858 HBM7008 (B7H4X4-1BB HBICE®) SYNERGIZES HBM7004 (B7H4XCD3 HBICE®) FOR SOLID TUMOR THERAPY

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Background B7H4 is a member of B7-family and its expression is not detected or barely detected in healthy tissues but highly expressed in multiple solid tumors. Thus, B7H4 is a good tumor associated antigen (TAA) for tumor therapy, especially used for construction of T cell engagers. CD3 T cell engager has shown promising efficacy in hematologic malignancies. However, the outcomes for solid tumors are still disappointing. One possible impediment to the efficacy could be that CD3 signal alone may cause rapid T cell exhaustion and apoptosis. 4-1BB(CD137) costimulation along with CD3 signal can significantly enhance T cell proliferation, cytotoxicity, as well as counteract T cell exhaustion and cell death. Combination B7H4xCD3 with B7H4x4-1BB T cell engagers may be a promising strategy for solid tumor therapy.

Methods Both B7H4xCD3 and B7H4x4-1BB bispecific antibodies were developed from Harbour BioMed heavy chain only antibodies (HCAb) based bispecific immune cell engager (HBICE®) platform. HBM7004 (B7H4xCD3 HBICE®) is composed of a bivalent B7H4 VH domain (2+1 format) to increase avidity driven cytotoxicity, and monovalent CD3 Fab domain with reduced activity to decrease systemic toxicity. *In vitro* cytotoxicity of HBM7004 was tested on multiple B7H4 positive tumor cell lines. HBM7008 (B7H4x4-1BB HBICE®) is composed of anti-B7H4 IgG1 and anti-4-1BB HCAb variable domains appended at C-terminus of Fc fragment (2+2 format). The synergistic effect of combination of HBM7004 and HBM7008 was studied in a series of assays.

Results HBM7004 (B7H4xCD3) showed potent *in vitro* efficacy to multiple tumor cell lines at high effector T cell: target cell (E: T) ratio. 2+1 HBICE® format showed much higher efficacy than monovalent B7H4 HBICE®. When the cytotoxicity assay was conducted in the co-culture with low E:T ratio, which mimicked the status in tumor microenvironment as measured by immunohistochemistry staining of T cell infiltration, HBM7004 could not kill the tumor cells. Combination with HBM7008 (B7H4x4-1BB) could restore HBM7004 cytotoxicity at low E:T ratio. It significantly reduced T cell apoptosis and increased T cell division, thus maintained T cell numbers after long-term co-culture with tumor cells. In addition to HBM7008 (B7H4x4-1BB), combination of HBM7004 with HER2x4-1BB HBICE® also had synergistic effect on killing SKBR3 cells which is B7H4 and HER2 double positive, indicating flexible combination approaches.

Conclusions Combination with HBM7008 (B7H4x4-1BB) could provide a secondary signal for T cell activation and significantly increase the efficacy of HBM7004 by reducing T cell apoptosis and increasing T cell division, providing a promising therapy solution for solid tumor therapy.

Ethics Approval The cancer tissue microarray was purchased from Fanpu Biotech, Inc. The company ensured ethical approval from the patients, and patient consent for publication.

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