A BISPESIFIC ANTIBODY TARGETING PD1 AND CD40 DISPLAYS POTENT ANTI-TUMOR EFFICACY THROUGH TWO MECHANISMS: PD1 BLOCKADE AND PD1-DEPENDENT CD40 AGONISM

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Background The discovery and development of first-generation immune checkpoint inhibitors (towards PD1 and CTLA-4) reached a major milestone for cancer therapy. CD40, as a member of the TNFR superfamily, plays a vital role in stimulating DC and B cell maturation and has been targeted to improve on existing immuno-oncology treatments. Due to potential systemic toxicity risks by targeting CD40 via agonist antibodies, new and emerging strategies have focused on localizing agonism to the tumor microenvironment.

Methods We have constructed a novel bispecific antibody towards PD1 and CD40, which were generated by fusing anti-CD40 scFvs to the C-terminus of an anti-PD1 IgG. Fc silencing was adopted to reduce effector function towards PD1- and CD40-expressing immune cells. Furthermore, anti-CD40 non-blocking antibodies were selected based on high agonist activity via receptor cross-linking but with non-ligand blocking properties to reduce any unwanted dampening of natural CD40-CD40L activity. Cell binding, receptor-ligand blockade, luciferase-based reporter assays, and primary B cell assays, were used for selecting and characterizing lead bispecific candidates. Mixed lymphocyte reactions were also performed to test for enhancing effects towards T cells. Finally, our lead candidates were evaluated in in vivo efficacy studies on MC38 and A375 tumor cell lines in syngeneic and PEMC-based models, respectively.

Results In vitro experiments showed that the lead anti-PD1 x CD40 bispecific could disrupt potently the interaction between PD1 and PD-L1. In addition, CD40 signal activation was detected using an NFκB luciferase reporter assay and in primary B cells, which was dependent on PD1-based cross-bridging/cross-linking. Thus, besides promoting the conditional activation of CD40 in the tumor microenvironment and tumor-draining lymph nodes where PD1 expression is upregulated, potential toxicity risks have been substantially reduced by screening away any superagonist traits when selecting for the anti-CD40 component. The anti-PD1 x CD40 bispecific also showed enhanced T cell responses than anti-PD1 alone or the combination of anti-PD1 and anti-CD40 antibodies in an MLR assay. Using in vivo tumor models, CD40-CD40L non-blocking, PD1-based bispecific candidates, displayed better anti-tumor efficacy than anti-PD1 alone or the combination of anti-PD1 and anti-CD40 antibodies. Furthermore, anti-PD1 x CD40 bispecific antibodies did not induce any untoward toxicity in MC38-bearing human-PD1/CD40 double knock-in mice.

Conclusions Collectively, these data demonstrate that anti-PD1 x CD40 bispecific antibodies are promising beyond PD1 inhibitors. As such, we have selected a final candidate that is undergoing CMC development for entering the clinic.

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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 Brainsmatics.