Preclinical Pharmacodynamic Characterization of STK-026: A Novel IL-12 Partial Agonist for Cancer with Maintained CD8 T cell activity, Reduced NK-mediated Toxicity and an Improved Therapeutic Window

Ievgen Koliesnik, Michael Totagrande, Ryan Burgess, Kim Quyen Tran, Michele Bauer, Bhargavi Jayaraman, Cindy Buffone, Priyanka Balasubrahmanyam, Jan Emmerich, Deepti Chaturvedi, Deepti Rokkam, Rene de Waal Malefyt, Heiko Greb, Navneet Ratti, Sandro Vivona, Robert A. Kastelein, Patrick Lupardus, Luis Zuniga, David B Rosen and Martin Oft
Synthekine, Menlo Park, CA

Abstract

Background: 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 (IL-12wt) or IL-12wt-Fc fusions are hampered by severe dose-limiting toxicities [1]. Preclinically, IL-12 toxicity is mediated by NK cell activation [2].

Here we report on a novel human IL-12 partial agonist (STK-026) with diminished binding to IL-12Rb1. STK-026 was designed to increase selectivity towards 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-12Rb1.

Methods: To understand the preclinical pharmacology of STK-026, we generated a half-life extended mouse surrogate of the IL-12 partial agonist (mSTK-026) for pharmacokinetic (PK) and pharmacodynamic (PD) assessment in healthy and tumor bearing mice. Further, PK and PD parameters of human STK-026 were analyzed in healthy cynomolgus macaques.

Results: At efficacious doses, systemic administration of a half-life extended version of wild type mouse IL-12 (mIL-12wt-Fc) induced significant weight loss and lethality with early proinflammatory cytokine release and systemic NK cell activation. Conversely, mSTK-026 was well tolerated, and avoided rapid NK cell activation seen with mIL-12wt-Fc. Both mSTK-026 and mIL-12wt-Fc showed robust single-agent anti-tumor efficacy in syngeneic tumor models however mSTK-026 demonstrated a >10-fold higher therapeutic index than mIL-12wt-Fc. Depletion of NK cells did not diminish anti-tumor efficacy of either IL-12 and efficacy of mSTK-026 was associated with intratumoral CD8 T cell activation and myeloid cell activation. In cynomolgus macaques, human STK-026 demonstrated antibody like PK, was well tolerated at all doses tested (highest: 5mg/kg) and induced no detectable systemic IL-6 or TNFα. Compared to hIL-12wt-Fc, STK-026 avoided early spikes in NK cell activity and systemic chemokine levels, reduced lymphocyte activation in peripheral tissues, and reduced ALT/AST induction, all at doses activating effector and memory CD8 T cells.

Conclusions: Overall, mSTK-026 retained anti-tumor efficacy without spikes of early NK activation and induction of severe toxicities seen with mIL-12wt-Fc. Similarly, human STK-026 avoided early spikes of NK activity in cynomolgus macaques and showed reduced signs of systemic toxicities compared to hIL-12wt-Fc. These data suggest that STK-026 is a novel immunotherapy approach with the potential to maintain anti-tumor efficacy while avoiding dose limiting toxicities classically associated with IL-12 therapy.

STK-026 and the mouse surrogate

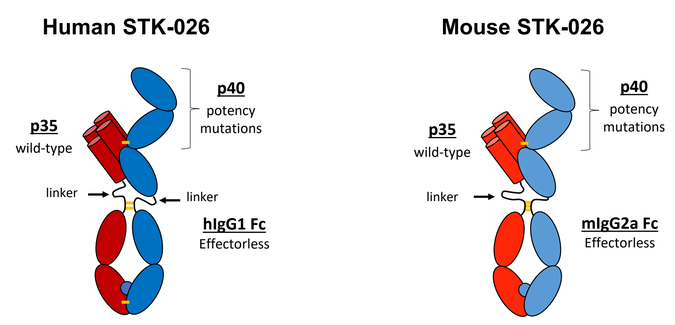

Figure 1: Human STK-026 is a novel human IL-12 partial agonist designed for diminished binding to human IL-12Rβ1. Surrogate Mouse mSTK-026 contains the identical p40 mutations in mouse IL-12.

Mouse STK-026 shows Reduced Toxicity and an Improved Therapeutic Window vs WT IL-12

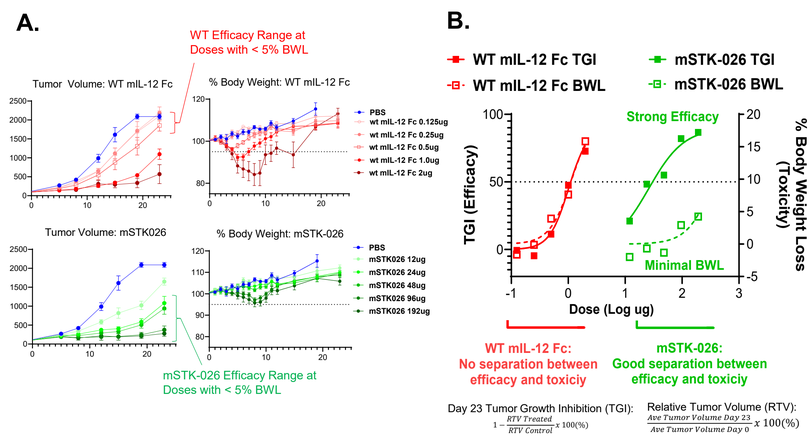

Figure 4: mSTK-026 shows excellent efficacy in the MC-38 tumor model without causing significant body weight loss at therapeutic doses.

A. Tumor Volumes and % Body Initial Weight of C57BL/6 mice implanted with MC-38 tumor cells and treated 1x/week with a titration of IL-12 molecules for 3 weeks, 8 animals/group (Day 0 = ~90 mm^3 starting TV). **B** Data from (A) overlayed as Day 23 Percent Tumor Growth Inhibition (Closed Symbols) and Percent Body Weight Loss (Open Symbols).

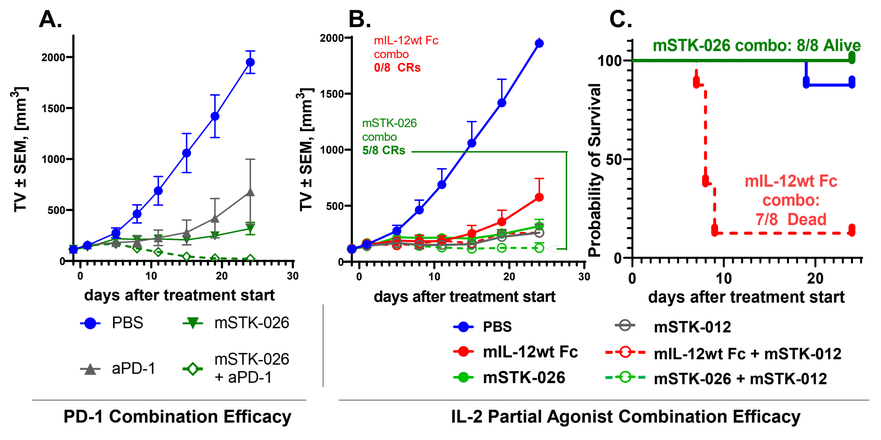

Figure 5: mSTK-026 shows combination benefit in efficacy with aPD-1. IL-2 partial agonist enhances mSTK-026 efficacy but is toxic in combination with mIL-12wt Fc.

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-2Rαβ-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 avoiding systemic NK cell activation and margination

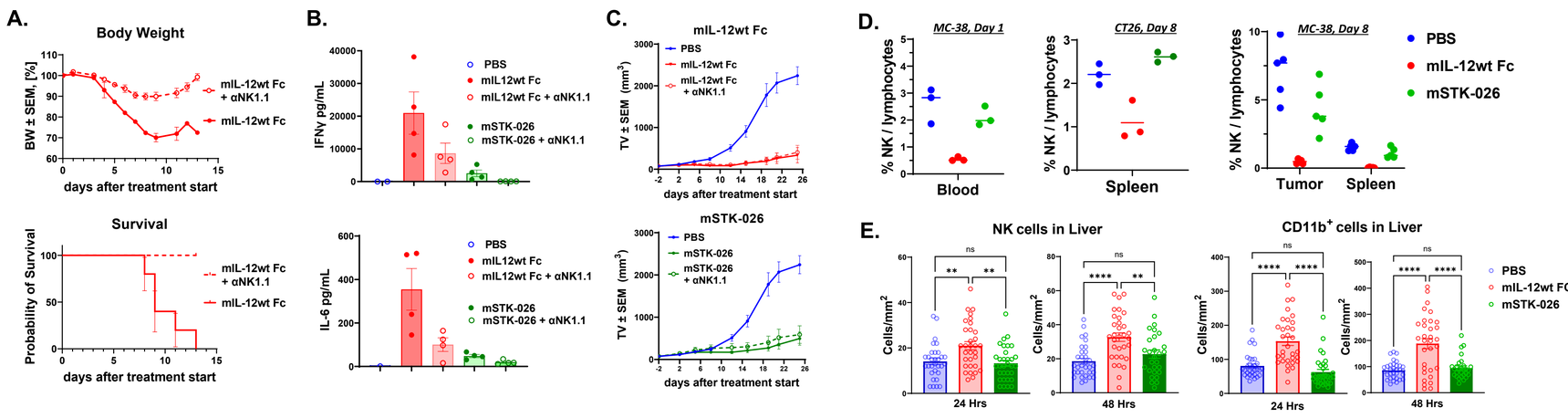

Figure 6: Toxicity of mIL-12wt Fc is dependent on NK cells, however both mIL-12wt Fc and mSTK-026 do not require NK cells for anti-tumor efficacy.

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**. NK cell frequencies measured by flow cytometry in peripheral blood, spleen or tumor at d1 or d8 post treatment. Doses are similar as in **C**. **E**. NKp46+ NK cell counts and CD11b+ myeloid cell counts from IHC analysis of liver at 24h-48h post treatment in MC-38 bearing mice which received 0.8μg of mIL-12wt Fc or 48μg of mSTK-026.

STK-026 has reduced activity on NK cells

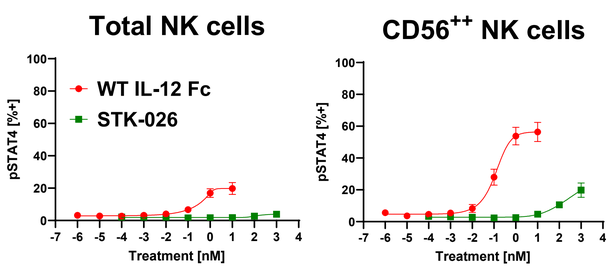

Figure 2: STK-026 has reduced signaling in NK cells vs WT IL-12.

Human PBMC were stimulated with cytokines and assessed for levels of phosphorylated STAT4 (pSTAT4). Shown are average values for 3 human donors analyzed for NK cell pSTAT4 levels.

STK-026 maintains activity on TCR stimulated T cells

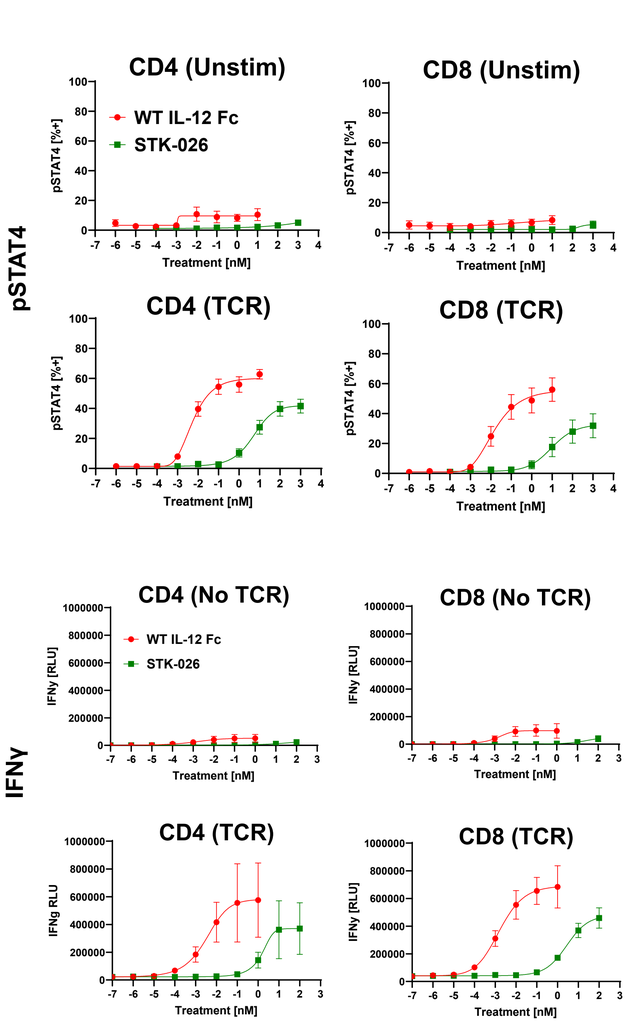

Figure 3: STK-026 shows preferential activity on activated T cells.

A. Human PBMC were incubated for 72h without (no TCR) or with (TCR) anti-CD3/CD28 antibodies and 10nM human IL-2, then treated with IL-12 Fc or STK-026 and assessed for pSTAT4 in CD4 and CD8 T cells. Shown are averages for 3 donors. **B.** Human PBMC were incubated with IL-2 without (no TCR) or with (TCR) anti-CD3/CD28 beads in the presence of IL-12 or STK-026 for 72h and analyzed for IFNg in supernatants. Average of 2 donor shown.

mSTK-026 maintains early activated CD8 TILs and promotes an activated effector phenotype

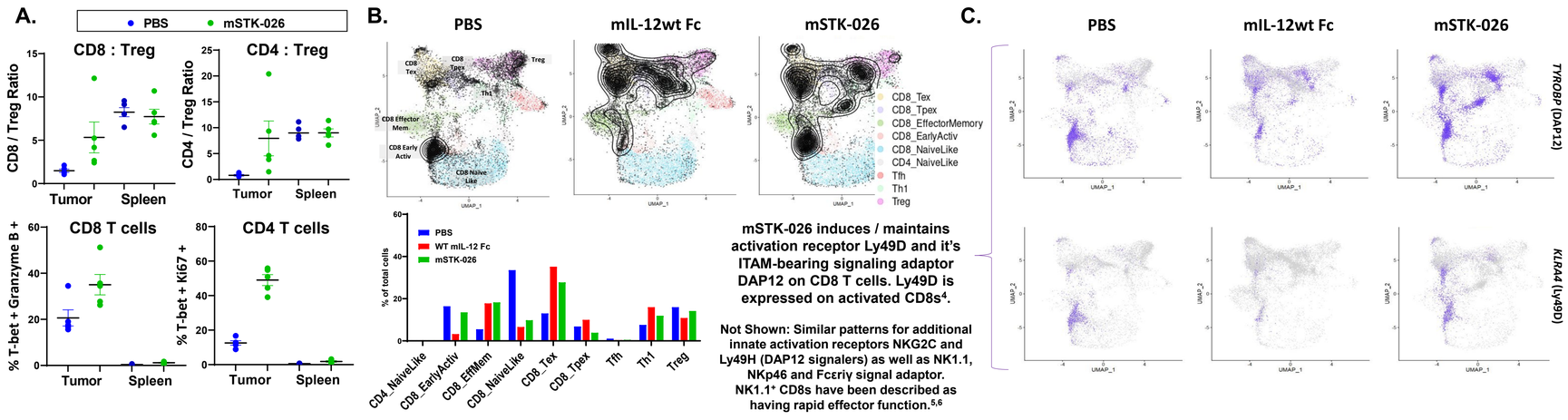

Figure 7: mSTK-026 activates CD8 TILs and maintains early activated TIL CD8s and maintains an activated effector phenotype

A. D8 FACS immunophenotyping of immune cells in C57BL/6 animals with MC-38 tumors treated with IL-12 molecules; **B,C**. UMAP visualization of T cells from scRNASeq of D7 CD45+ TILs from MC38 treated mice **B**. Density overlays and quantitation of T cell phenotypes. **C.** UMAP overlays of specific genes. Mice dosed with mIL-12wt Fc at 0.8μg/QWK, mSTK-026 at 48μg/QWK.

STK-026 activates CD8s in Cynos but, unlike WT IL-12, avoids early spikes in NK activation

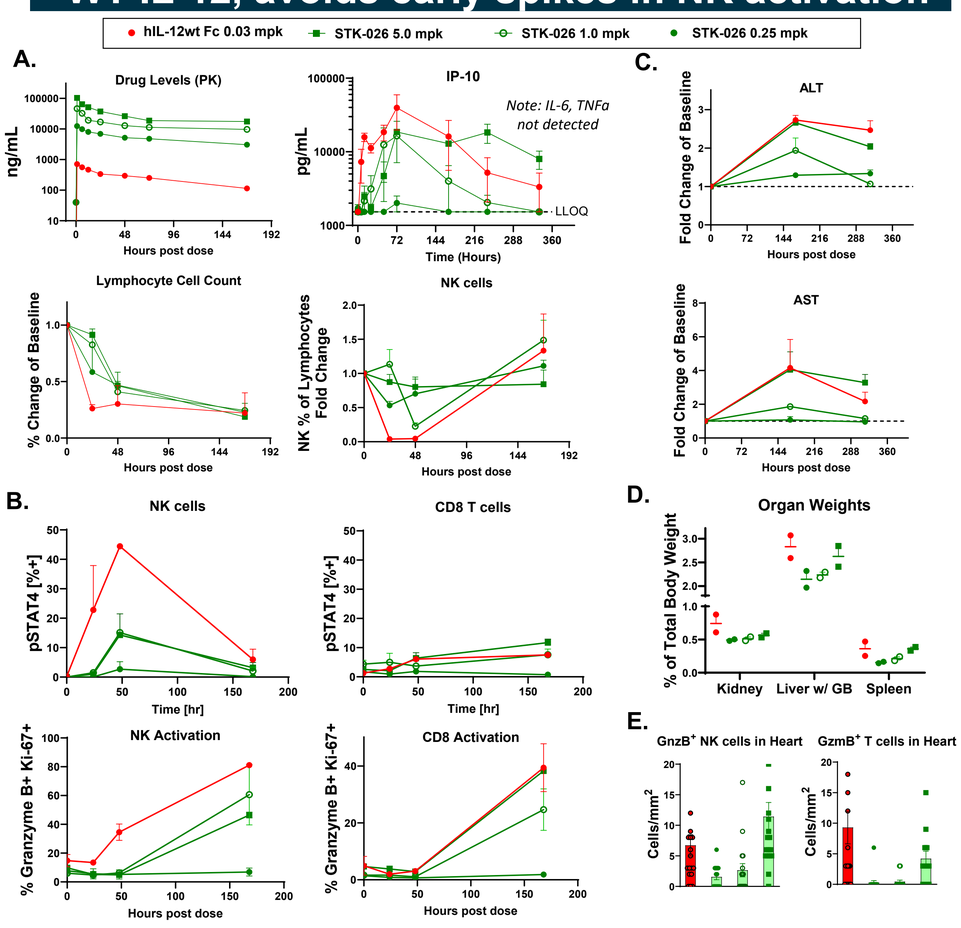

Figure 8: PK/PD responses to STK-026 in Cynomolgus Macaques (single dose)

A. STK-026 or IL-12wt Fc serum levels (PK), IP-10 serum chemokine levels, peripheral blood lymphocyte and NK changes after I.V. treatment of monkeys with WT IL-12 Fc of STK-026. **B.** Proximal (pSTAT4) or Distal (Ki67) biomarkers in NK or $CD8^+$ T cells. **C**. Serum Liver Enzymes ALT, AST. **D**. Organ weights at necropsy (D15). **E**. IHC analysis of $NKG2A^+$ Granzyme B^+ NK cells and $CD3^+$ Granzyme B^+ T cells in Heart.

Conclusions

1. **STK-026 is an engineered human IL-12 partial agonist**
2. **mSTK-026 shows improved therapeutic window for anti-tumor efficacy and tolerability vs wt mIL-12 Fc**
3. **STK-026 has reduced NK potency compared to wild type human IL-12-Fc and shows preferential activity on TCR activated T cells**
4. **mSTK-026 avoids systemic NK cell activation and effectively activates intratumoral CD4 and CD8 T cells**
5. **In contrast to mIL-12wt Fc, mSTK-026 maintains early activated CD8 TILs and maintains an activated phenotype in CD8 EM cells**
6. **STK-026 was well tolerated in Cynos with no clinical signs. STK-026 avoids early spikes of NK activation and chemokine induction in Cynos in comparison with IL-12wt Fc.**
7. **STK-026 demonstrates antibody-like PK in Cynos without CRS yet still activates effector T cells**
8. **STK-026 demonstrates CD8 T cell activation with reduced systemic liver enzymes and organ weight gain compared with IL-12wt Fc in Cynos**
9. **STK-026 represents a novel immunotherapy approach to maintain efficacy while avoiding classical toxicity associated with IL-12 therapy.**

References | 1) Atkins, *et al.;* (1997) *Clin Cancer Res* 3(3) 2) Carson, *et al.* (1999) *J Immunology* 162 (8) 3) Wang, *et al.* (2000) *Blood* 95 (10)
4) Shytikov, *et al.* (2021) *Front Immu* Jan (11) 5) Ruiz *et al.* (2014) *Nature Comm* 5 (5150) 6) Li et al. *Cancer Immunol Immunother* (2019) 68(8) 2018