

Novel IL-12 partial agonist for cancer immunotherapy avoids NK-cell mediated toxicity

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ABSTRACT

Interleukin-12 (IL-12) is a pro-inflammatory type 1 cytokine composed of the p35 and p40 subunits. It is produced by antigen-presenting cells to stimulate Th1 cells, cytotoxic CD8 T cells and NK cells. IL-12 has demonstrated potent anti-tumor properties in multiple preclinical models, however clinical applications of IL-12 have been hampered by severe dose-limiting toxicities including anemia, neutropenia, and severe infections as well as stomatitis and elevated transaminases¹.

Preclinically, IL-12 toxicity is mediated by NK cell activation². Here we report on a novel human IL-12 partial agonist (STK-026) that has diminished binding to IL-12Rb1. STK-026 is designed to more selectively engage antigen activated T-cells, which strongly upregulate IL-12Rb1 upon activation, and to reduce stimulation of NK cells or resting T cells, which express modest levels of IL-12Rb1³. To explore anti-tumor efficacy and toxicity in mouse syngeneic tumor models, we generated a half-life extended mouse surrogate of the IL-12 partial agonist (mSTK-026) and compared it to a similarly engineered half-life extended version of wild type mouse IL-12 (mIL-12wt Fc).

At efficacious doses, systemic administration of mIL-12wt Fc induced significant weight loss and lethality characterized by early proinflammatory cytokine release and systemic NK cell activation. Conversely, mSTK-026 was well tolerated and avoided the robust and rapid NK cell activation and peripheral NK count decreases seen with mIL-12wt Fc, suggestive of extravasation to tissues.

Both mSTK-026 and mIL-12wt Fc showed similar robust single-agent anti-tumor efficacy in syngeneic tumor models. Depletion of NK cells did not diminish anti-tumor efficacy. Efficacy for both molecules was characterized by CD8 T cell activation, myeloid cell reprogramming and antigen presentation. Moreover, combination of mSTK-026 with systemic immunotherapies further enhanced anti-tumor activity without compromising tolerability. Overall, mSTK-026 Fc retained anti-tumor efficacy without induction of severe toxicities compared to mIL-12wt Fc. These data suggest IL-12 partial agonists may represent a novel immunotherapy approach to maintain efficacy while avoiding classical toxicity associated with IL-12 therapy.

Human STK-026 and the mouse surrogate

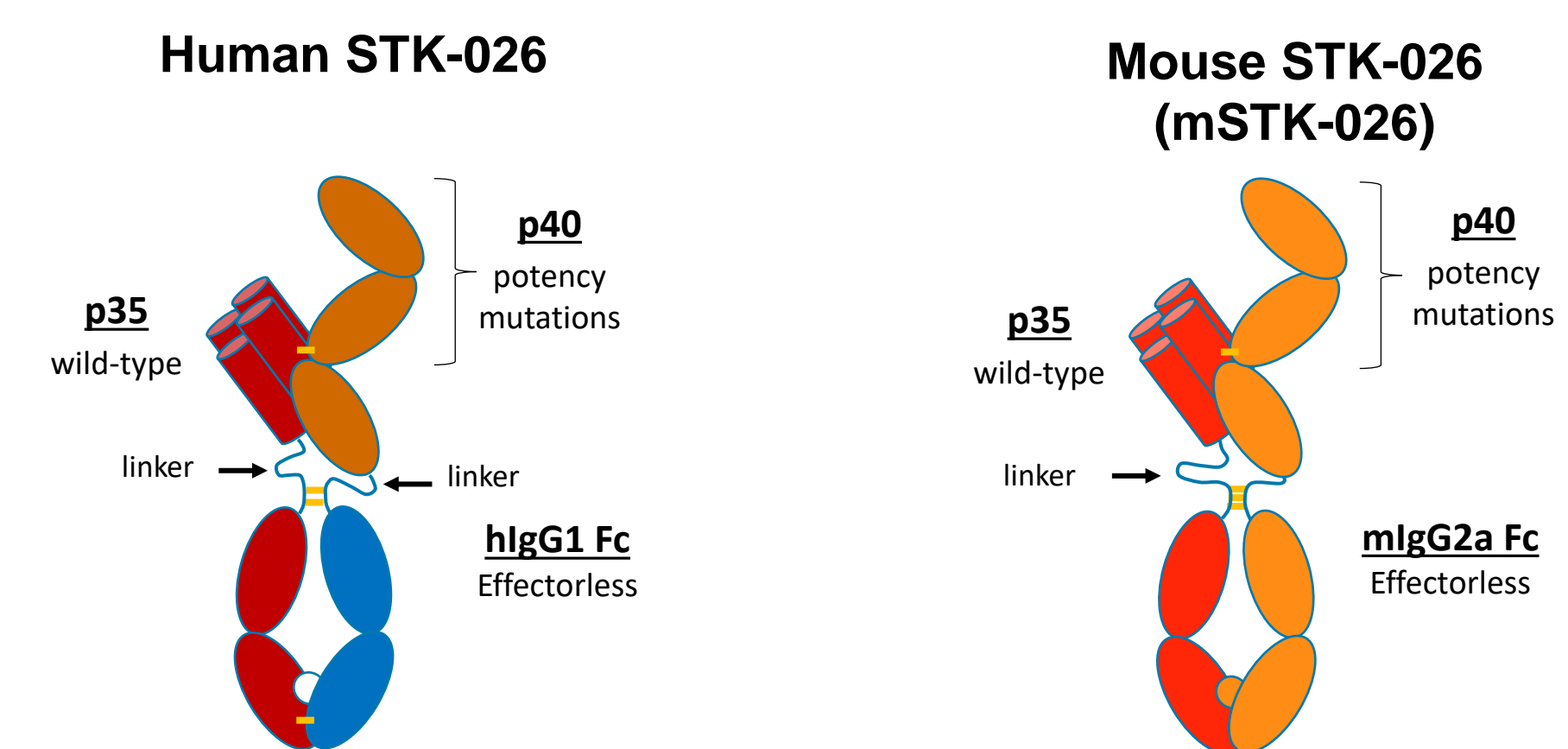


Figure 1: Human STK-026 is a novel human IL-12 partial agonist designed for diminished binding to human IL-12Rb1. Surrogate Mouse STK-026 contains the identical p40 mutations in mouse IL-12.

STK-026 has substantially reduced potency for NK cells

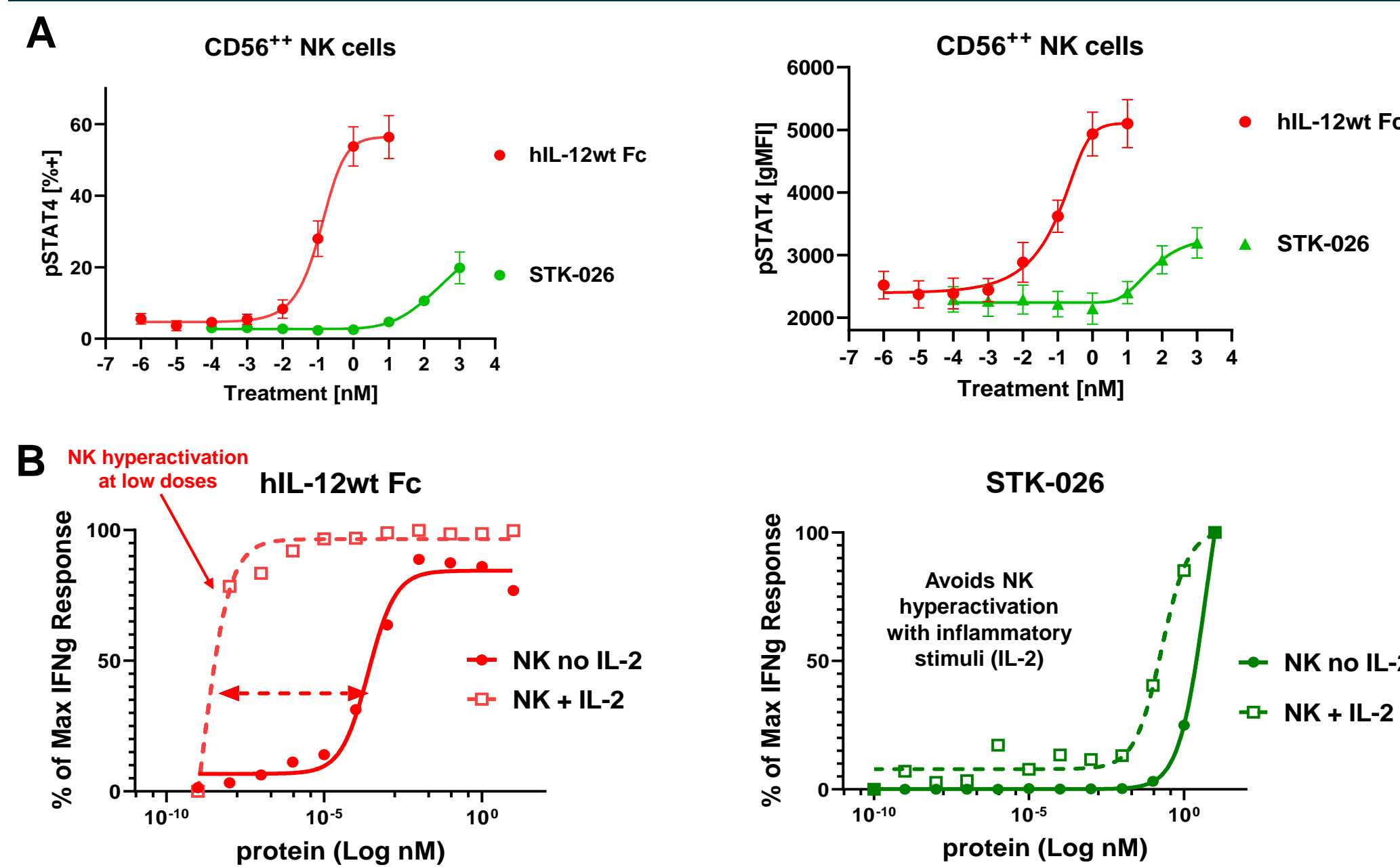


Figure 2: STK-026 has reduced NK potency and avoids NK cell hyperstimulation under inflammatory conditions.

A. Human blood was stimulated with IL-12 agonists for 15 minutes and analyzed by flow cytometry. Shown are phospho (p) STAT4 levels in CD56++ NK cells.; B. Human NK cells were incubated with IL-12 agonists +/- 100pM IL-2 for 48 hours and IFN γ levels were measured in the supernatants.

mSTK-026 has favorable PK/PD properties

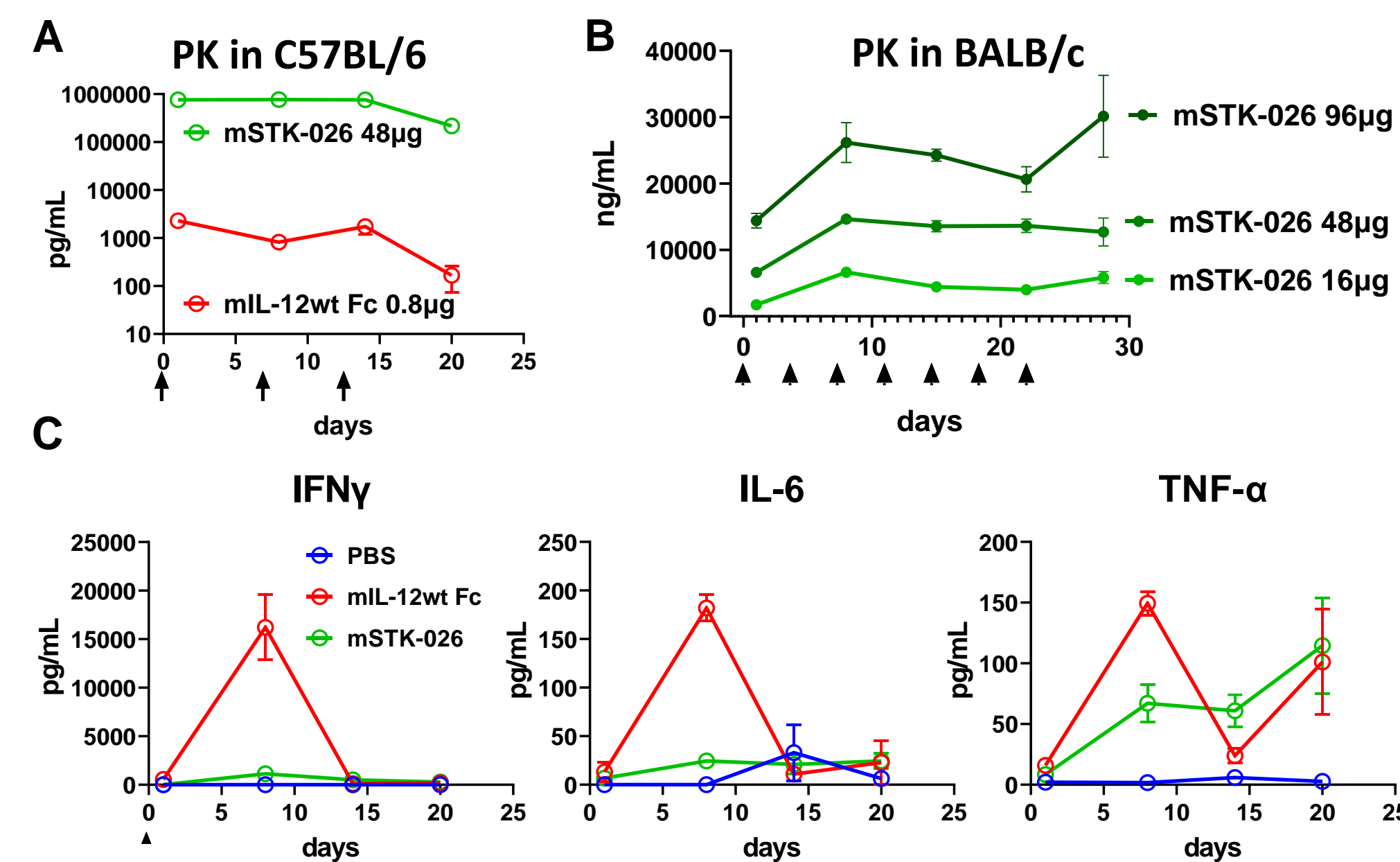


Figure 3: mSTK-026 demonstrates good PK and no signs of ADA after multiple doses. mSTK-026 does not cause acute cytokine induction.

A. and B. Concentration of mSTK-026 and IL-12wt Fc in mouse serum. Arrows indicate dosing schedule; C. Serum cytokines induced by the IL-12 molecules at therapeutic doses in C57BL/6 mice: mIL-12wt Fc at 0.8µg/QWK, mSTK-026 at 48 µg/QWK.

Mouse STK-026 shows anti-tumor efficacy without WT IL-12 associated toxicity

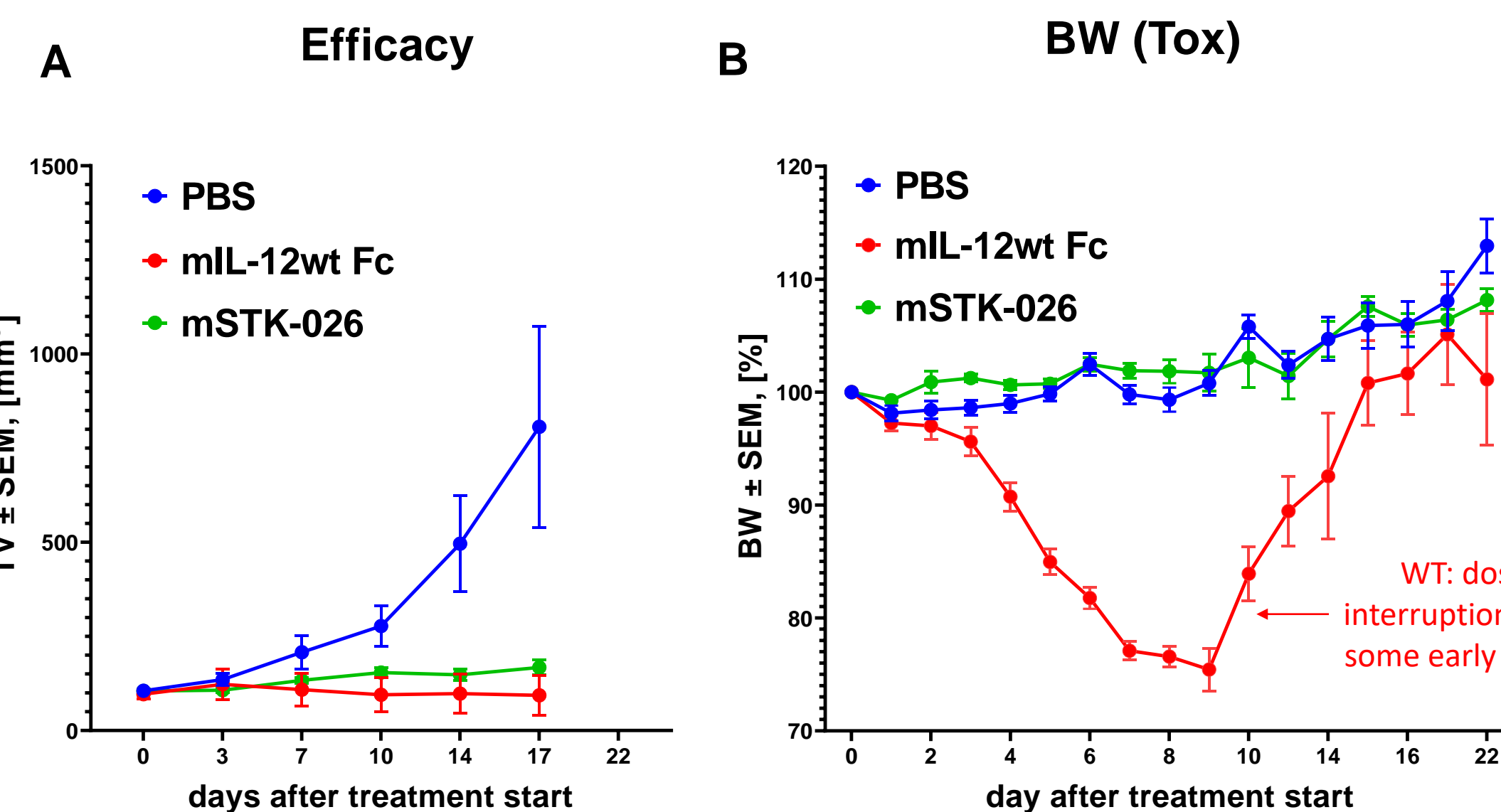


Figure 4: mSTK-026 shows excellent efficacy in the MC-38 tumor model without causing significant body weight loss at therapeutic doses.

A. Tumor Volume (TV) and B. Body Weight (BW) of C57BL/6 mice implanted with MC-38 tumor cells and treated 2x/week with IL-12 molecules, 5 doses in total. 8 animals/group (Day 0 = ~120 mm³ starting TV); mIL-12wt Fc dosed at 1.6µg 2x/week, mSTK-026 at 48µg 2x/week.

Combination immunotherapy with mSTK-026 shows potent combination activity and is well tolerated

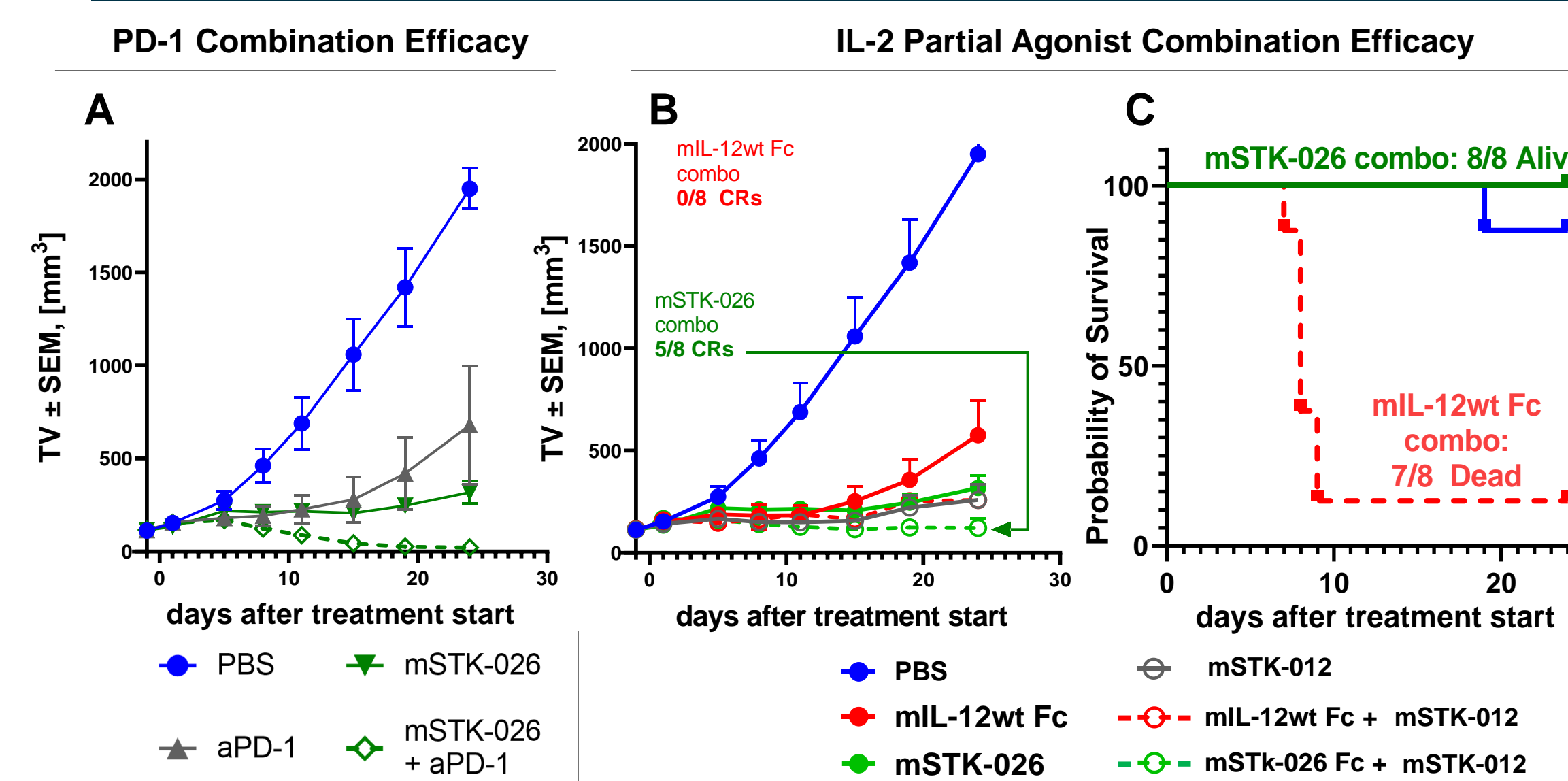


Figure 5: mSTK-026 shows combination benefit in efficacy with aPD-1. IL-2 partial agonist enhances mSTK-026 efficacy but is toxic in combination with mIL-12wt Fc.

TV of C57BL/6 mice implanted with MC-38 cells and treated with IL-12 agonists with either (A) aPD-1 antibody or (B) mSTK-012, an IL-2R β -selective IL-2 partial agonist; CRs were assessed at Day 26. C. Survival plot of mice treated with a combination of mSTK-012 and IL-12 molecules. mSTK-012 10µg 2x/week; mIL-12wt Fc 0.8µg 1x/week; mSTK-026 48µg 2x/week.

mSTK-026 avoids toxicity by sparing systemic NK cell activation and margination

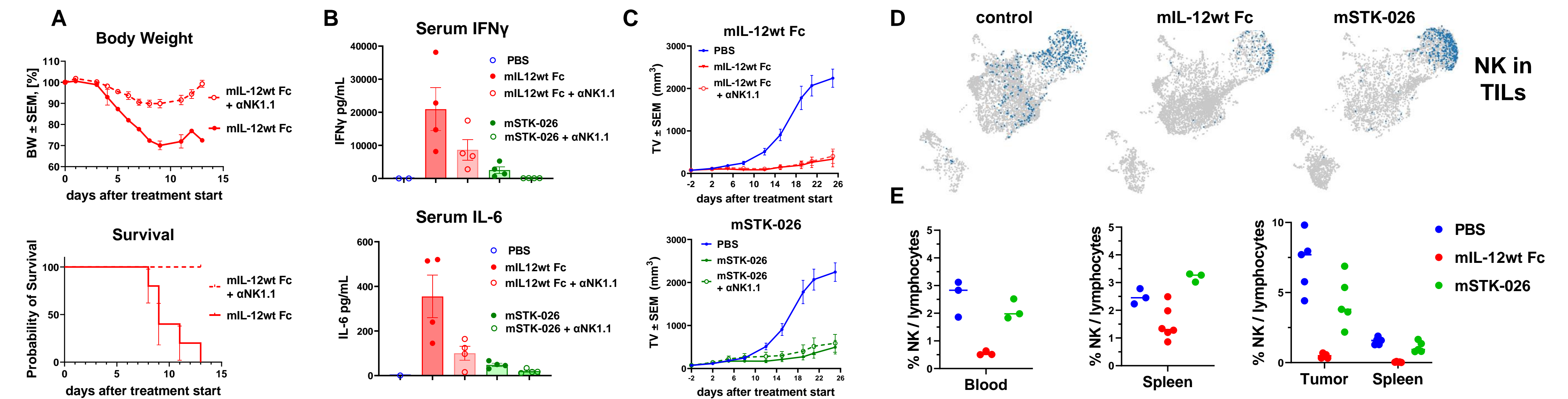


Figure 6: Toxicity of mIL-12wt Fc is dependent on NK cells, however both mIL-12wt Fc and mSTK-026 do not require NK cells for anti-tumor efficacy.

A. Body weight and survival of tumor-free C57BL/6 mice treated with mIL-12wt Fc at 1.6µg 2x/week with or without NK cell-depleting antibody (aNK1.1); B. Serum cytokines at d5 after IL-12 agonist administration at 1.6µg and 96µg of mIL-12wt Fc and mSTK-026 respectively; C. MC-38 tumor growth in C57BL/6 mice treated with IL-12 agonists and NK cell-depleting antibody. Dose: 1.6µg/1x/week of mIL-12wt Fc and 48µg/2x/week for mSTK-026; D. UMAP projections of NK cells from 10x Genomics single cell sequencing of MC-38 TILs after 2 doses of IL-12 agonists (0.8µg of mIL-12wt Fc and 48µg of mSTK-026). NK cells are defined as CD3⁺CD19⁻NK1.1⁺ (Cite-Seq) Klr1c1⁺ population; E. NK cell frequencies in peripheral blood (d1 after IL-12), spleen (d8 after IL-12) and tumor (count from the 10x Genomics, d10 after IL-12). Doses are the same as in D.

mSTK-026 increases and activates CD8 and CD4 TILs and promotes antigen presentation phenotype

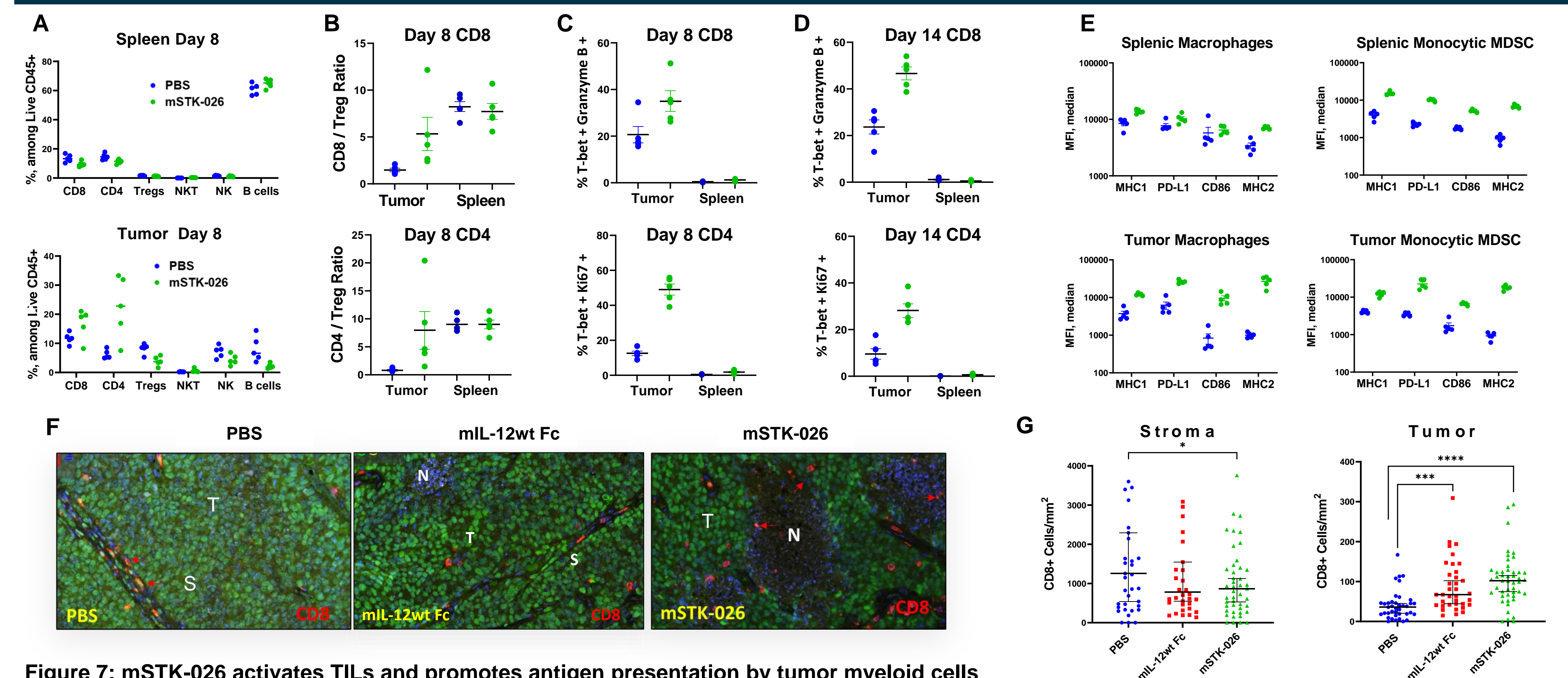


Figure 7: mSTK-026 activates TILs and promotes antigen presentation by tumor myeloid cells

A. Frequencies of immune cells in spleen (upper panel) and tumor (bottom panel) in C57BL/6 animals with MC-38 tumors treated with IL-12 molecules; B. Ratios of CD8/Treg (upper panel) and CD4/Tregs (bottom panel) from animals in A; C. Frequencies of T-bet⁺ Granzyme B⁺ cells among CD8 and CD4 cells; D. Same as C at day 14 post IL-12 administration; E. Myeloid cell phenotyping from spleen (upper panels) and tumor (bottom panels) at d8 post IL-12 treatment at 2x/week; Dose for A-E: mIL-12 wt Fc at 0.8µg/QWK, mSTK-026 at 48µg/QWK; F. IHC analysis of MC38 tumors collected at d28 after treatment start. G. Quantification of IHC analysis in F. Data merged from 3 animals/group. Dose: 1.6µg/1x/week of mIL-12wt Fc and 48µg/2x/week for mSTK-026. Each dot represents one IHC field. N-necrotic areas, S-tumor stroma, T-tumor bed.

Conclusions

1. STK-026 is an engineered human IL-12 partial agonist
2. STK-026 has reduced potency on NK cells compared to hIL-12wt Fc
3. mSTK-026 avoids systemic NK cell activation and effectively activates intratumoral CD4 and CD8 T cells
4. mSTK-026 has favorable PK/PD in mice and avoids cytokine release syndrome and the BW loss associated with mIL-12wt Fc
5. mSTK-026 shows potent anti-tumor efficacy and tolerability compared to mIL-12wt Fc
6. mSTK-026 shows combinatorial anti-tumor activity with anti-PD-1
7. mSTK-026 in combination with an IL-2 partial agonist is highly efficacious resulting in complete responses with substantially improved tolerability compared to combination with mIL-12wt Fc
8. STK-026 represents a novel immunotherapy approach to maintain efficacy while avoiding classical toxicity associated with IL-12 therapy.