Pharmacokinetic and pharmacodynamic study of NKTR-255, a polymer-conjugated IL-15, in cynomolgus monkey

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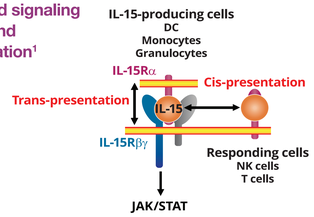
Introduction

Interleukin-15 (IL-15) is a common γc cytokine that activates and provides survival benefit to memory T and NK cells. IL-15 is predominantly produced by myeloid cells and its receptor is a heterotrimeric receptor consisting of the IL-15 receptor α subunit and IL-2/IL-15 receptor β, γ subunits. Exploiting the therapeutic value of native IL-15 has been challenging due to its unfavorable pharmacokinetic properties and undesirable tolerability profile. NKTR-255 is a polymer-conjugated human IL-15 that retains binding affinity to the α subunit of the IL-15 receptor and exhibits reduced clearance to provide a sustained pharmacodynamics response. Here we investigate the biological effects of NKTR-255 in naïve cynomolgus monkey.

IL-15-mediated signaling through cis- and trans-presentation[1]



IL-15 binds the unique IL-15Rα chain and presents to the IL-2/IL-15Rβγ complex on the same (cis) or adjacent cell (trans). Engagement of the IL-2/IL-15R βγ complex can induce JAK-STAT signaling, increasing survival and proliferation. This process is crucial for the proper support of IL-15 biology.[2]

Methods

***In vitro* assay:**

Cynomolgus monkey whole blood was stimulated with the indicated concentration of NKTR-255 or IL-15 for 20 minutes and the percentage of pSTAT5 positive populations in each of NK, CD4 T and CD8 T cells was determined by flow cytometry.

***In vivo* PK:**

Male cynomolgus monkeys received single IV doses of 0.05 mg/kg IL-15 or 0.1 mg/kg NKTR-255. Blood samples were collected to determine the plasma concentrations of NKTR-255 by enzyme-linked immunosorbent assay.

***In vivo* PD:**

Male cynomolgus monkeys received a single IV dose of NKTR-255 at 0.001, 0.01 or 0.1 mg/kg. Blood samples were collected to assess the effects of NKTR-255 on peripheral immune cell populations at multiple time points. Flow cytometry was used to measure STAT5 phosphorylation, Ki-67 expression, Granzyme B expression and frequency of cell populations.

Results

NKTR-255 induced dose-dependent phosphorylation of STAT5 in monkey whole blood (EC50 values NK cells: 6.9 ng/ml, CD8 T cells: 39 ng/ml, CD4 T cells: 53 ng/ml). The half-life and clearance of NKTR-255 were 26x longer and 38x lower, respectively, than of IL-15. NKTR-255 engaged the IL-15 signaling pathway, *in vivo*, demonstrating both robust and sustained STAT5 phosphorylation in lymphocytes. NKTR-255 drove the proliferation of total CD8 T cells and NK cells in a dose-dependent manner, with dramatic and durable increases observed in Ki67 positive population and absolute cell numbers (NK cells: 6.1 fold; CD8 T cells: 7.8 fold from baseline on day 5 at 0.1 mg/kg). These effects were strongly biased towards CD8 T cells and NK cells, with substantially less induction of CD4 T cells. The Ki67 response analyses of the T cell subpopulation revealed a higher response of memory populations than of naïve T cells. Among memory T cells, effector memory T cells showed the highest response over stem cell memory T cells and central memory T cells. NKTR-255 also increased the expression of Granzyme B in both NK and CD8 T cells, concomitant with an enhancement in target cell lysis. Finally, repeated administration of NKTR-255 sustained the Ki67 response of NK and CD8 T cells at levels similar to those observed at the single dose.

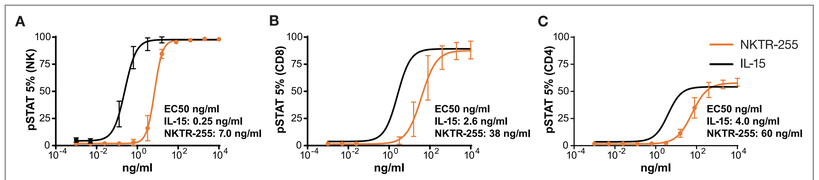
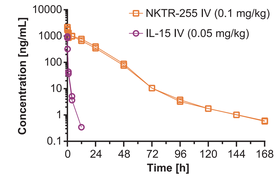


Figure 1. *In vitro*, NKTR-255 showed a dose-dependent phosphorylation of STAT5 in NK cells, CD8 T cells and CD4 T cells

Monkey whole blood was stimulated with the indicated concentration of NKTR-255 or IL-15 for 20 minutes and the percentage of pSTAT5 positive populations in each NK (A), CD8 T (B) or CD4 T cells (C) was determined by flow cytometry.

Results



Test Article	IV Dose [mg/kg]	CL [mL/hr/kg]	t½ [h]	Vd [mL/kg]
IL-15	0.05	161.4	1.2	112
NKTR -255	0.1	4.26	30.5	72.8

Figure 2. A single dose of NKTR-255 exhibited reduced clearance and longer half-life than IL-15

Monkeys received single IV doses of IL-15 at 0.05 mg/kg or NKTR-255 at 0.1 mg/kg. Blood samples were collected to determine the plasma concentrations of NKTR-255 or IL-15.

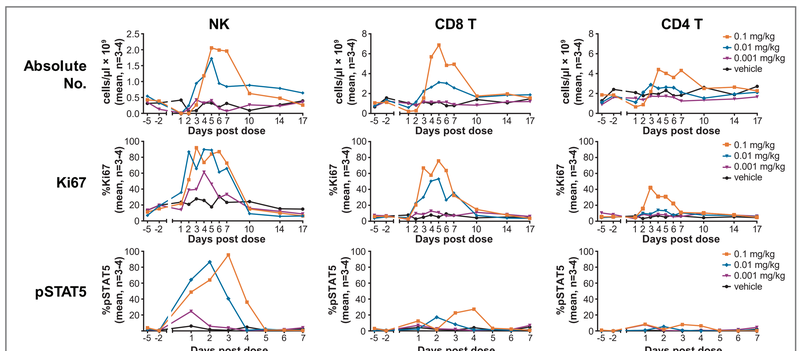


Figure 3. A single dose of NKTR-255 provided dominant effect to both NK cells and CD8 T cells, but not CD4 T cells

Monkeys received single IV doses of 0.001, 0.01 or 0.1 mg/kg of NKTR-255. Blood samples were collected to assess pSTAT5, Ki67 positive populations and the absolute number of peripheral NK, CD8 T or CD4 T cells by flow cytometry.

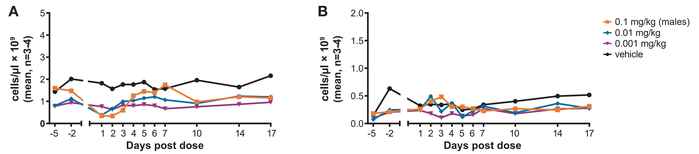


Figure 4. A single dose of NKTR-255 did not significantly impact B cells and monocytes

Monkeys received single IV doses of 0.001, 0.01 or 0.1 mg/kg of NKTR-255. Blood samples were collected to assess the absolute number of peripheral B cells (A) or monocytes (B) by flow cytometry.

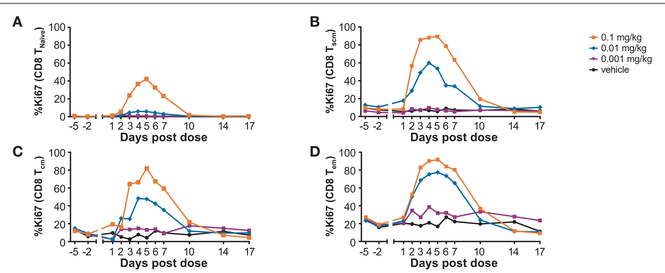


Figure 5. A single dose of NKTR-255 resulted in a higher response of memory CD8 T cell populations than of naïve CD8 T cells

Monkeys received single IV doses of 0.001, 0.01 or 0.1 mg/kg of NKTR-255. Blood samples were collected to assess Ki67 positive populations of naïve CD8 T cells (A), stem cell memory CD8 T cells (B), central memory CD8 T cells (C) or effector memory CD8 T cells (D) by flow cytometry.

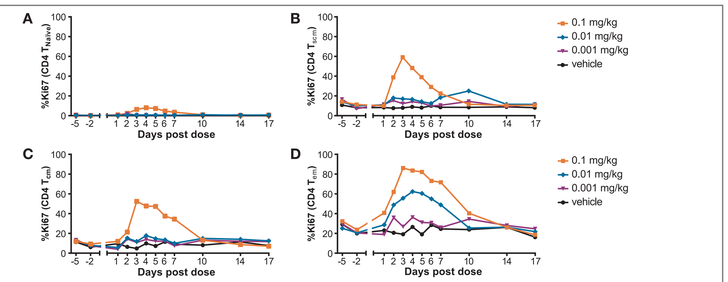


Figure 6. A single dose of NKTR-255 resulted in a higher response of memory CD4 T cell populations than of naïve CD4 T cells

Monkeys received single IV doses of 0.001, 0.01 or 0.1 mg/kg of NKTR-255. Blood samples were collected to assess Ki67 positive populations of naïve CD4 T cells (A), stem cell memory CD4 T cells (B), central memory CD4 T cells (C) or effector memory CD4 T cells (D) by flow cytometry.

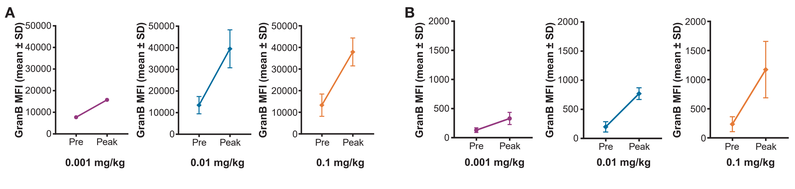


Figure 7. A single dose of NKTR-255 increased Granzyme B on peripheral NK and CD8 T cells

Monkeys received single IV doses of 0.001, 0.01 or 0.1 mg/kg of NKTR-255. Blood samples were collected to assess Granzyme B expression on peripheral NK (A) or CD8 T (B) cells by flow cytometry.

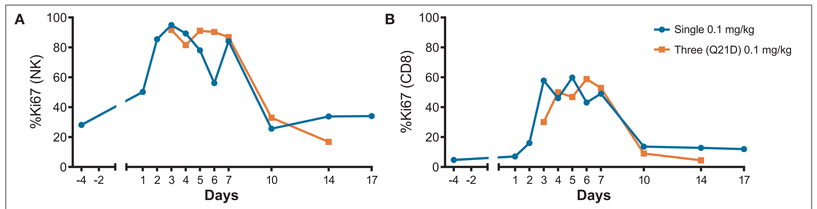


Figure 8. Repeat dosing of NKTR-255 did not reduce the magnitude of Ki67 responses in NK and T cells

Monkeys received single or three (Q21D) IV doses of 0.1 mg/kg of NKTR-255. Blood samples were collected after the first (single) or third (Q21Dx3) dose at the indicated time points and assessed for Ki67 positivity in NK cells (A) and CD8 T cells (B) by flow cytometry.

Conclusions

- NKTR-255 engages the JAK/STAT5 pathway with 10-fold less potency compared with IL-15
- A single dose of NKTR-255 exhibited reduced clearance and longer half-life than IL-15
- A single dose of NKTR-255 substantially enhances *in vivo* proliferation and activation of NK and CD8 T cells
- A single dose of NKTR-255 resulted in a higher response of memory populations than of naïve T cells
- A single dose of NKTR-255 increased levels of a cytotoxic enzyme in NK and CD8 T cells
- Repeat dosing of NKTR-255 does not reduce the magnitude of Ki67 responses in NK and T cells

References

1. Marcais et al., Regulation of mouse NK cell development and function by cytokines. *Front. Immunol.* 12 Dec 2013
2. Stonier and Schluns. Trans-presentation: a novel mechanism regulating IL-15 delivery and responses. *Immunol Lett.* 2010.

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