Development of fully human T-cell engaging bispecific antibodies targeting B7H4

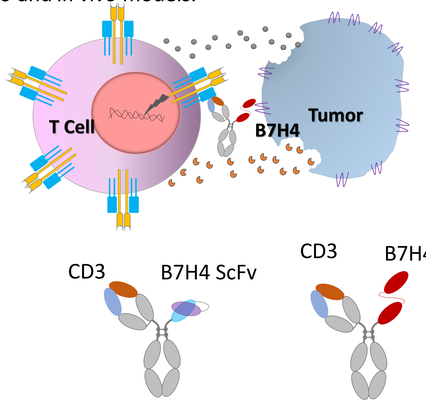
Xiaodong Wu,[1] Yongqiang Wang,[1] Gezi Jia,[1] Fei Chen,[1] Yuandong Wang,[1] Yunxing Yang,[1] Yun He,[1] Yiping Rong[1]

[1]Harbour BioMed Co., Ltd Email: Xiaodong.wu@harbourbiomed.com; jung.lee@harbourbiomed.com

Abstract

B7H4 (also known as B7x, B7-S1, and VTCN1) is a member of the B7-family and serves as an immune regulatory molecule to inhibit T cell activation. B7H4 expression in healthy tissues is relatively limited at the protein level, in contrast, it is highly expressed in several solid tumors such as breast cancer, ovary cancer, and endometrium cancer. Expression level of B7H4 in tumors is correlate with poor prognosis and has little overlap with PD-L1. The exclusive tumor expression pattern of B7H4 and PD-L1 makes B7H4 an attractive immunotherapeutic target.

Here we report the development of B7H4 X CD3 bispecific antibodies either with monovalent B7H4 ScFv or bivalent VH '2+1' format generated by the H2L2 or HCAB Harbour Mice® platform, respectively. Bispecific antibodies with reduced CD3-binding activity and eliminated FcγR reactivity could improve the safety while bivalent B7H4 binding domains could increase the avidity driven cytotoxicity. These selective B7H4 X CD3 bispecific antibodies demonstrated excellent activities in multiple *in vitro* and *in vivo* models.



Highlight

- ✓Bivalent 2+1 format with fully human VH generated by the HCAB Harbour Mice® platform increases the avidity driven cytotoxicity.
- ✓Weak-activity CD3 arm and FcγR reactivity eliminated Fc domain improve the safety.
- ✓The antibodies showed potent *in vitro* cytotoxicity efficacy and tumor growth inhibition in several mouse tumor models.

Results

B7H4 Expression on Tumor and Tumor Cell Line

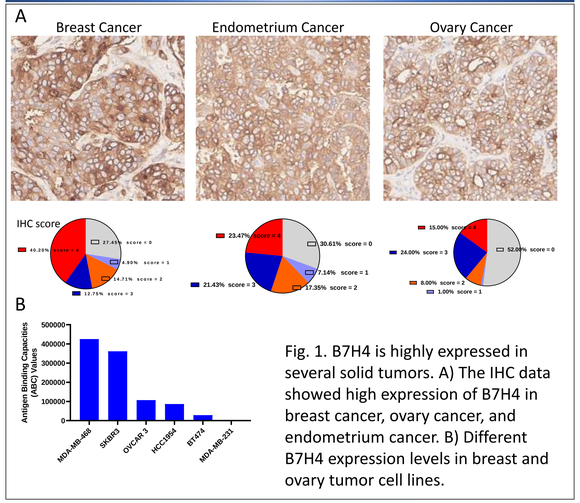
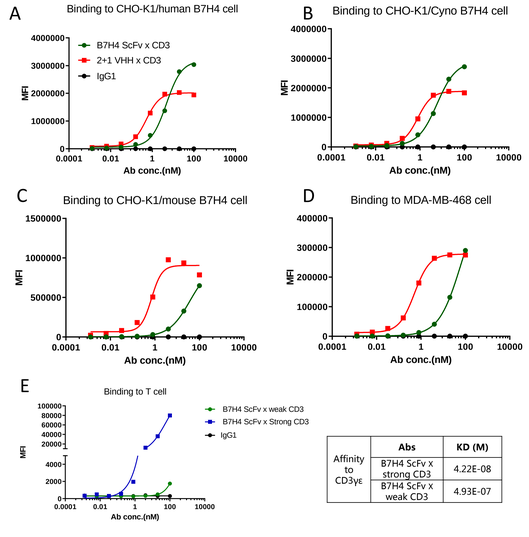


Fig. 1. B7H4 is highly expressed in several solid tumors. A) The IHC data showed high expression of B7H4 in breast cancer, ovary cancer, and endometrium cancer. B) Different B7H4 expression levels in breast and ovary tumor cell lines.

Binding to Human/cyno B7H7 and Tumor Cells



	Abs	KD (M)
Affinity to CD3γε	B7H4 ScFv x strong CD3	4.22E-08
	B7H4 ScFv x weak CD3	4.93E-07

Fig.2. Binding characterization of B7H4 X CD3 antibodies by FACS. (A) Binding to CHO-K1/h B7H4 cell. (B) Binding to CHO-K1/cyno B7H4 cell. (C) Binding to CHO-K1/m B7H4 cell. (D) Binding to MDA-MB-468 tumor cell. (E) Comparison of strong CD3 and weak CD3 arm binding to T cell.

Cytotoxic Activity against Various Tumor Cells

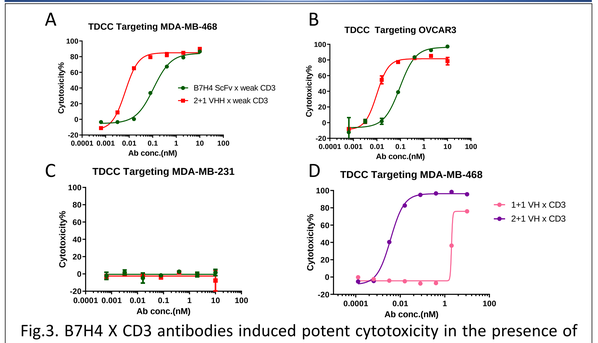


Fig.3. B7H4 X CD3 antibodies induced potent cytotoxicity in the presence of B7H4 expression on co-cultured cancer cells. A) Cytotoxicity on MDA-MB-468 breast cancer cells. B) Cytotoxicity on OVCAR3 ovary cancer cells. C) No cytotoxicity was observed on B7H4 negative MDA-MB-231 cells. D) Bivalent VH 2+1 format had more potent cytotoxicity in an avidity dependent manner.

Improved Safety Profile with Weak CD3

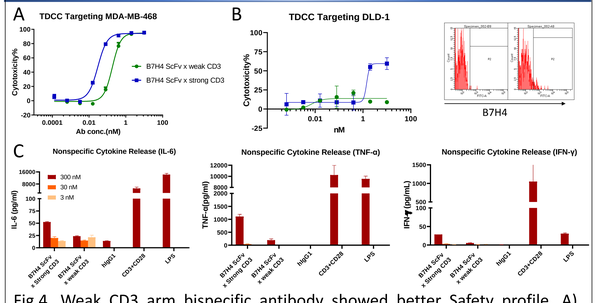


Fig.4. Weak CD3 arm bispecific antibody showed better Safety profile. A) Although strong CD3 antibody showed lower EC50 than weak CD3 antibody, they had same maximum cytotoxicity when targeting B7H4 high expression MDA-MB-468 cells. B) When targeting DLD-1 cells expressing trace B7H4, weak CD3 antibody showed no cytotoxicity indicating better safety against basic B7H4 expression tissues. C) weak CD3 antibody induced lower nonspecific cytokine release of IL-6, TNF-α, IFN-γ in absence of target cells.

Bystander Killing Efficacy

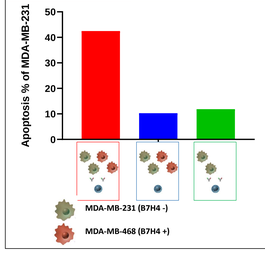


Fig.5. Bystander killing efficacy. B7H4 negative MDA-MB-231 cells were mixed with B7H4 positive MDA-MB-468 cells and cocultured with antibody and T cells. The apoptosis of MDA-MB-231 cells were detected by FACS. In presence of MDA-MB-468 cells the bispecific antibody could induce bystander killing effect against MDA-MB-231 cells .

In vivo Efficacy Study

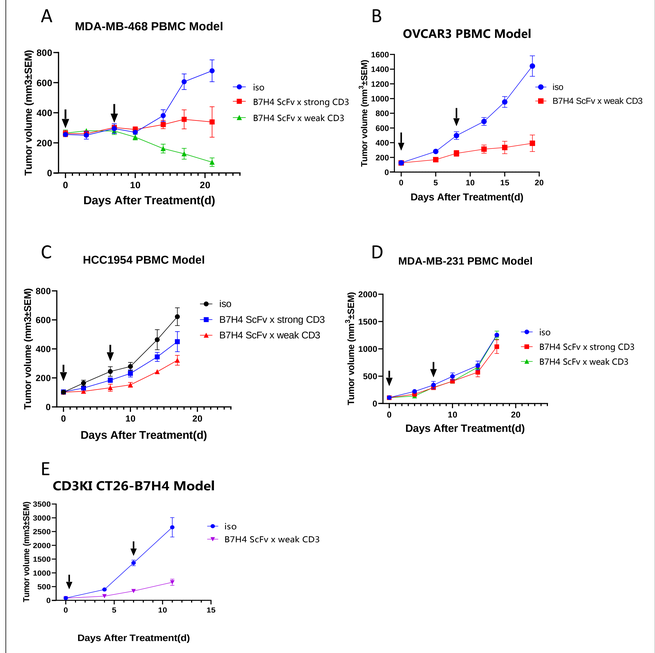


Fig.6. *In vivo* efficacy of B7H4 X CD3 bispecific antibodies on multiple tumors. NCG mice were inoculated with human PBMC and tumor cells with different B7H4 expression levels: A) MDA-MB-468 cells with high B7H4 expression; B) OVCAR3 cells with medium B7H4 expression; C) HCC-1954 cells with low B7H4 expression; D) MDA-MB-231 cells with no B7H4 expression. The efficacy was dependent on B7H4 expression. In B7H4 negative MDA-MB-231 model there was no efficacy. Interestingly, although strong CD3 antibody showed better *in vitro* killing efficacy, it had weaker *in vivo* efficacy. E) human CD3 knock-in mice were inoculated with CT-26 cells overexpressing human B7H4.

Conclusion

- ✓B7H4 is highly expressed in various solid tumors as a specific tumor associated antigen.
- ✓Bispecific antibodies derived from B7H4 H2L2 and HCAB showed potent *in vitro* and *in vivo* efficacy.
- ✓Bivalent 2+1 format increased the avidity driven cytotoxicity.
- ✓Weak-activity CD3 arm and eliminated FcγR reactivity improved safety.
- ✓Bispecific antibodies showed bystander killing efficacy which may help eliminate the heterogeneous tumors.