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**A BIPARATOPIC ANTI-HER2 ANTIBODY ENABLED WITH CONDITIONAL 4-1BB AGONISM INDUCES POTENT ANTI-TUMOR EFFICACY**

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**Background** HER2 is a well-established therapeutic target that is overexpressed in multiple cancers. Monoclonal antibodies (mAbs) targeting HER2 such as Herceptin (trastuzumab) and Perjeta (pertuzumab) have been used in the clinic for many years. Despite good outcomes, there remains an unmet medical need that requires the further development of novel agents for recurrent or metastatic patients. The combination of HER2 mAbs have shown synergistic activity with improved clinical benefit. Moreover, biparatopic antibodies that are composed of trastuzumab and pertuzumab binding domains have shown promising results in the clinic. 4-1BB is a potent stimulator of T cells and NK cells, and when activated, can improve effector and/or memory responses. However, inherent hepatotoxicity has been observed during the clinical development of 4-1BB agonists. PM1234 is a trisppecific antibody that binds to two different epitopes of HER2 (ECD4 and ECD2), and the CRD4 domain of 4-1BB. PM1234 stimulates immune cells such as T cells via HER2-mediated cross-bridging and 4-1BB activation, which results in potent anti-tumor efficacy. Moreover, Fc-effector function was shown to be essential for the *in vivo* anti-tumor efficacy of PM1234.

**Methods** PM1234 was generated as a biparatopic heterodimeric (1+1) IgG-like antibody composed of both trastuzumab and pertuzumab binding domains with anti-4-1BB VHHs fused to the C-terminus of the Fc. The immunomodulatory functions of PM1234 were evaluated using luciferase reporter cell assays, PBMC/primary T cell activation assays, and human 4-1BB KI mouse tumor models.

**Results** PM1234 displayed strong HER2 binding and signal inhibition activity due to its biparatopic binding nature. The binding mode of PM1234 may allow up to double the available HER2 binding domains that can facilitate 4-1BB cross-linking and activation and was thus more potent than non-biparatopic anti-HER2 x 4-1BB bispecifics (trastuzumab x 4-1BB and/or pertuzumab x 4-1BB). PM1234 retained Fc effector function towards HER2 but with negligible activity towards the 4-1BB-targeting arm. PM1234 showed more potent activity in *in vivo* CT26 and MC38 tumor models than the control molecules containing Fc-silencing mutations, the combination of trastuzumab and pertuzumab, and anti-HER2-ADC. Importantly, PM1234 induced immune memory and potent anti-tumor efficacy to both HER2+ primary tumors and distal tumors without HER2 expression.

**Conclusions** PM1234 exhibited potent anti-tumor activity with the induction of strong immunological memory to suppress both primary HER2+ tumors and distal tumors. The differentiation of PM1234 shows the next-generation potential of HER2-targeted therapies in this competitive space and provides an insight into further improvements for benefiting patients with HER2+ tumors.

**Ethics Approval** All mice were maintained under specified pathogen-free conditions, and all studies were approved by the Animal Care and Use Committee of HUST-Suzhou Institute for Brainmatics.

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