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An Fc-muted bispecific antibody targeting PD-L1 and 4-1BB induces antitumor immune activity in colorectal cancer without systemic toxicity

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Abstract

Background: Resistance to immune checkpoint inhibitor (ICI) therapy narrows the efficacy of cancer immunotherapy. Although 4-1BB is a promising drug target as a costimulatory molecule of immune cells, no 4-1BB agonist has been given clinical approval because of severe liver toxicity or limited efficacy. Therefore, a safe and efficient immunostimulatory molecule is urgently needed for cancer immunotherapy.

Methods: HK010 was generated by antibody engineering, and the Fab/antigen complex structure was analyzed using crystallography. The affinity and activity of HK010 were detected by multiple in vitro bioassays, including enzyme-linked immunosorbent assay (ELISA), surface plasmon resonance (SPR), flow cytometry, and luciferase-reporter assays. Humanized mice bearing human PD-L1-expressing MC38 (MC38/hPDL1) or CT26 (CT26/hPDL1) tumor transplants were established to assess the in vivo antitumor activity of HK010. The pharmacokinetics (PK) and toxicity of HK010 were evaluated in cynomolgus monkeys.

Results: HK010 was generated as an Fc-muted immunoglobulin (Ig)G4 PD-L1x4-1BB bispecific antibody (BsAb) with a distinguished Fab/antigen complex structure, and maintained a high affinity for human PD-L1 (KD: 2.27 nM) and low affinity for human 4-1BB (KD: 493 nM) to achieve potent PD-1/PD-L1 blockade and appropriate 4-1BB agonism. HK010 exhibited synergistic antitumor activity by blocking the PD-1/PD-L1 signaling pathway and stimulating the 4-1BB signaling pathway simultaneously, and being strictly dependent on the PD-L1 receptor in vitro and in vivo. In particular, when the dose was decreased to 0.3 mg/kg, HK010 still showed a strong antitumor effect in a humanized mouse model bearing MC38/hPDL1 tumors. Strikingly, HK010 treatment enhanced antitumor immunity and induced durable antigen-specific immune memory to prevent rechallenged tumor growth by recruiting CD8+T cells and other lymphocytes into tumor tissue and activating tumor-infiltrating lymphocytes. Moreover, HK010 not only did not induce nonspecific production of proinflammatory cytokines but was