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A NOVEL ANTI-MSLN X 4-1BB BISPECIFIC ANTIBODY WITH FC EFFECT FUNCTION AUGMENTS THE ANTITUMOR EFFICACY

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Background Mesothelin (MSLN) is a ~71kDa cell surface glycoprotein that is rarely expressed in normal tissues but over-expressed in a variety of cancers.¹ 4-1BB is not only expressed on the surface of activated T cells and NK cells but also a marker for Treg.² Moreover, 4-1BB shows high selectivity for human tumor-derived Tregs and is associated with worse survival outcomes in patients with multiple tumor types, such as bladder cancer, glioblastoma, prostate cancer, or renal clear cell cancer.³ Here, we developed a IgG1-based bi-specific antibody, HK013-1, targeting both MSLN and 4-1BB to achieve better antitumor therapeutic efficacy.

Methods We tested the binding ability of HK013-1 to tumor cells with different expression levels of MSLN, and tested the killing ability of HK013-1-mediated NK cells against these tumor cells in vitro. Moreover, the 4-1BB agonist activity of HK013-1 was detected using CD8+T cells co-cultured with MSLN+ or MSLN- cells. To confirm the safety of HK013-1, non-specific activation of 4-1BB signal mediated by Fc receptor and killing potency to CD8+T cells and Tregs induced by HK013-1 was evaluated. In vivo, we verified the ability to inhibit tumor growth of HK013-1 and examined the effects of HK013-1 on spleen and tumor CD8+T cells and Tregs.

Results HK013-1 could bind to various tumor cells that differentially expressed MSLN and induce NK cells to kill these cells. In co-cultured assay, HK013-1 increased IFN- γ production only in the presence of MSLN+ cells. Compared with anti-4-1BB parent antibody and urelumab, HK013 induced weaker Fc γ R-mediated 4-1BB activation. Furthermore, HK013-1 engaged NK cells to kill Treg but not CD8+T cells. In 4-1BB humanized transgenic mice, HK013-1 was revealed to reduce the proportion of Treg cells in tumor but had no effect on CD8+T cells, and CD8+T and Treg cells in the spleen. Compared with IgG4-based bi-specific antibody, IgG1-based HK013-1 showed a more significant anti-tumor effect in MC38/MSLN tumor model.

Conclusions IgG1-based HK013-1 prevents tumor development by directly killing tumor cells and depleting Treg to relieve immunosuppression. Preclinical studies have shown that IgG1-based HK013-1 has good antitumor activity and safety, which may further develop its clinical potential.

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