A HIGHLY POTENT ANTI-LAG-3-IL-2C THAT SELECTIVELY TARGETS TUMOR-SPECIFIC CD8+ T CELLS

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Background Interleukin 2 (IL-2) is an essential link in immune activation and heavily contributes to tumor eradication. Clinically, IL-2 has shown impressive efficacy in various tumor types. However, the abundant and ubiquitous expression of IL-2 receptors made IL-2 pleiotropic and hence limits its application as the sole agent for immunotherapy. LAG-3 emerged as the next-generation inhibitory immune checkpoint (ICP) following CTLA-4, PD-1, and PD-L1. Simultaneous blockade of LAG-3 and PD-1 has shown favorable clinical outcomes in PD-1/PD-L1 resistant melanoma patients. In addition to its ICP role, the expression pattern of LAG-3 has made it an appealing target to be used in combination with IL-2 as a bifunctional fusion protein for tumor immunotherapy as LAG-3 is highly expressed on tumor-specific CD8+ T cells. A LAG-3+-T-cell-targeting IL-2 has strong anti-tumor efficacy and minimal systemic toxicity, as well as the compatibility to be combined with PD-1 blockade. We herein proposed the use of an αLAG-3-IL-2c fusion protein which consists of a LAG-3 binding domain and IL-2c, a chimeric molecule that contains a fragment from IL-15 and does not bind to IL-2 receptor alpha, to achieve the aforementioned purpose.

Methods The in vitro potency was determined with pSTAT5 signaling assay and human PBMC proliferation assay. The in vivo efficacy was evaluated in syngeneic CT26 mouse model with a surrogate molecule. A humanized αLAG-3-IL-2c fusion protein was tested on hLAG-3-knocked-in (KI) mice with MC38 model, as well as in combination with mesothelin-targeted CAR-T on NSG mice with N87 gastric cancer xenograft model.

Results αLAG-3-IL-2c induced a greater pSTAT5 activation signal in LAG-3+ T cells than in LAG-3- T cells. Additionally, αLAG-3-IL-2c enhanced in vitro proliferation in LAG-3+ T cells compared to untargeted IL-2c by approximately 1000 folds. Furthermore, among the LAG-3+ cell population, αLAG-3-IL-2c expanded CD8+ T cells to a greater extent compared to CD4+ T cells. In the syngeneic CT26 model and hLAG-3 KI model, αLAG-3-IL-2c induced a much stronger anti-tumor response compared to LAG-3 mAb, IL-2c, or LAG-3 mAb in combination with IL-2c. Moreover, αLAG-3-IL-2c showed enhanced intratumoral CD8+ T cells proliferation and anti-tumor efficacy in a CART model, demonstrating the promising usage of αLAG-3-IL-2c to combine with CART therapy.

Conclusions Altogether, these results indicate that αLAG-3-IL-2c is a potent tumor-infiltrating CD8+ T agonist without notable peripheral toxicity.

Ethics Approval The protocol of animal studies has been reviewed and approved by IACUC.