STK-026, a detoxified IL-12 partial agonist is well-tolerated and sustains CD8+ T cell activity with repeat doses in cynomolgus macaques

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ABSTRACT# 941

Abstract

Background: Interleukin-12 (IL-12) is a pro-inflammatory cytokine produced by antigen-presenting cells which activates NK cells and cytotoxic CD8+ T cells and drives Th1 polarization in CD4+ T cells. In murine tumor models, IL-12 has potent T cell-mediated anti-tumor effects [1,2], but induces a systemic cytokine release syndrome (CRS) primarily via NK cell activation [2,3]. In patients, use of wildtype IL-12 is hampered by early dose-limiting toxicities, including CRS, hepatotoxicity, lymphopenia (especially NK cytopenia), neutropenia, and rapid tachyphylaxis on repeat dosing [4-6].

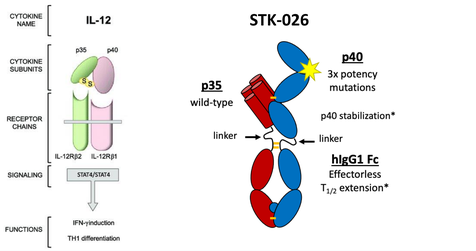
STK-026 is an Fc-fused human IL-12 partial agonist with diminished binding to IL-12Rb1, leading to decreased sensitivity to NK cells and increased selectivity towards T cells, which strongly upregulate IL-12Rb1 upon antigen activation. Previously, mouse STK-026 demonstrated strong anti-tumor immunity while avoiding acute NK activation and toxicity seen with WT mouse IL-12 [2]. Similarly, a single dose of human STK-026 in monkeys avoided early NK activation yet induced T cell proliferation [2]. Here, the results from a repeat-dose GLP toxicity study of STK-026 in cynomolgus monkeys are described.

Methods: Cynomolgus macaques were intravenously administered 0.5, 1.5 or 5 mg/kg STK-026, or vehicle, every other week for 3 cycles. Toxicity was evaluated by monitoring clinical symptoms, body weight, respiratory rate, cardiac function, clinical chemistry, and hematology. Immunological responses were assessed by blood immunophenotyping.

Results: All doses of STK-026 were well-tolerated with no drug-related clinical symptoms. Body weights and weekly peripheral lymphocyte, NK cell, platelet, and neutrophil counts remained stable upon treatment. Minor increases (<3-fold) in alanine aminotransferase and aspartate transaminase were observed 2 weeks after the first cycle of STK-026, while bilirubin remained stable, suggesting minimal liver-related toxicities. Total NK and T cell frequencies remained stable throughout the dosing. STK-026 induced transient NK cell proliferation only after the first dose. In contrast, STK-026 specifically, robustly and repeatedly increased and maintained CD38+Ki67+ and GranzymeB+Ki67+ memory CD8+ T cell activation at all dose levels without inducing PD-1. Similarly, STK-026 induced and maintained proliferation of CD4+ effector memory T cells. Lastly, STK-026 increased MHC-I and CD64 on monocytes and granulocytes, consistent with IFNγ pathway activation.

Conclusions: STK-026 is well-tolerated with minimal cytopenias or transaminase changes in cynomolgus macaques over 3 cycles of treatment up to 5 mg/kg. STK-026 induced transient NK cell proliferation, but sustained memory CD8+ T cell responses. These data indicate that STK-026 is a biased IL-12 partial agonist that could retain T cell responses while avoiding NK-mediated toxicities.

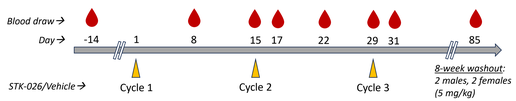
STK-026: An IL-12 partial agonist



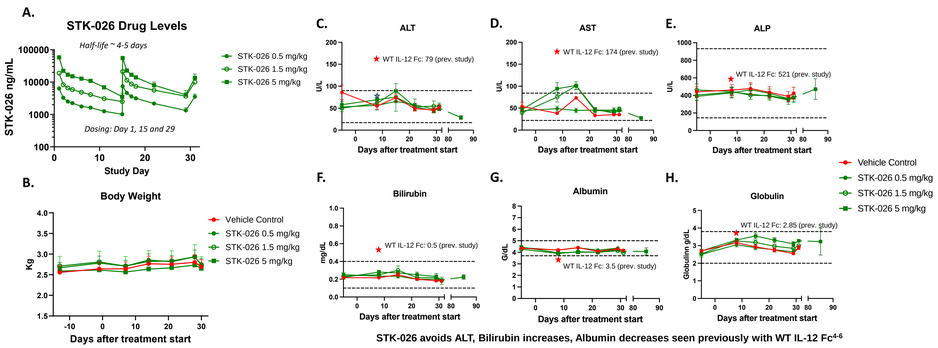
Study Design

GLP/Tox study in cynomolgus macaques: Treatment Groups

Group	Total animals	Males	Females	Treatment
1	6	3	3	Vehicle
2	6	3	3	STK-026 0.5 mg/kg
3	6	3	3	STK-026 1.5 mg/kg
4	10	5	5	STK-026 5 mg/kg



Prolonged exposure of high-dose STK-026 is well-tolerated in this repeat dose cynomolgus monkey study



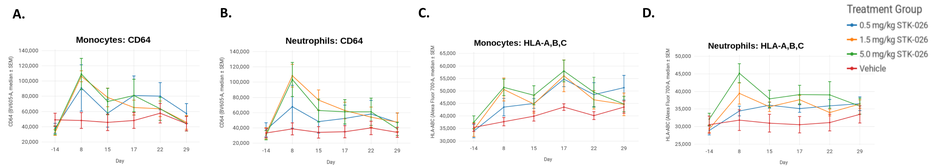
STK-026 avoids ALT, Bilirubin increases, Albumin decreases seen previously with WT IL-12 Fc[4-6]

(A) Pharmacokinetic analysis of STK-026 in the peripheral blood of cynomolgus monkeys. (B) The body weight of study animals remained stable through repeat doses. (C-H) Serum levels of alanine aminotransferase ALT (C), aspartate transaminase AST (D), and alkaline phosphatase ALP (E). Bilirubin (F), albumin (G) and globulin (H) levels showed minimal to no change with 3 cycles of STK-026 at all dose levels. Dotted lines indicate the minimum and maximum reference ranges for each analyte. Along with a lack of observed clinical symptoms, these data indicate that STK-026 was well-tolerated without body weight loss or liver toxicities with repeated doses of STK-026 in cynomolgus macaques.

STK-026 did not induce detectable cytopenias with repeat doses up to 5 mg/kg in cynomolgus monkeys

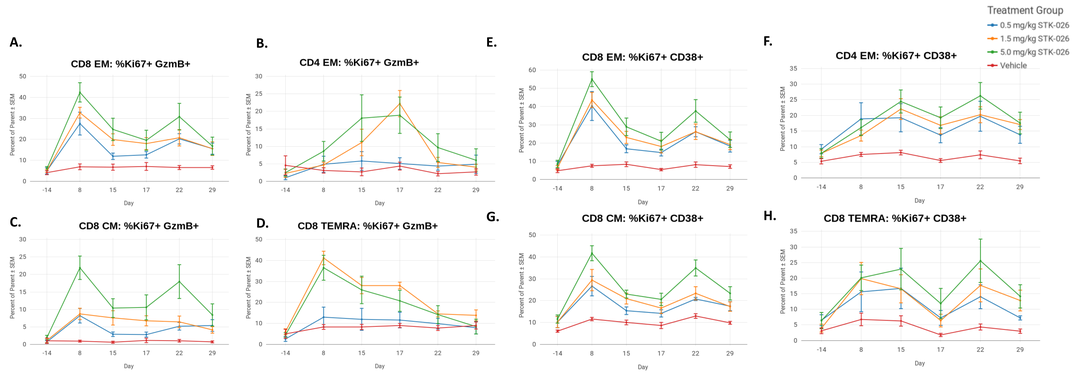
Hematology data show that STK-026 did not induce major changes in the counts of (A) lymphocyte, (B) neutrophils, (C) monocytes and (D) platelets. Dotted lines indicate the minimum and maximum reference ranges for each analyte. (E) Flow cytometry showed that the percentage of NKG2a+ NK cells and (F) CD3+ T cells among total lymphocytes showed minimal changes following STK-026 administration. Unlike WT-IL-12 or WT IL-12-Fc, this partial agonist does not induce cytopenias in major immune cell compartments, as seen by hematological analysis and flow cytometry.

CD64 and MHC class I IFNg pathway readouts are upregulated following the administration of STK-026



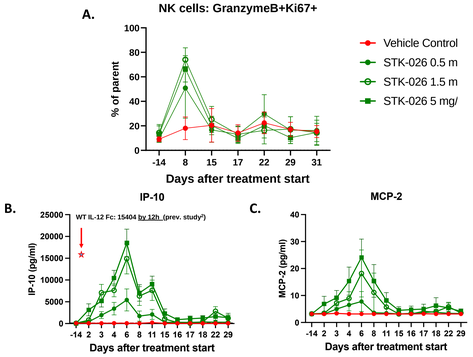
Flow cytometry analysis of peripheral blood showing median fluorescence intensities of IFNg target genes CD64 (Fcrγ1, Panels A,B) and HLA-A,B,C (MHC-I, Panels C,D) on CD14+ monocytes and CD87+ neutrophils. STK-026 induces the expression of CD64 on (A) monocytes and (B) neutrophils and MHC Class I on (C) monocytes and (D) neutrophils in a dose dependent manner.

STK-026 induces sustained activation and proliferation in CD4+ and CD8+ memory T cell subsets



Peripheral blood from STK-026 treated animals were analyzed for T cell activation by flow cytometry. Shown are frequencies of Ki67+GranzymeB+ or Ki67+CD38+ cells within CD8 effector memory (CD8 Tem) (A,E), CD4 Tem (B,F), CD8 central memory (CD8 Tcm) (C,G) and CD8 CD45RA+ terminal effector memory (CD8 TEMRA) (D,H) populations. STK-026 induces sustained T cell responses which peak approximately one week after each dosing cycle and are maintained above baseline levels

STK-026 induces transient proliferation in NK cells and systemic chemokines only after the first cycle



STK-026 induces transient NK cell proliferation and activation as seen by an increase in (A) Ki67+ Granzyme B+ NK cells. (B) Serum IP-10 and (C) MCP-2 were also induced by STK-026. These effects peaked only after the first dosing cycle, with minimal effects of subsequent cycles of STK-026.

No major toxicities in monkeys after 3 cycles of STK-026

STK-026 was well-tolerated in cynomolgus macaques after 3 cycles, with a No Adverse Event Level at or above 5 mg/kg

STK-026 Treatment-Related Findings:

- Macroscopic Observations: None
- Histopathology: Non-Adverse Minor liver inflammation
- Clinical Pathology: No Adverse Events
- ECG, Ophthalmology, Respiration: No Adverse Events
- Clinical Signs: No treatment related symptoms or interventions

Summary and Conclusions

- Repeat dosing of STK-026 was well-tolerated in cynomolgus macaques at all doses tested and caused no clinical symptoms or Adverse Events despite prolonged drug exposure and robust induction of CD8 T cell activation.
- STK-026 avoided classical IL-12 induced liver toxicity with only minor increases in AST, no bilirubin increases, and only minor non-adverse microscopic liver inflammation noted.
- STK-026 avoided classical IL-12 induced cytopenias with stable lymphocyte, monocyte, neutrophil, and platelet counts observed.
- STK-026 avoided early NK activation and chemokine induction seen with WT IL-12 and WT IL-12-Fc, yet STK-026 demonstrated signs of IFNγ pathway activation within one week.
- STK-026 induced sustained activation of CD4 and CD8 memory T cell subsets with each cycle of dosing. However, NK activation and systemic chemokine induction were transient (1st cycle only).
- Overall, STK-026 induced sustained CD8+ T cell responses, while avoiding (likely NK-mediated) classical IL-12 toxicities.

References

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