

A Novel Method for Generating Regulated Cytokine Therapeutics: Safety and Activity of a Conditionally Active cLAG3-IL2 in a Simple Antibody Format



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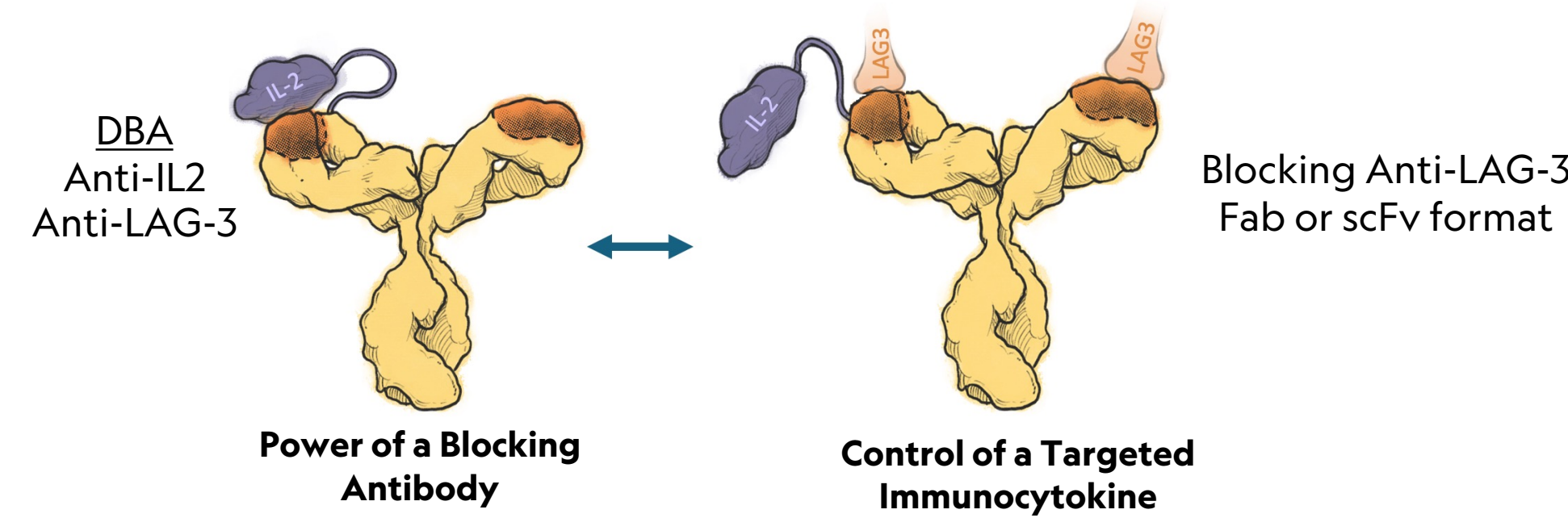
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Introduction

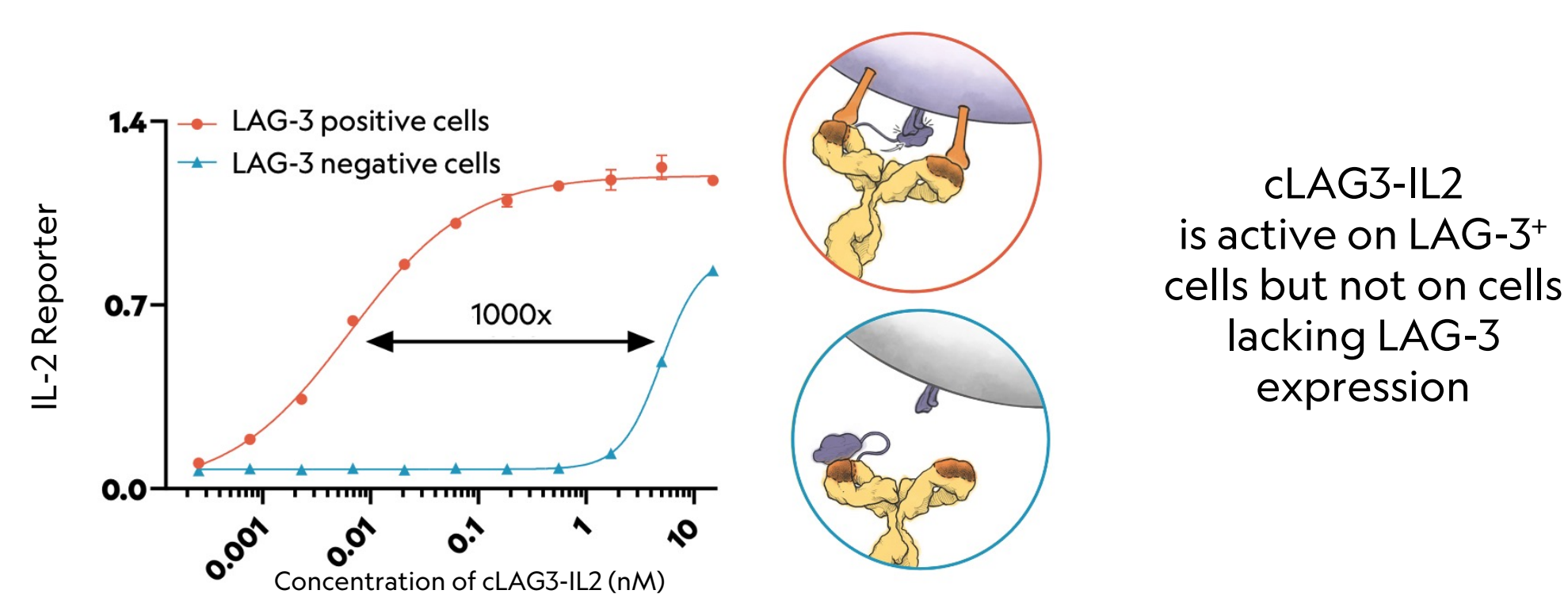
- IL-2 is a powerful cytokine, but it has seen limited use in the treatment of cancer due to its toxicity. Additional strategies to address these limitations are still needed.
- Our proprietary dual-binding antibody (DBA)-based platform allows the creation of conditionally active immunocytokines.
- Conditionally 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 DBA-Based Regulation

Dual Binding Antibody (DBA): Recognizes two distinct antigens

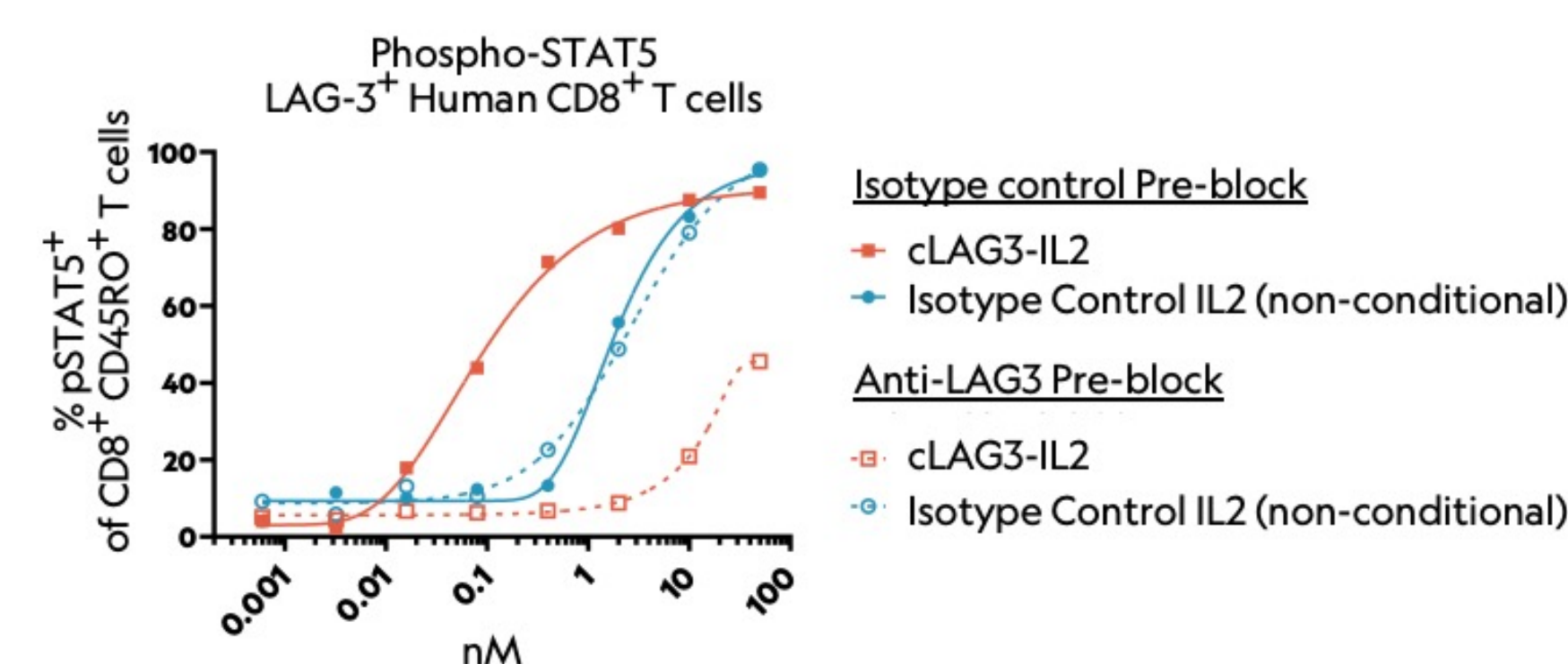


cLAG3-IL2 Preferentially Signals on LAG-3⁺ Cells



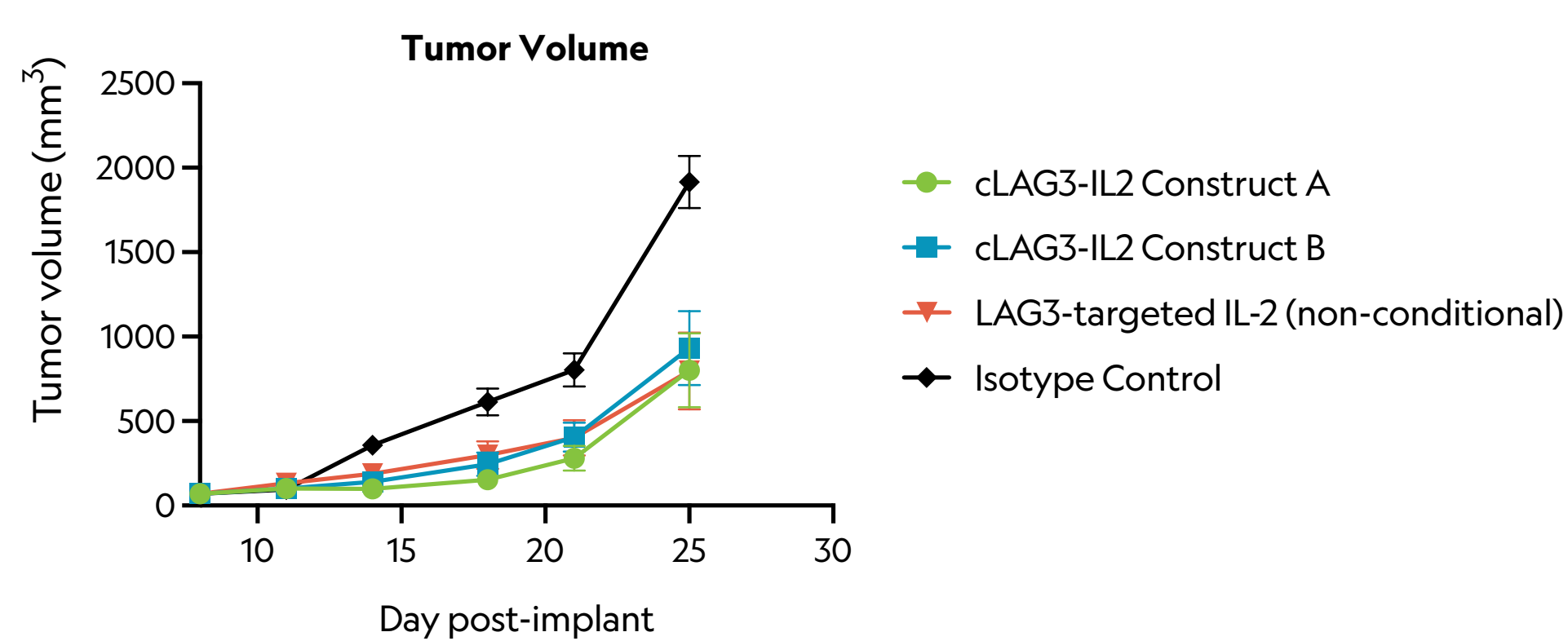
In vitro activity of cLAG3-IL2 on LAG-3-transfected (red) or mock transfected (blue) IL2 HEK-Blue reporter cells

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



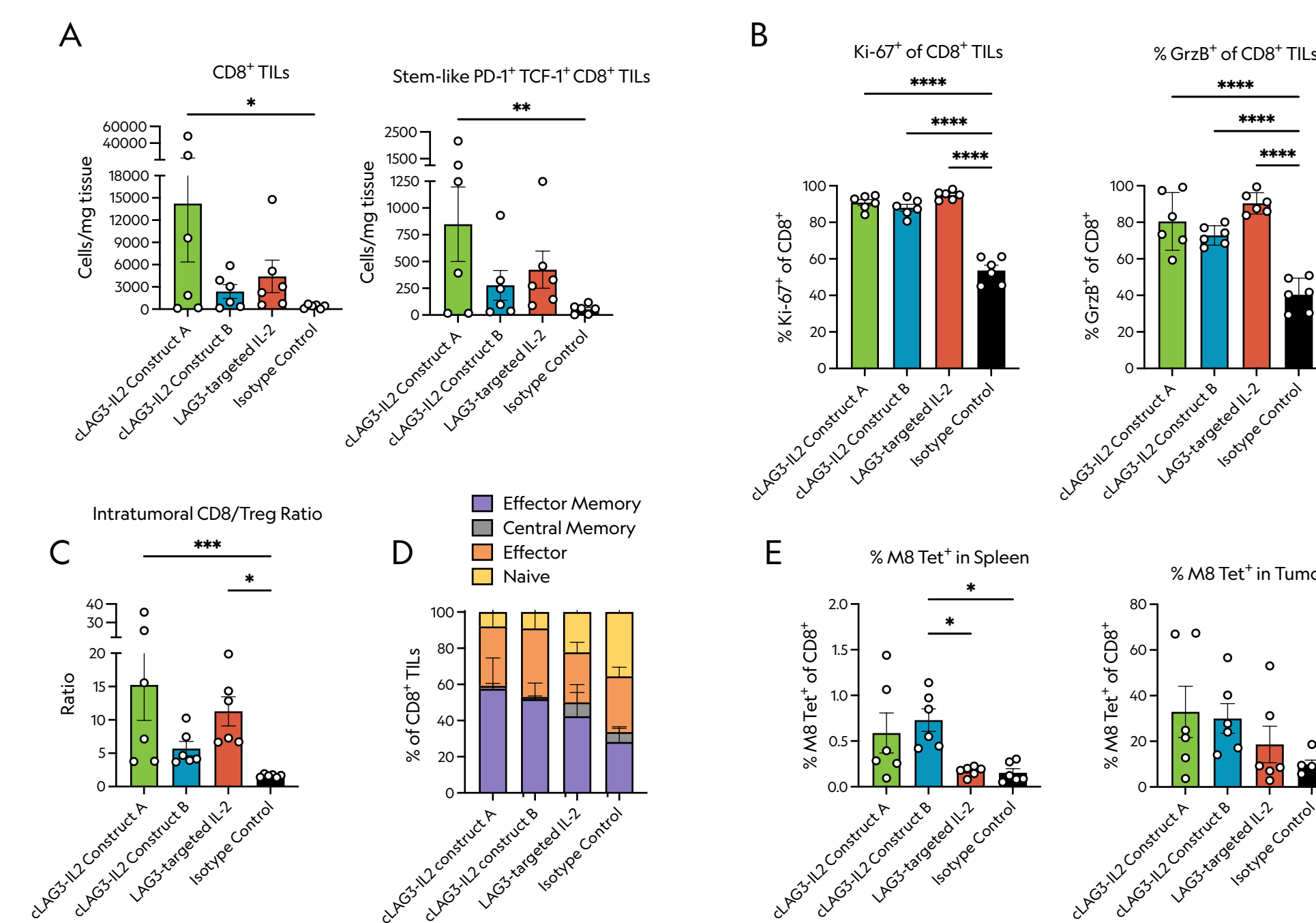
Human T cells were activated with anti-CD3/CD28 to induce LAG-3 expression. Cells were then blocked with either anti-LAG-3 or an isotype control prior to treatment with cLAG3-IL2 or non-conditional IL-2 for 20 min. Frequency of pSTAT5⁺ cells was determined by flow cytometry.

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



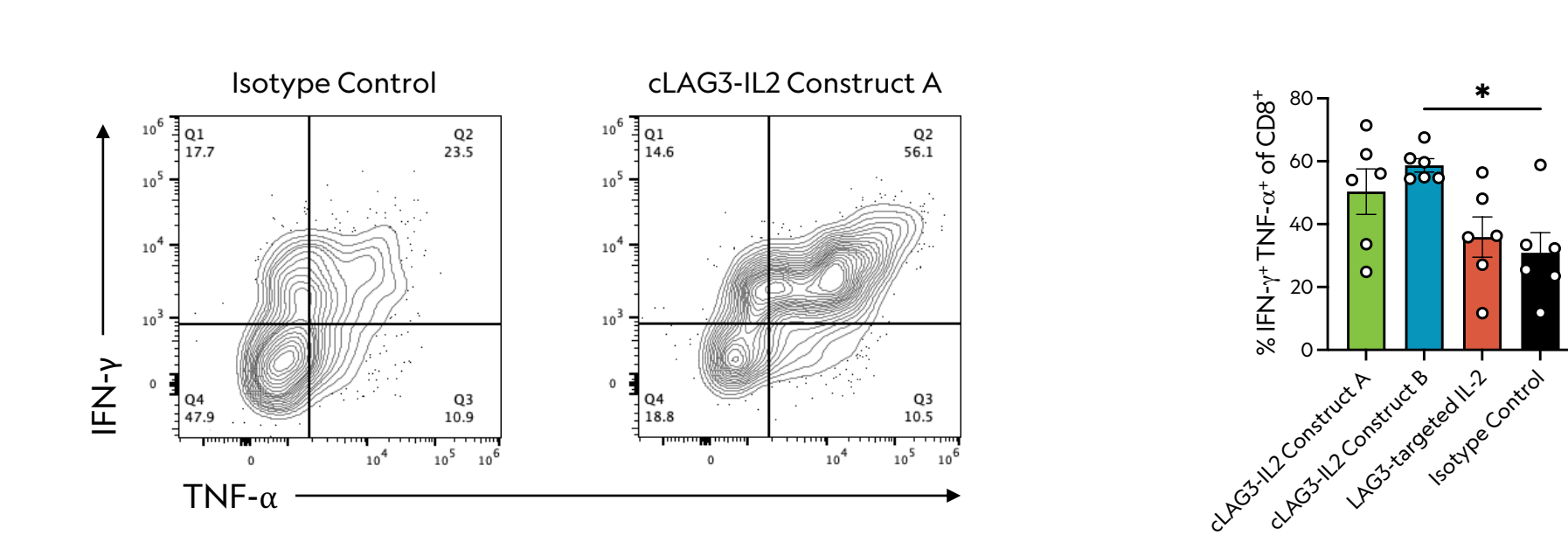
hPD-1/hLAG-3 KI mice bearing MC38 tumors were dosed I.V. on days 8 and 11 post implantation with cLAG3-IL2 (5 mg/kg), non-conditional LAG3-targeted IL-2 (2.5 mg/kg), or an isotype control antibody (5 mg/kg). Tumor volume was measured twice weekly. The number of tumor-free (TF) mice is indicated for each treatment group. Construct B has been tested at 1 mg/kg and demonstrated similar efficacy

Figure 3: cLAG3-IL2 Induces CD8⁺ TIL Expansion and Activation



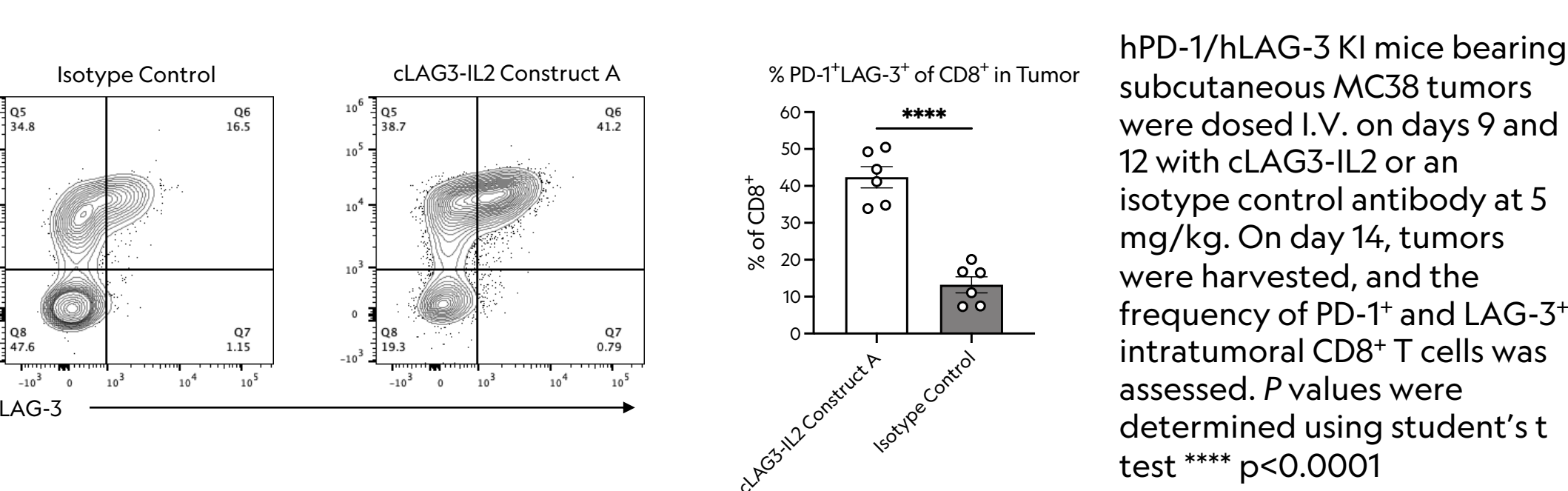
hPD-1/hLAG-3 KI mice bearing subcutaneous MC38 tumors were dosed I.V. as in Figure 2. On day 13, tissues were harvested to assess intratumoral CD8⁺ T cell and stem-like CD8⁺ T cell density (A), frequency of Ki-67⁺ and GrB⁺ CD8⁺ T cells (B), intratumoral CD8⁺/Treg ratio (C), frequency of CD8⁺ T cell subsets (D), and frequency of M8 tet⁺ T cells (E). P values were determined using one-way ANOVA with Tukey post hoc test. * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001

Figure 4: cLAG3-IL2 Enhances Tumor-Infiltrating CD8⁺ T Cell Cytokine Production



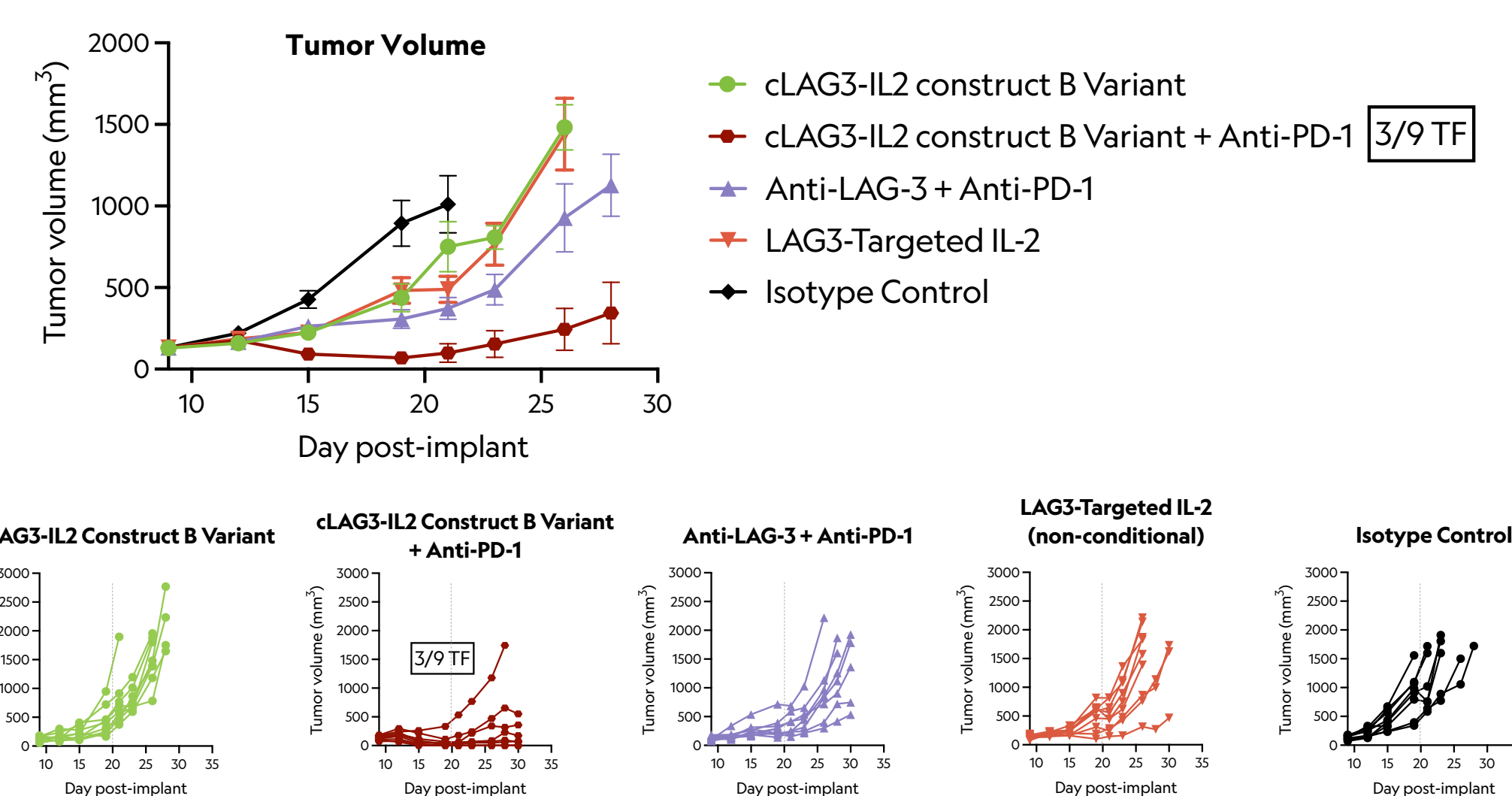
hPD-1/hLAG-3 KI mice bearing subcutaneous MC38 tumors were dosed I.V. as in Figure 2. On day 13, tumors were harvested, and IFN- γ , TNF- α , and GrzB expression were assessed following PMA/Ionomycin stimulation. Frequency of IFN- γ ⁺TNF- α ⁺ of CD8⁺ T cells. P values were determined using one-way ANOVA with Tukey post hoc test. * p<0.05

Figure 5: PD-1 and LAG-3 Expression are Increased on CD8⁺ TILs Following cLAG3-IL2 Treatment



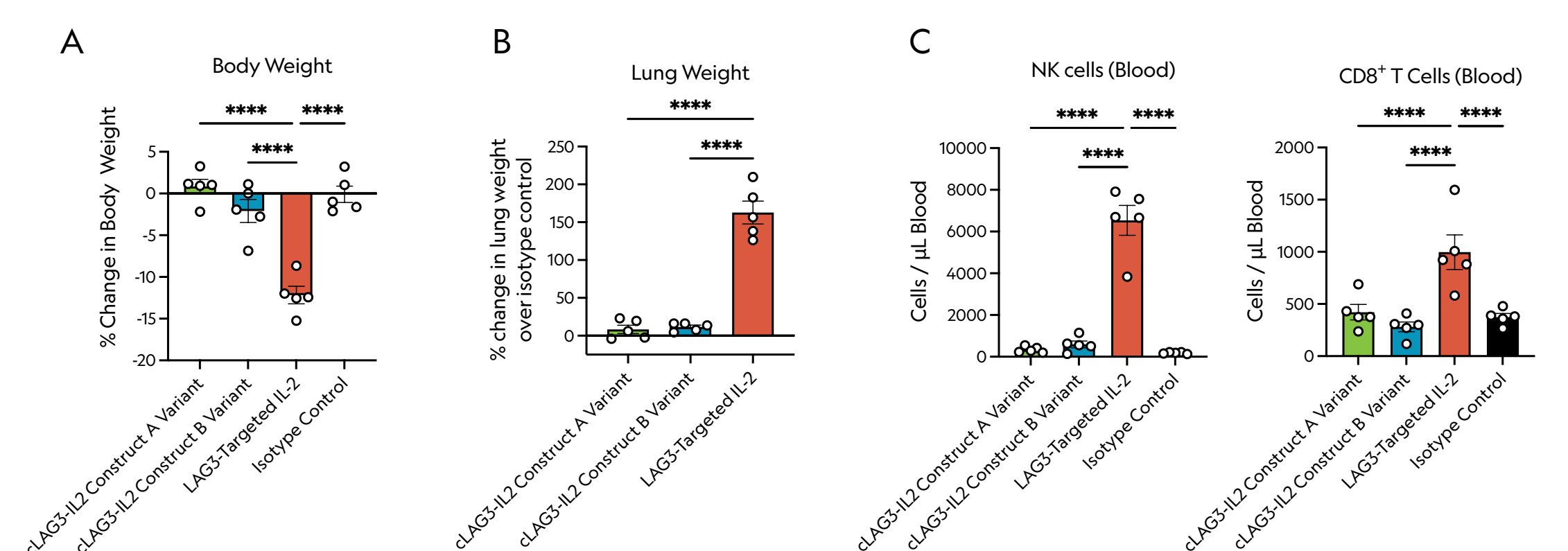
hPD-1/hLAG-3 KI mice bearing subcutaneous MC38 tumors were dosed I.V. on days 9 and 12 with cLAG3-IL2 or an isotype control antibody at 5 mg/kg. On day 14, tumors were harvested, and the frequency of PD-1⁺ and LAG-3⁺ intratumoral CD8⁺ T cells was assessed. P values were determined using student's t test **** p<0.0001

Figure 6: cLAG3-IL2 Combines with Anti-PD-1 to Drive Anti-Tumor Immunity



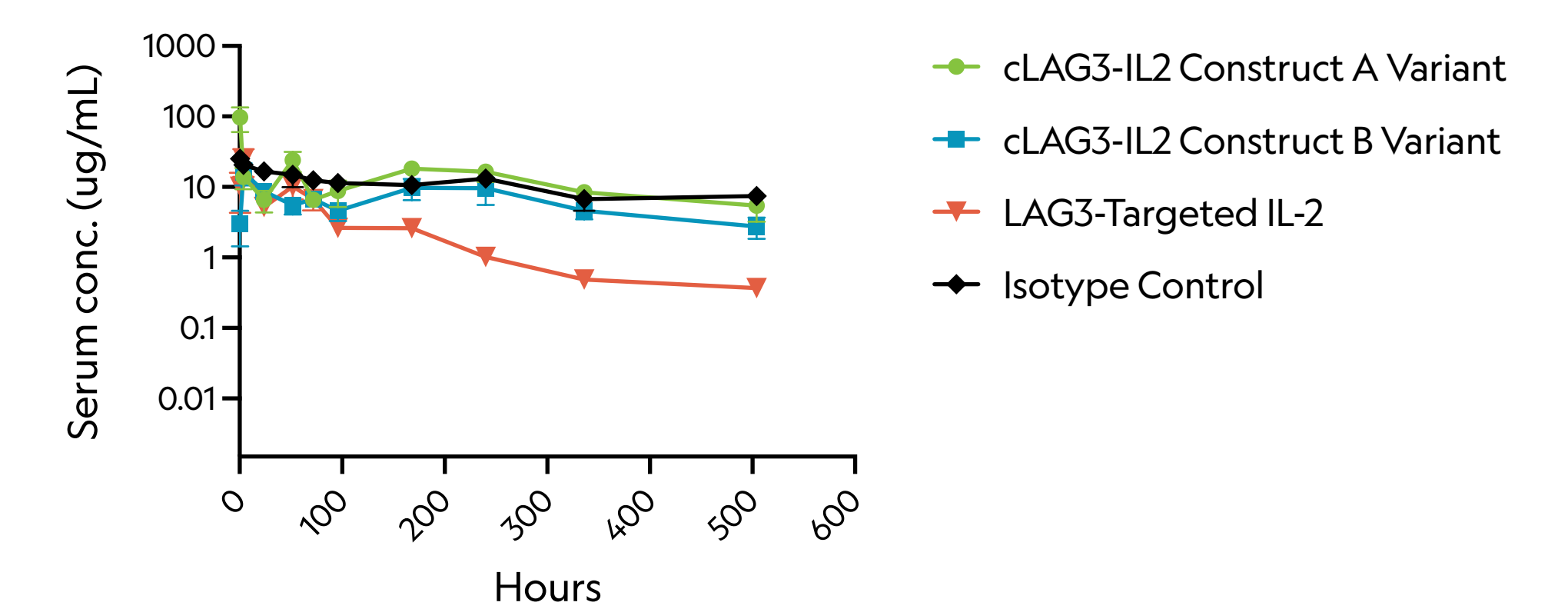
hPD-1/hLAG-3 KI mice bearing MC38 tumors were dosed I.V. on days 9 and 12 post implantation with cLAG3-IL2 (5 mg/kg) with or without anti-PD-1 (10 mg/kg), anti-LAG-3 (5 mg/kg) + anti-PD-1 (10 mg/kg), non-conditional LAG3-targeted IL-2 (2 mg/kg) or an isotype control (5 mg/kg). The number of tumor-free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

Figure 7: cLAG3-IL2 has Minimal IL-2 Activity in the Periphery at High Dose Levels



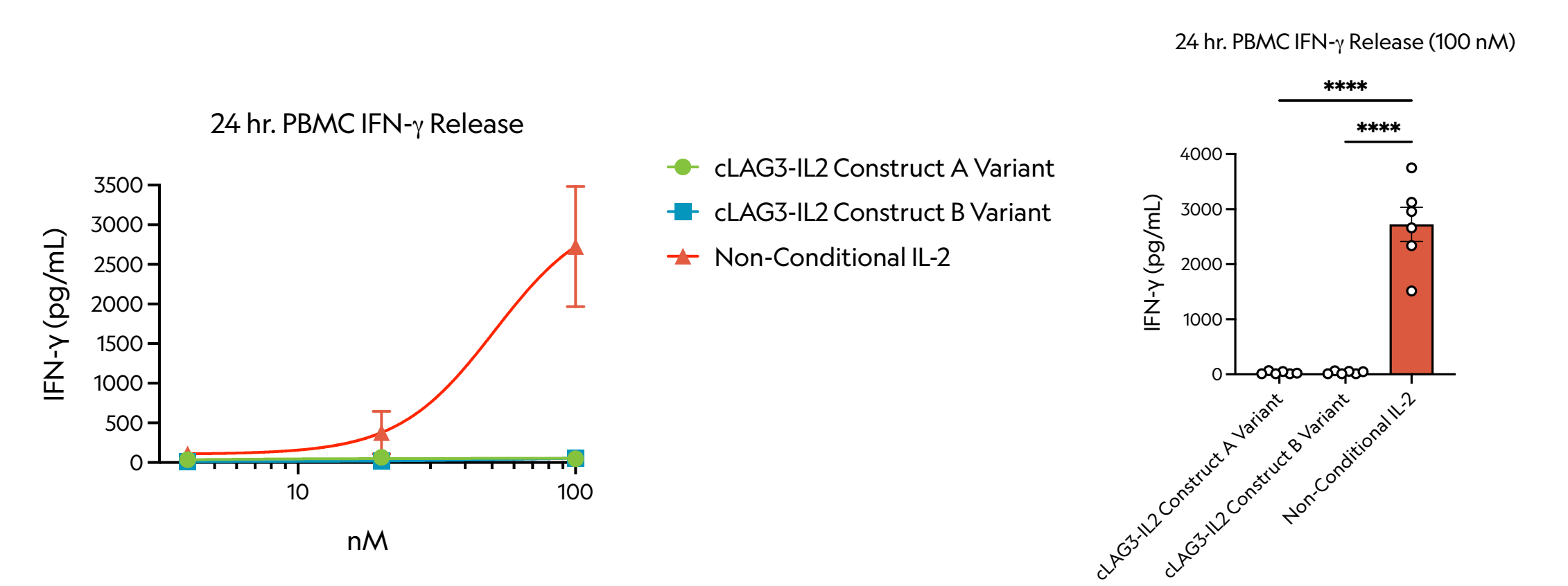
hPD-1/hLAG-3 KI mice were dosed I.V. twice (days 0 and 3) with cLAG3-IL2 (30 mg/kg), non-conditional LAG3-Targeted IL-2 (2.5 mg/kg), or an isotype control (30 mg/kg). 48 hrs. following the second dose (day 5), body weight (A), lung weight (B) and NK and CD8⁺ T cell counts in the blood (C) were assessed. P values were determined using one-way ANOVA with Tukey post hoc test. **** p<0.0001

Figure 8: cLAG3-IL2 Demonstrates Monoclonal Antibody-Like Pharmacokinetics



C57BL/6 mice were dosed at 1 mg/kg I.V. with the indicated treatments, and blood was collected at various time points. Serum concentration of each treatment was assessed using an MSD-based capture assay.

Figure 9: Minimal Cytokine Release from Human PBMCs treated with cLAG3-IL2



Total human PBMCs from healthy donors were treated as indicated. After 24 hours, supernatants were collected, and IFN- γ concentration was determined by MSD. Dose-response (A) and 100 nM concentration alone (B). Each point represents a unique donor. P values were determined using one-way ANOVA with Tukey post hoc test. **** p<0.0001

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.
- These data support the advancement of cLAG3-IL2 into IND-enabling studies.