1088 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 MC-38 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.

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