POSTER #892

# ABL503 (TJ-L14B), PD-L1×4-1BB bispecific antibody, induces superior anti-tumor activity by PD-L1-dependent 4-1BB activation with the increase of 4-1BB⁺CD8⁺ T cells in tumor microenvironment

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## Introduction

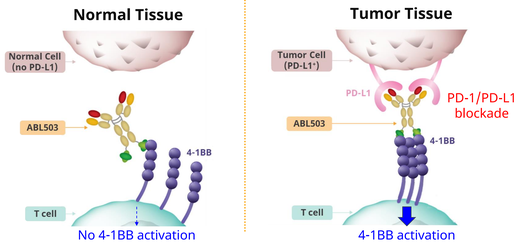
PD-1/PD-L1 inhibitor has revolutionized cancer treatment, but there are unmet clinical needs for PD-1/PD-L1 inhibitor-resistant/refractory patients. Activation of T cells in the tumor microenvironment (TME) by 4-1BB agonist antibodies is one of the promising approaches to complement the current limitation of PD-1/PD-L1 inhibitors. Although 4-1BB is a promising target for immunotherapy, clinical studies using 4-1BB agonist antibodies showed systemic immune cell activation resulting in dose-limiting hepatotoxicity. We generated ABL503 (TJ-L14B), a bispecific antibody (BsAb) that combines PD-1/PD-L1 pathway blockade and 4-1BB agonistic activity dependent on the PD-L1 engagement to limit unwanted toxicities while exerting a potent anti-tumor efficacy. Here, we reported the pre-clinical properties of ABL503 (TJ-L14B) in various studies.

## Background and Mode of Action


**ABL503 (TJ-L14B):** Full-length anti-PD-L1 mAb (Fc-silenced human IgG1, hIgG1) fused with scFv of anti-4-1BB mAb

[Functional activity]
1. 4-1BB clustering/activation only in PD-L1 engagement
2. PD-1/PD-L1 pathway inhibition

→ Enhanced anti-tumor activity by simultaneous 4-1BB activation and PD-1/PD-L1 blockade while minimizing the risks of peripheral toxicity

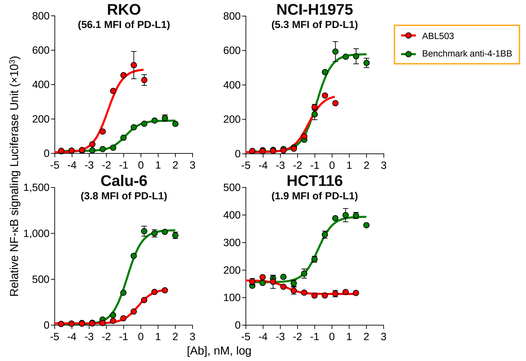



## Methods

- **PD-L1-dependent 4-1BB agonistic activity** was tested by 4-1BB bioassay with PD-L1-expressing tumor cells and 4-1BB signaling reporter cells. PD-L1 expression was measured by flow cytometry.
- **IFN-γ secretion** was measured in human PBMCs and Calu-6 co-culture system.
- **In vivo efficacy** was evaluated in B6-hPD-L1/h4-1BB knock-in mice implanted with MC38 tumor expressing a different level of hPD-L1.
- **Tumor-infiltrated mCD8⁺ or h4-1BB⁺ cells** were measured by IHC.
- **Pharmacodynamic changes in TILs and blood** were evaluated hPD-L1-expressing MC38 tumor-bearing B6-hPD-L1/h4-1BB knock-in mice.
- **In vitro tumor-killing activity** was assessed using an Autologous organoid-based Discovery for Immuno-Oncology drug platform from ORGANOIDSCIENCE by co-culturing cancer organoids from lung cancer patients with autologous PBMCs.
- **Cytokine (IL-6 and TNF-α) release** was measured in PBMCs from healthy donors stimulated with ABL503 (TJ-L14B).
- Statistical significances were analyzed by *t*-test, one-way ANOVA with Tukey's or Dunnett's multiple comparison test, or two-way ANOVA with Bonferroni's multiple comparison test in GraphPad Prism. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$; ns, not significant.

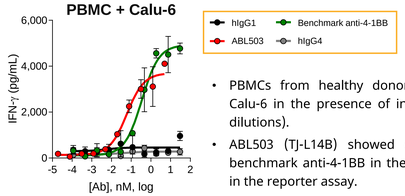
## Results

### *ABL503 shows PD-L1-dependent 4-1BB agonistic activity*



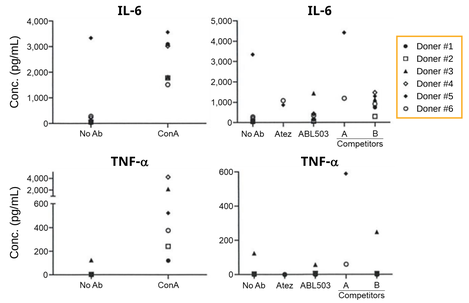

- 4-1BB signaling reporter cells and indicated tumor cells were cocultured in the presence of ABL503 or benchmark anti-4-1BB antibody (4-fold dilutions).
- ABL503 (TJ-L14B) showed 4-1BB agonistic activity in a PD-L1-dependent manner, while benchmark anti-4-1BB induced 4-1BB activation regardless of PD-L1.
- Higher PD-L1 expression leads to more potent 4-1BB activation by ABL503 (TJ-L14B).

### *PD-L1 on immune cells also contribute to the activity of ABL503*



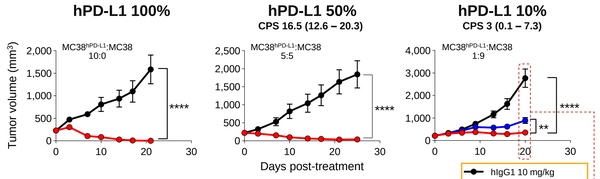

- PBMCs from healthy donors were co-cultured with Calu-6 in the presence of indicated antibodies (4-fold dilutions).
- ABL503 (TJ-L14B) showed comparable activity with benchmark anti-4-1BB in the presence of PBMC unlike in the reporter assay.

### *ABL503 shows favorable safety profile*



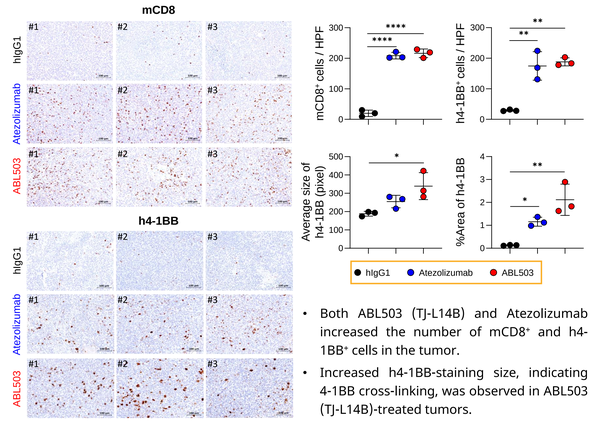

- PBMCs from healthy donors were stimulated with indicated antibodies, including Atezolizumab (Atez), ABL503 (TJ-L14B), and two competitor BsAbs (A & B). No Ab control and ConA were used for negative and positive control, respectively. Cytokine release was measured by ELISA.
- No cytokine release by ABL503 (TJ-L14B) was observed.

### *ABL503 elicits anti-tumor response across different PD-L1 levels*



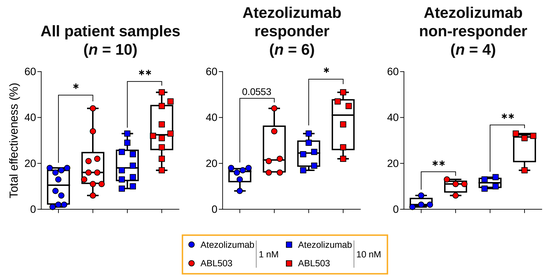

- ABL503 (TJ-L14B) shows antitumor efficacy in MC38$^{hPD-L1}$ tumor model, even in the model in which only 10% of tumor cells express hPD-L1 (MC38$^{hPD-L1\ low}$).
- ABL503 (TJ-L14B) shows better activity over Atezolizumab in MC38$^{hPD-L1\ low}$ tumor model.

### *ABL503 increases CD8⁺ and 4-1BB⁺ cells in the MC38$^{hPD-L1\ low}$ tumor*



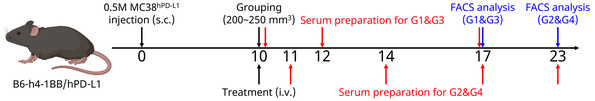

- Both ABL503 (TJ-L14B) and Atezolizumab increased the number of mCD8⁺ and h4-1BB⁺ cells in the tumor.
- Increased h4-1BB-staining size, indicating 4-1BB cross-linking, was observed in ABL503 (TJ-L14B)-treated tumors.

### *ABL503 shows superior activity over Atezolizumab in organoid system*

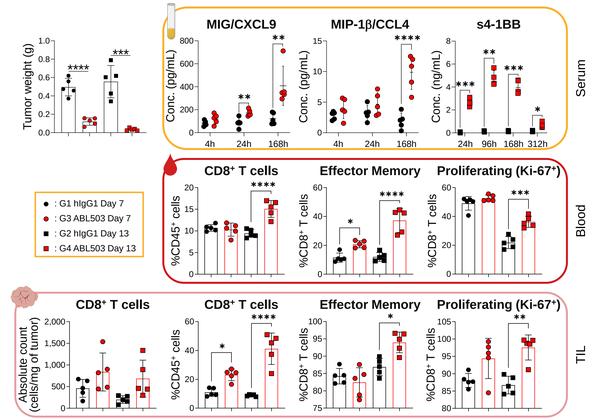



- Cancer organoids ($n$ = 10) from lung cancer patients co-cultured with autologous PBMCs for 3 days in the presence of Atezolizumab or ABL503 (TJ-L14B) (1 or 10 nM).
- ABL503 (TJ-L14B) showed enhanced tumor-killing activity over Atezolizumab, even in organoids from Atezolizumab non-responder.

### *Pharmacodynamic changes by ABL503*




| Group | Treatment | Dose (mg/kg) | Group size | Analysis |
|---|---|---|---|---|
| G1 | hIgG1 | 7.5, i.v. | $n$ = 5/group | Cytokine, Immune phenotyping (Blood & TIL) |
| G2 | | | | s4-1BB, Immune phenotyping (Blood & TIL) |
| G3 | ABL503 | 10, i.v. | | Cytokine, Immune phenotyping (Blood & TIL) |
| G4 | | | | s4-1BB, Immune phenotyping (Blood & TIL) |




- Pharmacodynamics changes were assessed in peripheral blood and tumor from MC38$^{hPD-L1}$-bearing B6-hPD-L1/h4-1BB mice at 7 and 13 days after a single treatment of ABL503 (TJ-L14B).
- ABL503 (TJ-L14B) showed anti-tumor activity accompanied by an increase of MIG/CXCL9, MIP-1β/CCL4, and soluble 4-1BB (s4-1BB) in the serum.
- ABL503 (TJ-L14B) increased frequency of CD8⁺ T cells both in peripheral blood and tumor with enhanced proliferation and increased effector memory population.

## Summary

- ✓ ABL503 (TJ-L14B) shows PD-L1-dependent 4-1BB agonistic activity, which is expected to minimize peripheral toxicity as shown in the cytokine release assay and requires PD-L1 on both tumor and immune cells for its optimal activity.
- ✓ ABL503 (TJ-L14B) shows anti-tumor efficacy across the hPD-L1 levels. Especially, ABL503 (TJ-L14B) shows superior anti-tumor efficacy over Atezolizumab in the tumor model with low hPD-L1 expression.
- ✓ ABL503 (TJ-L14B) increases 4-1BB⁺ cells and CD8⁺ T cells in the TME, accompanied by proliferation and phenotypic changes to effector memory type. These changes are also reflected in blood.
- ✓ ABL503 (TJ-L14B) increases MIG/CXCL9, MIP-1b/CCL4, and s4-1BB in the serum, which may be used for PD markers in clinical trials.
- ✓ ABL503 (TJ-L14B) exhibits enhanced tumor-killing activity over Atezolizumab in patient-derived lung cancer organoid system.
- ✓ ABL503 (TJ-L14B) is currently undergoing a phase 1 clinical trial in the U.S. (NCT04762641).

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