Abstract No. 3265

NKTR-255, a polymer-conjugated IL-15 enhances anti-tumor NK cell responses and synergizes with monoclonal antibodies to provide long-term survival in human lymphoma models

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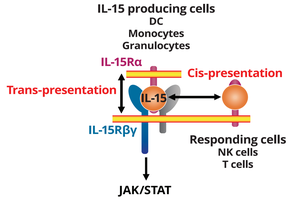
Background

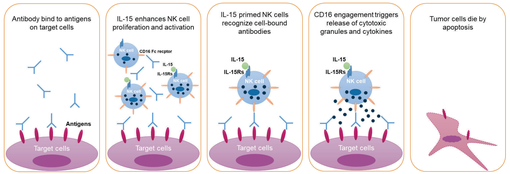
IL-15 is a cytokine that activates and provides survival benefit to NK cells. Exploiting the therapeutic value of native IL-15 has been challenging due to its unfavorable pharmacokinetic properties and tolerability. NKTR-255 is a polymer-conjugated human IL-15 that retains binding affinity to the alpha subunit of IL-15 receptor and exhibits reduced clearance to provide a sustained pharmacodynamic response. NKTR-255 has potential for providing an enhanced immunotherapeutic effect when combined with monoclonal antibodies that mediate tumor killing by antibody-dependent cellular cytotoxicity (ADCC). Here we investigate the pharmacological properties of NKTR-255 on NK cells and the therapeutic effect of NKTR-255 when combined with tumor-directed monoclonal antibodies in a B-cell lymphoma model.

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 cells (trans). Engagement of the IL-2/IL-15Rβγ complex can induce JAK-STAT signaling, increasing survival and proliferation. This process is crucial for the proper support of IL-15 biology[2]

Antibody-Dependent Cellular Cytotoxicity (ADCC) by NK Cells



Antibody-dependent cellular cytotoxicity (ADCC) is a crucial mechanism in tumor depletion by tumor-targeted antibodies. CD16 Fc receptors on NK cells recognize tumor cell-bound antibodies and the CD16 engagement triggers release of cytotoxic granules and cytokines to kill tumor cells. IL-15 enhances NK-mediated ADCC[3]

Results

In vitro studies demonstrated that NKTR-255 dose-dependently induced phosphorylation of STAT5 and proliferation in KHYG-1 cells (EC50 values for pSTAT5: 0.2 ng/ml, proliferation: 5 ng/ml) and human primary NK cells (EC50 values for pSTAT5: 2.4 ng/ml, proliferation: 39 ng/ml). In addition, NKTR-255 and CD16 cross-linking synergistically enhanced proliferation, CD69 and CD107a expression and Granzyme B production in NK cells. The properties of NKTR-255 to boost NK cell proliferation and activation with CD16 cross-linking translated into enhanced in vitro ADCC function.

In the Daudi B-cell lymphoma model, NKTR-255 combined with a tumor-directed antibody, either daratumumab (anti-CD38 Ab) or rituximab (anti-CD20 Ab), synergistically provided long-term survival benefit in a NKTR-255 dose-dependent manner. Furthermore, NKTR-255 (0.3 mg/kg) treatment not only significantly increased NK cell numbers in bone marrow, but robustly enhanced their Granzyme B, CD16 expression. NKTR-255 (0.3 mg/kg) and daratumumab treatment was the most effective in reducing the number of Daudi cells in the bone marrow.

Results

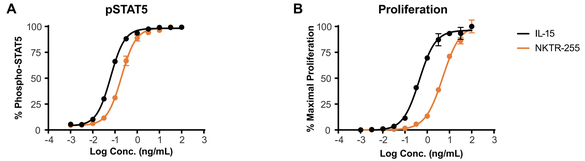


Figure 1. NKTR-255 showed a dose-dependent phosphorylation of STAT5 and increased proliferation in the human NK cell line, KHYG-1.

KHYG-1 was stimulated with the indicated concentration of rhIL-15 or NKTR-255 for 10 minutes and the induction of pSTAT5 relative to total STAT5 protein was measured using the pSTAT5/total STAT5 multiplexed MSD® assay (A). Cell proliferation following 48 hours culture was assessed by quantification of adenosine triphosphate levels using CellTiter-Glo 2.0 (B).

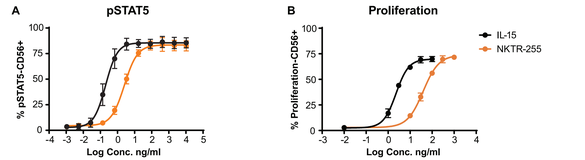


Figure 2. NKTR-255 showed a dose-dependent phosphorylation of STAT5 and increased proliferation in human NK cells.

Human PBMCs were stimulated with the indicated concentration of rhIL-15 or NKTR-255 for 20 minutes and the pSTAT5+ population within CD56+ NK cells was measured by flow cytometry (A). NK cell proliferation following 5 days culture with CFSE-labeled human PBMCs was assessed by flow cytometry (B).

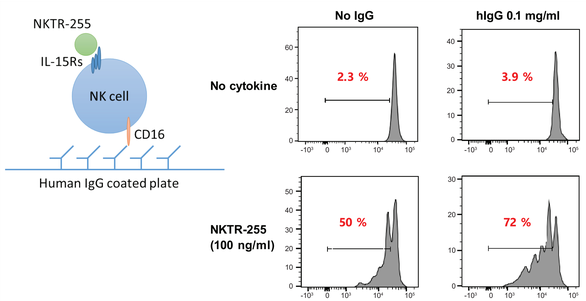


Figure 3. NKTR-255 and CD16 cross-linking synergistically enhanced NK cell proliferation.

CFSE-labeled human PBMCs were cultured with 100 ng/ml NKTR-255 on non-coated or 0.1 mg/ml human IgG-coated plates for 5 days. NK cell proliferation following the culture was assessed by flow cytometry.

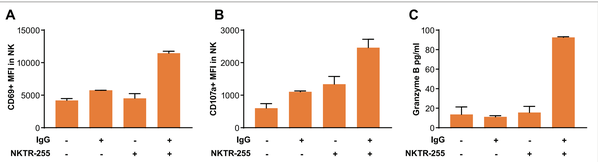


Figure 4. NKTR-255 and CD16 cross-linking synergistically enhanced NK cell activation.

Human PBMCs were cultured overnight with 100 ng/ml NKTR-255 on non-coated or 0.1 mg/ml human IgG-coated plates. CD69 (A) and CD107a (B) expression on NK cells were assessed by flow cytometry. Secreted Granzyme B protein (C) was measured from culture supernatant by ELISA.

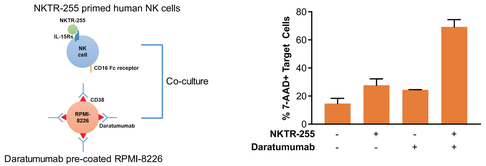


Figure 5. NKTR-255 enhanced daratumumab-mediated ADCC to multiple myeloma cell line, RPMI-8226.

Purified human NK cells were cultured overnight with or without 1 µg/ml NKTR-255. The NK cells (effector cells) were co-cultured with CFSE-labeled RPMI-8226 (target cells) pre-coated with or without daratumumab (100 ng/ml) at the effector:target ratio of 10:1 for 3 hours at 37°C. The ability of NK cells to lyse target cells was evaluated by detecting 7-AAD-stained RPMI-8226.

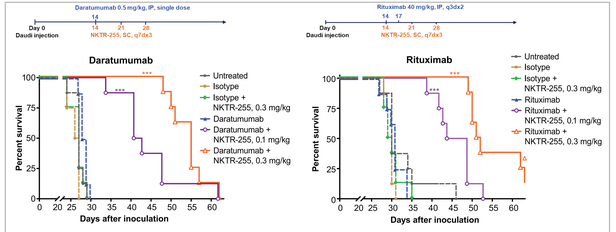


Figure 6. NKTR-255 enhanced therapeutic efficacy of the tumor-targeted antibodies with ADCC mechanisms in the Daudi B-cell lymphoma model

SCID mice (n=8/group) inoculated IV with Daudi cells were treated with a single dose of daratumumab (0.5 mg/kg, 14 days after inoculation) or two doses of rituximab (40 mg/kg, 14 and 17 days after inoculation) and three doses of NKTR-255 (14, 21 and 28 days after tumor inoculation). The survival endpoint was measured by hind limb paralysis onset and body condition. *** – NKTR-255 combination with rituximab or daratumumab significantly increases median survival compared to single-agent treatments ($p<0.05$, Log-Rank test).

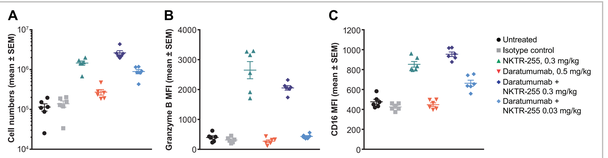


Figure 7. NKTR-255 expanded and activated NK cells in the bone marrow compartment.

SCID mice (n=6/group) inoculated with Daudi cells were treated with a single dose of daratumumab (14 days after inoculation) and two doses of NKTR-255 (14 and 21 days after inoculation). Cell number (A), Granzyme B (B) and CD16 (C) in bone marrow NK cells were assessed by flow cytometry three days after the second dose of NKTR-255.

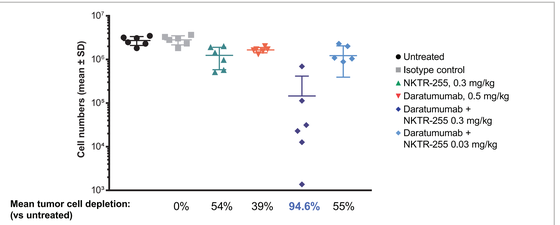


Figure 8. NKTR-255 and daratumumab synergized to deplete bone marrow resident tumor cells.

SCID mice (n=6/group) inoculated with Daudi cells were treated with a single dose of daratumumab (14 days after inoculation) and two doses of NKTR-255 (14 and 21 days after inoculation). Daudi cell numbers in the bone marrow were assessed by flow cytometry three days after the second dose of NKTR-255.

Conclusions

- NKTR-255 dose-dependently induces pSTAT5 and proliferation in human NK cells.
- NKTR-255 and CD16 cross-linking synergistically enhances NK cell proliferation and activation.
- The properties of NKTR-255 to boost NK proliferation and activation with CD16 cross-linking translates into enhanced in vitro ADCC function.
- NKTR-255 has a potential to be broadly applied with the tumor-directed antibodies that trigger ADCC to enhance their therapeutic efficacies.
- In the bone marrow, an optimal growth environment for hematologic malignancies, NKTR-255 combination treatment enables effective ADCC mechanism-based tumor cell killing by simultaneously increasing NK cell numbers and their activity.

References

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3. Wei et al. NK cell-mediated antibody-dependent cellular cytotoxicity in cancer immunotherapy. *Front. Immunol.* 27 Jul 2015.

Presented at AACR 2019 in Atlanta, GA