NKTR-255: an IL-15-based therapeutic with optimized biological activity and anti-tumor efficacy

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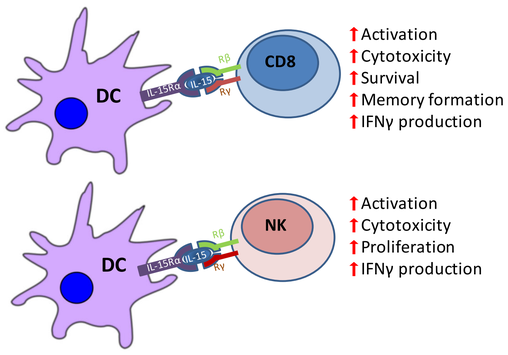
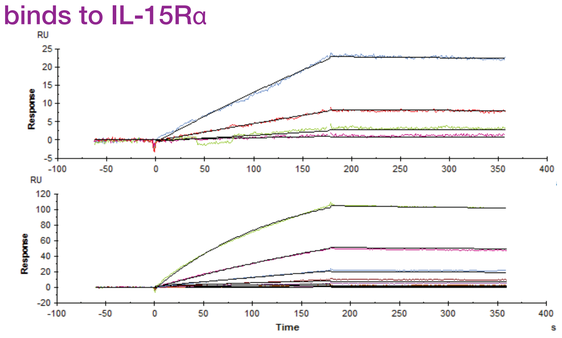
Interleukin-15 has been identified as a promising candidate for use as an immuno-oncology therapeutic, but the native cytokine has poor drug-like properties. NKTR-255 is a novel immunotherapeutic agent consisting of polymer-engineered IL-15 designed to optimally engage the IL-15 receptor complex and provide durable pathway activation *in vivo*. Here we show that NKTR-255 has greatly improved plasma and tumor exposure relative to IL-15, induces NK and CD8 T-cell activation and proliferation, and has single-agent efficacy in syngeneic tumor models.



Binding kinetics and affinity of NKTR-255 for IL-15Rα were measured by surface plasmon resonance using immobilized IL-15Rα. Cell-based potency was determined by treating CTLL-2 cells with NKTR-255 at a range of concentrations and measuring phosphorylation of STAT5 in cell lysate by immunoassay. Pharmacokinetic analysis was performed following single-dose intravenous administration of IL-15 and NKTR-255 in normal mice and in mice bearing subcutaneous B16F10 and CT-26 tumors, with analytes quantified in tumor and plasma by ELISA. Immunophenotyping studies were performed by flow cytometry on lymphocytes from peripheral blood of NKTR-255-treated and IL-15-treated normal mice and from spleen, tumor, and draining lymph node of treated mice carrying subcutaneous TRAMP-C2 tumors. Efficacy was determined by measuring tumor volume of subcutaneous TRAMP-C2 and CT-26, with q5dx3 treatment with NKTR-255.

NKTR-255 binds to IL-15Rα, and induces STAT5 phosphorylation in CTLL-2 cells with subnanomolar EC_{50}. Following intravenous administration, NKTR-255 demonstrates a greatly reduced clearance rate compared to IL-15, with plasma $t_{1/2}$ of 22-26h versus <1h for IL-15. Tumor exposure of NKTR-255 was 50-fold greater than IL-15 in B16F10-bearing C57/Bl6 mice and 110-fold greater in CT-26-bearing Balb/c mice. Immunophenotyping studies in normal mice showed an induction of Ki-67 and CD122 expression in NK cells, indicating proliferation and activation. In tumor-bearing mice, NKTR-255 treatment resulted in an increased CD8:CD4 and CD8:Treg ratio in tumor and spleen, and an increased frequency of CD8+TNF+IFNγ+ T-cells. Tumor growth inhibition was observed in both CT-26 and TRAMP-C2 models.

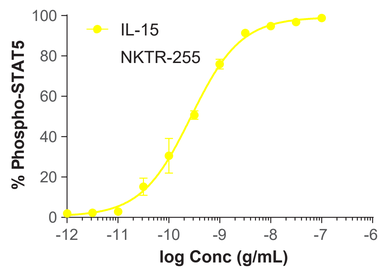
NKTR-255 binds to IL-15Rα



IL-15	5.78x10^5	1.49 x 10^{-4}	0.26
NKTR-255	8.29x10^4	1.62 x 10^{-4}	1.96

- Retention of binding to IL-15Rα is an important determinant of the biological activity of NKTR-255
- Surface Plasmon Resonance was used to determine the binding kinetics and affinity of NKTR-255 for IL-15Rα

NKTR-255 is bioactive, with sub-nanomolar EC_{50}

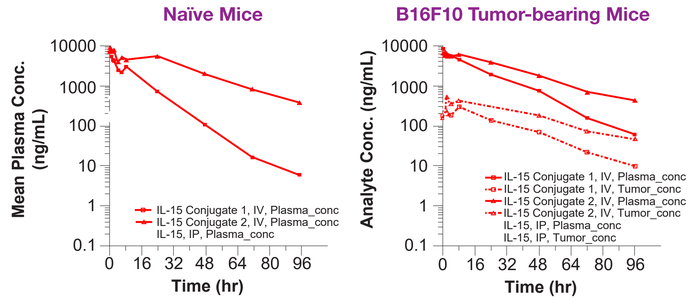


IL-15	0.27	21
NKTR-255	5.6	430

CTLL-2 cells were treated with test article for 10 minutes, then cells lysed and STAT5 phosphorylation assessed using a MesoScaleDevice immunoassay.

Compared to IL-15, NKTR-255 has greatly improved plasma and tumor exposure

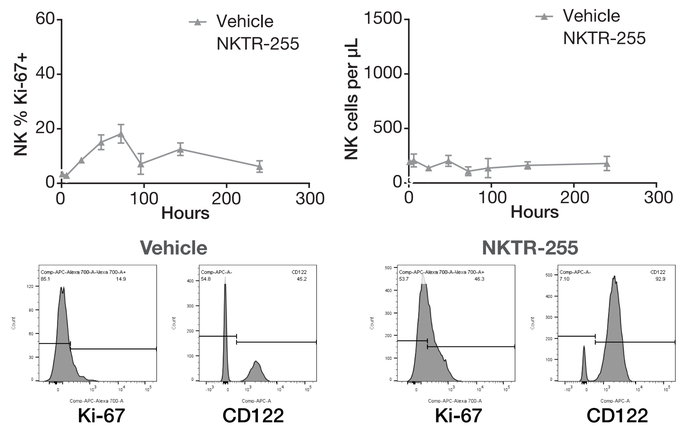
Pharmacokinetic data are shown for IL-15 and two PEG-IL-15 conjugates with linkers tuned to provide different PEG release kinetics. All were administered i.v. at 0.5 mg/kg.



- Unmodified IL-15 is cleared rapidly, so frequent administration of a high dose is required to achieve therapeutically relevant exposure
- NKTR-255 achieves >100x greater tumor exposure for the same dose

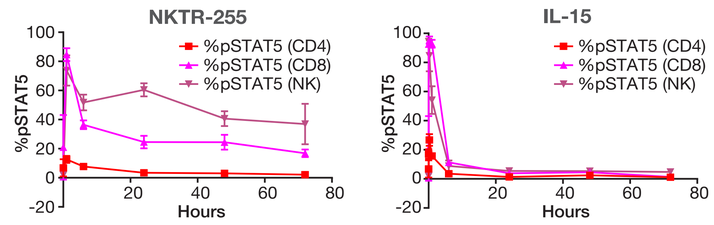
NKTR-255 induces NK cell proliferation and activation

A single i.v. dose of NKTR-255 induces expression of proliferation marker Ki-67 on NK cells peaking at 72hr, with a corresponding increase in frequency of NK cells in blood. Expression of NK activation marker CD122 was also increased – histograms shows 72 hour timepoint.



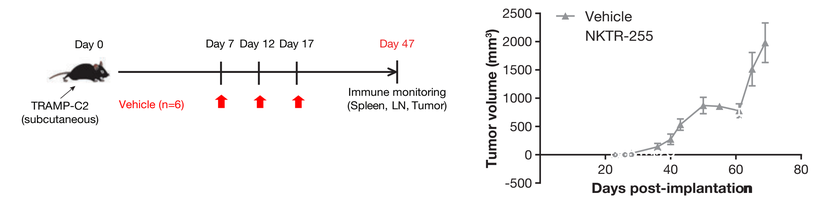
NKTR-255 induces sustained signaling in lymphocytes *in vivo*

Mice received a single i.v. dose of 0.3 mg/kg NKTR-255 (left) or IL-15 (right), then STAT5 phosphorylation in lymphocyte subpopulations from whole blood was assessed by flow cytometry.

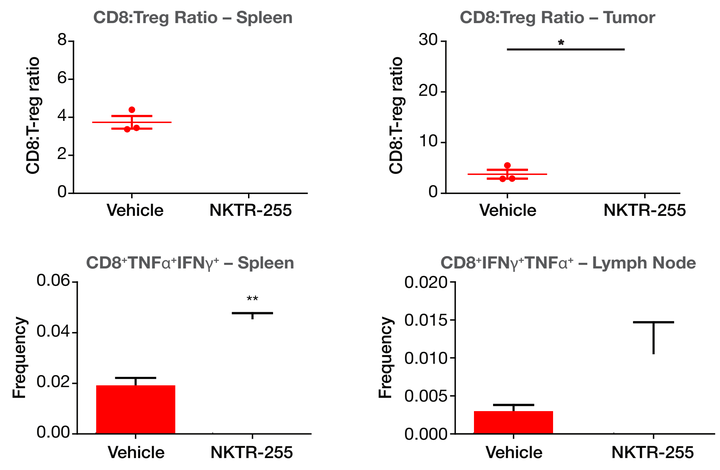


- Signaling induced by IL-15 is gone by 6 hours
- NKTR-255 sustains signal in NK and CD8 cells for more than 3 days

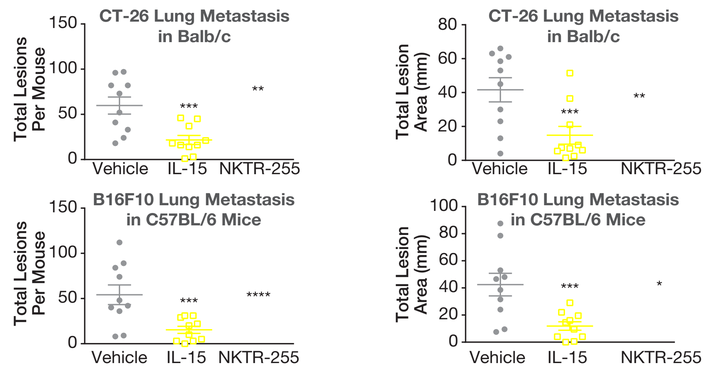
Immunophenotypic changes induced by NKTR-255 in tumor-bearing mice



NKTR-255 treatment results in reduced tumor growth rate, an increase in the CD8:Treg ratio in spleen and tumor, and an increase in the frequency of IFNγ⁺/TNF⁺ CD8 T-cells



NKTR-255 reduces tumor burden in CT-26 and B16F10 lung metastasis models



Tumors injected i.v. on day 0. NKTR-255 dosed i.v. at 0.3 mg/kg on d1, 5, 10; IL-15 dosed at 0.3 mg/kg i.p. on d1,2,3,4,5,8,9,10,11,12. Metastases counted on d13

NKTR-255 treatment results in sustained IL-15 activity, which induces CD8 T-cell and NK cell activation and proliferation, and produces long-lived immunophenotypic changes in tumor-bearing mice. The design of NKTR-255 enables a potential drug-like therapeutic strategy for accessing IL-15-based immunotherapy.

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