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

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Background 4–1BB (CD137) is not only expressed on the surface of activated T cells and NK cells, but also a marker for Treg cells. Mesothelin (MSLN) is a ~71 kDa cell surface glycoprotein that is rarely expressed in normal tissues but over-expressed in many types of tumors. Here, we developed a bispecific antibody (bsAb) targeting both MSLN and 4–1BB with an intact Fc fragment from human IgG1. It can simultaneously exert the cytotoxic effect of CD8+T cells and NK cells on tumor cells expressing MSLN to achieve better antitumor efficacy.

Methods HK013-G1 with IgG-scFv format was constructed with IgG1 and its affinity was optimized to making it highly effective in tumor localization. Next, we evaluated the binding activities of HK013-G1 to tumor cells with different MSLN expression levels and its effects on 4–1BB+ cell activation mediated by MSLN-crosslinking. Subsequently, the cell-killing abilities of NK induced by HK013-G1 were quantified. The risk of cytokine release storm (CRS) of HK013-G1 was detected in vitro. And the anti-tumor activity was evaluated in CT26/MSLN tumor model. Finally, PK and safety analysis of HK013-G1 were undertaken in cynomolgus monkeys following intravenous infusion (IV) at 3 or 30 mg/kg.

Results Affinity-optimized HK013-G1 has an order of magnitude greater affinity for MSLN than 4–1BB. HK013-G1 is able to bind different MSLN-expressing cancer cells and bridge MSLN+ cells and 4–1BB+ cells. In luciferase reporter assay, the bsAb-induced 4–1BB activation is dependent on expression level of MSLN. While incubated with CD8+T cells, HK013-G1 increased IFN-γ production only in the presence of MSLN+ cells. Furthermore, HK013-G1 could exert its affects via NK cell. Also, HK013-G1 was shown no stronger ability to inducing CRS in vitro. In CT26-hMSLN tumor models, HK013-G1 showed a dose-dependent anti-tumor activity and more significant growth inhibition effect than HK013-G4. In addition, HK013-G1 could protect mice against tumor re-challenge. For the IV administration of HK013-G1, it displays prolonged half-life and no CRS and hepatotoxicity were observed in NHP.

Conclusions HK013-G1, a 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. Moreover, HK013-G1 is well tolerated in cynomolgus monkeys. These results show that this bsAb has the potential to develop into a new clinical therapy for cancer types with high-level MSLN expression.

http://dx.doi.org/10.1136/jitc-2023-SITC2023.1192