Pre-clinical investigation of NKTR-255, a polymer-conjugated IL-15 with a potent NK cell dependent anti-tumor efficacy

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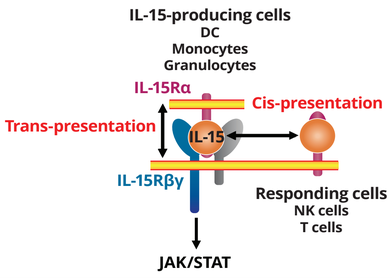
BACKGROUND

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 βγ 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 recombinant IL-15 that retains binding affinity to the α subunit of the IL-15 receptor and exhibits reduced clearance to provide a sustained pharmacodynamic response. Here we investigate the pharmacological properties of NKTR-255 on NK cells and the effect of NKTR-255 in NK cell-dependent tumor models.

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* assays:** Mouse whole blood was stimulated with the indicated concentration of NKTR-255 or IL-15 for 20 minutes. Enriched mouse splenic NK cells were stimulated with NKTR-255 overnight and used as effectors in a standard flow-based cytotoxic assay against YAC-1 (a mouse T lymphoma cell line) target cells.

***In vivo* PD assays:** Mice received single or three times (weekly) IV doses of 0.03 or 0.3 mg/kg of NKTR-255. Blood and spleen samples were collected to assess the NK cell population and function. Flow cytometry was used to measure pSTAT5, Ki-67, Mcl-1, Granzyme B, and CD16 in NK cells. Purified splenic NK cells from NKTR-255 treated mice were co-cultured with YAC-1 to measure cytotoxic function.

***In vivo* efficacy models:** In the CT26 mouse model, 1x10^5 cells were administered intravenously on Day 0, treatment was initiated on Day 1 at 0.03, 0.1, or 0.3 mg/kg IV q7d, and on Day 13 lungs were scored for metastases. In the 4T1 spontaneous metastasis mouse model, 5x10^5 cells were implanted in the mammary fat pad on Day 0, treatment was initiated on Day 5 at 0.3 mg/kg IV q7d, and on Day 14, metastases were determined from culture of single lung cell isolates. In the Daudi B cell lymphoma model, SCID mice were intravenously injected on day 0 with 1x10^7 Daudi cells. On days 14 and 17 mice were treated with rituximab (40 mg/kg IP), and/or NKTR-255 (0.3 mg/kg SC). Survival rate was determined with onset of hindlimb paralysis as a surrogate parameter.

RESULTS

NKTR-255 showed a dose-dependent phosphorylation of STAT5 in the mouse NK cells with a EC50 of 42 ng/ml. Engagement of the IL-15 pathway enhanced cytotoxic function in mouse NK cells to kill YAC-1 cells at all effector ratios.

A single administration of NKTR-255 *in vivo* increased the pSTAT5+ population and the Ki67+ population of NK cells. The absolute number of NK cells was also increased at 4 days post treatment by 6.6-fold and 100-fold at 0.03 and 0.3 mg/kg, respectively. In addition, a single dose of NKTR-255 resulted in enhanced Mcl-1, Granzyme B, and CD16 expression and cytotoxic function, illustrating sustained effects of NK cell activation.

Repeated administration of NKTR-255 sustained the proliferation and activation of NK cells at levels similar to those observed at the single dose. Finally, pretreatment of NKTR-255 resulted in sustained cytotoxic function in both *ex vivo* and *in vivo* studies.

In the disseminated CT26 model, NKTR-255 treatment resulted in a significant increase of NK cells in lung and a dose-dependent reduction in the number of lung metastases in a NK cell-dependent manner. In the 4T1 spontaneous metastasis model, NKTR-255 also showed a significant anti-metastatic effect although it did not affect primary tumor growth. Finally, NKTR-255 synergistically provided long-term survival benefit when administered with rituximab in the Daudi B cell lymphoma model.

RESULTS *(continued)*

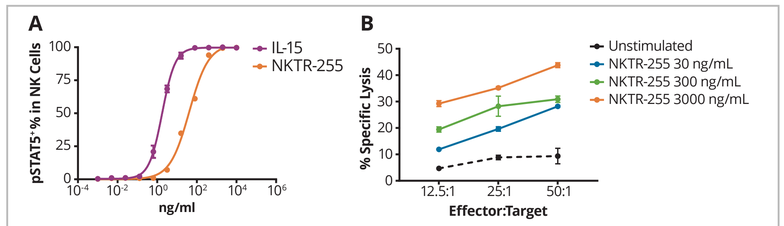


Figure 1. *In vitro*, NKTR-255 showed a dose-dependent phosphorylation of STAT5 and enhancement of cytotoxic function in mouse NK cells

Mouse whole blood was stimulated with the indicated concentration of NKTR-255 or IL-15 for 20 minutes (A). Enriched mouse splenic NK cells were stimulated with the indicated concentration of NKTR-255 over night and used as effectors in a standard flow-based cytotoxic assay against YAC-1 target cells (B).

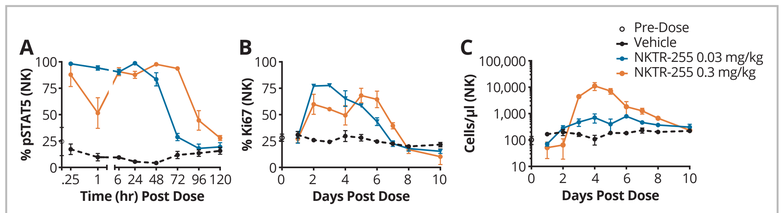


Figure 2. A single dose of NKTR-255 increased the pSTAT5+ population, the Ki67+ population, and the absolute number of peripheral NK cells in normal mice

Mice received single 0.03 or 0.3 mg/kg IV doses of NKTR-255. Blood samples were collected to assess pSTAT5 (A), Ki67 positive peripheral NK population (B), and the absolute number of peripheral NK cells (C) by flow cytometry.

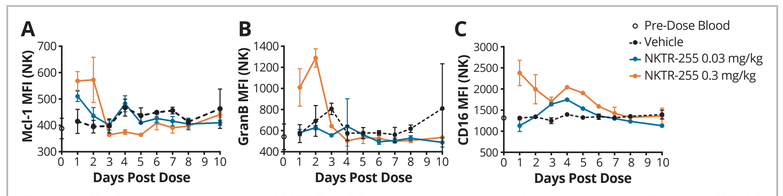


Figure 3. A single dose of NKTR-255 increased Mcl-1, Granzyme B, and CD16 expression on peripheral NK cells in normal mice

Mice received single IV doses of 0.03 or 0.3 mg/kg of NKTR-255. Blood samples were collected to assess Mcl-1 (A), Granzyme B (B), and CD16 (C) on peripheral NK cells by flow cytometry.

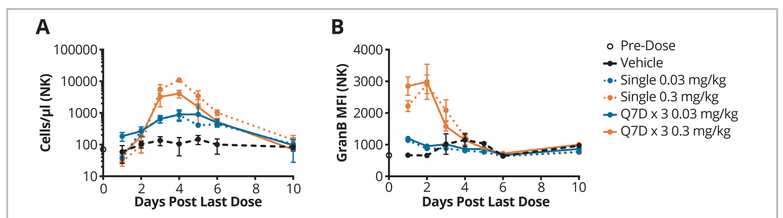


Figure 4. Repeat dosing of NKTR-255 did not reduce the magnitude of NK cell proliferation and activation

Mice received single or three times (weekly) IV doses of 0.03 or 0.3 mg/kg of NKTR-255. Blood samples were collected at each time point following the previous dose to assess the absolute number of NK cells (A) and Granzyme B expression on NK cells (B) by flow cytometry.

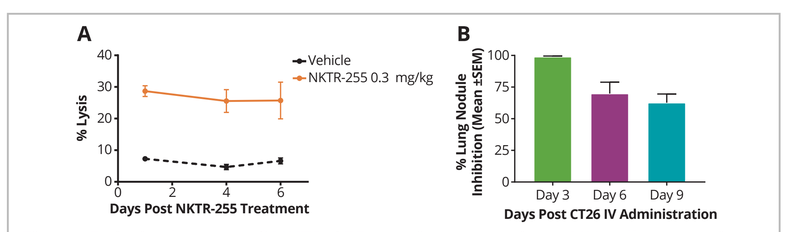


Figure 5. A single dose of NKTR-255 provided sustained NK cell cytotoxic function

After a single dose of NKTR-255 at 0.3 mg/kg IV, mouse splenic NK cells were collected on Day 1, 4 or 6 and used as effectors in a standard flow-based cytotoxic assay against YAC-1 target cells (A). After a single dose of NKTR-255 0.3 mg/kg IV, CT26 1x10^5 cells were administered intravenously on Days 3, 6 or 9. Lungs were scored for metastases on Day 13 post CT26 IV administration (B).

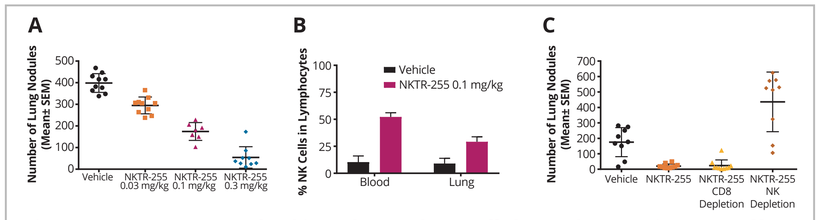


Figure 6. NKTR-255 resulted in enhanced NK cell-dependent anti-tumor effect in disseminated CT-26 lung metastasis model

In the CT26 model, 1x10^5 cells were administered intravenously on Day 0, treatment was initiated on Day 1 at 0.03, 0.1, or 0.3 mg/kg IV q7d, and on Day 13 lungs were scored for metastases (A). Blood and lung samples were collected 5 days after the second injection to assess the NK cell population (B). Anti-asialo GM1 Ab or anti-CD8 Ab was injected at Day -1, 3, 8 to deplete NK cells or CD8 T cells, respectively (C).

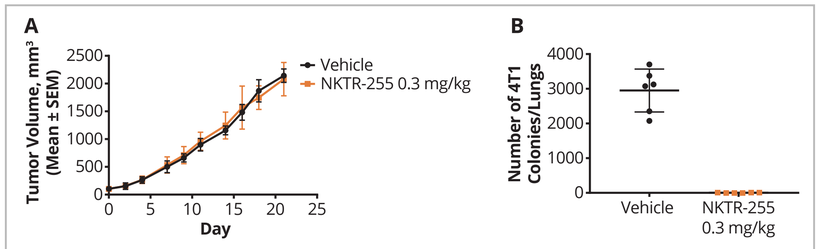


Figure 7. NKTR-255 suppressed spontaneous lung metastasis in 4T1 model

In the 4T1 spontaneous metastasis model, 5x10^5 cells were implanted in the mammary fat pad on Day 0, treatment was initiated on Day 5 at 0.3 mg/kg IV q7d. Primary tumor volume was measured over time (A). Lung metastases were assessed from culture of single lung cell isolates on Day 14 (B).

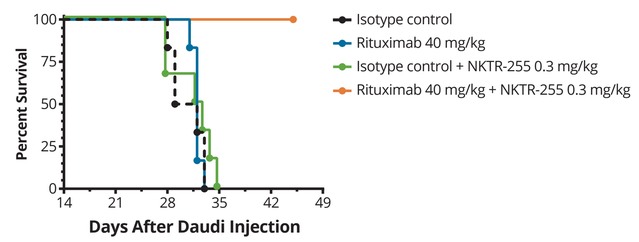


Figure 8. NKTR-255 improved rituximab efficacy in Daudi B Cell lymphoma model

SCID mice were intravenously injected on Day 0 with 1x10^7 Daudi cells. On Day 14 and 17 mice were treated with rituximab (40 mg/kg IP), and/or NKTR-255 (0.3 mg/kg SC). Survival rate was determined by measuring the onset of hindlimb paralysis as a surrogate parameter.

CONCLUSIONS

- NKTR-255 engages the JAK/STAT5 pathway and enhances NK cell function with 10-fold less potency compared with IL-15
- A single dose of NKTR-255 substantially enhances *in vivo* proliferation and activation of NK cells
- Repeat dosing of NKTR-255 does not reduce the magnitude of NK cell responses
- A single dose of NKTR-255 provides sustained cytotoxic function for NK cells
- The properties of NKTR-255 to boost NK proliferation and activation translates into enhanced anti-metastatic activity in mouse tumor models
- NKTR255 also demonstrates synergistic activity with rituximab to provide long-term survival in the Daudi B cell lymphoma model

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.