A novel T-cell engaging bispecific antibody, ABL103, shows potent anti-tumor effect via B7-H4 mediated 4-1BB activation in TME

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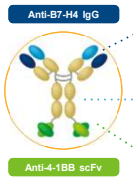
INTRODUCTION

- B7-H4 (B7 homolog 4) is a single-pass type I transmembrane protein in the B7 family and its ligation to T-cells inhibits T-cell growth, cytokine secretion, and cytotoxicity.
- B7-H4 is nominally expressed in normal human tissues but highly overexpressed in various cancers including breast, ovarian, and endometrial cancer.
- Although CD137(4-1BB) is a potent co-receptor augmenting T-cell receptor-mediated activation and proliferation, clinical development of agonistic 4-1BB antibody for therapeutic use has not been successful, specifically due to hepatotoxicity. Therefore, conditional T-cell activation in tumor microenvironment is a key for eliciting potent immune response with no/reduced risk of toxicity

General Feature of ABL103 (B7-H4 Grabody-T)



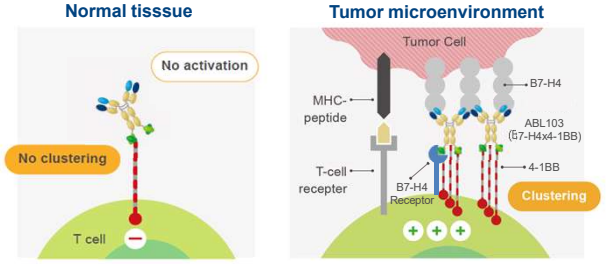
Potential Indication
- B7-H4+ cancer patients (TNBC, Ovarian, NSCLC, etc.)

Function
- B7-H4 expression-dependent T-cell activation
- Blocks B7-H4-mediated immunosuppression

Clinical benefits
- Long-term clinical efficacy due to tumor specific immune-memory
- Significantly lower toxicity than anti-4-1BB mAb (Urelumab)
- Potential treatment option for patients with low response to conventional immuno-therapy (e.g., PD-1/PD-L1)

Current stage
- IND enabling study including preclinical toxicity study and CMC development
- US IND submission planned on 3Q, 2023

MODE OF ACTION



SUMMARY

- **ABL103** ***binds to B7-H4 and 4-1BB simultaneously*** **with high affinity**
- **ABL103 specifically** ***activates 4-1BB signaling only in the presence of B7-H4*** **expressing cells**
- **ABL103 effectively** ***rescues T-cell activity*** **from B7-H4-mediated suppression**
- **ABL103** ***strongly inhibits tumor growth*** **and induces prolonged anti-tumor effect with** ***immunological memory***

RESULTS

ABL103 binds to B7-H4 and 4-1BB simultaneously in a dose-dependent manner

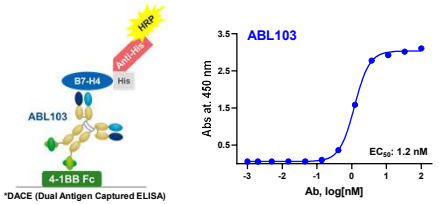


Fig.1. Target antigen binding analysis by Dual Antigen Captured ELISA
To evaluate the antigen binding activity, ABL103 was subjected to DACE. Briefly, plate was coated with human 4-1BB Fc then blocked with PBSB (BSA in PBS). Three-fold dilutions of ABL103 starting from 100 nM were added to each well and incubated for 1 hr at 37 ℃. Bound ABL103 was detected by incubating with his tag fused human B7-H4, followed by HRP-labeled anti-His antibody.

ABL103 binds to B7-H4 specifically but not other B7-family Protein

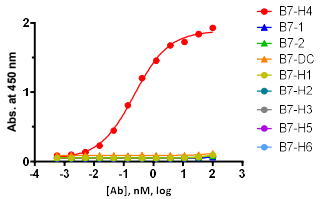


Fig.2. Protein binding assay against B7-family proteins
To confirm the specific binding of ABL103 to B7-H4, plate was coated with each protein (100 ng/well) then blocked with PBSB (BSA in PBS). Three-fold dilutions of ABL103 starting from 100 nM was added to each well and incubated for 1 hr at 37 ℃. Bound ABL103 was detected by HRP conjugated Anti-human Fc antibody

ABL103 binds to the Ig-like V domain of B7-H4

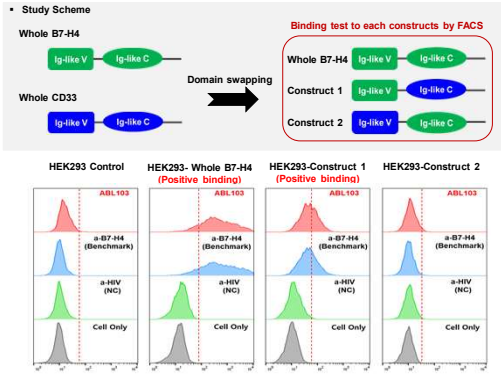
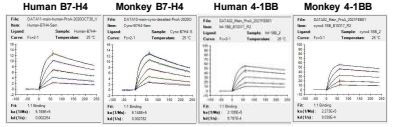


Fig.3. Identification of B7-H4 binding domain by FACS analysis
To determined B7-H4 binding domain by ABL103, plasmid vectors for whole B7-H4, construct 1 (IgV domain of B7-H4 and IgC domain of CD33) or construct 2 (IgV domain of CD33 and IgC domain of B7-H4) were constructed. After transfection to HEK293 cells, ABL103 was incubated with each cell and then measured for cell binding activity by FACS analysis

ABL103 shows comparable binding affinities to human and monkey targets (B7-H4 and 4-1BB)



	Species	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)
B7-H4	Human	5.169×10^{5}	2.254×10^{-3}	4.361×10^{-9}	13.79
	Monkey	6.145×10^{5}	2.782×10^{-3}	4.527×10^{-9}	13.60
4-1BB	Human	2.132×10^{5}	9.706×10^{-4}	4.555×10^{-9}	58.84
	Monkey	2.239×10^{5}	8.986×10^{-4}	4.015×10^{-9}	57.93

Fig.4. Affinity measurement for both targets by using surface plasmon resonance
To confirm the target binding affinity, ABL103 was captured on a protein A chip. Recombinant B7-H4 (Human/Monkey) or 4-1BB (Human/Monkey) protein was flowed across the chip at concentration range from 100 nM to 6.25 nM for B7-H4 or 250 nM to 15.625 nM for 4-1BB at 30 ul/min for 60 seconds, followed by a dissociation phase of 180 seconds.

T cells are rescued from B7-H4-mediated suppression by ABL103 and M40413 (B7-H4 binding part of ABL103)

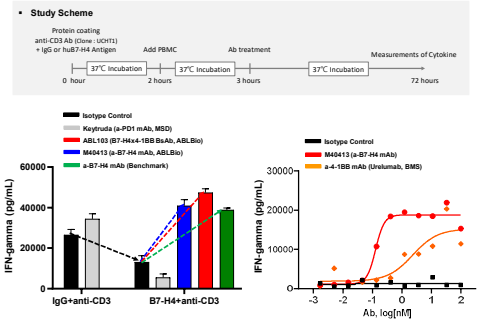


Fig.5. PBMC-based in vitro T-cell activity test
To determine the T-cell checkpoint blockade activity antibodies were incubated with PBMCs in the plates coated with anti-CD3 antibody and B7-H4. IgG was used as a negative control of B7H4. After 72 hrs of incubation, the secreted IFN-gamma was measured. M40413 is a parental B7-H4 antibody of ABL103. T cells were rescued from B7-H4-mediated suppression by M40413 or ABL103 more efficiently than anti-PD1 mAb (Keytruda) or anti-4-1BB mAb (Urelumab)

ABL103 induces more significant cell lysis than Urelumab in the presence of B7-H4 expressed cancer cell line

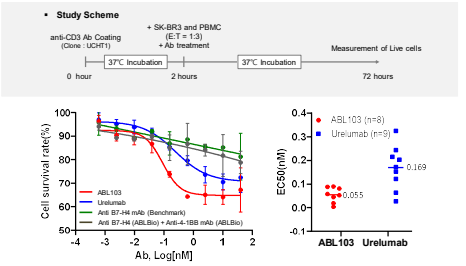


Fig.6. PBMC based in vitro target cell lysis
PBMCs were plated with SK-BR3 (E:T=1:3) and incubated with serially diluted antibodies for 72 hrs. The % of target cell lysis were measured by cell counting kit (CC8, CK04-20). ABL103 induced dose dependent target cell lysis more efficiently than Urelumab, agonistic anti-4-1BB mAb (Benchmark) or combination of a-B7-H4 mAb and anti-4-1BB mAb (left graph. EC50s of each antibody produced from 8~9 different PBMC donors are displayed (right panel)

ABL103-mediated 4-1BB activation depends on B7-H4 expression

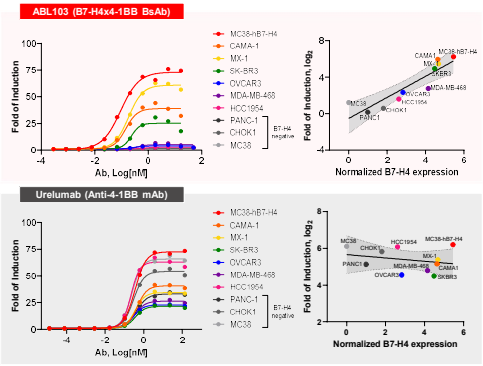
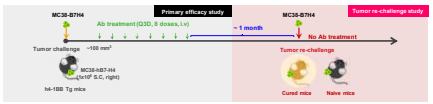


Fig.7. Correlation between expression level of B7-H4 and 4-1BB activation
ABL103 or anti-4-1BB mAb (Urelumab) was analyzed for its in vitro 4-1BB activity by using Promega kit system. In brief, various cell lines were added to 96 well assay plate and incubated with ABL103 (starting from 50 nM diluted 3-fold) or Urelumab (starting from 133 nM diluted for 6-fold) and GloResponseTM NFkB-luc2/4-1BB Jurkat cell line. After 6 hr incubation, BioGloTM reagent was added to each well and luminescence was measured using microplate reader.

ABL103 completely eliminates tumors and protects mice from re-challenge with MC38-B7-H4 tumor



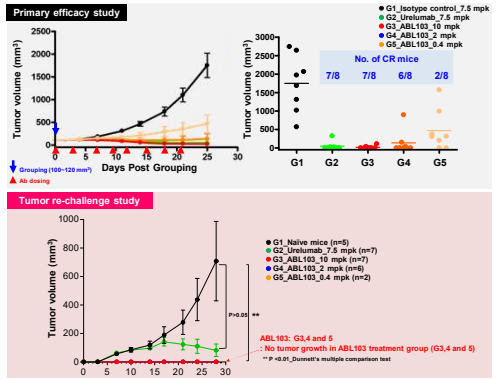


Fig.8. in vivo efficacy test in 4-1BB transgenic mice with MC38-hB7-H4 tumor
To test the in vivo efficacy of ABL103, 4-1BB TG mice were injected to right flank with MC38-hB7-H4 tumor cells (1×10^6, S.C.) then enrolled into five groups when the tumor size reached ~103 mm3. All test articles were injected total 8 times (I.V., Q3D, upper graph). After 1 month from the final treatment, 22 cured mice in administrated groups and 5 naïve mice were implanted MC38-hB7-H4 tumor cells (1×10^6, left flank) for re-challenge experiment. Overall, ABL103 showed strong in vivo efficacy and more potent long-term memory protection than Urelumab.

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