ABL103, A novel T-cell engaging bispecific antibody, exhibits potent *in vitro* and *vivo* antitumor activity and low toxicity via B7-H4 dependent 4-1BB activation in tumor microenvironment

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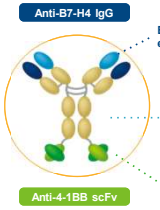
Abstract

B7-H4 (B7x, VTCN1), a member of the B7-family, is overexpressed in majority of cancer patients with ovarian, endometrial and breast cancers. B7-H4 expression was also observed in tumor associated macrophages and implicated as an immune checkpoint regulating T cell responses. Although B7-H4 expression in normal tissues is quite limited, tumor-specific immune response by ABL103 would minimize potential adverse effects. ABL103, B7-H4 Grabody-T, is a First-in Class bispecific antibody targeting B7-H4 and 4-1BB and augments T cell function by a dual mechanism, i.e., 1) blocks the B7-H4 mediated T cell inhibition, and 2) elicits superior T cell activation through B7-H4-dependent 4-1BB clustering. Our data showed that simultaneous binding of B7-H4 and 4-1BB by ABL103 led to potent in vitro T cell activation only in the presence of B7-H4 expressing tumor cells. In the established tumor model, ABL103 potently inhibited tumor progression in a dose-dependent manner and showed higher rate of complete remission (CR) at 2 and 10 mg/kg dose groups. Moreover, mice were protected from the tumor re-challenge 3 months after cessation of ABL103 treatment, suggesting long-term memory has been established. In 4-week pilot tox study using cynomolgus monkeys, ABL103 was tolerable up to 100 mg/kg dosed weekly with no ABL103-related toxicity observations.

To understand the relationship of target expression, expression of B7-H4, CD4, CD8, PD-L1, and 4-1BB was examined in 142 ovarian cancer patient biopsies. About 83% of ovarian cancer patient tissues showed B7-H4 positive staining and 62% of them showed strong positive. In addition, the staining of 4-1BB as well as CD4 and CD8 T cells were observed in both tumor nest and stroma, suggesting that cross-linking of B7-H4 and 4-1BB is feasible in tumor microenvironment.

Overall, ABL103 has a strong *in vitro* and *vivo* anti-tumor activity and good safety profile via B7-H4-dependent 4-1BB activation. This strongly suggests ABL103 is a promising therapeutic agent potentially benefitting patients with B7-H4 overexpression.

General Feature of ABL103



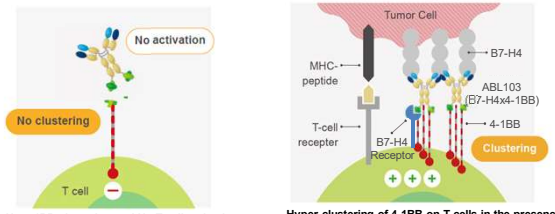
Potential Indication
- B7-H4+ cancer patients (TNBC, Ovarian, NSCLC, etc.)

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

Mode of Action



- No 4-1BB clustering and No T-cell activation in the absence of B7-H4 expression
- No Liver toxicity
- Hyper-clustering of 4-1BB on T-cells in the presence of B7-H4 expression, leading to T-cell activation
- Blocks B7-H4 and B7-H4 receptor ligation
- Strong & Localized anti-tumor effect

Conclusion

- 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***
- ABL103 was ***well tolerable up to 100 mg/kg*** in 4-week pilot tox study

Results

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

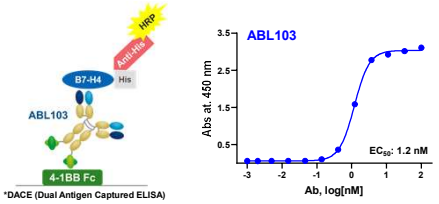
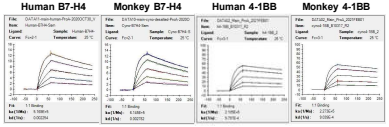


Fig.1. Target antigen binding analysis by Dual Antigen Captured ELISA
To evaluate the antigen binding activity, ABL103 was subjected to DACE. Briefly, plate was coated with human 4-1BB Fc then blocked with PBSB (BSA in PBS). Three-fold dilutions of ABL103 starting from 100 nM were added to each well 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 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	$5.169x10^5$	$2.254x10^{-3}$	$4.361x10^{-9}$	13.79
	Monkey	$6.145x10^5$	$2.782x10^{-3}$	$4.527x10^{-9}$	13.60
4-1BB	Human	$2.132x10^5$	$9.706x10^{-4}$	$4.555x10^{-9}$	58.84
	Monkey	$2.239x10^5$	$8.986x10^{-4}$	$4.015x10^{-9}$	57.93

Fig.2. 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)

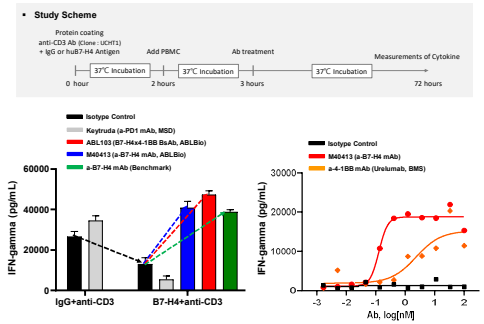


Fig.3. PBMC-based in vitro T-cell activity test
To determine the T-cell checkpoint blockade activity antibodies were incubated with PBMCs in the plates coated with anti-CD3 antibody and recombinant B7-H4 protein. IgG was used as a negative control of B7H4. After 72 hrs of incubation, the secreted IFN-gamma was measured. M4043 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 expressing cancer cell line

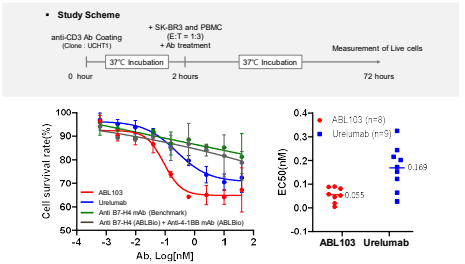


Fig.4. PBMC-based in vitro target cell lysis
PBMCs were plated with SK-BR3 (E:T=1:3) and incubated with serially diluted antibodies for 72 hrs. The % of target cell lysis were measured by cell counting kit (CCB, CK04-20). ABL103 induced dose dependent target cell lysis more efficiently than Urelumab, 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 I

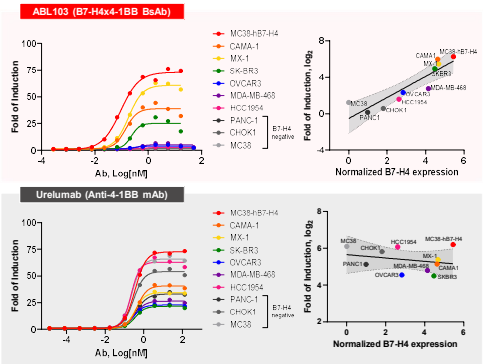


Fig.5. Correlation between expression level of B7-H4 and 4-1BB activation I
ABL103 or anti-4-1BB mAb (Urelumab) was analyzed for its in vitro 4-1BB activity by using Promega kit system. In brief, various cell lines were added to 96 well assay plate and incubated with ABL103 (starting from 50 nM diluted 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-mediated 4-1BB activation depends on B7-H4 expression II

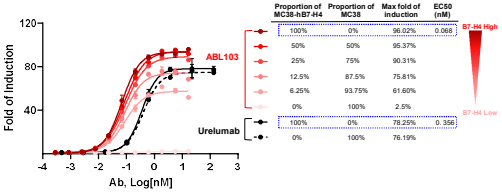


Fig.6. Correlation between expression level of B7-H4 and 4-1BB activation II
ABL103 or anti-4-1BB mAb (Urelumab) was analyzed for its in vitro 4-1BB activity by using Promega kit system. In brief, mixtures of parental MC38 and B7-H4 overexpressed MC38 (MC38-hB7-H4) with indicated proportion were added 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 in advanced tumor model and protects mice from re-challenge of MC38-B7-H4

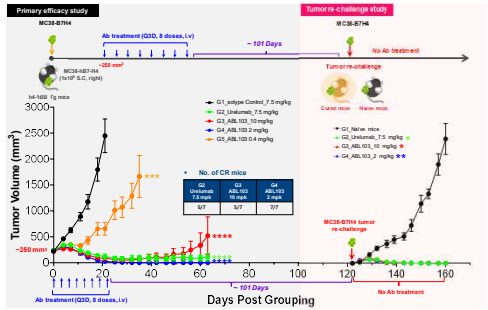
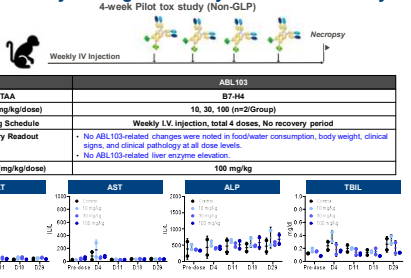


Fig.7. in vivo efficacy test in 4-1BB transgenic mice with MC38-hB7-H4 tumor
To test the in vivo efficacy of ABL103, 4-1BB TG mice were injected to right flank with MC38-hB7-H4 tumor cells ($1x10^6$, S.C.) then enrolled into five groups when the tumor size reached ~250 mm3. All test articles were injected total 8 times (I.V., Q3D, left graph). After 101 days from the final treatment, 17 cured mice in administrated groups and 3 naïve mice were implanted MC38-hB7-H4 tumor cells ($1x10^6$, left flank) for re-challenge experiment. Overall, ABL103 showed stronger in vivo efficacy than Urelumab and potent long-term memory protection. [Statistical analysis via t-test, ***p<0.001, ****P<0.0001 (Left) *P<0.05, **P<0.01 (right)]

ABL103 was well tolerated up to 100 mg/kg in 4-week repeat dose cynomolgus monkey non-GLP tox study



	ABL103
TAA	B7-H4
Dose (mg/kg/dose)	10, 30, 100 (n=2/Group)
Dosing Schedule	Weekly I.V. injection, total 4 doses, No recovery period
Primary Readout	• No ABL103-related changes were noted in food/water consumption, body weight, clinical signs, and clinical pathology at all dose levels. • No ABL103-related liver enzyme elevation.
NOAEL (mg/kg/dose)	100 mg/kg

Fig.8. in vivo efficacy test in 4-1BB transgenic mice with MC38-hB7-H4 tumor
To determine the potential toxicity of ABL103, Cynomolgus monkeys received vehicle or ABL103 at dosages of 0, 10, 30 or 100 mg/kg/dose, respectively (QW, Total 4 times). Parameters evaluated during the study included viability (mortality and morbundity), clinical observations including body weight, qualitative food consumption, clinical pathology, gross pathology, organ weight and histopathology. Intravenous injection of ABL103 to female cynomolgus monkeys at 10, 30, or 100 mg/kg/dose once weekly for 4 doses was well tolerated.

B7-H4 expression profiling and TIL analysis by IHC study using 142 of ovarian tumors

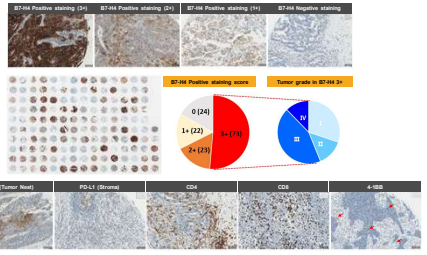


Fig.9. B7-H4 expression and TIL analysis in 142 of ovarian tumors
(A) Immunohistochemistry was applied to investigate the expression of B7-H4 in 142 ovarian caner tissues. 118 (83%) cases showed B7-H4 positive (52% of 3+, 16% of 2+, 15% of 1+) and grade III and IV were more than half in 3+ B7-H4 positive tumor. (B) Representative immunohistochemistry staining of PD-L1, CD4, CD8 and 4-1BB in tumor nest or stroma (CRO : Tristar)

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