Gradual lymphocyte activation with IL-12 partial agonist STK-026 maintains anti-tumor efficacy and escapes acute NK-mediated cytokine release and toxicities associated with WT IL-12

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ABSTRACT #964

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Interleukin-12 (IL-12) is a pro-inflammatory cytokine composed of p35 and p40 subunits produced by antigen-presenting cells to stimulate Th1 cells, cytotoxic CD8 T cells, and NK cells. IL-12 has potent anti-tumor properties in multiple preclinical models. However, clinical applications of wild type IL-12 (WT IL-12) or WT IL-12 Fc fusions are hampered by early severe dose-limiting toxicities and later tachyphylaxis [1]. Preclinically, IL-12 toxicity is mediated by NK cell activation [2].

STK-026 is an Fc-fused human IL-12 partial agonist (STK-026) with diminished binding to IL-12Rb1. STK-026 exhibits selectivity for antigen activated T cells and avoids NK cell induced toxicities. Here we demonstrate that STK-026, or its murine surrogate mSTK-026, provides gradual, prolonged, and efficacious T cell and NK cell activation, achieving anti-tumor efficacy with minimal toxicity, while avoiding acute NK-cell mediated toxicity.

Pharmacokinetic/pharmacodynamic responses and overall toxicity to human STK-026 were evaluated in healthy cynomolgus macaques. The data demonstrate that STK-026 exhibits reduced release of IL-12 related toxicity associated .

NK-dependent toxicity limits the use of WT IL-12

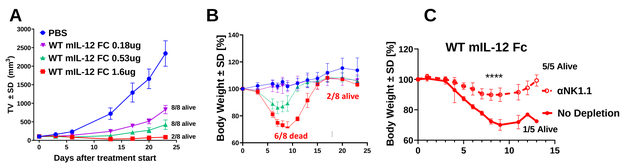


Figure 1: Wild Type (WT) IL-12 Fc demonstrates dose-dependent efficacy in MC38 tumor model, but is associated with NK cell activity

A: MC-38 tumor volume (TV) and body weight of C57BL/6 mice treated with WT mIL-12-Fc. **B:** Dose-dependent effect of WT mIL-12-Fc on tumor volume and body weight. **C:** Body weight and survival of healthy C57BL/6 mice treated with 1.6 ug WT mIL-12-Fc 2x/week, with or without NK cell-depleting antibody (aNK1.1).

STK-026 design leverages T cell IL-12R upregulation

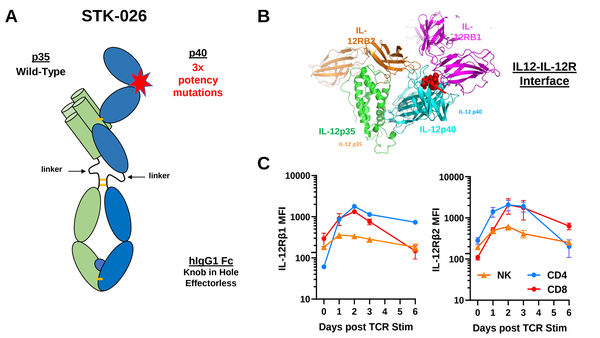


Figure 2: STK-026 is a human IL-12 partial agonist with reduced binding to IL-12Rb1

A: Representation of STK-026, highlighting mutations in p40, wild type p35, and half-life extension via effectorless KiH-Fc. **B:** Model of quaternary IL-12, IL-12R complex. **C:** Time course of IL-12Rb1 and IL-12Rb2 on CD3/CD28 stimulated human PBMCs.

STK-026 has reduced NK cell potency vs WT IL-12 Fc

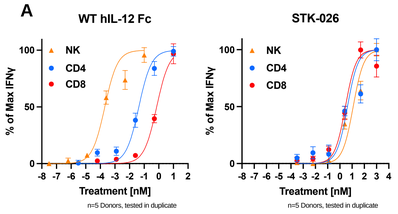


Figure 3: STK-026 parity in NK and T cell responses

A: Interferon gamma (IFNγ) secretion by cells pre-stimulated with anti-CD3/anti-CD28 + IL-2, treated with IL-2 + WT IL-12 Fc or STK-026.

Mouse STK-026 maintains anti-tumor efficacy

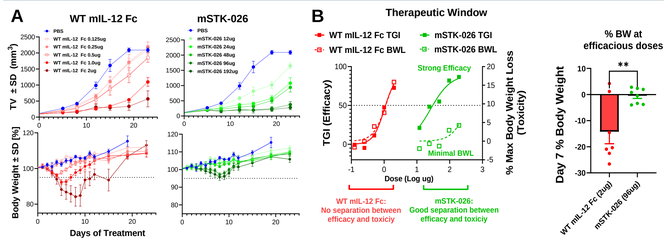


Figure 4: Mouse STK-026 maintains potent anti-tumor efficacy, without causing significant body weight loss at therapeutic doses

A: MC-38 tumor growth and body weights of tumor bearing mice across a dose titration of WT mIL-12 Fc and mSTK-026. **B:** Tumor Growth Inhibition (TGI) (study day 23) vs. max % body weight loss by dose (study day 7).

Mouse STK-026 avoids acute NK hyperactivation and allows gradual, sustained NK and CD8 T cell activation

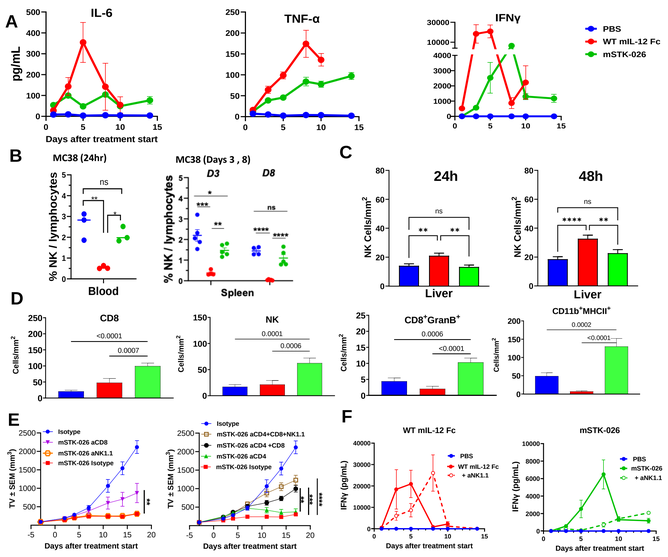


Figure 5: mSTK-026 avoids IL-12-induced cytokine release syndrome yet retains activation of T cells, NK cells and APCs

A: Cytokines of healthy C57BL/6 mice treated with mSTK-026 (96 ug/wk) or mIL-12 Fc (1.6 ug/wk). **B** and **C:** NK cell composition in blood, spleen, and liver. **D:** TILs from MC-38 tumors harvested at day 26. **E** and **F:** MC-38 tumor growth and serum IFNγ of mice treated with mSTK-026 and anti-NK1.1/CD4/CD8 depleting antibodies.

mSTK-026 reverses "cold" B16F10 tumor model by activating T cells and Macrophages

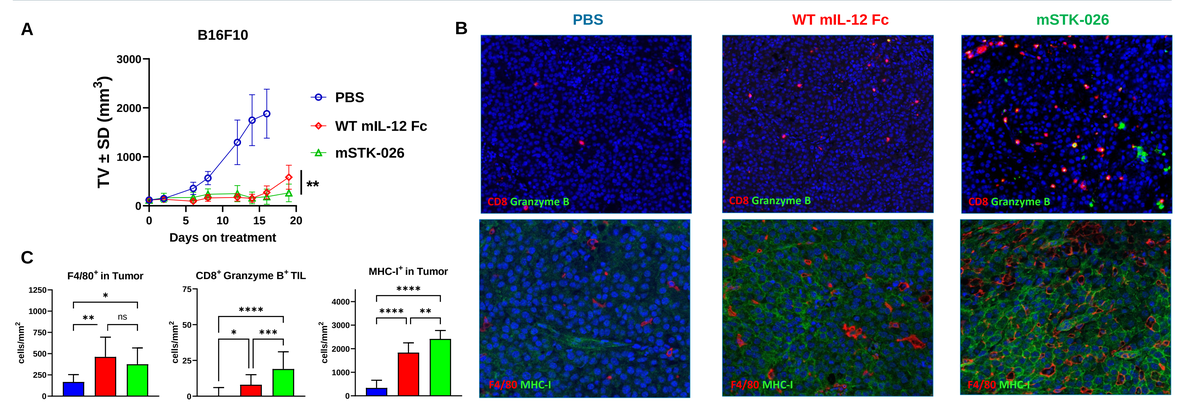


Figure 6: Mouse STK-026 effectively controls "cold" tumors by activating T cells, priming macrophages for MHC-I expression

A: B16F10 tumor growth of tumor bearing mice treated with WT mIL-12 Fc (0.5 ug/week) and mSTK-026 (48 ug/week). **B** and **C:** Immunohistochemistry image and TIL quantification from B16F10 tumors, illustrating elevated CD8 T cell and macrophage activation on treatment (day 19).

STK-026 induces gradual lymphocyte activation with reduced cardiac inflammation in NHP

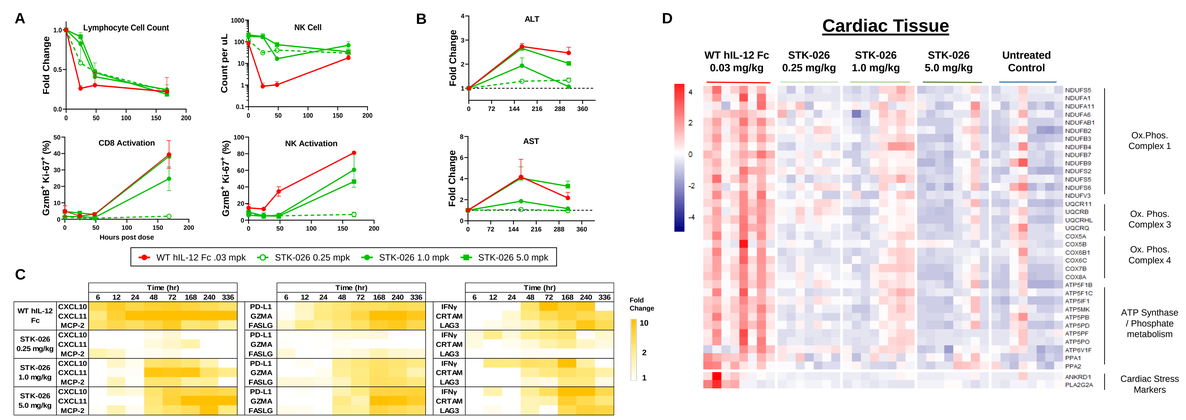
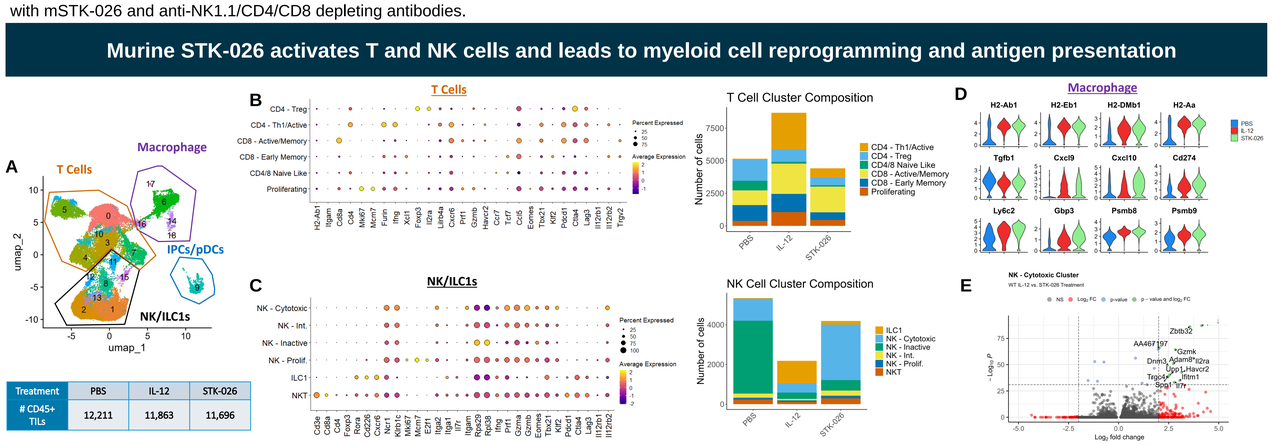


Figure 7: STK-026 is active in cynomolgus macaques while avoiding toxicity associated with wild type IL-12

A: Lymphocyte, NK cell counts, markers of activation in blood from dose-range finding cynomolgus *in vivo* study (single dose, 1M/1F per group). **B:** ALT/AST liver enzyme measurements during study. **C:** Serum cytokines, chemokines, and activation-induced proteins measured via Olink. **D.** Nanostring analysis of heart tissues, highlighting genes associated with oxidative phosphorylation and cardiac stress (4 regions/animal).

Murine STK-026 activates T and NK cells and leads to myeloid cell reprogramming and antigen presentation



Treatment	PBS	IL-12	STK-026
# CD45+ TILs	12,211	11,863	11,696

Figure 6. scRNA-Seq reveals activated NK and T cell phenotype, with increased myeloid reprogramming and antigen presentation

A: UMAP projection of combined tumor CD45⁺ cells (MC38, day 8 tumors); cell counts tabulated per treatment. **B:** T cell subcluster gene expression and total composition. **C:** NK cell and ILC1 subcluster gene expression and total composition. **D:** Cluster 6 (Mo) macrophage select gene expression associated with antigen presentation. **E:** Differential gene expression volcano plot of Cytotoxic NK cluster, WT mIL-12 Fc vs. STK-026.

Conclusions

1. **STK-026 is a human IL-12 partial agonist engineered with half-life extension.**
2. **Human STK-026 demonstrates parity in NK and T cell potency under inflammatory conditions.**
3. **mSTK-026 maintains the potent anti-tumor efficacy of WT mIL-12 without the acute cytokine storm associated body weight loss.**
4. **Single cell RNASeq analysis of MC38 TILs reveals activity consistent with IL-12, with reduced NK and T cell exhaustion, and increased effector cell survival and infiltration genes.**
5. **mSTK-026 effectively activates intratumoral CD4 and CD8 cells in "cold" tumor types, resulting in myeloid activation consistent with IFNγ secretion.**
6. **STK-026 avoids acute circulating lymphocyte activation in non-human primates and WT IL-12 associated cardiac toxicities, while maintaining measurable pharmacodynamic cytokines and chemokines.**

1) Atkins MB, *et al.* Phase I evaluation of intravenous recombinant human interleukin 12 in patients with advanced malignancies. Clinical Cancer Research. 1997; 3 (3): 409-17.

2) Carson W, *et al.* A fatal cytokine-induced systemic inflammatory response reveals a critical role for NK cells. J Immunology. 1999; 162 (8): 4943–4951.