The PD-L1 x 4-1BB Bispecific Antibody ABL503 Shows Potent Anti-Tumor Effect through Tumor-Directed T Cell Activation

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Abstract

Blockade of PD-1/L1 axis created a new paradigm for cancer treatment but there is still unmet need for PD-1/L1 treatment resistant/refractory patients. Activation of tumor necrosis factor receptor (TNFR) such as 4-1BB which could break the immune tolerance represents the next generation of anti-cancer agents in immune-oncology field. However, clinical studies using anti-4-1BB agonist antibody showed immune cell activation not only in tumor tissues but also in the peripheral, leading to dose-limiting liver toxicity.

Bispecific antibody is one of the approaches to overcome current limitations. Here we report the generation and characterization of ABL503, a bispecific antibody targeting two immune modulators, PD-L1 and 4-1BB.

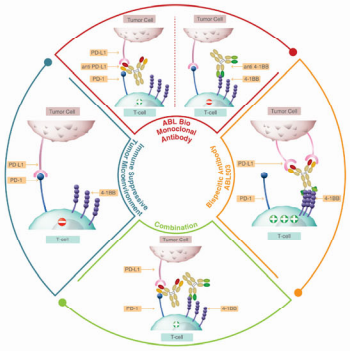
Functional evaluation data by using robust cell-based assays indicate that ABL503 retains full checkpoint blockade activity of PD-1/PD-L1 signaling axis. Furthermore, the activation of 4-1BB signaling of ABL503 solely depends on PD-L1 expression on tumor cells. Notably, when comparing with a benchmark anti-4-1BB agonistic antibody, the benchmark antibody showed the activation of 4-1BB signaling regardless of PD-L1. Moreover, ABL503 shows superior activity in T cell activation than the benchmark anti-4-1BB antibody in the presence of PD-L1 expressing tumor cells.

In a preclinical study using a humanized mouse model, tumor growth inhibition induced by ABL503 was significantly greater than that observed with the combination of each targeting monoclonal antibodies.

In addition, ABL503 displays normal range of serum half-life in C57BL/6 mice compared to that of general monoclonal antibody. Collectively, our data demonstrate that PD-L1 and 4-1BB dual targeting bispecific antibody, ABL503, shows potent anti-tumor effect through PD-L1 dependent T-cell activation potentially minimizing the risk of peripheral toxicity. These compelling data support further clinical development of ABL503, a bispecific antibody targeting PD-L1 and 4-1BB, that will be entered into a phase 1 trial in early 2020.

Background/ Rationale

- Major Issues of Agonistic 4-1BB Ab in the clinic: Liver Tox
- Selected ABL Bio's anti-4-1BB monoclonal antibody which can not induce clustering of 4-1BB in T cell by itself
- ABL Bio engineered 4-1BB T cell Engager BsAb to activate T-cells only at the tumor microenvironment
- The unique feature of ABL Bio's anti-4-1BB antibody can tightly control systemic 4-1BB activation in the format of bispecific antibody



ABL503, PD-L1x4-1BB bispecific antibody via tumor-immune cell interaction induces

1) Clustering 4-1BB only in presence of PD-L1 to minimize the risk of peripheral toxicity
2) Enhancing anti-tumor activity through simultaneous 4-1BB mediated T cell co-stimulation and PD-1/PD-L1 checkpoint inhibition

Results

- ABL503 retains full checkpoint blockade activity of PD-1/PD-L1 signaling axis

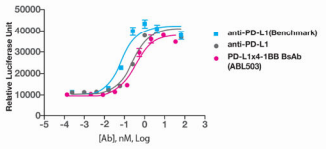


Figure 1.PD-1/PD-L1 checkpoint blockage activity was assessed in a Jurkat PD-1 reporter cell line co-cultured with PD-L1 expressing CHO cells. ABL503 retains checkpoint blockade activity similar to anti-PD-L1 mAb building block and benchmark anti-PD-L1 mAb.

- ABL503 activates PD-L1 dependent 4-1BB signaling

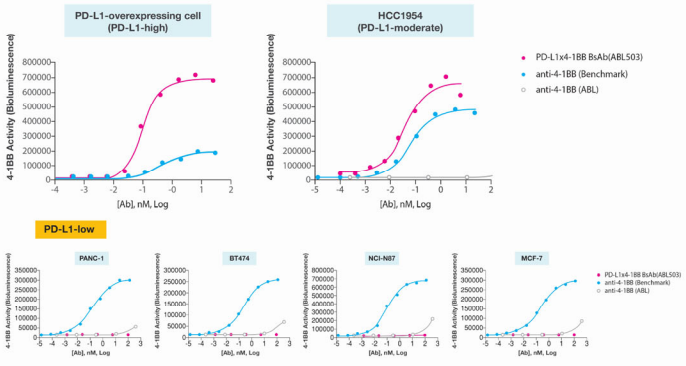
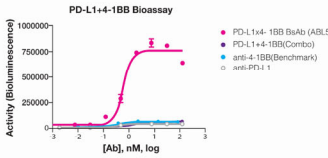


Figure 2. 4-1BB expressing Effector cells have luciferase report gene expression under 4-1BB signaling. Effector cells were co-culture with tumor cells expressing different amount of PD-L1. Anti-4-1BB benchmark activates 4-1BB signals regardless of PD-L1 presence.

- ABL503 effectively enhances T cell activation by blocking PD1/PD-L1 and clustering 4-1BB



Binding Affinity by SPR

Test article	Antigen	KD(M) (Mean±SD)
ABL503	PD-L1	2.778±0.449 x10^{-9}
	4-1BB	1.145±0.120x10^{-8}

Figure 3. PD-1 and 4-1BB co-expressing effector cells has suppressed luciferase activity by co-culture with PD-L1 expressing target cells. Suppressed reporter gene expression was recovered by simultaneous activation of PD-1/PD-L1 blockage and 4-1BB activation with ABL503.

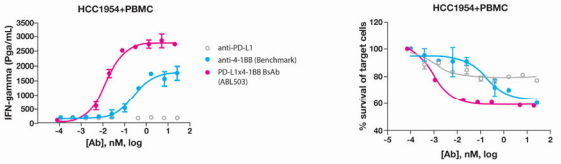
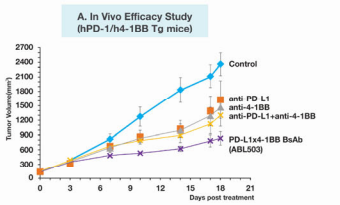
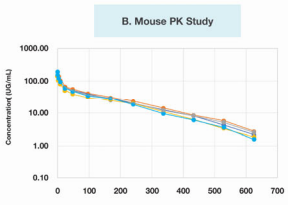


Figure 4. PBMC were co-cultured for 4 days with PD-L1 expressing cancer cells (HCC1954). Cytokine secretion(IFN-gamma, Left) and cell death (% survival of target cells, Right) were measured to represent the activation of cytotoxic T cell by ABL503.

- Non-Clinical Study of ABL503





Clone		i.v (10mg/kg)	Half Life (hr)	Tmax (h)	Cmax (ug/mL)	AUClast (h*ug/mL)	AUCINF_obs (h*ug/mL)	Vz_obs (mL/kg)	Cl_obs (mL/h/kg)
ABL503	Mean	133.5	0.05	181.6	12485.1	12908.5	0.781	140.7	
	SD	6.9	0.00	9.1	1181.4	1271.2	0.075	11.5	

Figure 5A. ABL503 shows superior anti-tumor activity to the combination of each targeting mAbs. Double transgenic mice (hPD-1/h4-1BB) were inoculated with human PD-L1 expression MC38 cells and treated with 10 mg/kg of all monoclonal antibodies and 13.3 mg/kg of bispecific antibody Q3D for 5 doses (n=7 mice/group).

Figure 5B. ABL503 displays mAb-like normal range of serum-half life in mice. PK was analyzed in 5 female C57BL/6 mice of about 8 weeks of age. Animals were injected with 10 mg/kg of ABL503. Plasma samples were taken at the indicated timepoints and analyzed by dual antigen capture ELISA (DACE). Under this dosing regimen, the half-life was estimated to be 5.6 days.

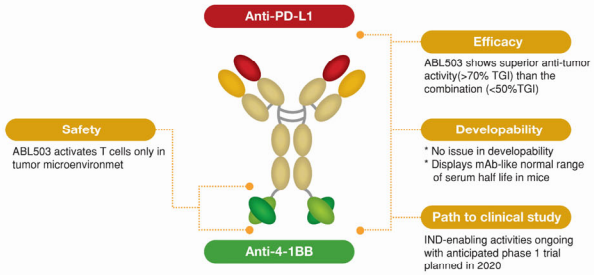
- Developability Assessment

	Content	Purity	Thermal Stability	Hydrophobicity	Solubility	pI	Heparin Binding (Non-specific clearance related PK)	Protein Binding	Potency
In vitro	Method	SEC	PTS	HIC	Visual inspection	cIEF	Heparin affinity column	ELISA (DACE)	Cell based assay
	ABL503	●	●	●	●	●	●	●	●
	Content	Purity (%)	Purity (%, intact IgG)	Purity (%, HC+LC)	Charge heterogeneity	Protein Binding			
Stressed condition	Method	SEC	CE-SDS (Non-Reduced)	CE-SDS (Reduced)	cIEF	ELISA (DACE)			
	ABL503	●	●	●	●	●			

● Pass ● Monitor ● Warning

ABL503 shows no issues in developability.

Summary



Well integrated Teams in ABL Bio

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