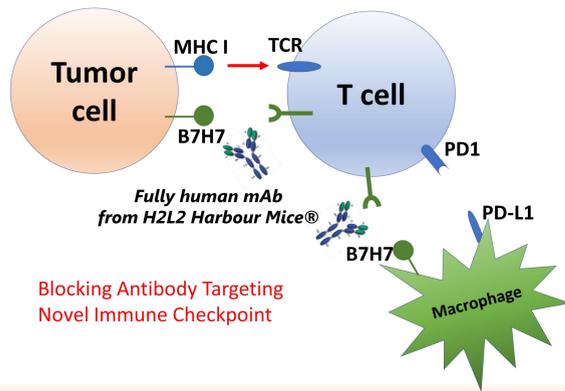


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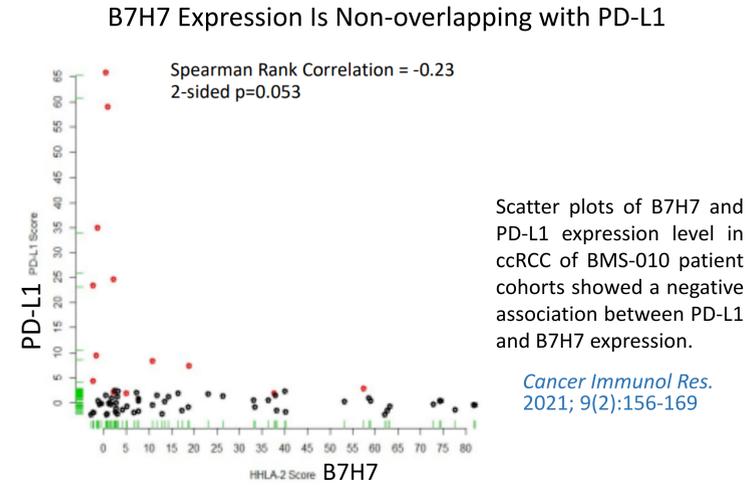
## Abstract

- Developing novel immune checkpoint inhibitors in combination with current immune-oncology (IO) therapeutics can provide a more efficient therapy and expand indications.
- B7H7, (or HHLA2), is a novel B7 family member that;
  - Is highly expressed on a variety of human cancers such as colon, pancreas, kidney, breast, bladder, lung cancers and associated with metastatic disease and poorer survival of cancer patients.
  - Provides co-inhibitory function on T cell activation.
- Harbour BioMed has discovered functional antibodies targeting this novel B7 family member with potent anti-tumor activity.
  - Generated by the H2L2 Harbour Mice<sup>®</sup> platform, of fully human antibody with Fc silenced mutation.
  - Promoted T cell activation *in vitro*, dependent on B7H7 expression on APC cells.
  - Showed potent anti-tumor activity in murine tumor models.
- Anti-B7H7 antibody may present a promising novel anti-tumor agent as monotherapy and/or combination therapy with currently established IO agents.



## Highlight

- ✓ B7H7 is a novel immune checkpoint of B7 family. B7H7 was highly expressed on a variety of cancers and its expression was reported non-overlapping with PD-L1 (*Cancer Immunol Res.* 2021;9(2):156-169). Anti-B7H7 antibody may present a novel anti-tumor therapy complementary to PD-L1/PD1 based therapy.
- ✓ Anti-B7H7 antibodies were generated from utilizing the H2L2 Harbour Mice<sup>®</sup> platform, of fully human antibody.
- ✓ Anti-B7H7 antibodies show significant T cell activation effect *in vitro* and tumor growth inhibition in several mouse tumor models.



## Results

### B7H7 Expression on Tumor and Tumor Cell Line

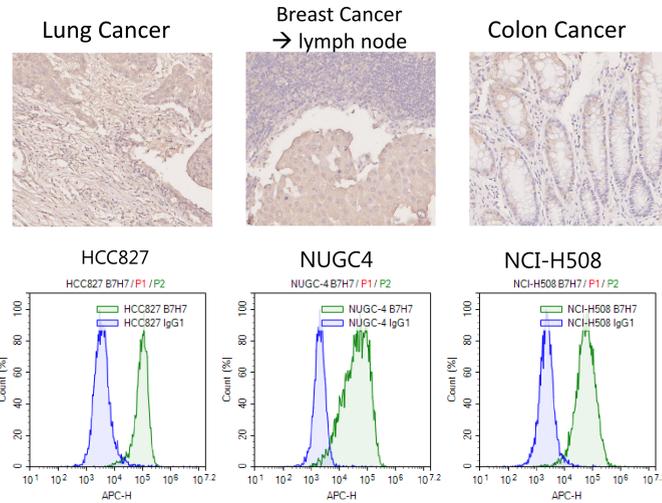


Fig.1. B7H7 highly expressed on virous cancers as well as cancer cell lines.

### B7H7 Function on T Cell Activation

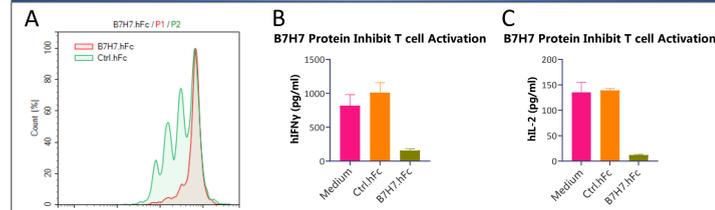


Fig.2. B7H7 inhibiting T cell activation. B7H7-hFc protein was coated on a plate with plate-bound anti-CD3 and soluble anti-CD28 mAbs to activate T cells for 3 days. (A) T cell proliferation, (B) IFN- $\gamma$  and (C) IL-2 production.

## Results

### Binding to Human/cyno B7H7 and Tumor Cells

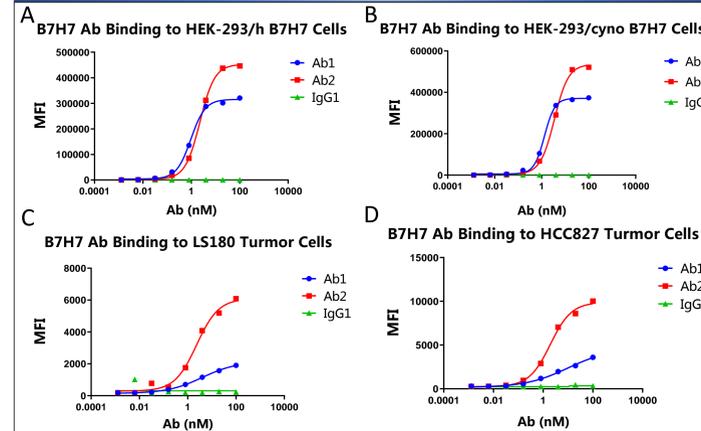


Fig.3. Binding characterization of anti-B7H7 antibodies by FACS. (A) Binding to HEK-293/h B7H7 cells. (B) Binding to HEK-293/cyno B7H7 cells. (C) Binding to LS-180 cells. (D) Binding to HCC-827 cells.

### Specificity Study

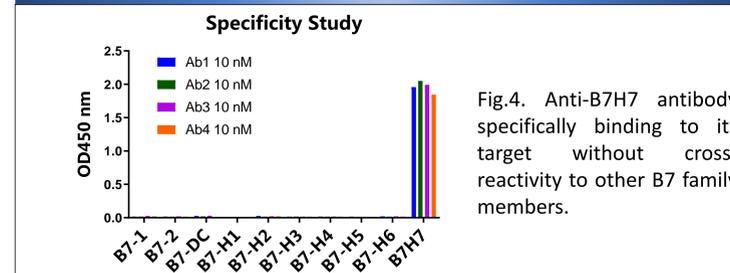


Fig.4. Anti-B7H7 antibody specifically binding to its target without cross-reactivity to other B7 family members.

### Functional Effect of anti-B7H7 Antibodies on T Cell Activation

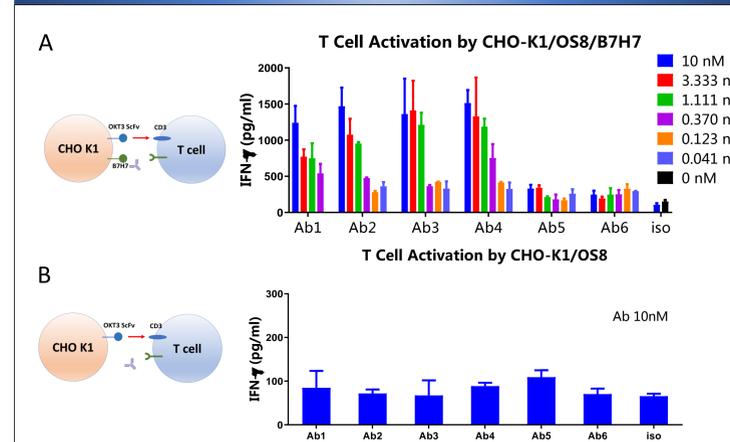


Fig.5. Anti-B7H7 antibodies restoring T cell activation. Human donor T cells were isolated and activated by (A) CHO-K1 cells overexpressing OKT3 ScFv and B7H7 or (B) CHO-K1 cells overexpressing OKT3 ScFv alone as artificial APC cells. Supernatants were collected after 3 days' co-culture and tested for IFN- $\gamma$  production. Anti-B7H7 antibodies can promote IFN- $\gamma$  production only in the presence of B7H7 expression. Ab5, Ab6 are non-functional antibodies.

### Receptor Blocking assay

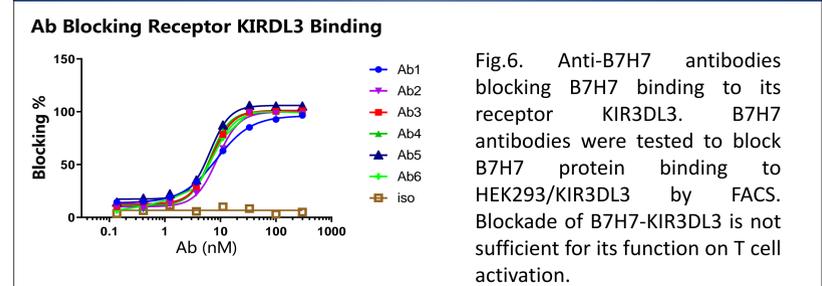


Fig.6. Anti-B7H7 antibodies blocking B7H7 binding to its receptor KIR3DL3. B7H7 antibodies were tested to block B7H7 protein binding to HEK293/KIR3DL3 by FACS. Blockade of B7H7-KIR3DL3 is not sufficient for its function on T cell activation.

### In vivo Efficacy Study

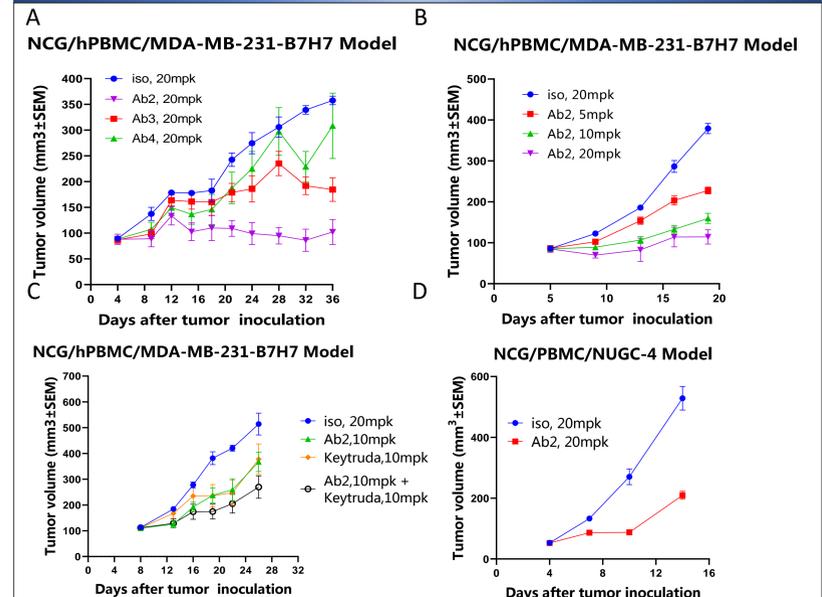


Fig.7. In vivo anti-tumor efficacy of anti-B7H7 antibodies. NCG human PBMC mice were inoculated with MDA-MB-231 cells overexpressing B7H7. The mice were treated with (A) 20mpk BIW of Ab2, Ab3 or Ab4; (B) dose titration of Ab2; (C) combination of Ab2 and Keytruda. (D) NCG human PBMC mice were inoculated with NUGC-4 cells and treated with 20mpk Ab2. Tumor growth and body weight was recorded twice a week.

## Conclusion

- ✓ B7H7 is highly expressed in various cancers and acts as an immune check point regulator to inhibit T cell activation.
- ✓ Anti-B7H7 antibodies generated from the H2L2 Harbour Mice<sup>®</sup> potentially promote T cell activation dependent on B7H7 expression.
- ✓ Anti-B7H7 antibodies showed potent anti-tumor activities in murine tumor models.
- ✓ The B7H7 expression is associated with worse survival in many human cancers and its expression is exclusive to PD-L1 expression. Anti-B7H7 antibody may present a novel anti-tumor therapy agent, besides the PD-L1/PD1 antibodies.