A NOVEL MSLN × 4-1BB BISPECIFIC ANTIBODY FOR SOLID TUMOR

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Background: Mesothelin (MSLN) is a 70 KD glycosylphosphatidylinositol (GPI)-anchored cell surface glycoprotein that is rarely expressed in normal tissues but overexpressed in a variety of cancers, including mesothelioma, ovarian cancer, pancreatic cancer and breast cancer et.al. 4-1BB is a member of the tumor necrosis factor receptor superfamily that functions as a co-stimulatory molecule. Agonistic antibodies bind to 4-1BB, triggering a signaling cascade leading to T-cell activation and expansion of cytotoxic CD8+ T lymphocytes. Here, we developed two bispecific antibodies (bsAbs) targeting both MSLN and 4-1BB with an intact Fc fragment from human IgG1 or IgG4, named HK013-G1 and HK013-G4 respectively. We suspected that HK013-G1 can simultaneously exert the cytotoxic effect of CD8+ T cells and NK cells on tumor cells expressing MSLN to achieve better antitumor efficacy.

Methods: Both HK013-G1 and HK013-G4 were constructed by fused a single-chain variable fragment (scFv) targeting hu4-1BB to the C terminus of an anti-MSLA nanobody. And their affinity was optimized to making it highly effective in tumor localization. Next, we tested the killing ability of bsAbs-mediated PBMC or NK92 against tumor cells with different expression levels of MSLN in vitro. And the IFN-γ secretion was detected when CD8+ T cells co-cultured with MSLN+ or MSLN- cells in the presence of antibodies. Also, the 4-1BB agonist activity of bsAbs was measured in a luciferase report gene assay. To confirm the safety of HK013-G1, non-specific activation of 4-1BB signal mediated by Fc receptor and CRS was evaluated in vitro. Finally, we compared the antitumor activity of two bsAbs in both MC38/hMSLN and CT26/hMSLN tumor model and hepatotoxicity as well as cardiotoxicity was evaluated.

Results: Affinity-optimized HK013-G1 has an order of magnitude greater affinity for MSLN (KD≈10−9 M) than 4-1BB (KD≈10−4 M). HK013-G1 induced stronger PBMC against tumor cells than MOARB009 while HK013-G4 does not. Also, HK013-G1 could only mediate the killing of NK92 on MSLN-positive tumor cells. In co-cultured assay, HK013-G1 had superior ability to stimulate CD8+ T cell secretion of IFN-γ than urelumab in the presence of MSLN. In luciferase reporter assay, the bsAbs-induced 4-1BB activation is dependent on expression level of MSLN. In addition, HK013-G1 was shown no stronger ability to inducing non-specific activation of 4-1BB signal mediated by Fc receptor and CRS in vitro. Compared with HK013-G4, HK013-G1 showed a more significant anti-tumor effect in both MC38/hMSLN and CT26/hMSLN tumor model. And, HK013-G4 showed significant hepatotoxicity in mice while HK013-G1 not. Moreover, HK013-G1 can protect mice against tumor re-challenge.

Conclusions: HK013-G1, an MSLN × 4-1BB bsAb with human IgG1 Fc fragment, prevents tumor development by killing tumor cells directly via effector functions mediated by NK and cytotoxic T cells. More importantly, HK013-G1 showed no stronger toxic side effects both in vitro and in vivo. These results show that HK013-G1 has the potential to develop into a new clinical therapy for cancer types with MSLN expression.

Abstract citation ID: tbad014.003