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

Anwita Biosciences
Zoey Huang zhuang@anwitabio.com
Ziyang Zhong zzhong@anwitabio.com
300 Industrial Rd. San Carlos, CA 94070

Zoey Huang, Jianing Huang, Fan Ye, Sandra Chen, Danny Huang, Michael Hua, Ella Li, Jenny Jiang, Hanna Lin, Shirley Shi, Henry He, Mingxing Yang, Qiang Fu, Mingyan Yang, Mengting Feng, Ziyang Zhong

sitc 2022 SITC NOVEMBER 8-12 BOSTON MASSACHUSETTS
Abstract Number: 1088

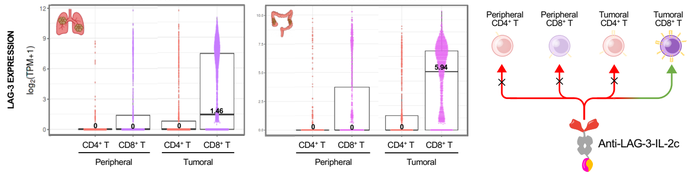
INTRODUCTION

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[1]. 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[2].

We have previously engineered a chimeric IL-2 which contains a fragment from IL-15 and does not bind to IL-2Rα. We performed further protein engineering to generate an IL-2c, a potency optimized No-α-IL2. IL-2c is demonstrated to bypass systemic stimulation of regulatory T cells and natural killer cells, while it retains its agonistic function to IL-2Rβγ expressing immune cells upon accumulation on cell surface with a cell target.

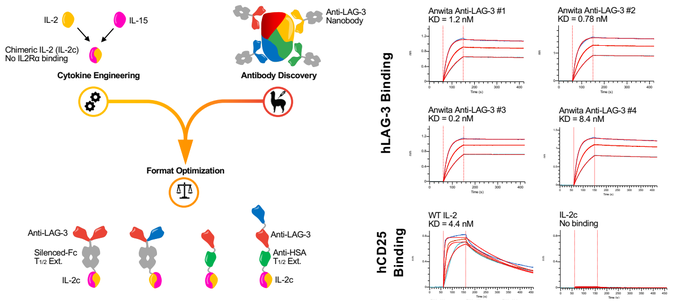
To achieve such specific local accumulation, we engineered a fusion protein consists of an anti-LAG-3 antibody and IL-2c. LAG-3 emerged as the next-generation inhibitory immune checkpoint following CTLA-4, PD-1, and PD-L1[3]. Simultaneous blockade of LAG-3 and PD-1 has shown favorable clinical outcomes in PD-1/PD-L1 resistant melanoma patients[4]. In addition, LAG-3 is highly expressed on tumor-specific CD8⁺ T cells[5,6], which makes it an appealing target to be used in combination with IL-2c as a bifunctional fusion protein for tumor immunotherapy. The engineered anti-LAG-3-IL-2c shows selective activation towards activated CD8⁺ T cells and consequently efficacious antitumor activity in multiple tumor models without obvious clinical sign of toxicity. Furthermore, anti-LAG-3-IL-2c is demonstrated to have synergy with anti-PD-1 antibody, as well as an extraordinary ability to promote CAR-T functionality, suggesting its vast potential for many applications.

MODE OF ACTION FOR DRUG DESIGN



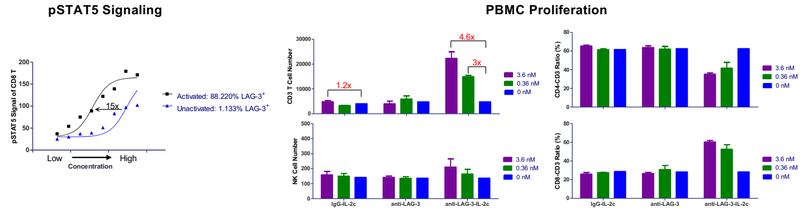
- LAG-3 is highly expressed on intratumoral cytotoxic CD8⁺ T cells
- A fusion protein consists of anti-LAG-3 and IL-2c, which has modified potency towards IL-2R, would selectively target and stimulate intratumoral CD8⁺ T cells, hence promote antitumor efficacy with minimized systemic toxicity

MOLECULAR DESIGN



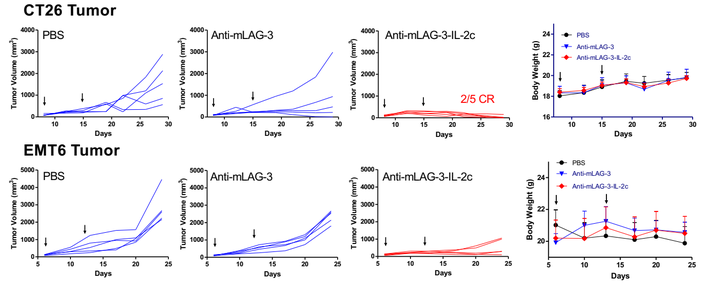
- Anwita IL-2c, which has no binding to IL-2Rα (CD25), was created by fusion of IL-2 and IL-15 sequences.
- Multiple humanized anti-LAG-3 nanobodies with unique binding epitopes were developed for IL-2c fusion
- Various molecular formats including mono- and bi-paratopic fusion were created and optimized to achieve optimal biological performance

SELECTIVE TARGETING OF ACTIVATED CD8⁺ T CELL



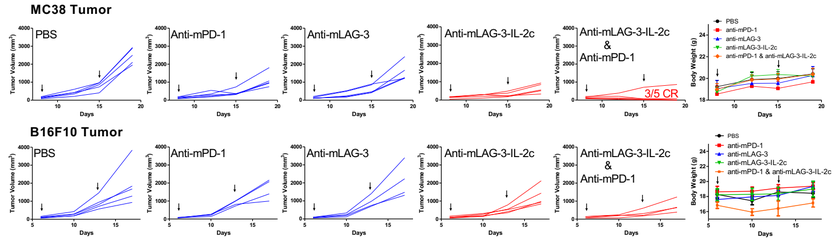
- Anti-LAG-3-IL-2c is a more potent inducer of pSTAT5 signaling in activated CD8⁺ T cells than unactivated CD8⁺ T cells
- Anti-LAG-3-IL-2c is effective at expanding activated PBMC while untargeted IL-2c is ineffective
- Anti-LAG-3-IL-2c preferentially targets and expands activated CD8⁺ T cells than CD4⁺ T cells
- NK proliferation is minimally induced by anti-LAG-3-IL-2c

ANTI-mLAG-3-IL-2c EFFECTIVELY INHIBITS TUMOR GROWTH



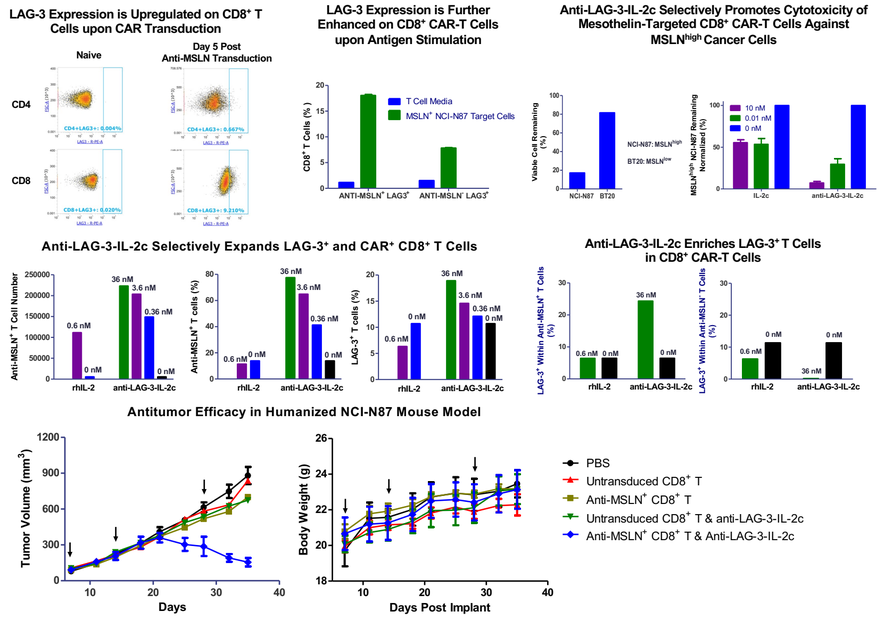
- Anti-mLAG-3-IL-2c demonstrated potent antitumor efficacy as mono-agent therapy in multiple tumor models including CT26 with 2/5 CR and EMT6 with no obvious body weight loss or other clinical signs of toxicity

ANTI-mLAG-3-IL-2c SYNERGIZES WITH ANTI-mPD-1



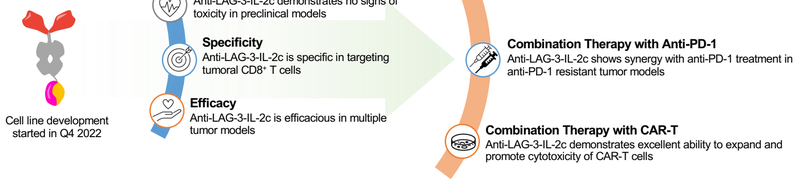
- Anti-mLAG-3-IL-2c and anti-mPD-1 showed synergy in tumor suppression with no obvious body weight loss or other clinical signs of toxicity in MC38 tumor model with 3/5 CR and B16F10 tumor model

ANTI-LAG-3-IL-2c PROMOTES MESOTHELIN-TARGETED CAR-T FUNCTIONALITY



- LAG-3 expression is upregulated on CD8⁺ T cells after anti-MSLN CAR transduction and is further enhanced on mesothelin-targeted CD8⁺ CAR-T cells upon antigen stimulation
- Mesothelin-targeted CD8⁺ CAR-T cells have selective cytotoxicity, which is further strengthened by treatment with anti-LAG-3-IL-2c, against tumor cells with high mesothelin expression
- Anti-LAG-3-IL-2c robustly expands mesothelin-targeted CD8⁺ CAR-T cells and enriches LAG-3⁺ CAR-T cells in vitro
- Anti-LAG-3-IL-2c shows potent antitumor efficacy in humanized mice engrafted with mesothelin-targeted CD8⁺ CAR-T cells

CONCLUSIONS



References
1. Jiang et al. *Oncoimmunology*. 2016
2. Krieg et al. *Proc Natl Acad Sci*. 2010
3. Long et al. *Genes Cancer*. 2018
4. Tawbi et al. *N Engl J Med*. 2022
5. Guo et al. *Nat Med*. 2018
6. Zhang et al. *Nature*. 2018

Download this poster →