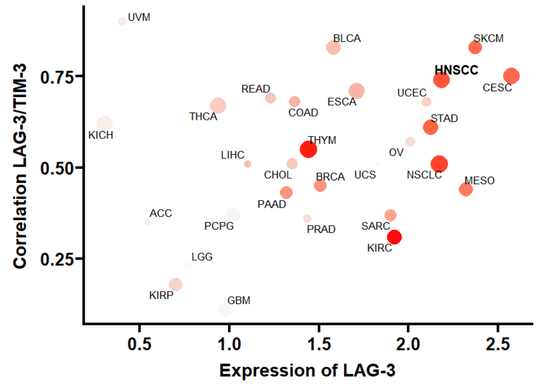


(A) Human PBMCs were pre-stimulated with staphylococcal enterotoxin B (SEB) for three days and then re-stimulated with SEB for another two days in the presence of Tisle, LBL-007 and Surze or the combinations of Tisle+LBL-007 and Tisle+Surze. INFγ was measured to reflect T cell activation.

(B) The anti-tumor activity of the combination of Ch15mt (anti-mouse PD-1) and LBL-007 (anti-human LAG-3) was evaluated in the MC-38 model using human LAG-3 knock-in mice.

(C) The anti-tumor activity of the combination of Ch15mt and Surze (anti-human TIM-3) was evaluated in CT26 model using human TIM-3 knock-in mice.

In silico analyses indicate strong clinical potential of the triple combination in solid tumors such as HNSCC



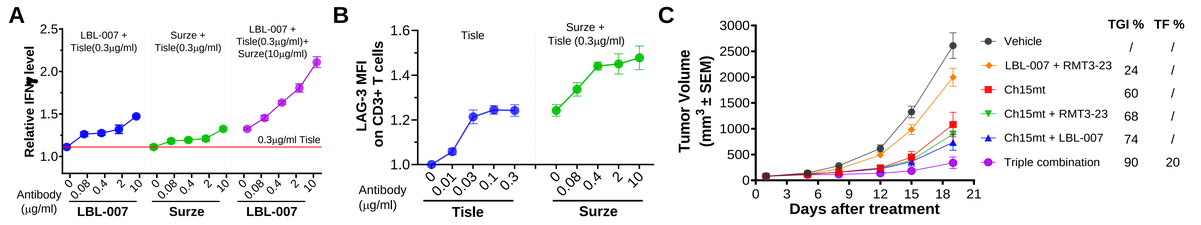
To predict the potential responsiveness of the triple combination of tislelizumab (anti-PD-1), LBL-007 (anti-LAG-3) and surzebiclimab (anti-TIM-3) in solid tumors, several gene signatures across different tumor types were analyzed using the dataset from TCGA sources (tumor type abbreviation refers to TCGA library). The following signatures are shown, with HNSCC (head and neck squamous cell carcinoma) being one of the most susceptible tumor types.

1) LAG-3 expression level across different solid tumors (X-axis); 2) LAG-3&TIM-3 expression's correlation (Y-axis) to indicate LAG-3/TIM-3 co-expression; 3) PD-L1 expression (size big to small); 4) Early effector T cell signature (transparency 1-0) to reflect effector T cell infiltration; 5) Tumor Inflammation signature (color red to gray) to reflect tumor microenvironment.

Methods

- To evaluate the rationale for the triple blockade of PD-1/LAG-3/TIM-3 , LAG-3, and TIM-3 expression was analyzed in *in-vitro* activated T cells, syngeneic mouse models and cancer patients receiving anti-PD-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.
- The potential response of the triple combination of Tislelizumab (anti-PD-1), LBL-007 (anti-LAG-3) and Surzebiclimab (anti-TIM-3) was predicted using 5 gene signatures, including the expression of LAG-3, TIM-3 and PD-L1, early effector T cell signature, tumor inflamed signature. Relevant gene expression and immune signatures were ranked across different solid tumor types in the TCGA database.

Triple combination of anti-PD-1, anti-LAG-3, and anti-TIM-3 antibodies further enhanced *in-vitro* T cell activation and improved *in-vivo* anti-tumor activity



(A) Human PBMCs were pre-stimulated with SEB for 3 days and then incubated with SEB for another 2 days in the presence of indicated concentrations of Tisle, Surze, and indicated concentrations of Tisle, Surze and LBL-007 or the combinations, INFγ was measured to indicate T cell activation.

(B) Same as (A), but LAG-3 expression on T cells treated with Tisle or the combination of Tisle and Surze was assessed by flow cytometry.

(C) The anti-tumor activity of the triple blockade of PD-1/LAG-3/TIM-3 was evaluated in the MC-38 syngeneic model using human LAG-3 knock-in mice. Tumor-bearing mice were randomized and treated with ch15mt (anti-mouse PD-1, 0.5 mg/kg), LBL-007 (3 mg/kg), RMT3-23 (anti-mouse TIM-3, 10 mg/kg), dual or triple combinations. Tumor growth inhibition (TGI) and tumor-free (TF) rates were measured at the end of the study.

Summary and Conclusions

- The immune checkpoint proteins LAG-3 and TIM-3 were upregulated in tumor-infiltrating T cells by anti-PD-1 treatment, representing a potential resistance mechanism.
- Simultaneous blockade of PD-1/LAG-3, PD-1/TIM-3 and PD-1/LAG-3/TIM-3 resulted in enhanced T cell activation *in-vitro* and improved anti-tumor activity *in-vivo.*
- The data suggest that the triple combination is a promising strategy to enhance anti-PD-1 therapy and has great therapeutic potential in multiple solid tumors including HNSCC.
- A Ph2 study evaluating tislelizumab in combination with LBL-007 and/or surzebiclimab in the first-line treatment of recurrent or metastatic HNSCC (NCT05909904) is currently recruiting.

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