

Targeting Highly attenuated IL18 (HT18) to PD-1: A Next Generation Checkpoint Inhibitor with Enhanced Anti-tumor Activity

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Background

IL-18 is a danger induced cytokine that serves to promote the activity and survival of antigen presenting cells, NK cells and subsets of T cells. The cytokine is strongly regulated by a negative feedback loop that includes the induction of IL-18BP and IL-37, which act to bind IL-18 and IL-18R α , respectively. In the tumor microenvironment (TME), the IL-18R α β complex is expressed on PD-1⁺ progenitor exhausted T cells that are responsive to PD-1 antagonism. Targeting an IL-18BP resistant variant of IL-18, which is attenuated for safety, but retains activity when brought into close proximity of the IL-18 receptor complex is hypothesized to maintain survival of the α PD-1 responsive T cell pool. Thus, we developed a first-in-class variant of IL-18 termed "HT18" fused to an α PD-1 antibody (α PD1-HT18_{cis}). HT18 is 10,000-fold attenuated but retains nearly full activity of native cytokine when delivered to PD-1⁺ cells.

Findings

- HT18 did not bind IL-18BP nor IL-18R α in trans but can bind strongly to the IL-18R α β complex
- Targeting HT18 to PD-1 augmented T cell mediated proinflammatory cytokine release in-vitro
- Strong efficacy was observed with α PD1-HT18_{cis} in multiple aggressive mouse tumor models including a complete response (CR) rate ranging from 60%-100% without weight loss or other signs/symptoms of toxicity
- Immune memory and evidence supporting epitope spreading was observed in both mouse tumor models that induce both weak and strong emergency myelopoiesis
- TGI was associated with about a 6-fold increase in TIL numbers relative to the PD-1 antibody benchmark with significant increases in CD8⁺ T cells, effector and central memory T cells, reduced tumor associated macrophages (TAM) and skewing towards a proinflammatory anti-tumor cellularity

Unique mechanism renders HT18 active when targeted to PD-1⁺/IL-18R⁺ cells but 10,000-fold attenuated in circulation

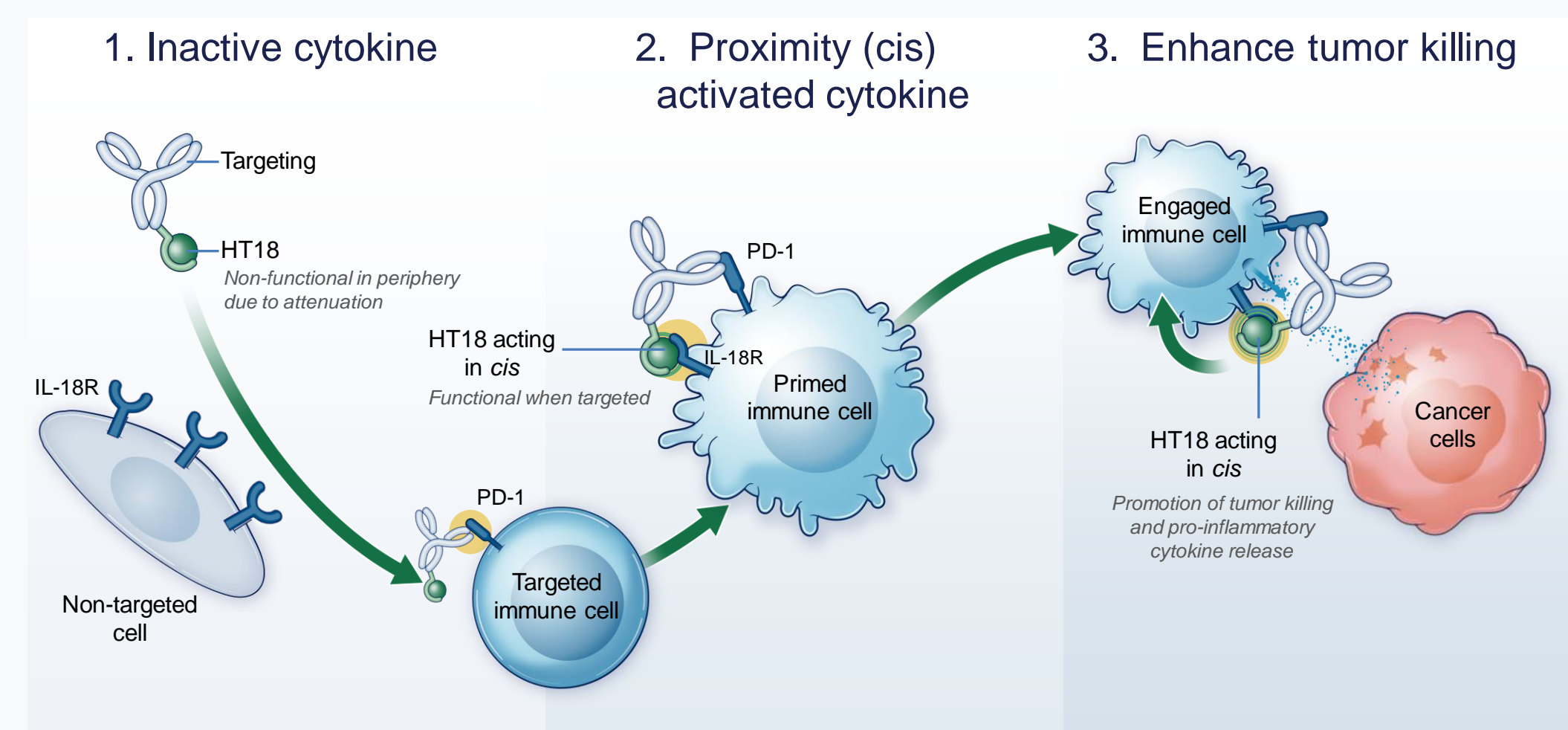


Figure 1. α PD1-HT18_{cis} is 10,000 less active than IL-18 unless bound to cells that express both PD-1-IL-18R. In the tumor, PD-1⁺/IL-18R⁺ T cells are functional and respond to PD-1 checkpoint inhibition.

Safe and effective delivery of IL-18 to PD-1⁺ T cells necessitates resistance to IL-18BP and attenuation

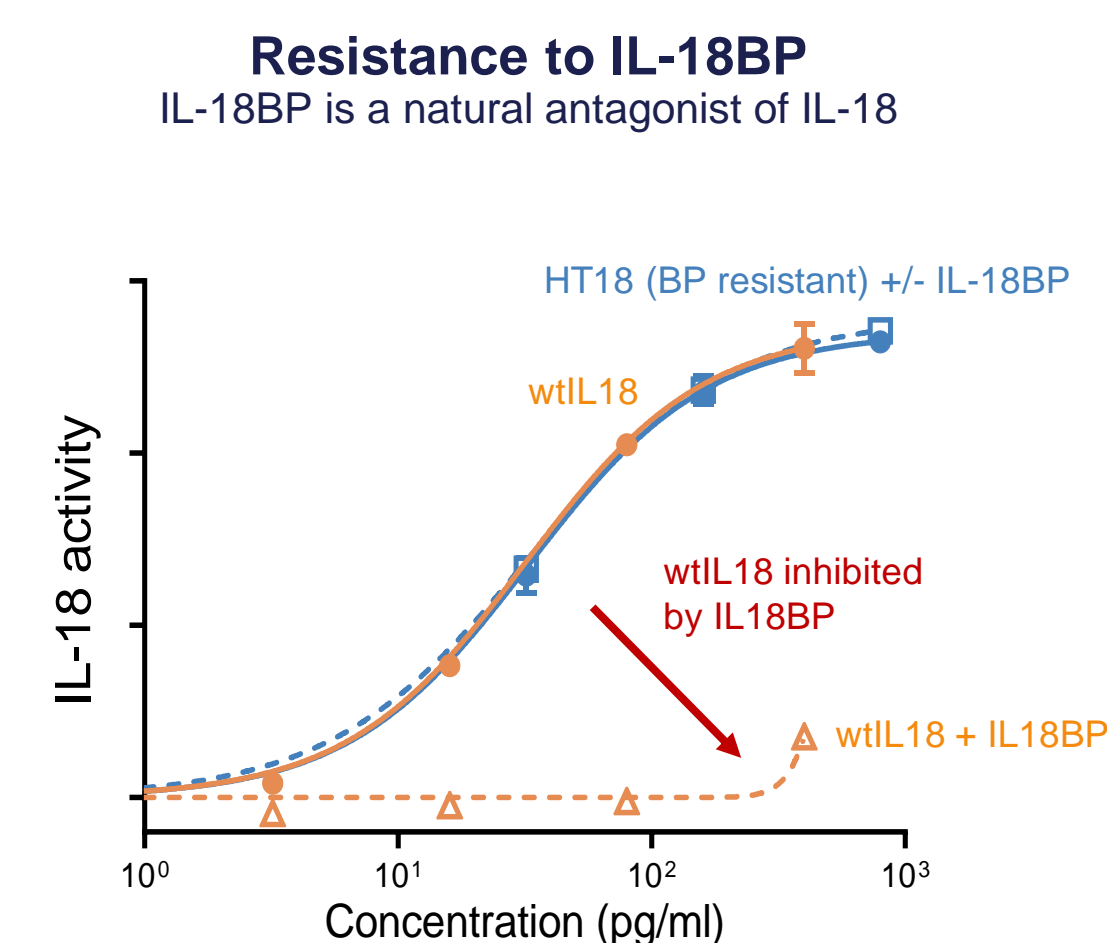


Figure 2. Impact of 100-fold excess IL-18BP on activity of wtIL-18 and HT18 activity in HEK-Blue-IL-18 reporter assay

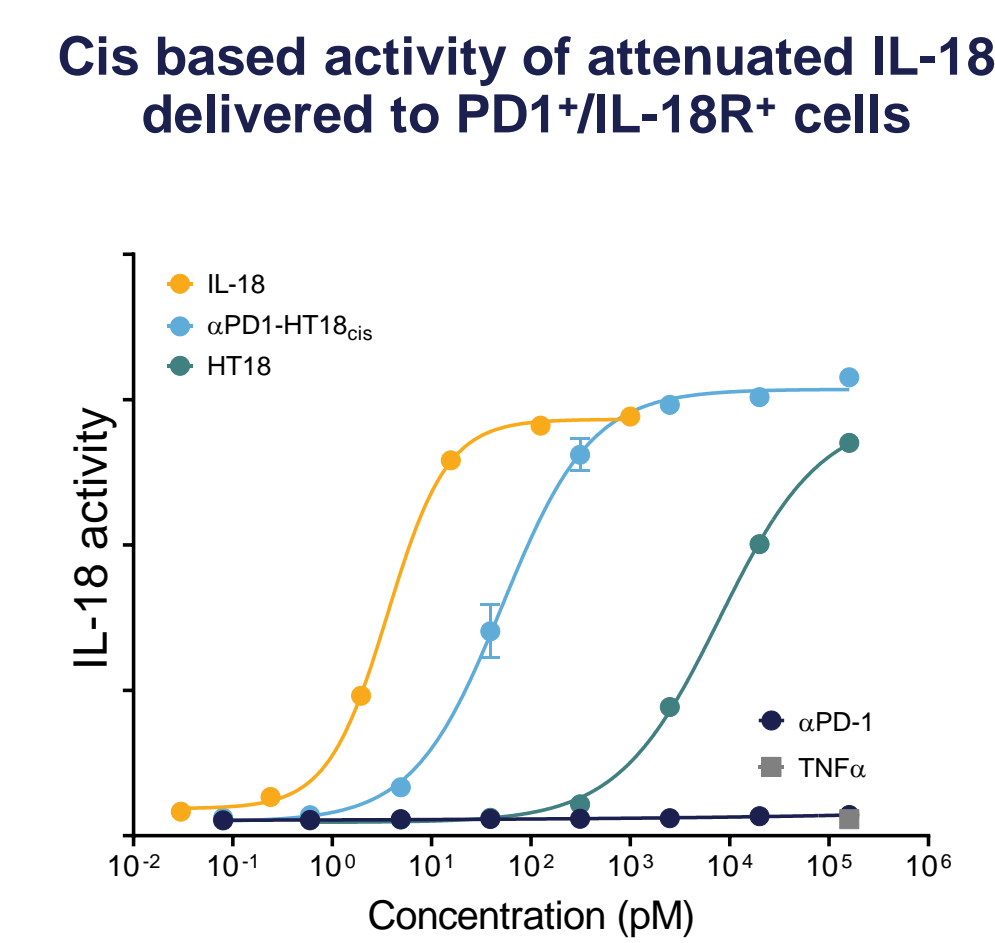


Figure 3. Impact of targeting HT18 to PD-1⁺ HEK-Blue-IL-18. Exemplary test articles normalized across >5 experiments

Affinity of IL-18 and HT18 for IL-18R α and the IL-18R α β complex

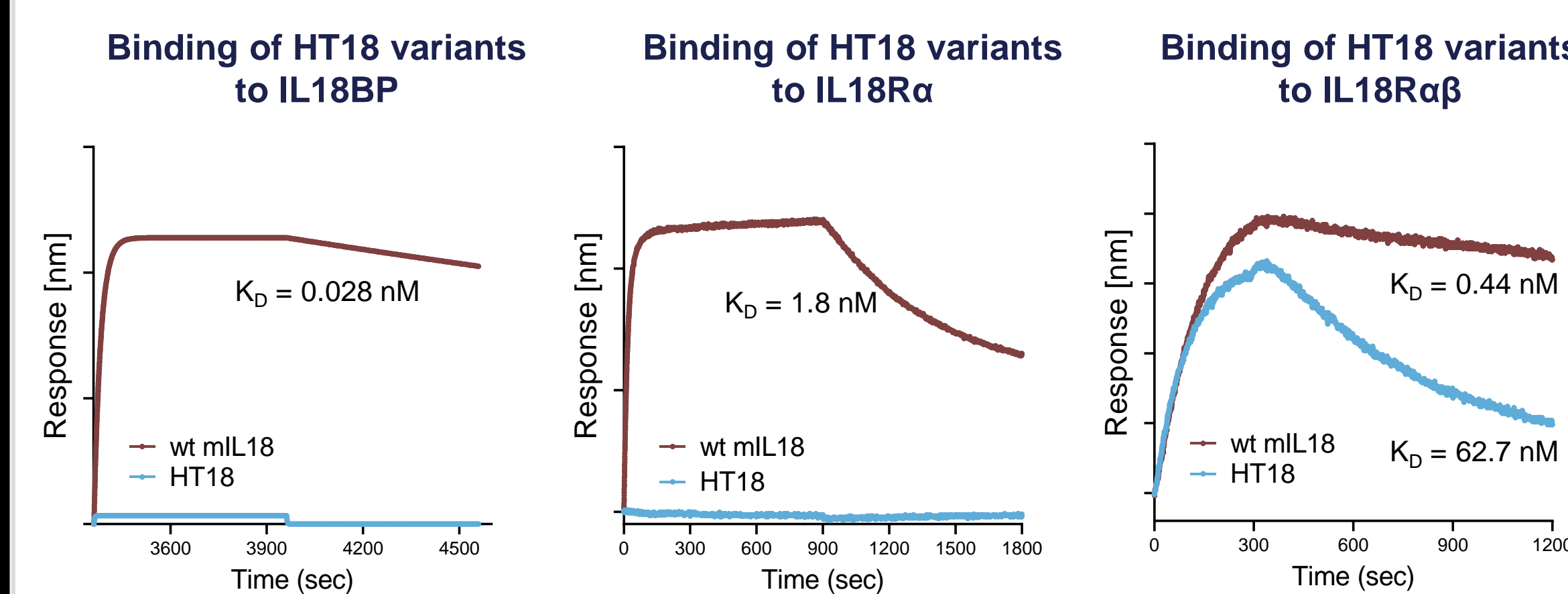


Figure 4. Equilibrium dissociation constants (KD) of monovalent human Fc fusions of wt-mIL-18 and HT18 for mIL-18BP, IL-18R α and IL-18R α β . For mIL-18BP, human Fc fusions of wt-mIL-18 and HT18 were immobilized on AHC biosensors. For IL-18R α and IL-18R α β mouse Fc fusions of IL-18R α or IL-18R α β were immobilized on AMC biosensors.

α PD1-HT18_{cis} augments T cell activity

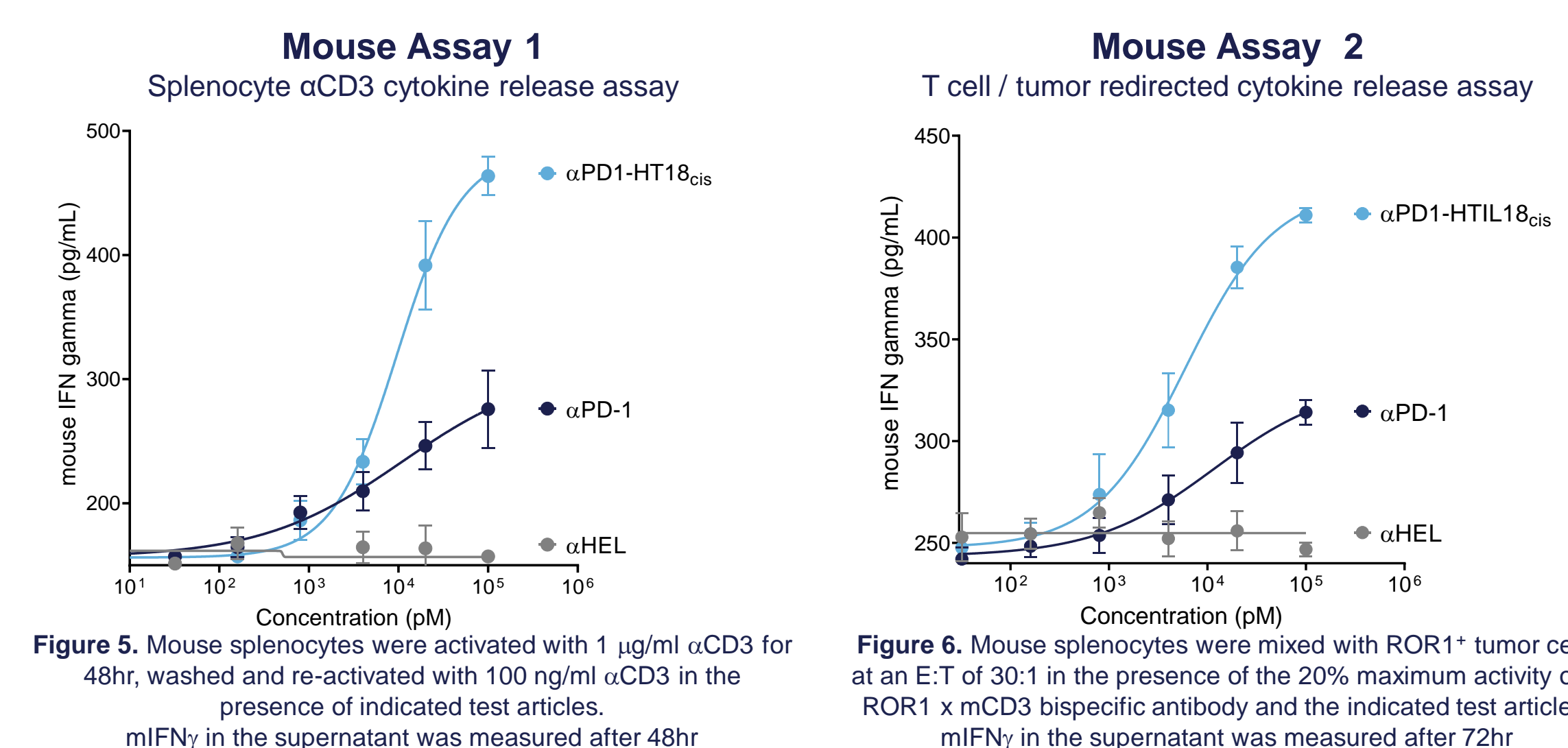


Figure 5. Mouse splenocytes were activated with 1 μ g/ml α CD3 for 48hr, washed and re-activated with 100 ng/ml α CD3 in the presence of indicated test articles. mIFN γ in the supernatant was measured after 48hr

Figure 6. Mouse splenocytes were mixed with ROR1⁺ tumor cells at an E:T of 30:1 in the presence of the 20% maximum activity of a ROR1 x mCD3 bispecific antibody and the indicated test articles. mIFN γ in the supernatant was measured after 72hr

IL-18 preserves and augments tumor reactive T cells that are α PD1-HT18_{cis} induced both memory and epitope spreading in immunogenic colon carcinoma model

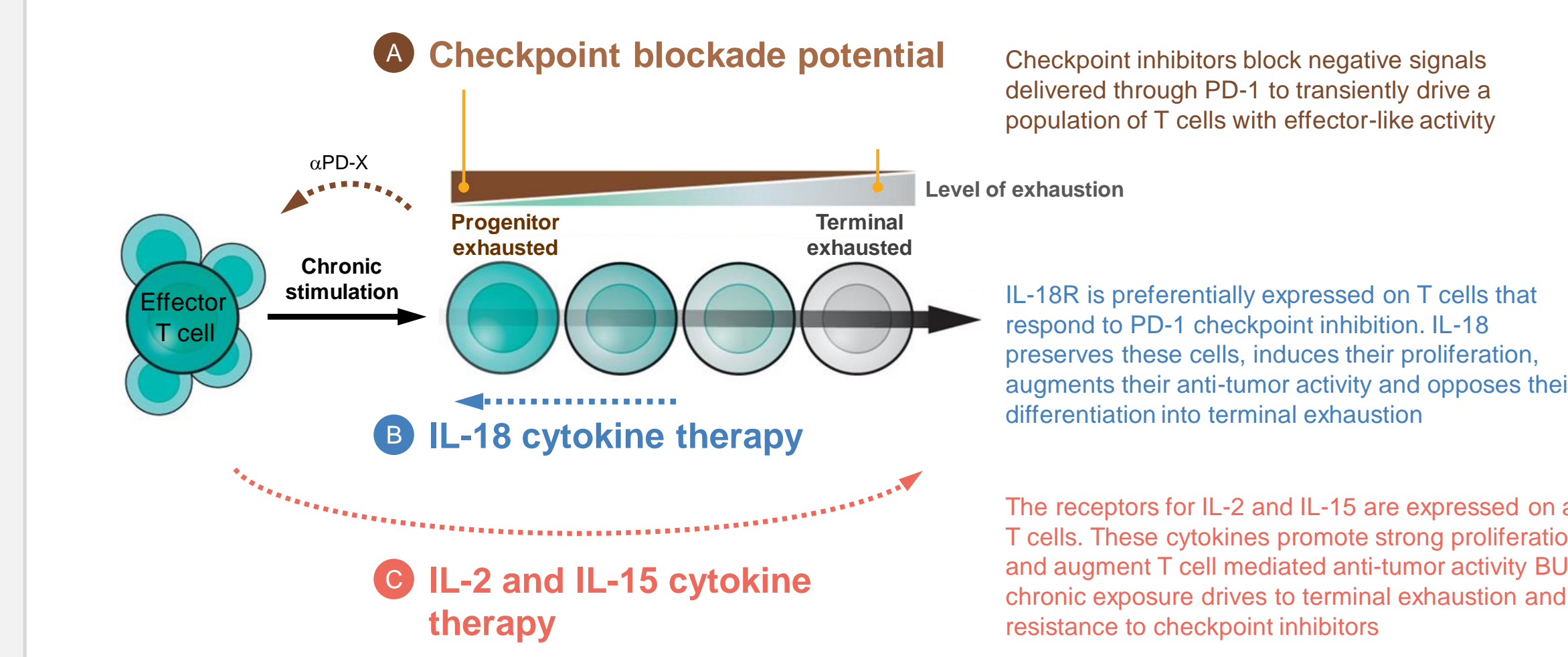


Figure 7. Response of T cell subsets to checkpoint inhibition, IL-2, and IL-15. Only IL-2 and IL-15 have evolved to both promote and limit immune responses. IL-18 is a "danger signal" that has evolved to preserve immune cell activity

α PD1-HT18_{cis} but not α PD-1-IL-18 results in strong tumor efficacy without weight loss

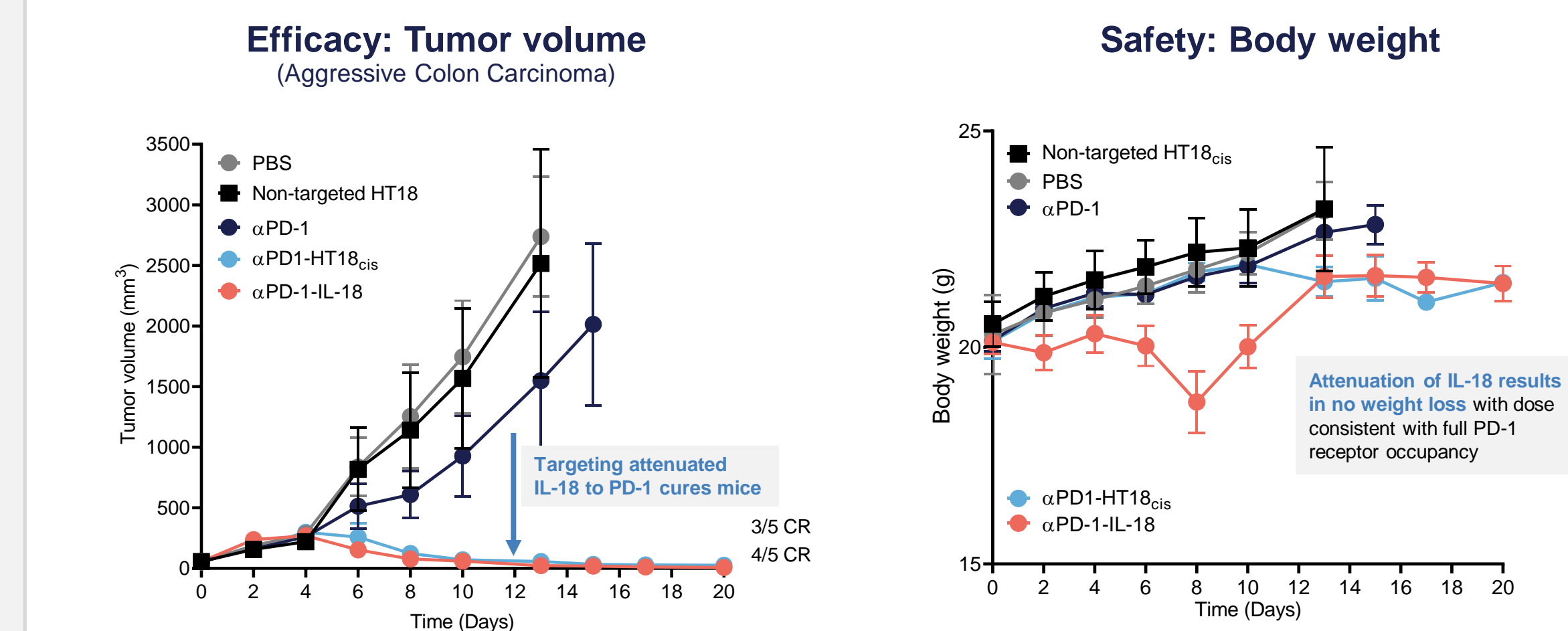


Figure 8. C57BL/6 mice were injected subcutaneously with MC38i, an aggressive MC38 clone. When mean tumor volume ranged between 75-100 mm³, mice were randomized into groups of 5 and treated with PBS, α PD-1, α PD1-HT18_{cis} or α PD-1-IL-18 Days 0 and 4 at 15mg/kg

α PD-1-IL-18 induced far greater serum IFN γ but weaker immune memory than α PD1-HT18_{cis}

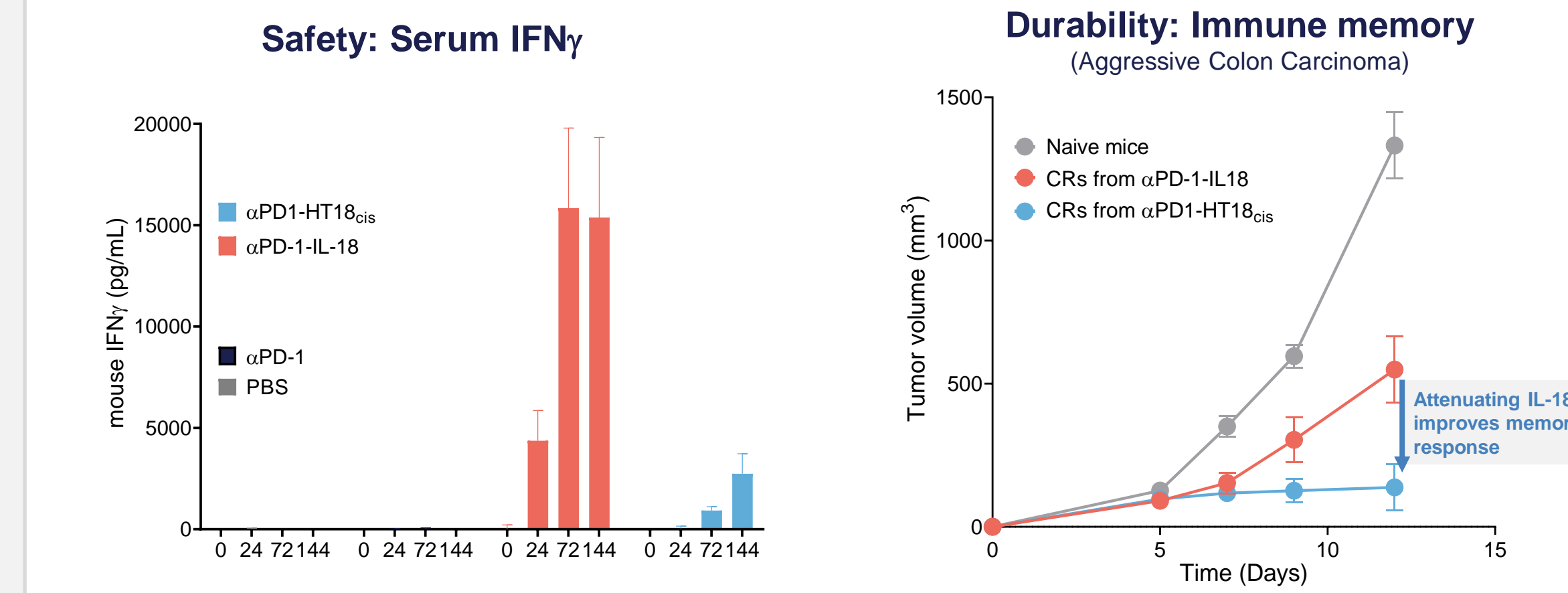


Figure 9. C57BL/6 mice were injected subcutaneously with MC38i. Serum was collected at 0, 24, 72 and 144 hours after the Day 0 treatment and mouse IFN γ measured

Figure 10. C57BL/6 mice that harbored MC38i tumors and experienced a CR following treatment with α PD1-HT18_{cis} or α PD-1-IL-18 were re-challenged with MC38i after 90 days

α PD1-HT18_{cis} induced 100% cure rate in immunogenic colon carcinoma model

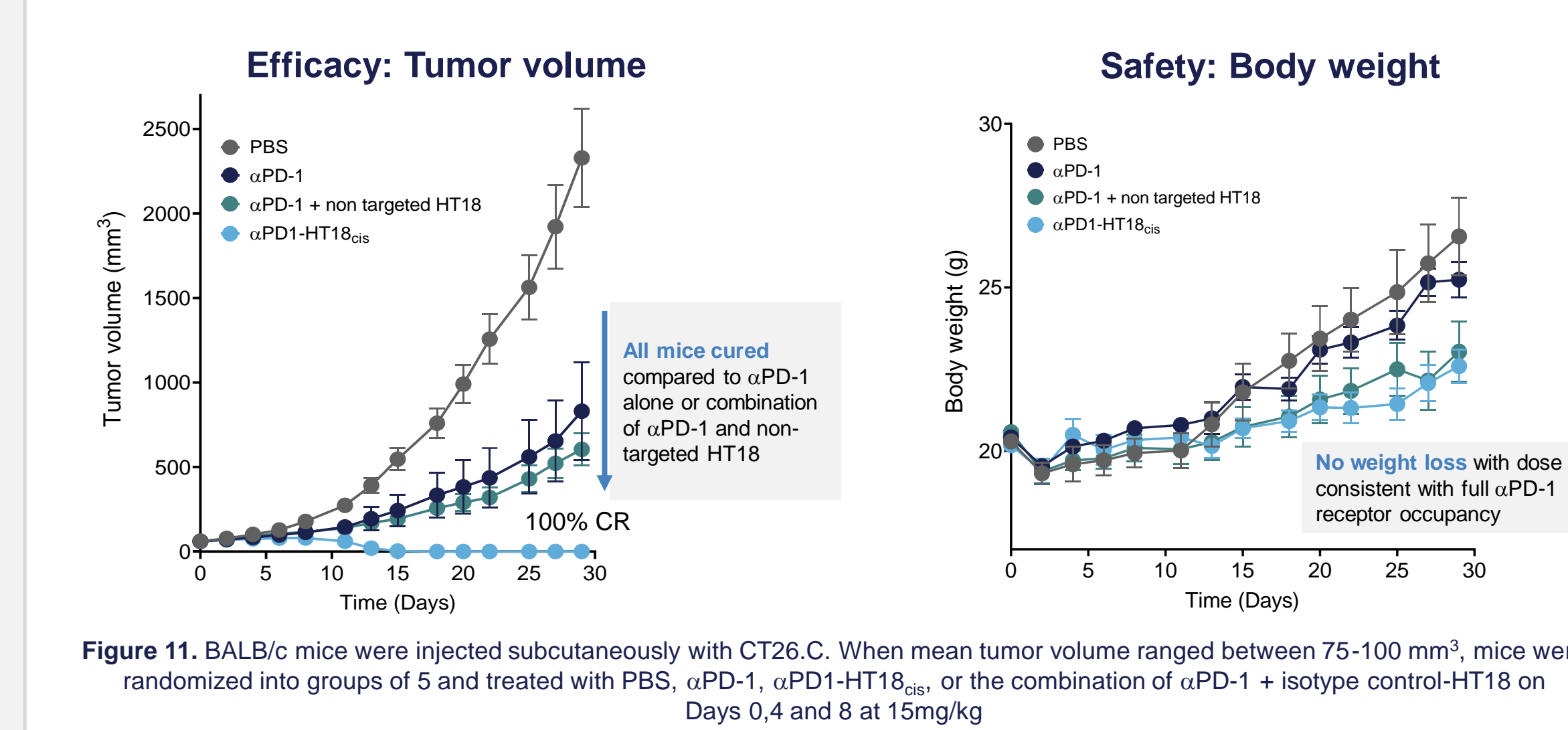


Figure 11. BALB/c mice were injected subcutaneously with CT26.C. When mean tumor volume ranged between 75-100 mm³, mice were randomized into groups of 5 and treated with PBS, α PD-1, α PD1-HT18_{cis} or the combination of α PD-1 + isotype control-HT18 on Days 0, 4 and 8 at 15mg/kg

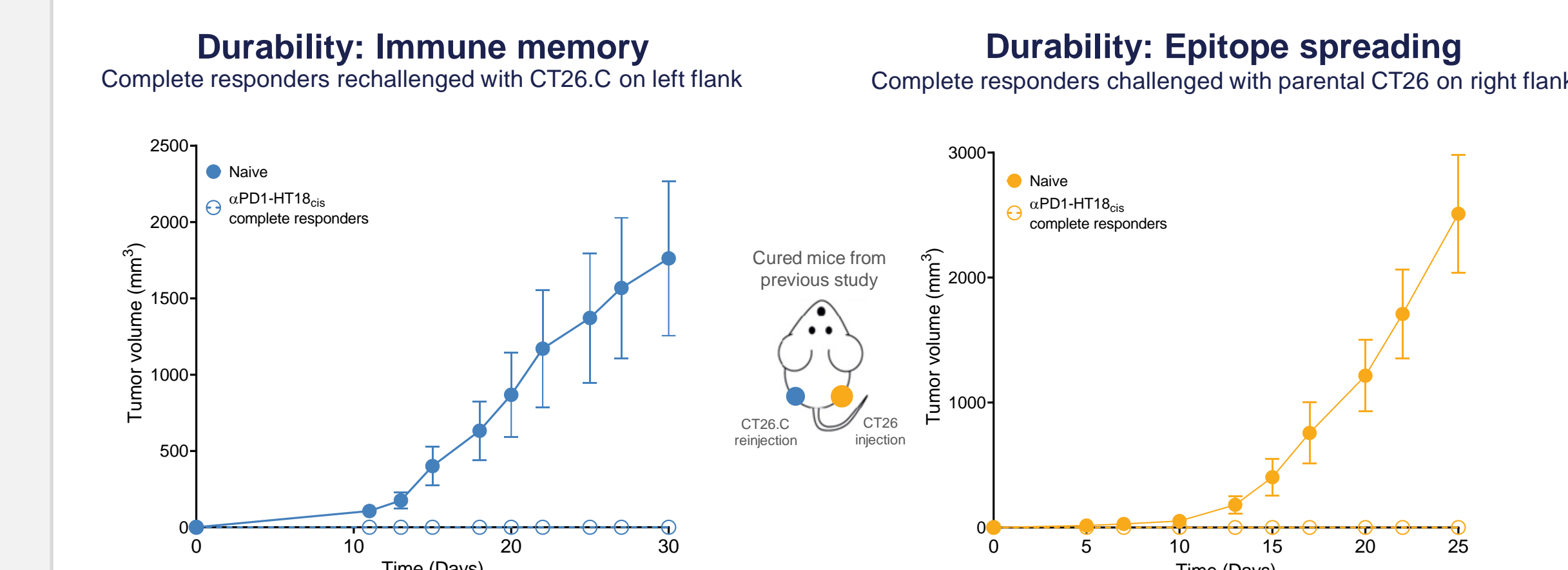


Figure 12. BALB/c mice that harbored CT26.C tumors and experienced a CR following treatment with α PD1-HT18_{cis} were rechallenged with CT26.C after 65 days on the left flank and parental CT26 after 70 days on the right flank. As a means of comparison, a group of 5 naive mice were subjected to the equivalent tumor inoculation

α PD1-HT18_{cis} induces strong efficacy and memory in an aggressive model of melanoma

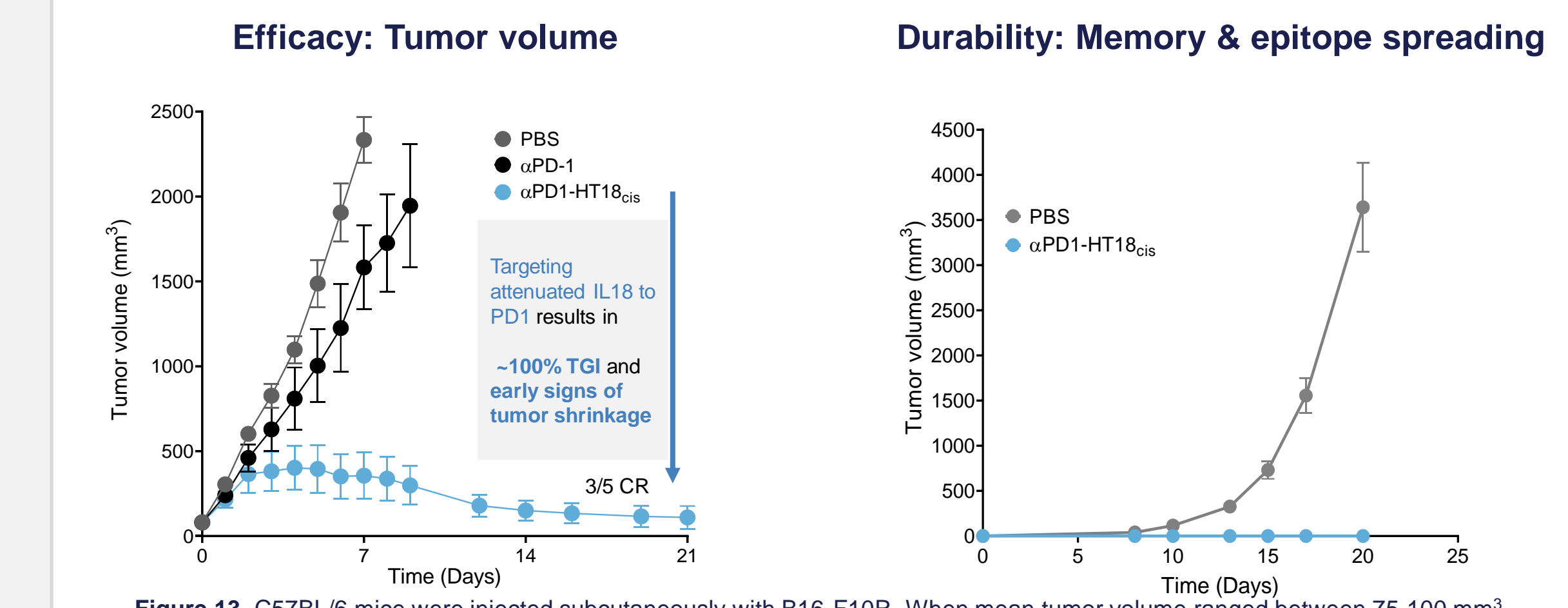


Figure 13. C57BL/6 mice were injected subcutaneously with B16-F10R. When mean tumor volume ranged between 75-100 mm³, mice were randomized into groups of 5 and treated with PBS, α PD-1, α PD1-HT18_{cis} on Days 0, 3 and 6 at 15mg/kg. Mice that experienced a CR following treatment with α PD1-HT18_{cis} were rechallenged with B16-F10 after 60 days

α PD1-HT18_{cis} induces strong immune infiltration in aggressive murine melanoma tumors

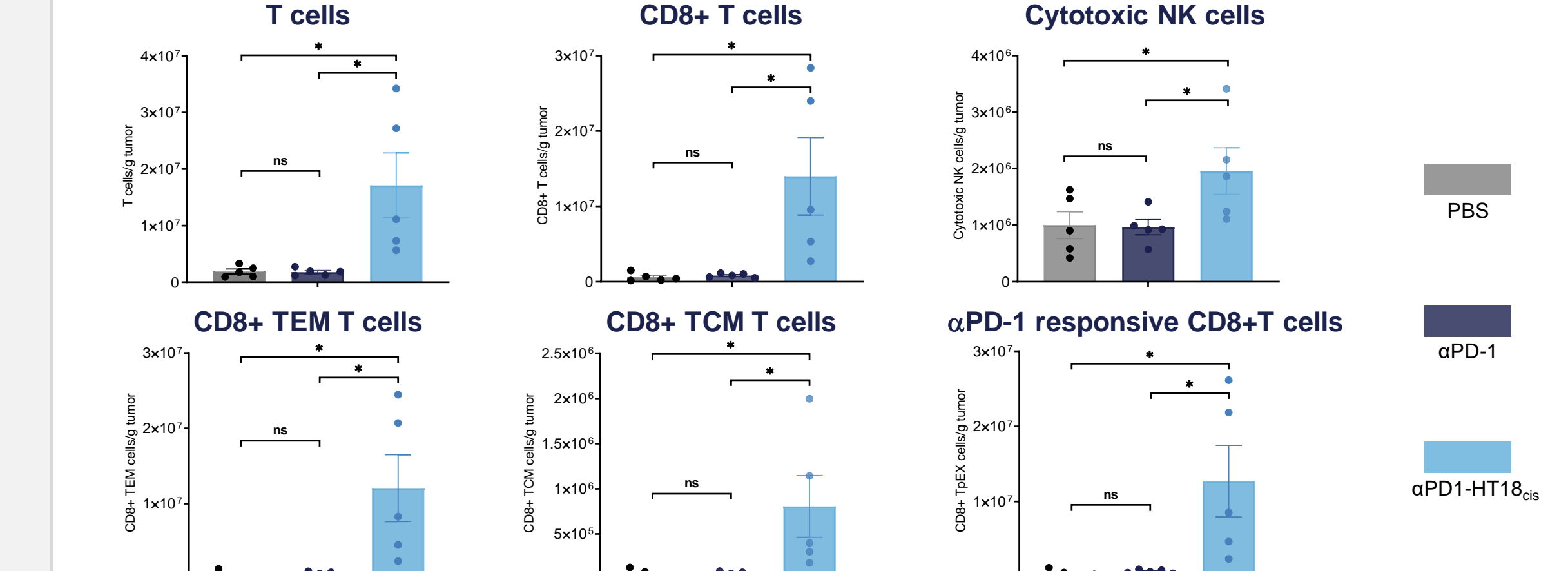


Figure 14. C57BL/6 mice harboring B16-F10R tumors were treated on Days 0 and 3. Mice were euthanized on Day 5, tumors collected and dispersed into single cell suspensions for flow cytometric assessment of TIL (CD45⁺ cells). TEM and TCM were defined CD44⁺/CD62L⁻ and CD44⁻/CD62L⁺, respectively within the CD8⁺ pool. α PD-1 responsive CD8⁺ T cells were defined as PD1⁺/TCF1⁺ cells within the CD8⁺ pool

TAM and inflammatory ratios in aggressive melanoma tumor model

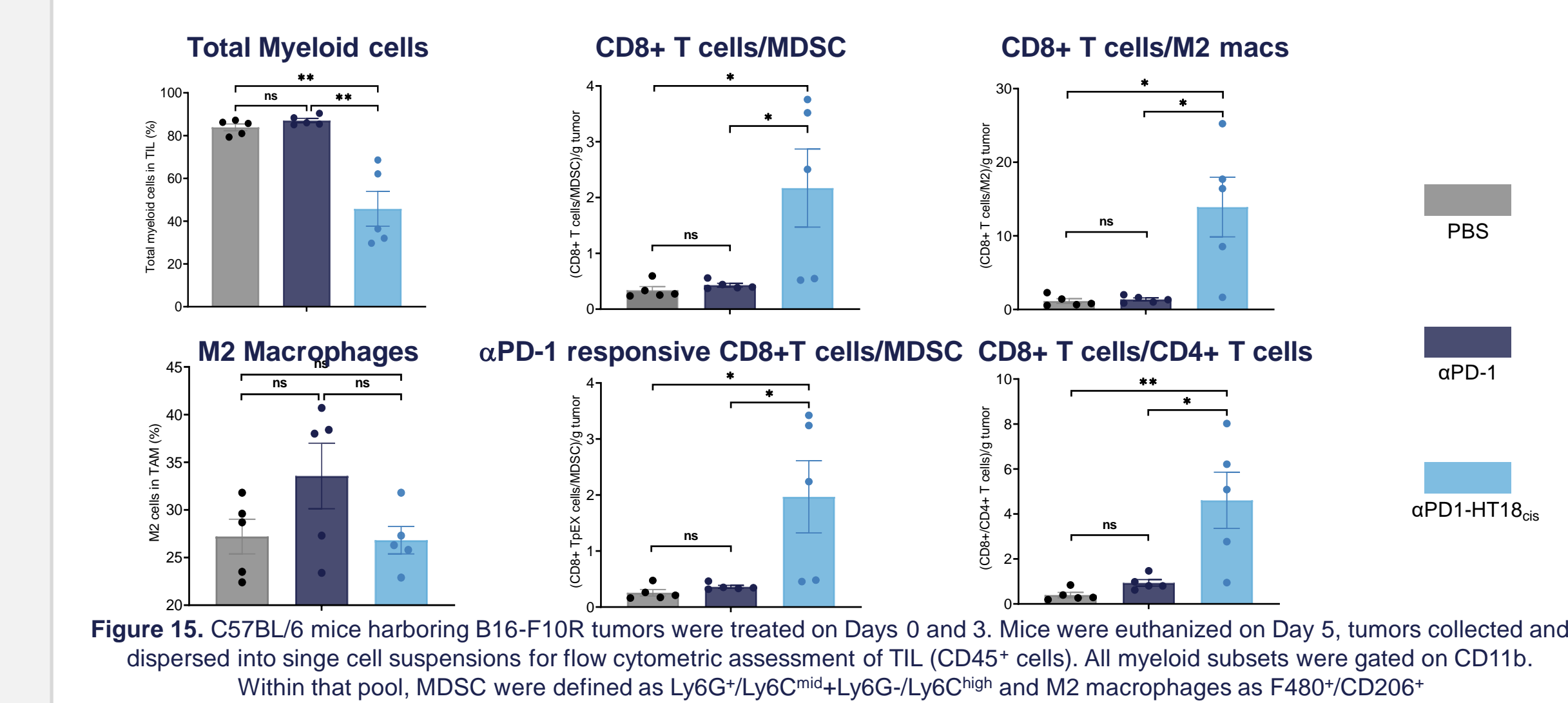


Figure 15. C57BL/6 mice harboring B16-F10R tumors were treated on Days 0 and 3. Mice were euthanized on Day 5, tumors collected and dispersed into single cell suspensions for flow cytometric assessment of TIL (CD45⁺ cells). All myeloid subsets were gated on CD11b. Within that pool, MDSC were defined as Ly6G⁺/Ly6C^{hi} and M2 macrophages as F480⁺/CD206⁺