LAG3-targeted IL15/IL15Rα-Fc (LAG3 x IL15) fusion proteins for preferential TIL expansion

Matthew J. Bernett, Suzanne Schubbert, Michael Hackett, Lukasz J. Ochyl, Lizett E. Scott, Christine Bonzon, Rumana Rashid, Kendra N. Avery, Irene W. L. Leung, Nicole Rodriguez, Connie Ardila, Umesh S. Muchhal, Norman J. Barlow, Rena Bahjat, John R. Desjarlais

*Contact: mbernett@xencor.com

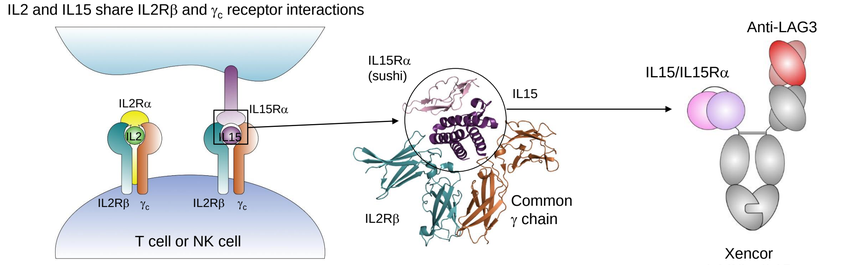
AACR 2022
Abstract #2080

Introduction

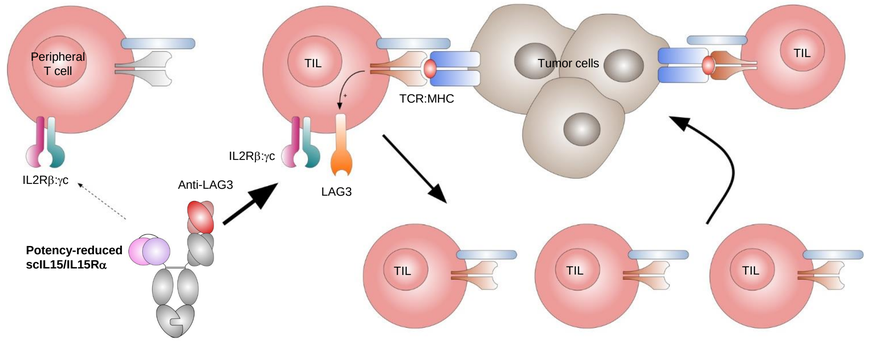
- IL2 and IL15 are potent cytokines that cause activation and proliferation of T and NK cells. Having evolved for local activity at very low concentrations, they suffer from low tolerability and very fast clearance that limits therapeutic window when given systemically as therapeutics.
- Tumor-infiltrating lymphocytes (TIL) are known to express multiple immune checkpoints (CP) such as PD1 and LAG3, and these have limited peripheral expression in normal human PBMCs.
- We hypothesized that we could selectively target tumor-reactive TIL by combining a reduced potency IL15/IL15Rα heterocomplex with a Fab-based LAG3-targeting arm to bias binding and activation to LAG3-positive TILs, potentially improving therapeutic index.
- LAG3 was chosen as the CP targeting-arm due to its frequent co-expression with PD1, bias to CD8+ T cells, ability to easily combine with anti-PD1 agents, and recent promising results with anti-LAG3 agents in the clinic.

LAG3 x IL15 bispecific Fc fusions are engineered for optimal activity to LAG3⁺ TIL with minimal peripheral activity

Rationale and design of LAG3 x IL15



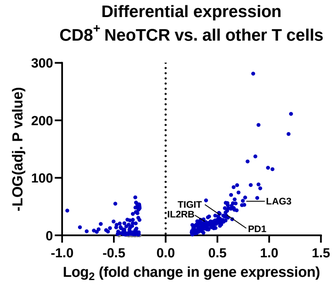
LAG3 x IL15 are designed to selectively deliver IL15 to LAG3⁺ TIL while avoiding peripheral lymphocytes

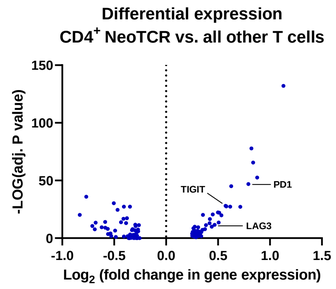


- **Potency-reduced** IL15/IL15Rα(sushi domain) and a Fab arm targeting LAG3 are attached to Xencor's heterodimeric Fc domain
- The Fc domain is further modified to eliminate FcγR interactions and contains Xtend™ Fc technology to promote longer half-life and extended pharmacodynamics (PD)
- Fab domain targets IL15/IL15Rα to LAG3⁺ TIL; minimal peripheral activity on LAG3⁻ cells due to reduced potency IL15 arm and low or lack of LAG3 expression on normal cells

LAG3 is CD8-biased and LAG3 x IL15 demonstrates potent activity and high selectivity for LAG3⁺ T cells in vitro

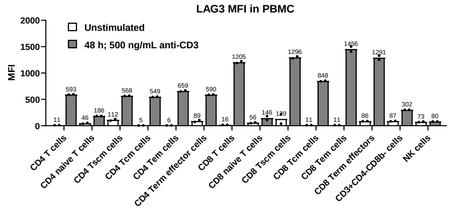
LAG3 is CD8-biased and more highly expressed on CD8⁺ neoantigen specific T cells compared to PD1



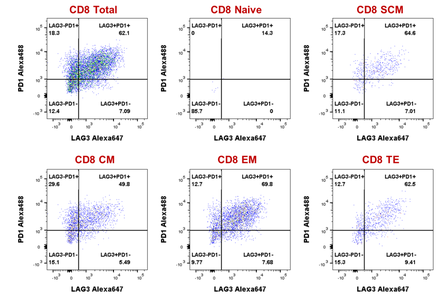


Data from F. J. Lowery et al., Science 10.1126/science.abl5447 (2022). Data from scRNA-seq and TCR-seq on CD8+ and CD4+ T cells within metastatic cancers from 10 patients across multiple solid tumor types.

LAG3 expression is low on normal huPBMC but can be upregulated on T cells activated with anti-CD3

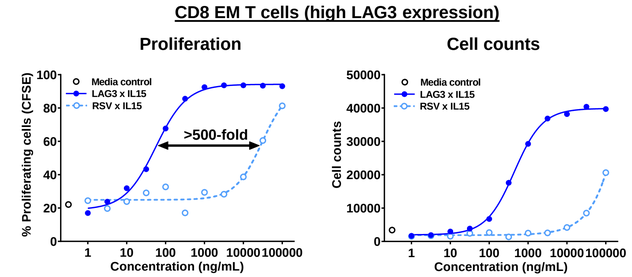


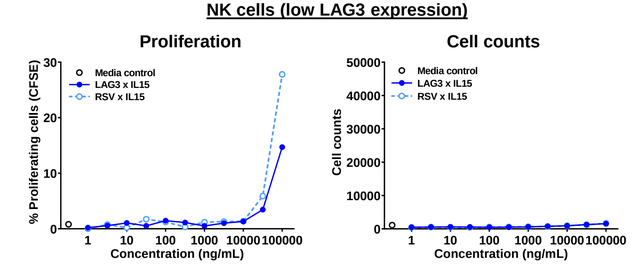
On activated huPBMC, LAG3 expression is highest on CD8 effector memory T cells and LAG3 is largely coexpressed with PD1



Human PBMC were activated with 500 ng/mL plate-bound anti-CD3 (OKT3) for 48 h and then analyzed by flow cytometry for LAG3 and PD1 expression.

LAG3 x IL15 induces proliferation and increases LAG3⁺ cell counts; has pronounced selectivity (>500-fold) for LAG3⁺ cells in vitro

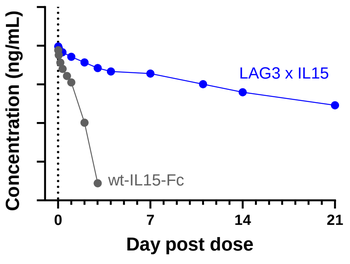




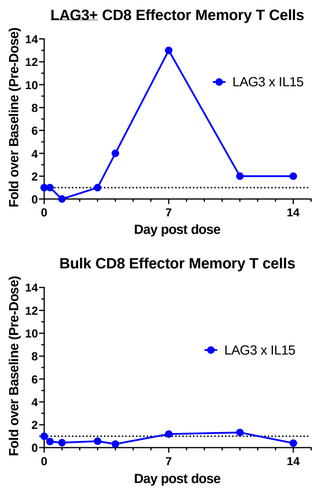
LAG3 x IL15 was constructed along with RSV x IL15 (anti-RSV Fab-arm) as a control for untargeted activity. Human PBMC were activated with 500 ng/mL plate-bound anti-CD3 (OKT3) for 48 h, labeled with CFSE, treated with targeted-IL15 molecules for 4 days at 37 C, and then analyzed by flow cytometry.

LAG3 x IL15 has superior in vivo PK and shows selective targeting of LAG3⁺ peripheral T cells in NHP

LAG3 x IL15 has superior PK compared to wt-IL15-Fc in NHP

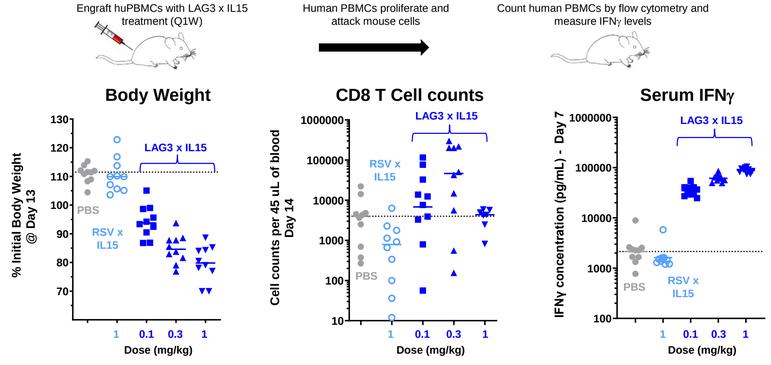


LAG3 x IL15 has selectivity for LAG3⁺ cells in NHP



A single dose of LAG3 x IL15 or IL15/IL15Rα-Fc (wild-type IL15) was administered to cynomolgus monkeys (NHP). Drug concentrations (PK, top) and lymphocyte counts (PD, right) were monitored over time.

LAG3 x IL15 promotes T cell proliferation and IFNγ production in huPBMC-engrafted NSG mice (GVHD model)



Summary

- LAG3 x IL15 bispecific Fc fusions were constructed and show high selectivity for LAG3⁺ cell populations in multiple in vitro and in vivo models.
- LAG3 x IL15 shows a promising profile of selective delivery to LAG3⁺ cells with minimal peripheral activity and may help to preferentially expand LAG3⁺ TIL in patients with cancer, while potentially avoiding systemic toxicity due to off-target activation and expansion of peripheral lymphocytes.