



A Novel Method for Generating Regulated Cytokine Therapeutics: Safety and Activity of a Conditionally Active cLAG3-IL2 Capable of Delivering IL-2 to LAG-3⁺ Cells While Remaining Inert on LAG-3⁻ Cells

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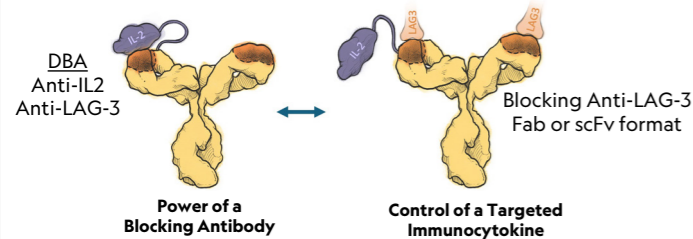


Introduction

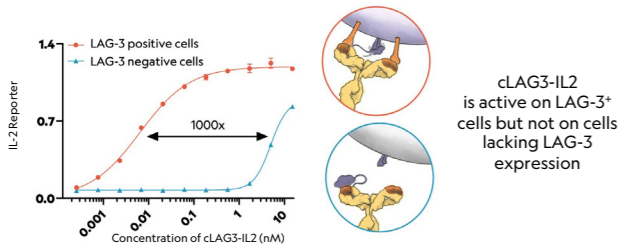
- IL-2 is a powerful cytokine, but it has seen limited use in the treatment of cancer due to its toxicity. Additional strategies to address these limitations are still needed.
- Our proprietary dual-binding antibody (DBA)-based platform allows the creation of conditionally active immunocytokines.
- Conditionally active LAG3-IL2 (cLAG3-IL2) specifically targets IL-2 to LAG-3-expressing antigen-experienced T cells while remaining inactive on the majority of IL-2R⁺ cells. This combines the IL-2 cis-targeting activity of a LAG3-IL2 immunocytokine when bound to LAG-3 with the "offness" of an IL-2 neutralizing antibody when unbound.
- In vitro activity of cLAG3-IL2 on reporter cell lines and LAG-3⁺ human T cells demonstrates conditional IL-2 signaling dependent on LAG-3 binding.
- In syngeneic mouse tumor models, cLAG3-IL2 inhibits tumor growth while avoiding clinical signs of IL-2 toxicity, even at high doses.
- cLAG3-IL2 drives the expansion and activation of tumor-specific CD8⁺ T without increasing peripheral IL-2R⁺ NK cell or T cell numbers.

Conditional IL-2 Cis-Targeting Using Dual-Binding Antibody-Based Regulation

Dual Binding Antibody (DBA): Recognizes two distinct antigens
Bonum's regulated therapeutics utilize standard antibody and linker components
DBA-cytokine regulation domains are portable to multiple formats

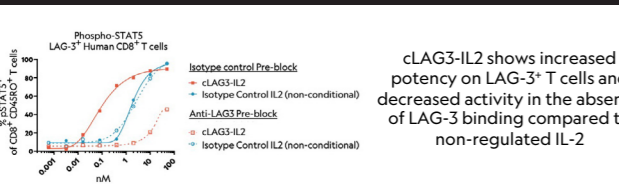


cLAG3-IL2 Preferentially Signals on LAG-3⁺ Cells



In vitro activity of cLAG3-IL2 on LAG-3-transfected (red) or mock transfected (blue) IL2 HEK-Blue reporter cells

Figure 1: cLAG3-IL2 Preferentially Signals on LAG-3⁺ Human CD8⁺ T Cells



Human T cells were activated with anti-CD3/CD28 to induce LAG-3 expression. Cells were then blocked with either anti-LAG-3 or an isotype control prior to treatment with cLAG3-IL2 or non-conditional IL-2 for 20 min. Frequency of pSTAT5⁺ cells was determined by flow cytometry.

Figure 2: cLAG3-IL2 Demonstrates Anti-Tumor Activity in the MC38 Tumor Model

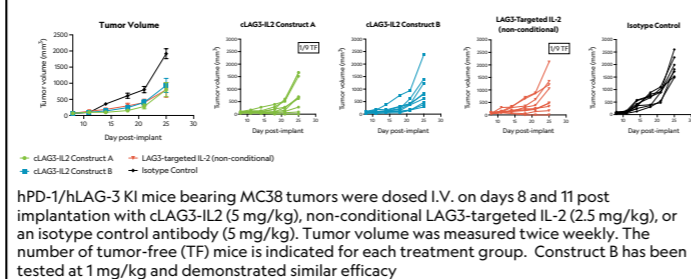


Figure 3: cLAG3-IL2 Avoids IL-2-Mediated Toxicity and Does Not Expand Peripheral IL-2R⁺ Cells

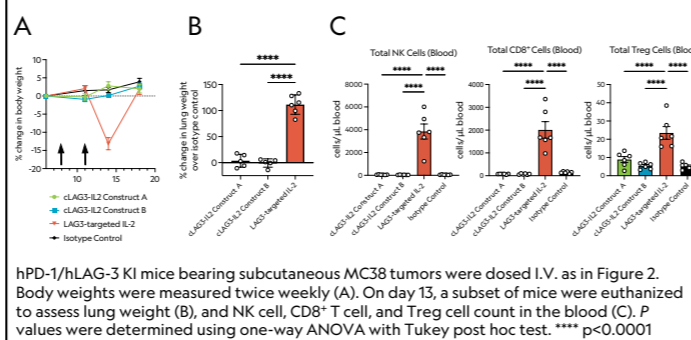


Figure 4: cLAG3-IL2 Induces CD8⁺ TIL Expansion and Activation

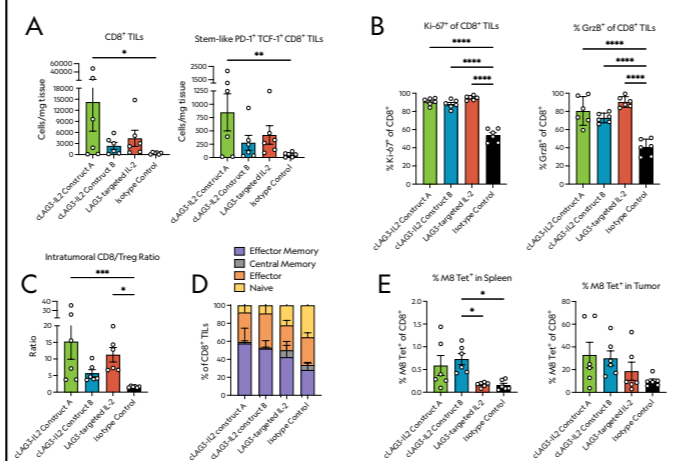


Figure 5: cLAG3-IL2 Enhances Tumor-Infiltrating CD8⁺ T Cell Cytokine Production

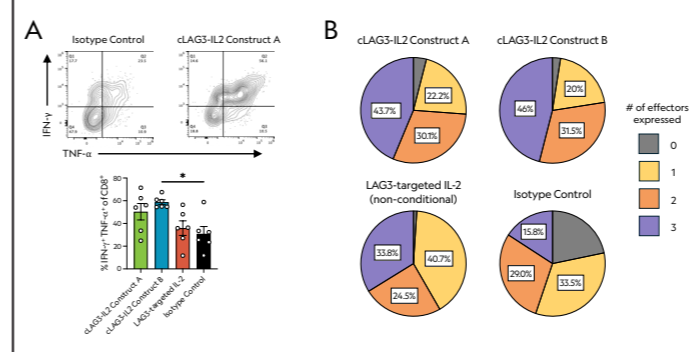


Figure 6: PD-1 and LAG-3 Expression are Increased on CD8⁺ TILs Following cLAG3-IL2 Treatment

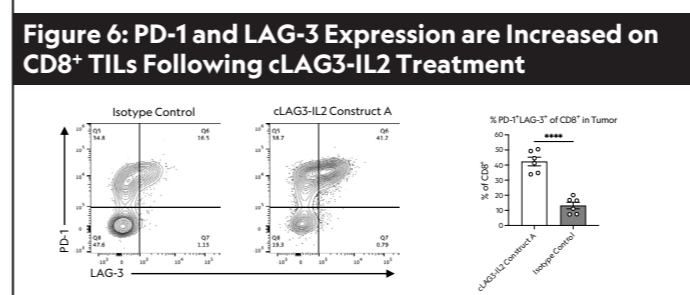
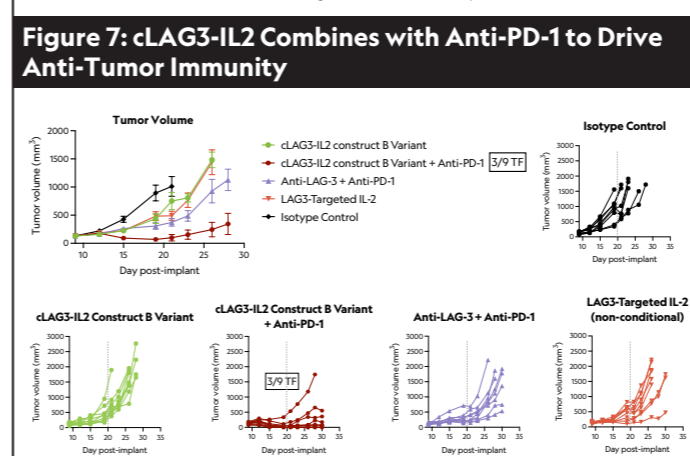


Figure 7: cLAG3-IL2 Combines with Anti-PD-1 to Drive Anti-Tumor Immunity



hPD-1/hLAG-3 KI mice bearing MC38 tumors were dosed I.V. on days 9 and 12 post implantation with cLAG3-IL2 (5 mg/kg) with or without anti-PD-1 (10 mg/kg), anti-LAG-3 (5 mg/kg) + anti-PD-1 (10 mg/kg), non-conditional LAG3-targeted IL-2 (2 mg/kg) or an isotype control (5 mg/kg). The number of tumor-free (TF) mice is indicated for each treatment group.

Figure 8: cLAG3-IL2 has Minimal IL-2 Activity in the Periphery at High Dose Levels

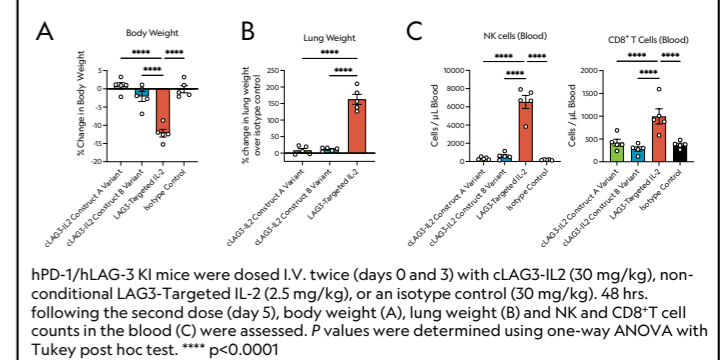


Figure 9: cLAG3-IL2 Demonstrates Monoclonal Antibody-Like Pharmacokinetics

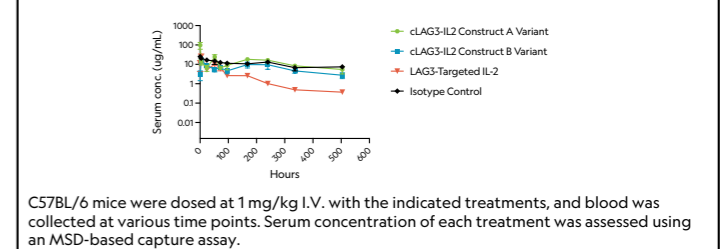
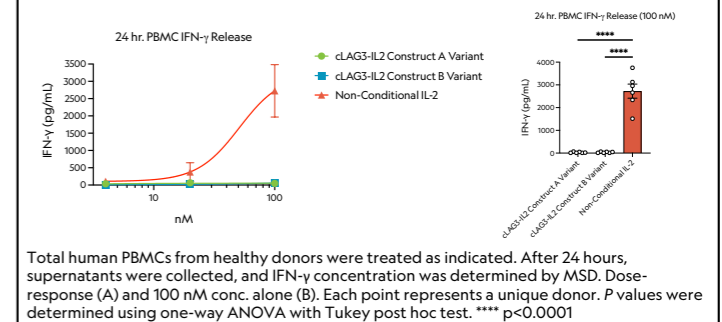


Figure 10: Minimal Cytokine Release from Human PBMCs treated with cLAG3-IL2



Summary

- Using a completely novel DBA-mediated regulation strategy, Bonum's conditionally active immunocytokines combine potent, cis-targeted cytokine delivery with antibody-mediated cytokine neutralization when unbound.
- Our conditionally-active cLAG3-IL2 constructs demonstrate dramatic LAG-3-dependent regulation in vitro and in vivo, a lack of toxicity at high doses, antibody-like PK, and excellent developability properties.
- This data supports the advancement of cLAG3-IL2 into IND-enabling studies.