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.

**RESULTS**

**IL-15-mediated signaling through cis- and trans-presentation**

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-15Rβγ complex can induce JAK-STAT5 signaling, increasing survival and proliferation. This process is crucial for the proper support of IL-15 biology.

**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 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, 1x10^7 cells were administered intravenously on Day 0. Treatment was initiated on Day 1 at 0.03, 0.1, or 0.3 mg/kg of NKTR-255. Blood and spleen samples were collected to assess pSTAT5, Ki-67 positive peripheral NK population, and the absolute number of peripheral NK cells (C) by flow cytometry.

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. Finally, NKTR-255 synergistically provided long-term survival benefit when administered with rituximab in the Daudi B cell lymphoma model.

**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**