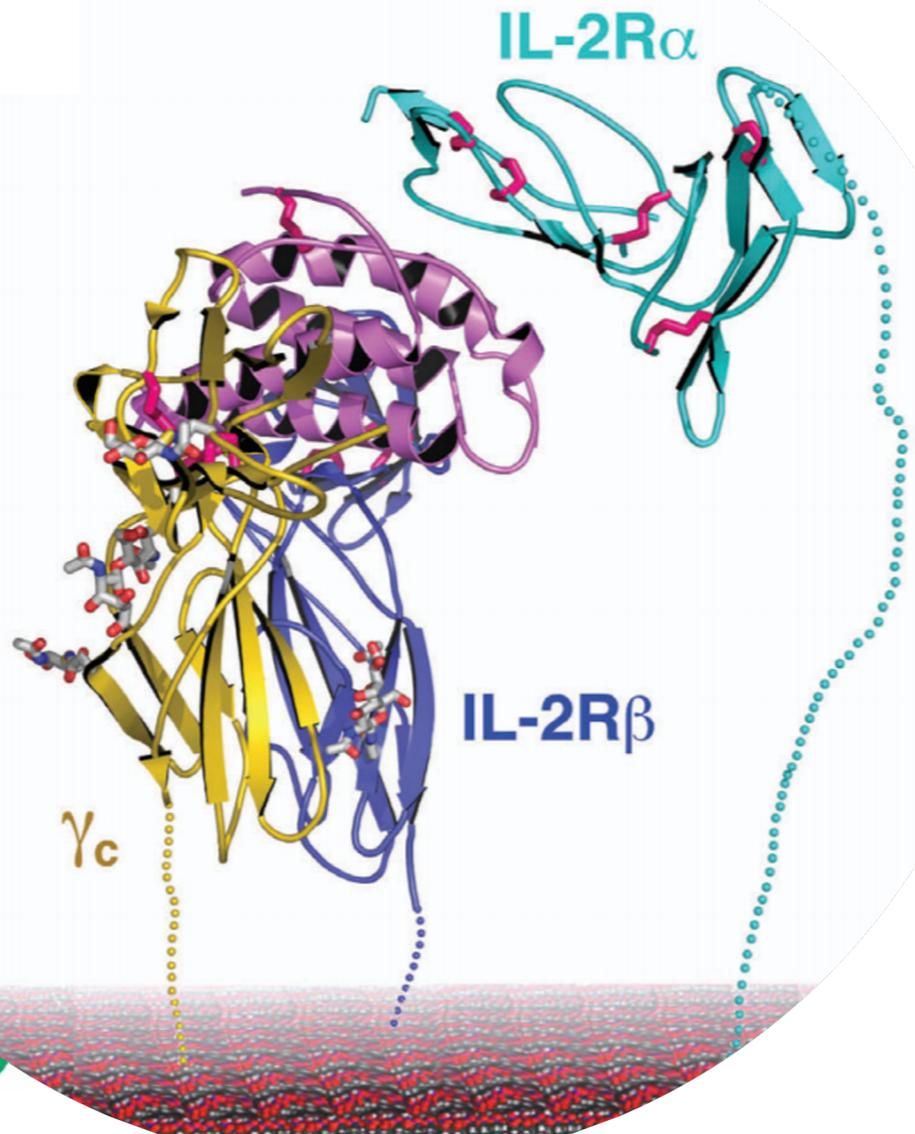


# AACR 2021 Poster # 1744



**STK-012, an alpha/beta selective IL-2 mutein for the activation of antigen-activated T cells in solid tumors**

*Jan Emmerich, Navneet Ratti, Michele Bauer, Sandro Vivona, Marie Semana, Bhargavi Jayaraman, Scott McCauley, Romina Riener, Rene De Waal Malefyt, Paul-Joseph Aspuria, Deepti Rokkam, Patrick Lupardus, Rob Kastelein, Martin Oft.*

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# STK-012, an alpha/beta selective IL-2 mutein for the activation of antigen-activated T cells in solid tumors

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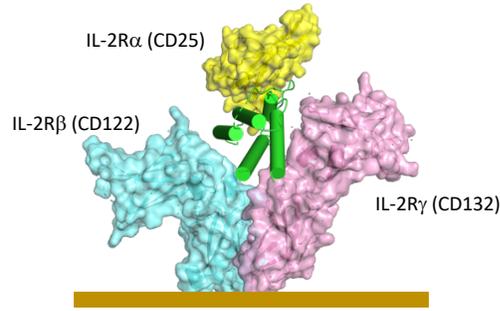
Synthekine, Menlo Park, CA

Interleukin-2 (IL-2) is a potent stimulator of T and NK cell proliferation, survival, and cytotoxic function. High dose IL-2 induces complete responses as a single agent in certain cancers. Its use is limited due to toxicities such as severe hypotension and vascular leak syndrome (VLS). A better understanding of the mechanisms of IL-2 efficacy and toxicity and development of more selective therapies are needed to improve the use of IL-2. Next generation IL-2 therapeutics have been engineered to bias activity towards the dimeric form of the IL-2 receptor, which consists of IL-2R $\beta$  (CD122) and IL-2R $\gamma$  (CD132), and away from the high affinity trimeric form of the receptor, which also includes IL-2R $\alpha$  (CD25). This approach is designed to stimulate NK cells and naïve effector T-cells, which express the dimeric, and not the trimeric, form of the receptor. However, this approach largely misses antigen-activated and tumor-specific T-cells, which have high expression of both CD25 and CD122. In addition, this approach expands peripheral NK cells, which have been shown in mice to cause VLS (1-3). To specifically stimulate antigen-activated CD25+ effector T cells in cancer patients and avoid systemic NK and naïve T cell activation, we have developed a pegylated, CD25/CD122-selective IL-2 mutein (STK-012) and its mouse surrogate (STK-014). Here we present efficacy and safety of STK-014 compared to wild type IL-2 (wtIL-2) and a CD122/CD132 biased IL-2 (non- $\alpha$ -IL-2). We also present safety of STK-012 in non-human primates (NHP). STK-012 / STK-014 selectively induced STAT5 phosphorylation and proliferation in antigen activated T cells but not in NK cells and naïve T cells. In mice, STK-014 showed reduced toxicity compared to wtIL-2 and non- $\alpha$ -IL-2. In particular, wtIL-2 and non- $\alpha$ -IL-2 induced VLS while STK-014 did not. Moreover, STK-014 demonstrated complete responses both as single agent and in combination with a PD-1 antibody in syngeneic tumor models. In general, STK-014 demonstrated improved efficacy compared to wtIL-2 and non- $\alpha$ -IL-2. STK-014 dramatically increased intratumoral T cells and intratumoral cytotoxic activity compared to wtIL-2 and non- $\alpha$ -IL-2 while avoiding T cell activation in the spleen. STK-014 treatment drastically increased the CD8+ T cell to Treg ratio within the tumor when compared to control tumors (25-fold), but also in comparison to wtIL-2 (2.75-fold) and to non- $\alpha$ -IL-2 (5.2-fold). In NHP, STK-012 was well tolerated at doses supra-*efficacious* in mice. Weekly dosing of STK-012 led to continuous elevated serum concentrations, demonstrating selectivity for CD25/122+ T cells. STK-012 selectively induced memory T cell expansion, including CD28+ CD95+ CD8 T cells. In summary, STK-012 avoids IL-2 mediated toxicity and may enable the specific expansion of antigen activated memory T cells in cancer patients, leading to durable tumor response.

# Evolution of IL-2 Therapeutics

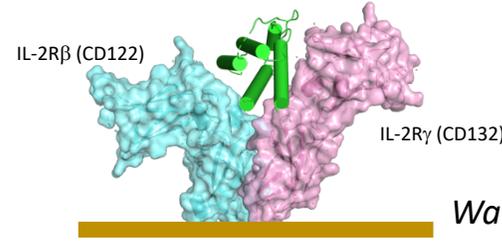
## High Affinity Receptor

Expressed on **antigen activated CD8s and Tregs**



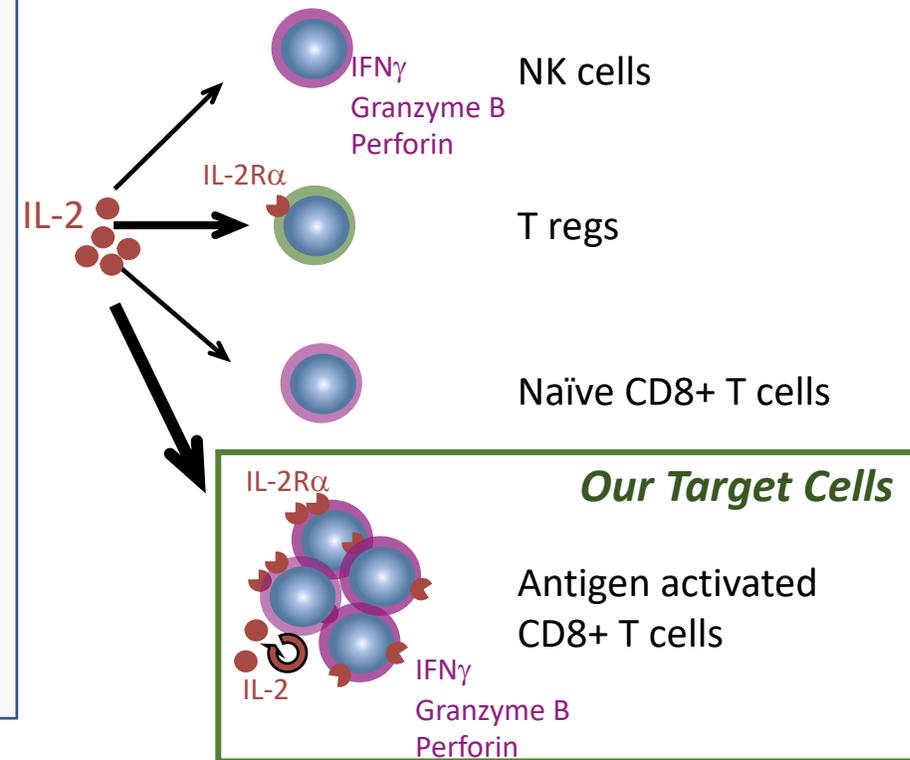
## Medium Affinity Interaction

Receptor complex most commonly expressed on NK cells and naïve (and not activated) T cells)



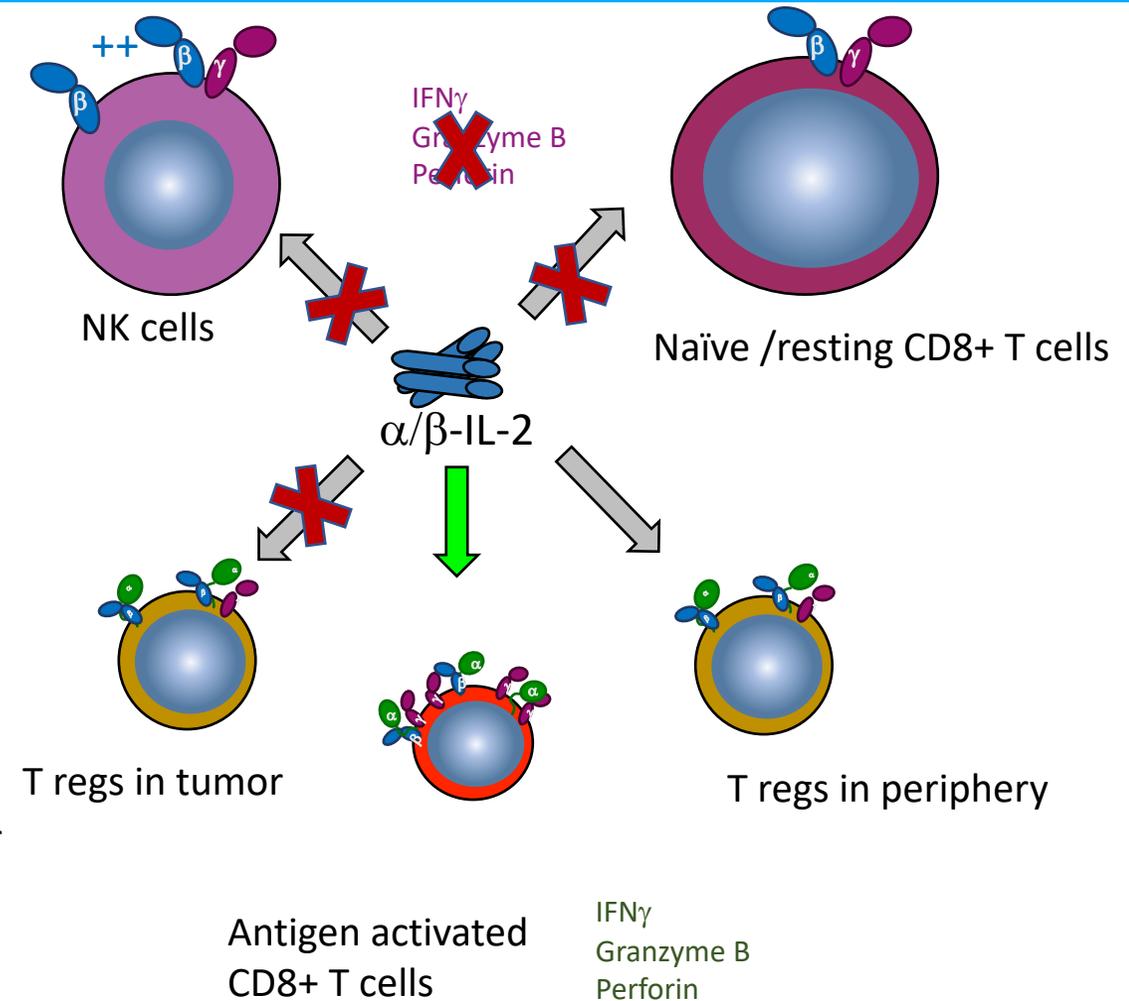
Wang et al (Garcia), Science 2005

- High dose wild type IL-2 has single agent activity including durable complete responses in melanoma and RCC patients
- IL-2 has significant, life threatening toxicity (small therapeutic window)
  - Capillary leak syndrome (CLS), hypotension mediated by NK and T cells
  - Perception that IL-2 binding to CD25 on endothelial cell or eosinophil may cause CLS through direct activation
- IL-2 activates T cells and NK cells, but also regulatory T cells and antigen activated T cells
- IL-2 preferentially activates CD25+ T cells (Tregs AND **antigen activated CD8+ T cells**)
- Current approaches: Avoid T reg activation by biasing away from CD25. As a byproduct, this strategy avoids **ANTIGEN ACTIVATED CD8+ T CELL ACTIVATION**
  - “Super 2”, NKTR-214, THOR-707, NL-201, ALKS 4230
  - Limited pre-clinical and clinical single agent activity
  - Substantially lower clinical doses than cyno MTD AND High Dose IL-2



# $\alpha/\beta$ -IL-2 Designed to Be Selective for Antigen Activated T-Cells

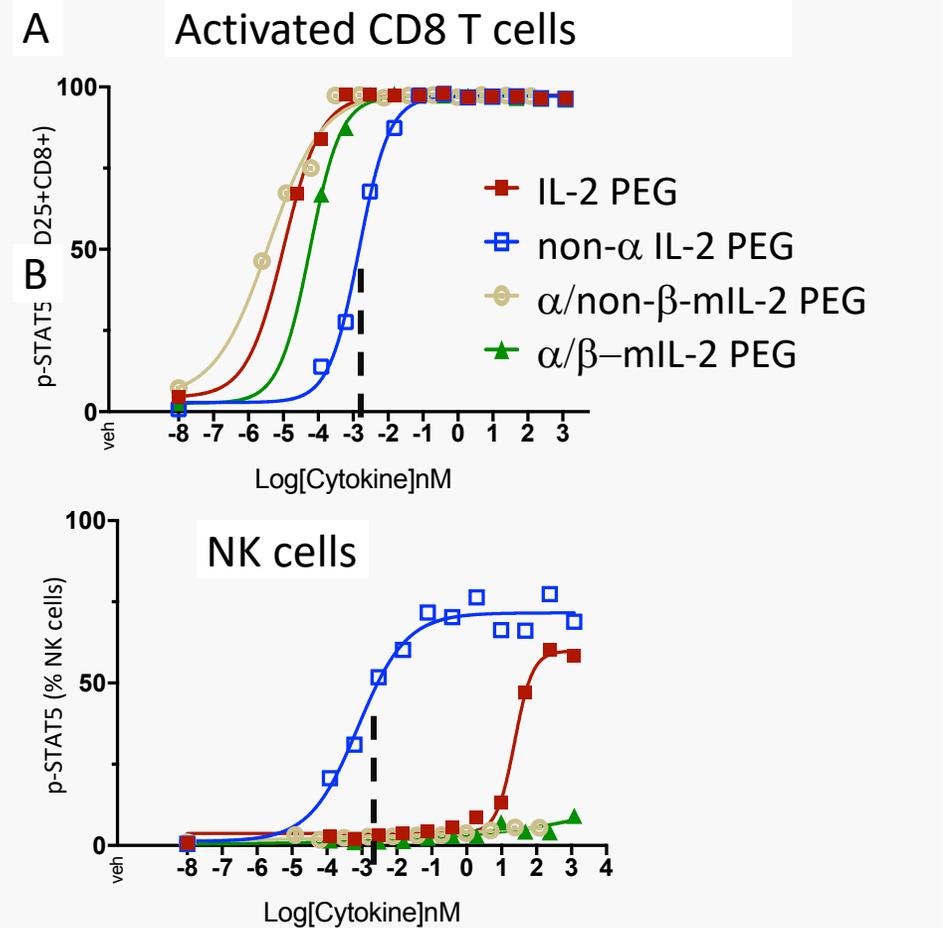
- **Design Target I: Avoid NK cells and Naïve T cells to avoid toxicity**
  - Both cell types lack IL2R $\alpha$
  - Low expression of IL2R $\gamma$   $\rightarrow$  less sensitive to IL2 with reduced gamma affinity
- **Design Target II: Proliferate and Activate Antigen Activated T Cells to enhance efficacy**
  - These cells have high expression of IL2R $\alpha$ , IL2R $\beta$ , and IL2R $\gamma$
  - High expression of IL2R $\gamma$   $\rightarrow$  still sensitive to IL2 with reduced gamma affinity
- **Design Target III: Avoid Tregs in the tumor microenvironment**
  - Competitive disadvantage of Treg versus CD8 $^+$  T cells in the tumor due to low expression of IL2R $\gamma$  expression on Tregs



**Fig.1 Target Selectivity of  $\alpha/\beta$ -IL-2**

# $\alpha/\beta$ -IL-2 is highly selective for activated T cells

<b>Synthetic Approach</b> <ul style="list-style-type: none"> <li>• STK-012                     <ul style="list-style-type: none"> <li>• Synthekine's IL-2 clinical candidate</li> <li>• Selective for <math>R\alpha+</math> / <math>R\beta+</math> T cells</li> <li>• Modulated <math>R\gamma</math> binding</li> </ul> </li> <li>• <math>\alpha/\beta</math>-mIL-2-PEG                     <ul style="list-style-type: none"> <li>• Mouse surrogate for STK-012</li> </ul> </li> </ul>	
<b>Currently Used in Cancer</b> <ul style="list-style-type: none"> <li>• IL-2                     <ul style="list-style-type: none"> <li>• approved in melanoma and RCC (Proleukin®)</li> </ul> </li> </ul>	
<b>1st Generation "improved" IL-2 in Cancer</b> <ul style="list-style-type: none"> <li>• non-<math>\alpha</math>-IL-2                     <ul style="list-style-type: none"> <li>• Engineered for reduced IL2R<math>\alpha</math> binding, IL-2 maintains binding selective for <math>R\beta/\gamma</math></li> <li>• Proxy for NKTR-214, THOR-707, NL-201, etc.</li> </ul> </li> </ul>	
<b>Current Approach in AI</b> <ul style="list-style-type: none"> <li>• non-<math>\beta</math>-IL-2 (Treg proliferation)                     <ul style="list-style-type: none"> <li>• Engineered for reduced/no <math>R\beta</math> binding IL-2 maintains binding selective for <math>R\alpha</math></li> <li>• Proxy for N88D, N88R, V91K, D20T, etc.</li> </ul> </li> </ul>	



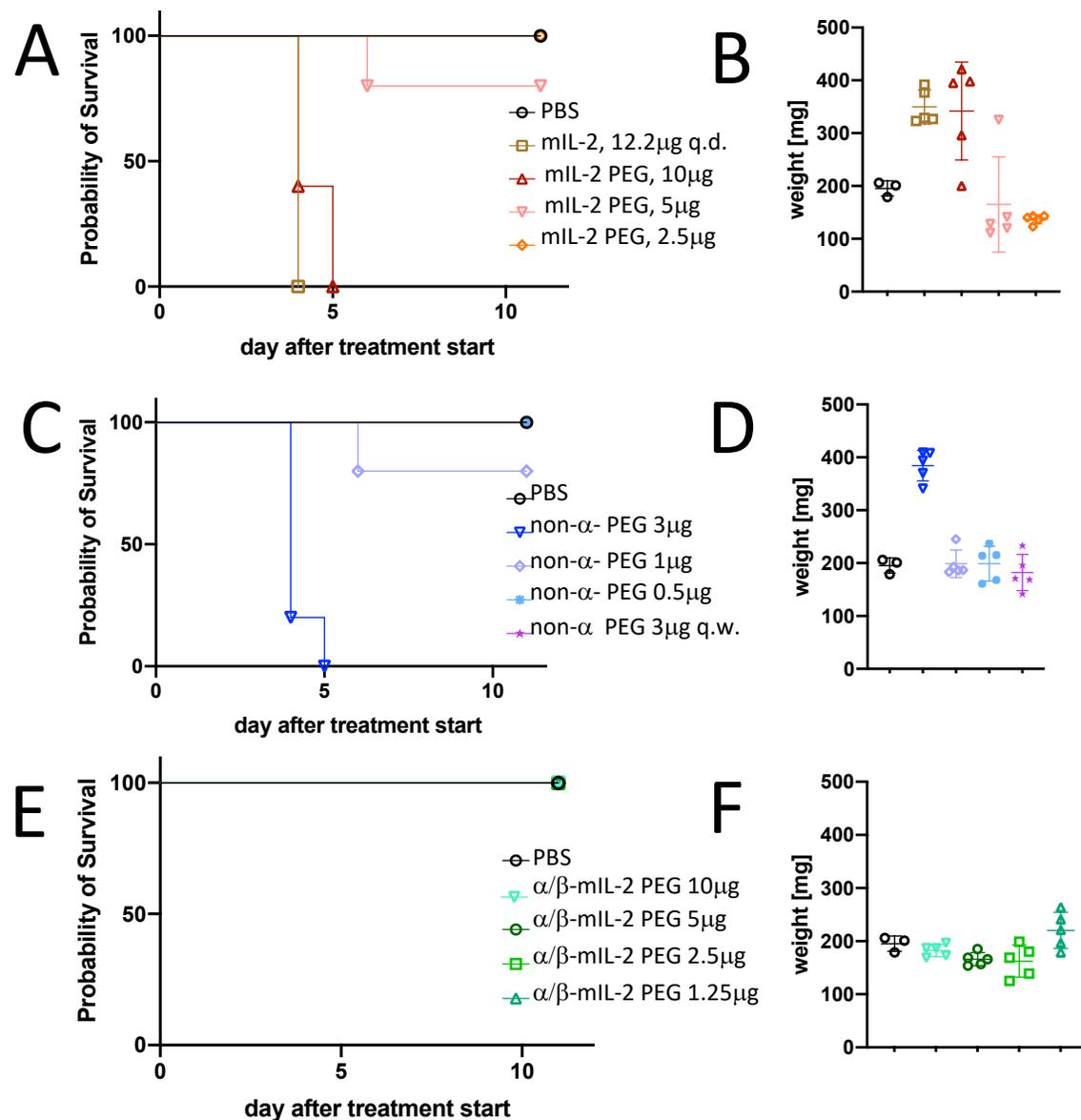
**Fig.2 Selectivity of wt-IL-2 and  $\alpha/\beta$ -IL-2-PEG but not non- $\alpha$ -IL-2-PEG for TCR activated T cells (A,B) STAT5 phosphorylation in response to IL-2 variants, (A) CD8 T cells, activated for 3 days with CD3/28 or (B) NK cells were stimulated for 20min with IL-2. Note: IL-2 has high selectivity for activated T cells vs. NK cells, but non- $\alpha$ -IL-2 has equal EC50 on both cell types.  $\alpha/\beta$ -IL-2 does not directly stimulate NK cells**

# $\alpha/\beta$ -IL-2 Does Not Induce Lethality and VLS in Mice, Unlike wt IL-2 and non- $\alpha$ -IL-2

**Fig. 3. Acute toxicity, lethality and vascular leak syndrome in wt-mIL-2 and non- $\alpha$ -IL-2-PEG but not  $\alpha/\beta$ -mIL-2-PEG treated mice (A,C,E) Survival and wet lung weight (B,D,F) of mice treated every second day (unless indicated) with the indicated doses of wt-mIL-2 (A,B) non- $\alpha$ - (C,D)  $\alpha/\beta$ -mIL-2 (E,F). (q.d. daily dosing, q.w. weekly dosing).**

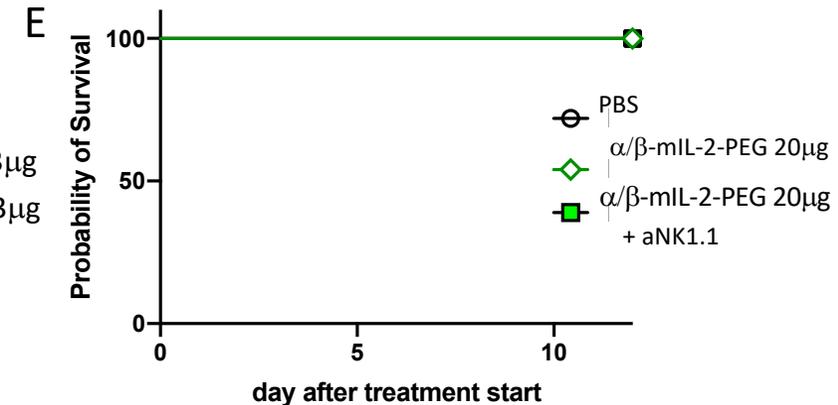
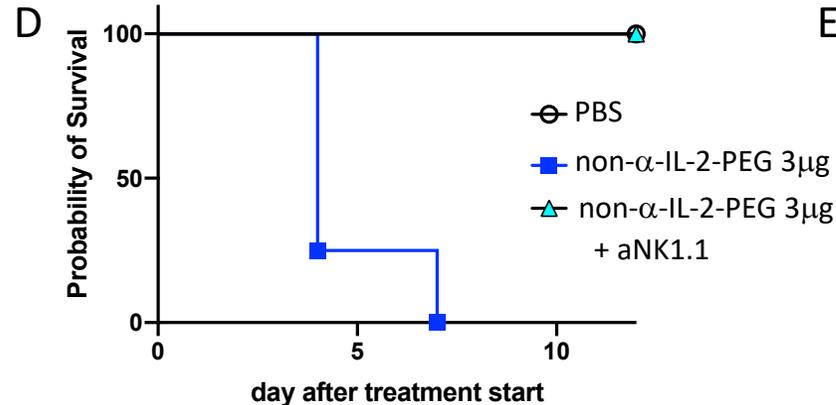
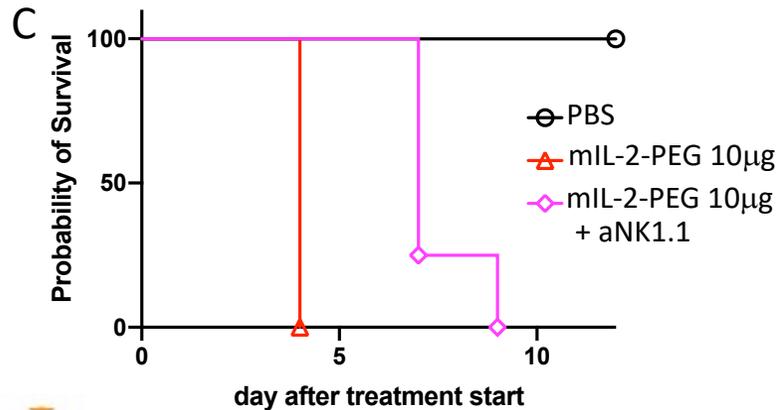
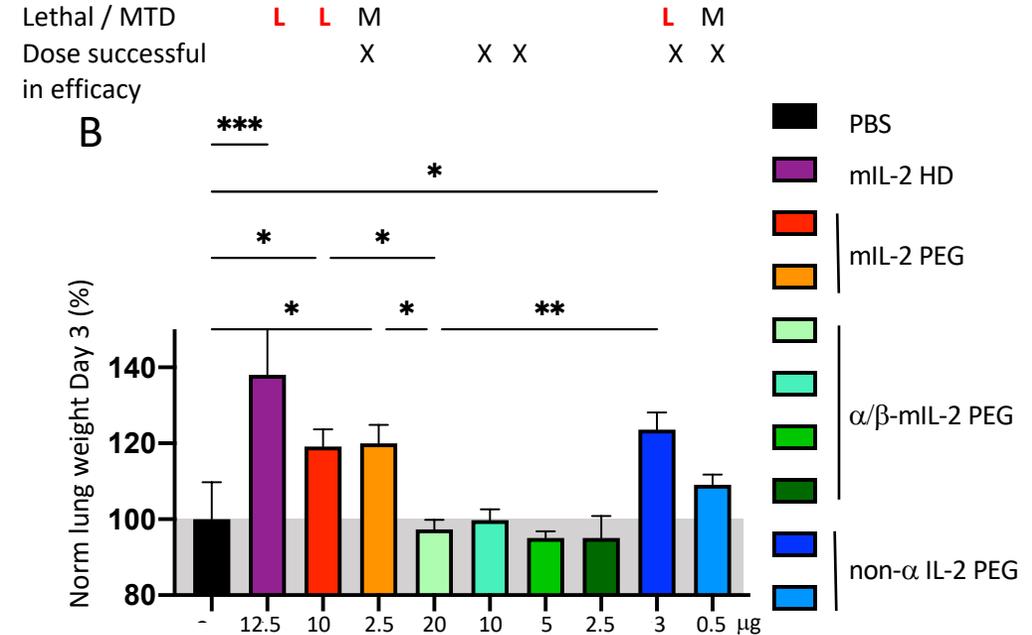
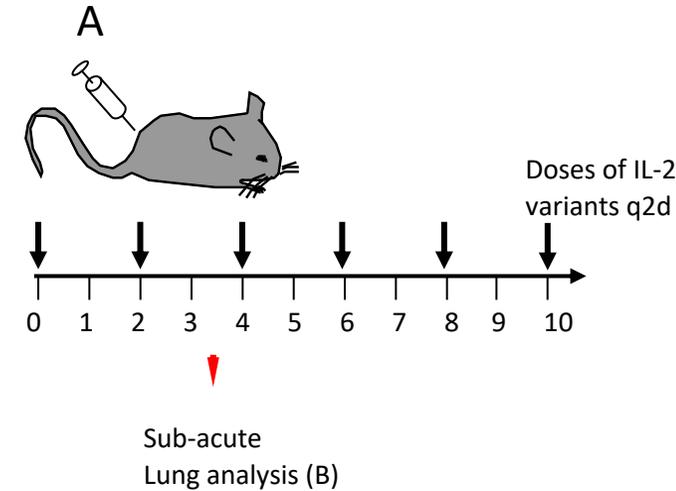
Lungs were harvested at the time of death or at the end of the study. Wet lung weights were determined by subtracting the lung wet after desiccation from the fresh lung weight

All IL-2 PEG molecules were covalently conjugated with a 40kD polyethylene glycol (PEG).

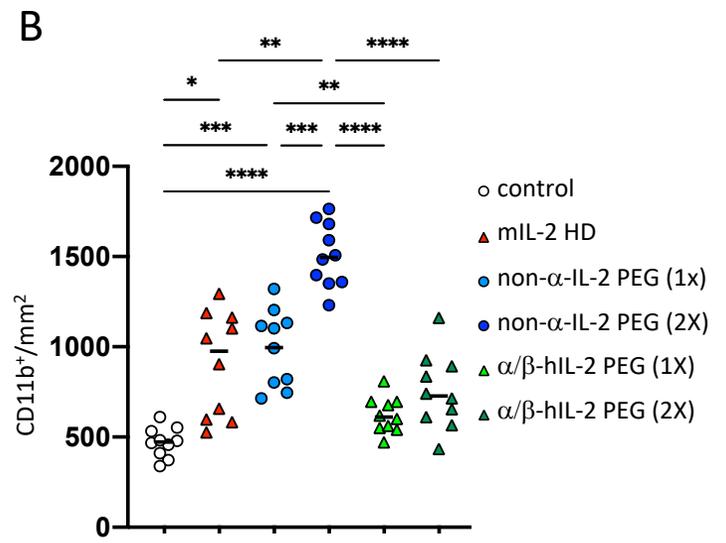
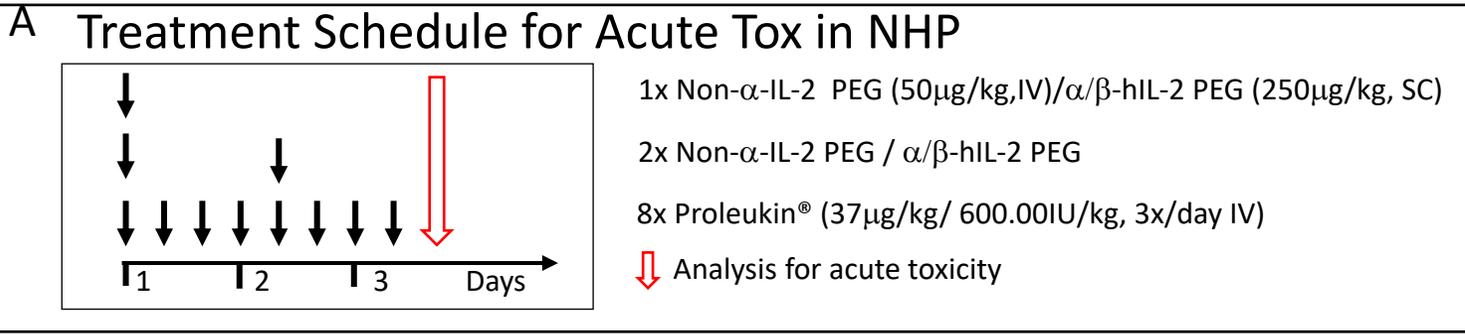


# Acute IL-2 Toxicity of WT IL-2 and non- $\alpha$ -IL-2-PEG is Abrogated by Depleting NK Cells

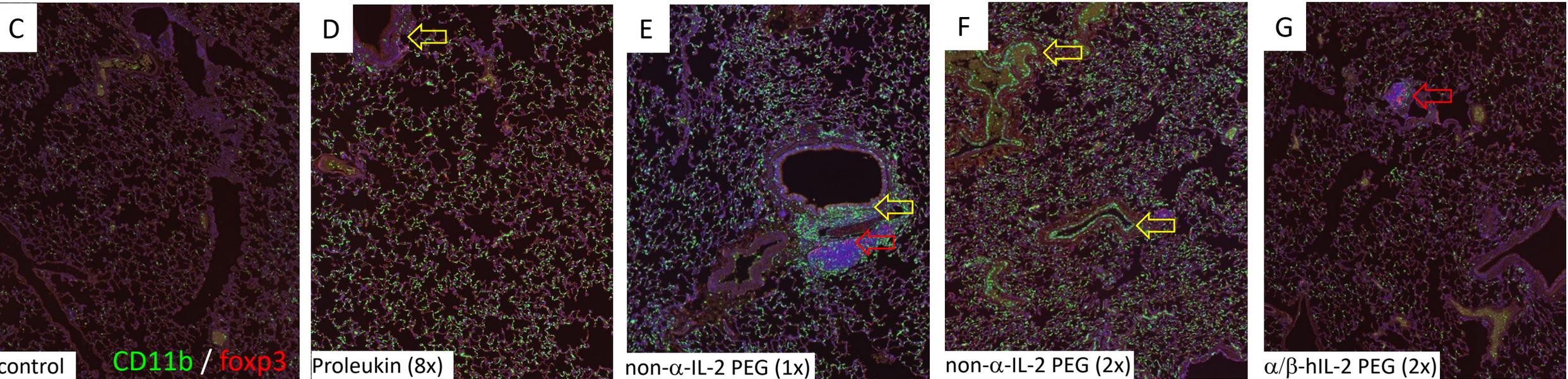
**Fig.4 NK Dependent Acute Immune toxicity to wt-mIL-2, or non- $\alpha$ -IL-2-PEG but not  $\alpha/\beta$ -mIL-2-PEG (A) Treatment schedule, lung weights (B) and survival (C-E) and of mice in IL-2 induced acute toxicity model. (B) Subacute lung weights of mice on day 3 of treatment with IL-2 variants ( $\mu\text{g}$ ) indicating vascular leak syndrome (VLS) in wt-IL-2 and non- $\alpha$ -IL-2 cohorts ; (C-E) Survival of mice on IL-2 variants (every other day, 10 $\mu\text{g}$  mIL-2 PEG, 3 $\mu\text{g}$  non- $\alpha$ -IL-2 or 20 $\mu\text{g}$   $\alpha/\beta$ -mIL-2-PEG) with or without NK cell depletion (anti-NK1.1)<sup>1</sup>. NK cell depletion avoids lethality induced by non- $\alpha$ -IL-2.**



# STK-012 ( $\alpha/\beta$ -IL-2) Does Not Induce Acute Lung Inflammation in Non-Human Primates (NHP), Unlike IL-2 and Non- $\alpha$ -IL-2

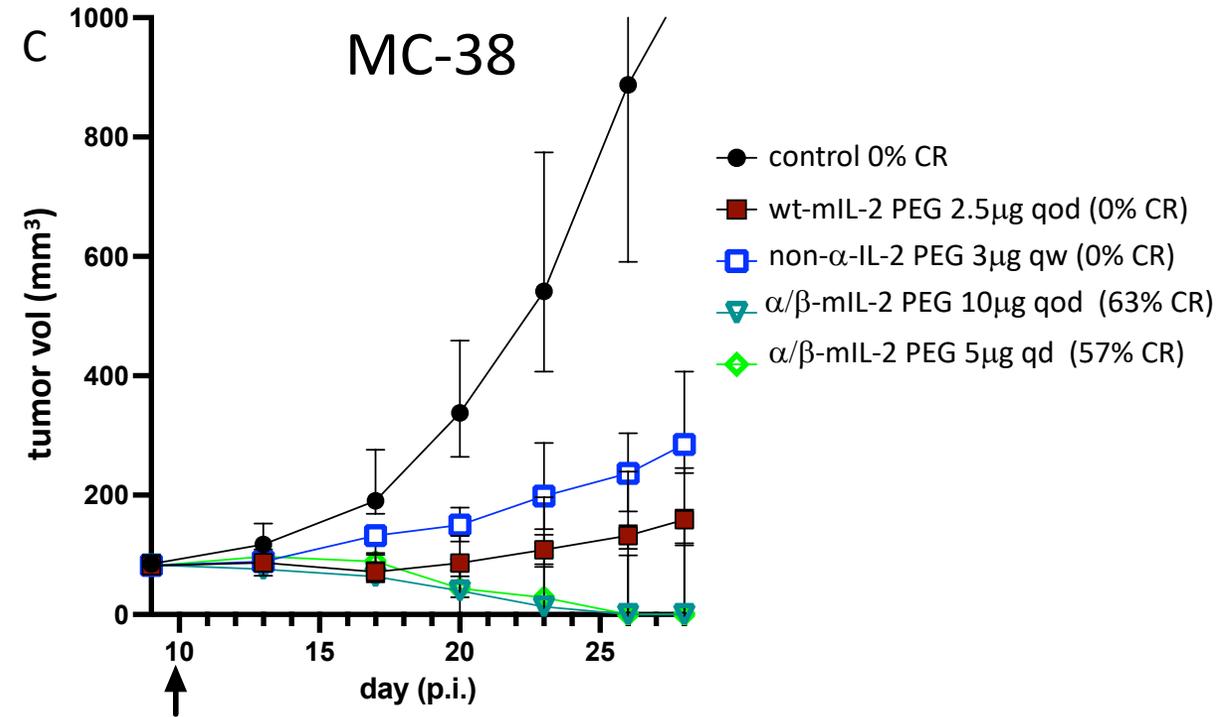
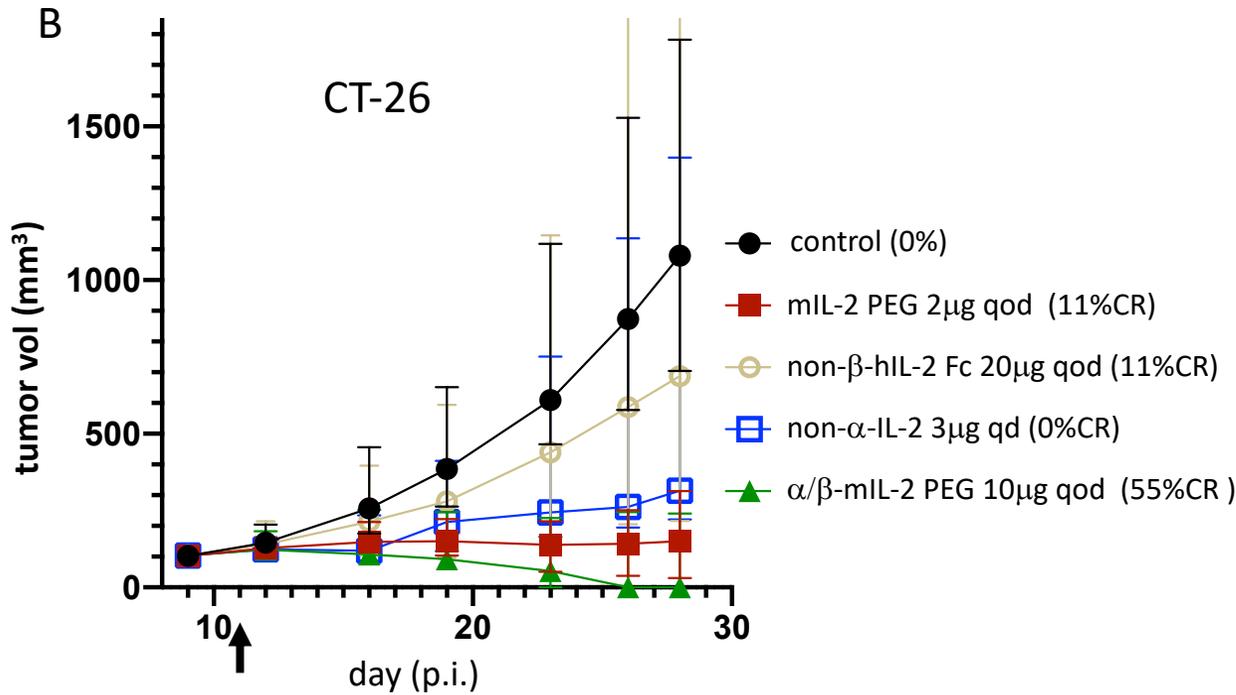
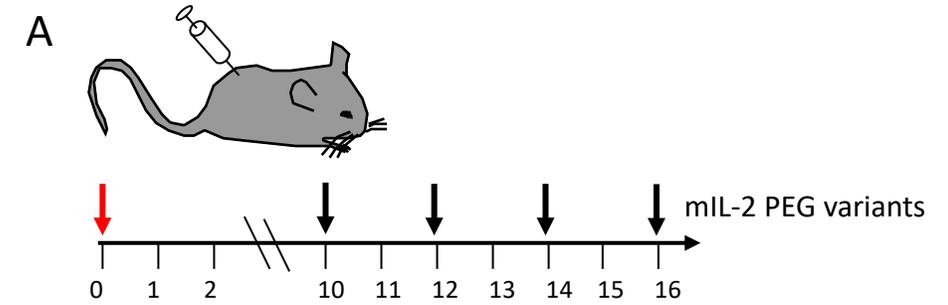


**Fig.5 Acute Immune toxicity to HD-IL-2, or non- $\alpha$ -IL-2 PEG but not to  $\alpha/\beta$ -hIL-2 PEG (A)** Treatment schedule of hIL-2 (Proleukin<sup>®</sup>, every 8h),  $\alpha/\beta$ -hIL-2-PEG or non- $\alpha$ -IL-2-PEG (1x or 2x) in NHP; **(B-G)** immunofluorescence (IF) for CD11b (green) and Foxp3+ Treg (red) in NHP acute lung injury model on day 3, **(B)** quantitation of CD11b+ cells (NK cells, macrophage, Granulocytes), **(C)** control lung, **(D)** Proleukin<sup>®</sup>, **(E,F)**, non- $\alpha$ -IL-2 PEG, and **(G)**  $\alpha/\beta$ -hIL-2 PEG. Yellow arrow: subendothelial CD11b+ infiltrate; red arrow: Foxp3+ Treg.



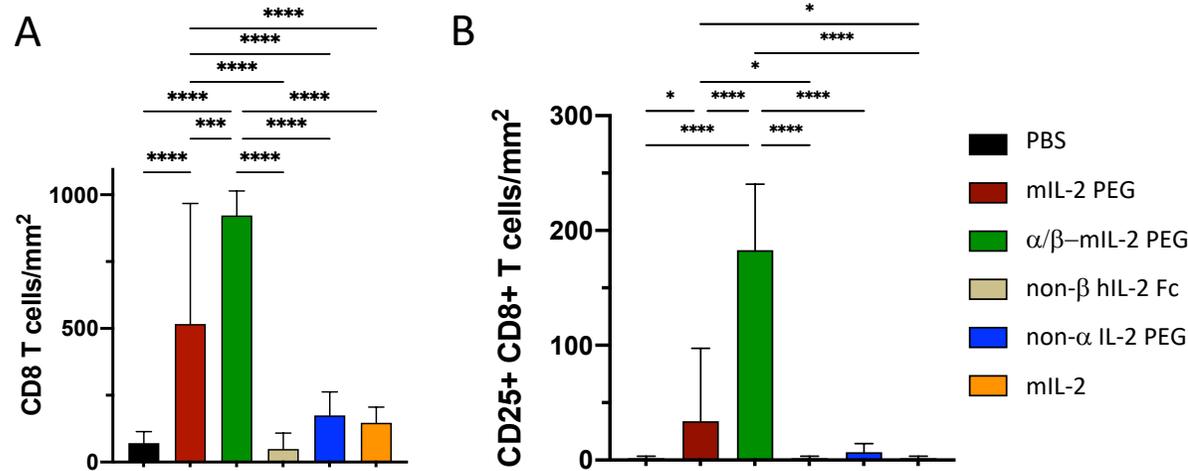
# $\alpha/\beta$ -mIL-2 PEG Demonstrates Superior Efficacy Compared to non- $\alpha$ -IL-2 PEG and mIL-2 PEG

**Fig.6  $\alpha/\beta$ -IL-2 PEG Enhances Tumor and CD8+ T cell response** (A) treatment schema, (B) tumor growth of CT-26 syngeneic colon cancer, (C) Tumor growth of MC38 tumors treated with IL-2 variants (2.5 $\mu$ g mIL-2 PEG every other day (MTD), 3 $\mu$ g non- $\alpha$ -IL-2 PEG weekly (MTD) or 10 $\mu$ g  $\alpha/\beta$ -mIL-2 PEG every other day). wt-mIL-2 PEG, non- $\alpha$ -IL-2 and non- $\alpha$ -IL-2 PEG were dosed at MTD (Fig. 3;  $\downarrow$  tumor injection;  $\downarrow$  dosing start; tumor volumes plotted post tumor inoculation; qd: daily; qod: every other day; qw: weekly).



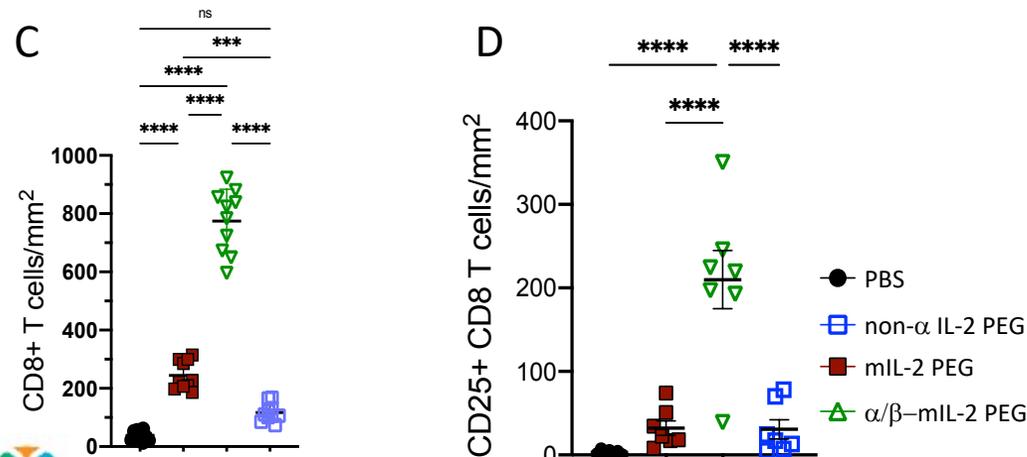
# $\alpha/\beta$ -mIL-2 Selectively Increases and Activates the CD8+ TILs in the Tumor

## CT-26 colon cancer model

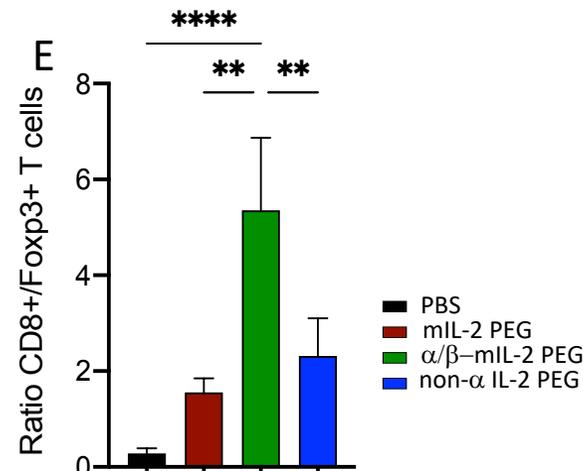


**Fig.7  $\alpha/\beta$ -IL-2-PEG Enhances Tumor and CD8+ T cell response (A,B)** CT-26 tumor infiltrating T cells, **(A)** quantitation of intratumoral CD8+ T cells and **(B)** CD25+ CD8+ T cells in response to IL-2s (IHC of Day 20 tumor from Fig.6B); **(C-F)** MC38 tumors, **(C)**, Intratumoral CD8+ T cells and **(D)** CD25+ CD8+ T cells in the tumor, **(E)** CD8+ T cell to Treg ratio in the tumor **(F)** GranzymeB+ cells in relation to Tregs in the spleen of mice treated with IL-2 variants (Quantitation of immunohistochemistry for CD8, CD25, granzyme B, Foxp3 respectively). High level of granzyme B<sup>+</sup> cells in the spleen induced by non- $\alpha$ -IL-2 may indicate peripheral toxicity of non- $\alpha$ -IL-2 (Fig. 4).

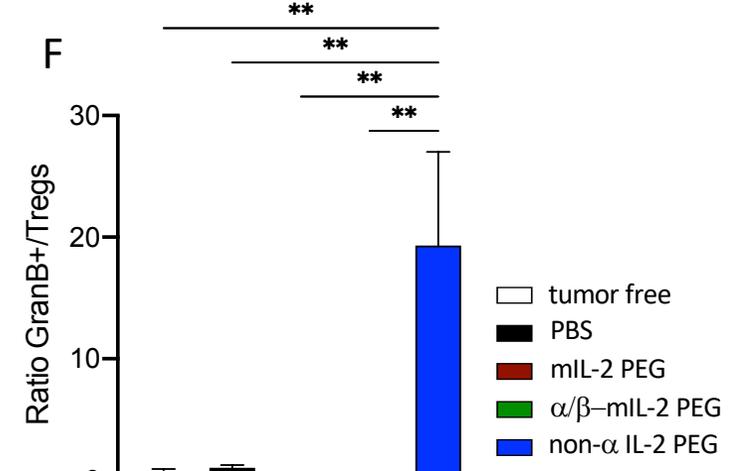
## MC38 colon cancer model



## Tumor



## Spleen



# IL-2 directed to antigen activated T cells - Summary

- $\alpha/\beta$ -mIL-2 and STK-012 are highly selective for antigen activated T cells
  - No detectable activity in NK cells and resting T cells
- No IL-2 related acute toxicity and vascular leak induced by  $\alpha/\beta$ -mIL-2 and STK-012
  - Acute NK cell dependent lung and liver toxicity observed with wt-IL-2 and non- $\alpha$ -IL-2
- $\alpha/\beta$ -IL-2 has strongly increased anti-tumor activity in syngeneic tumor models compared to wt-IL-2, non- $\alpha$ -IL-2 or non- $\beta$ -IL-2
  - Complete responses as monotherapy (in large tumors)
  - Efficacy correlates with strongly increased intratumoral CD8<sup>+</sup> T cells and CD25<sup>+</sup> CD8<sup>+</sup> T cells
  - Strongest increase of intratumoral CD8 / Treg ratio compared to other IL-2s
- In summary, STK-012 avoids IL-2 mediated acute toxicity which may enable specific expansion of antigen activated memory T cells in cancer patients, enabling a more durable anti-tumor response.