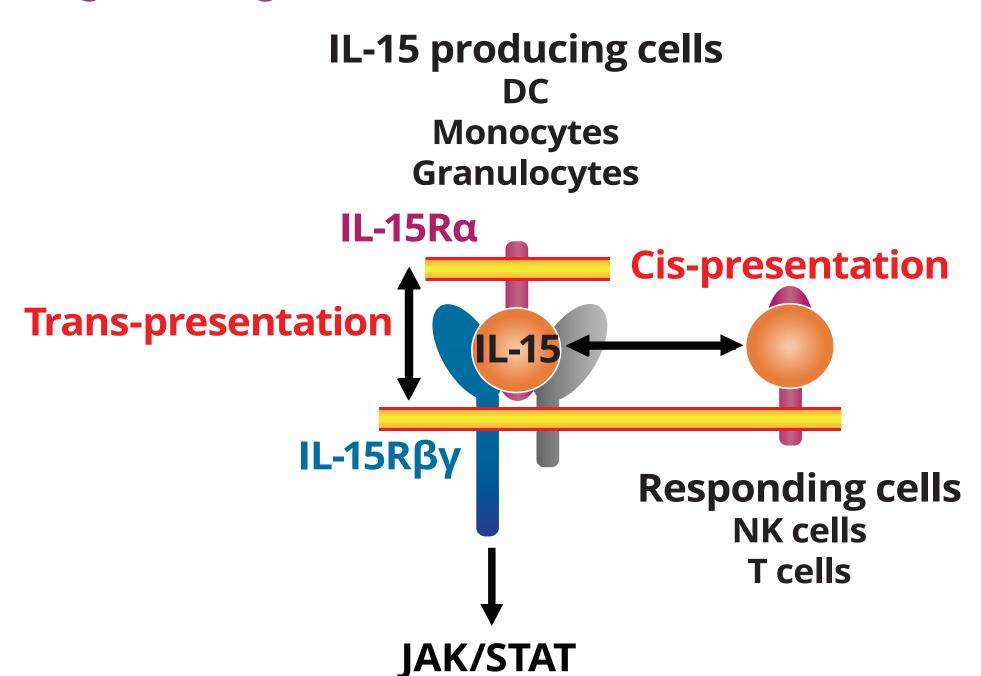
NKTR-255, a polymer-conjugated IL-15 enhances anti-tumor NK cell responses and synergizes with monoclonal antibodies to provide long-term survival in human lymphoma models

Background

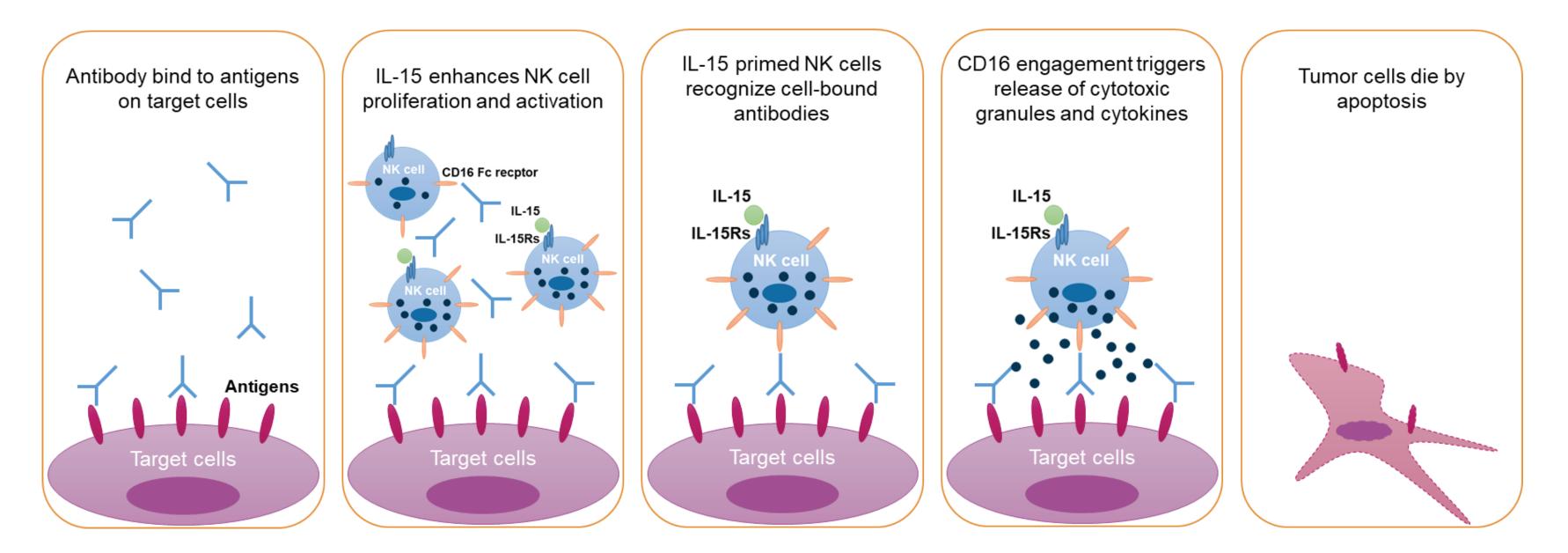
IL-15 is a cytokine that activates and provides survival benefit to NK cells. Exploiting the therapeutic value of native IL-15 has been challenging due to its unfavorable pharmacokinetic properties and tolerability. NKTR-255 is a polymer-conjugated human IL-15 that retains binding affinity to the alpha 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-Presentation¹



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 biology²

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 ADCC³

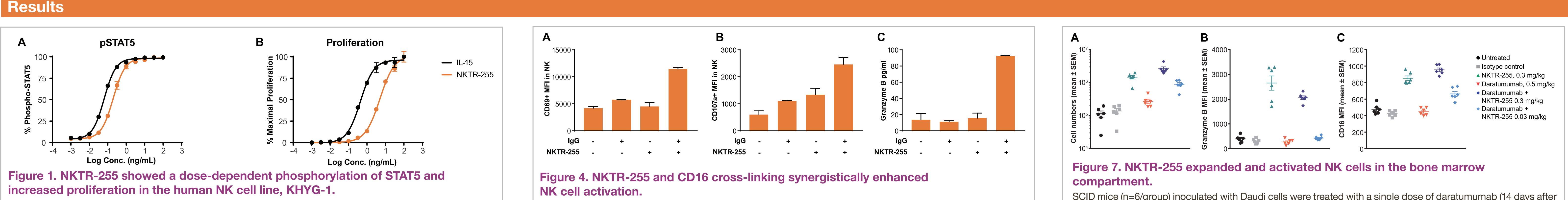
Results

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.

Takahiro Miyazaki, Saul Kivimäe, Rhoneil Pena, Peiwen Kuo, Marlene Hennessy, Murali Addepalli, Neha Dixit, Wildaliz Nieves, Sara Sheibani, Mekhala Maiti, Laurie VanderVeen, Joanna Wilczek, Loui Madakamutil, Jonathan Zalevsky

Nektar Therapeutics, San Francisco, California



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).

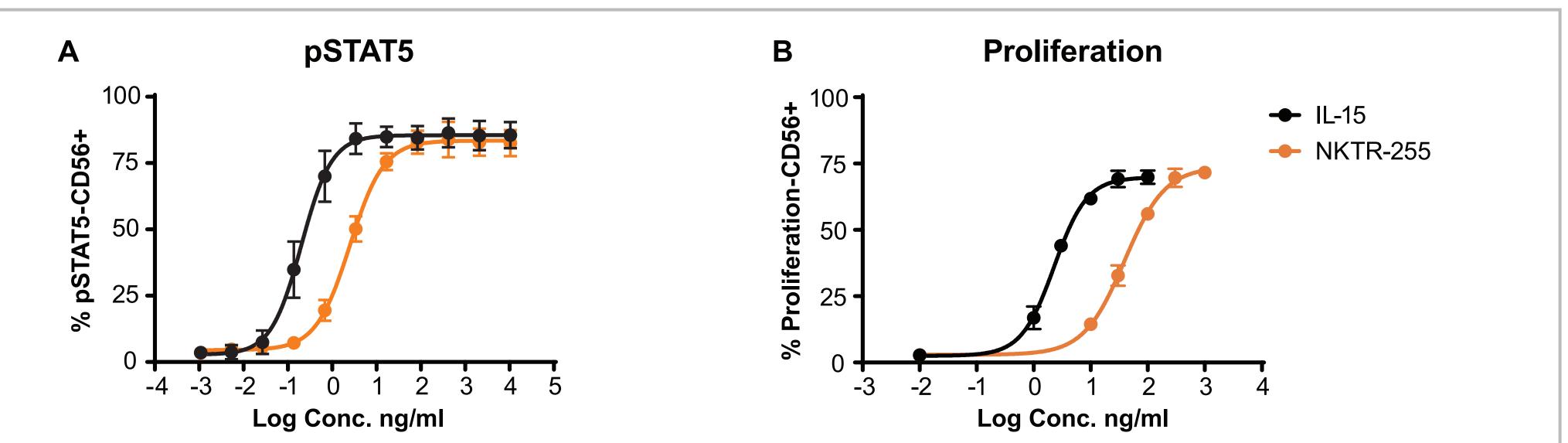
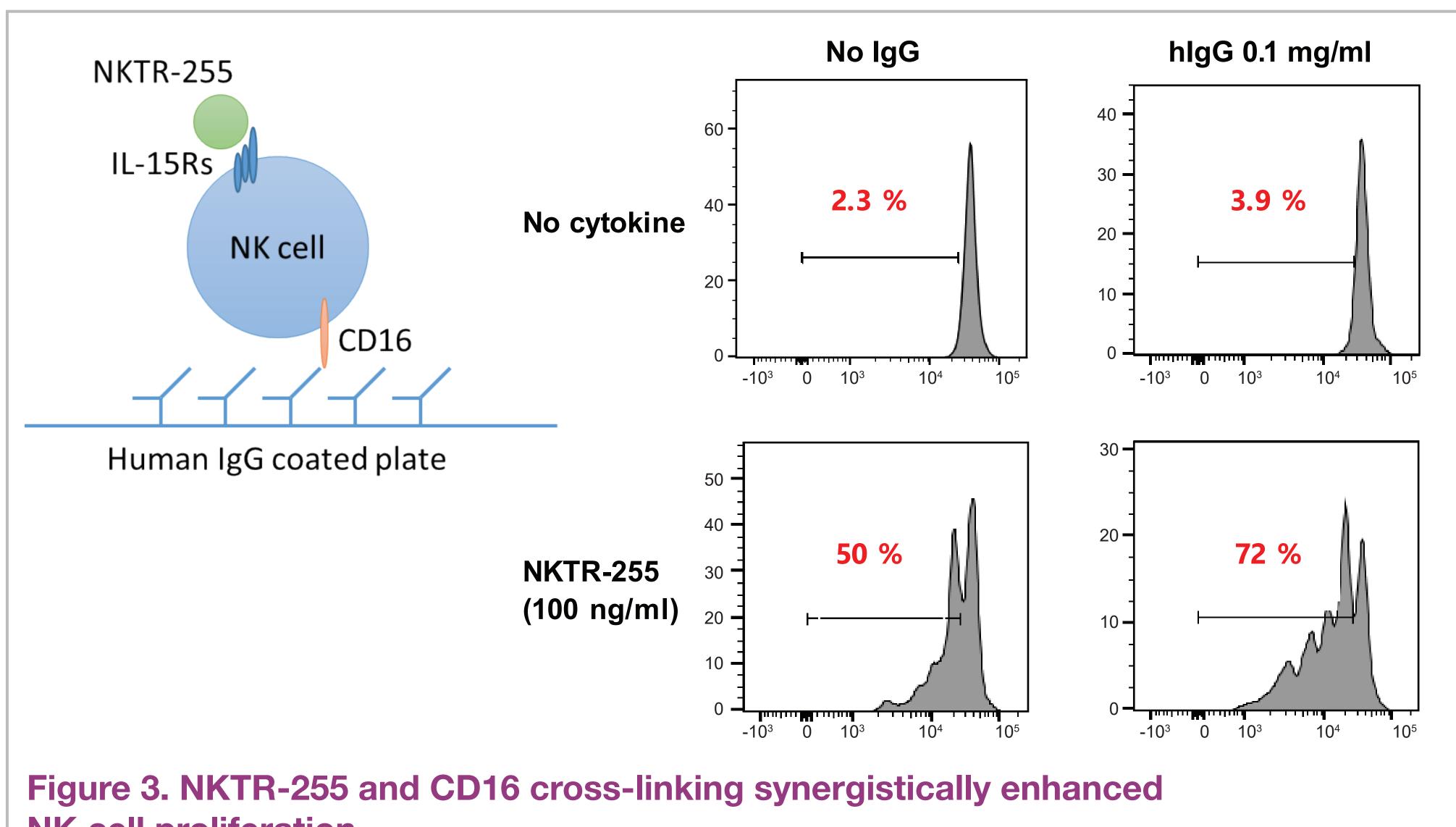


Figure 2. NKTR-255 showed a dose-dependent phosphorylation of STAT5 and increased proliferation in human NK cells.

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).



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.

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.

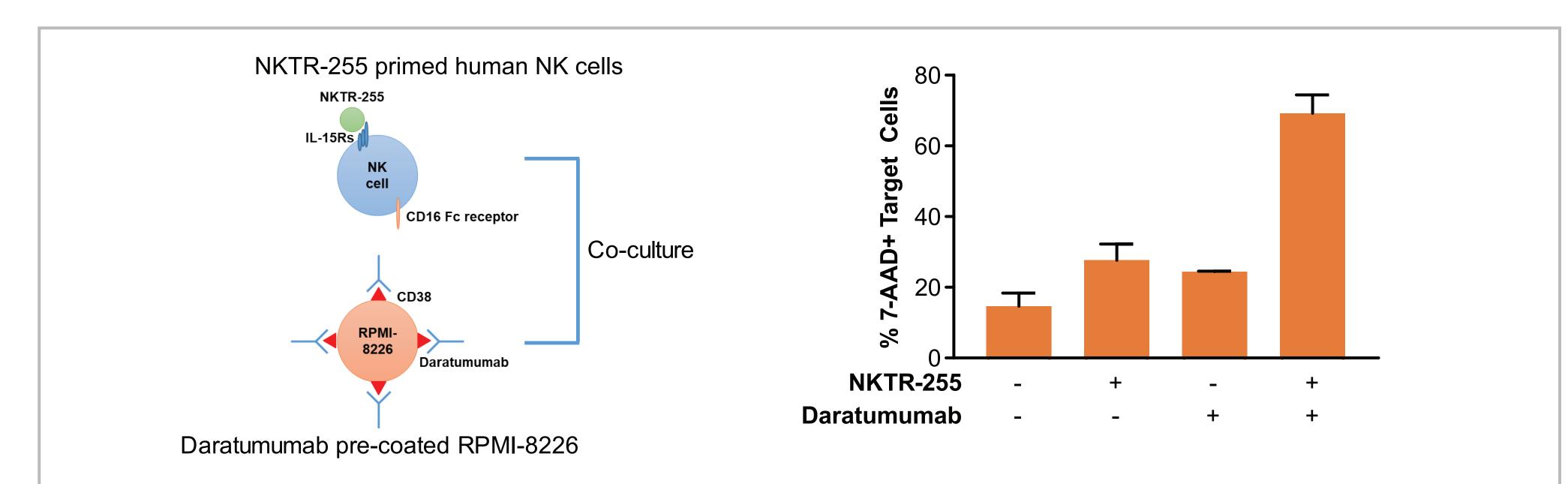


Figure 5. NKTR-255 enhanced daratumumab-mediated ADCC to multiple myeloma cell line, RPMI-8226.

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.

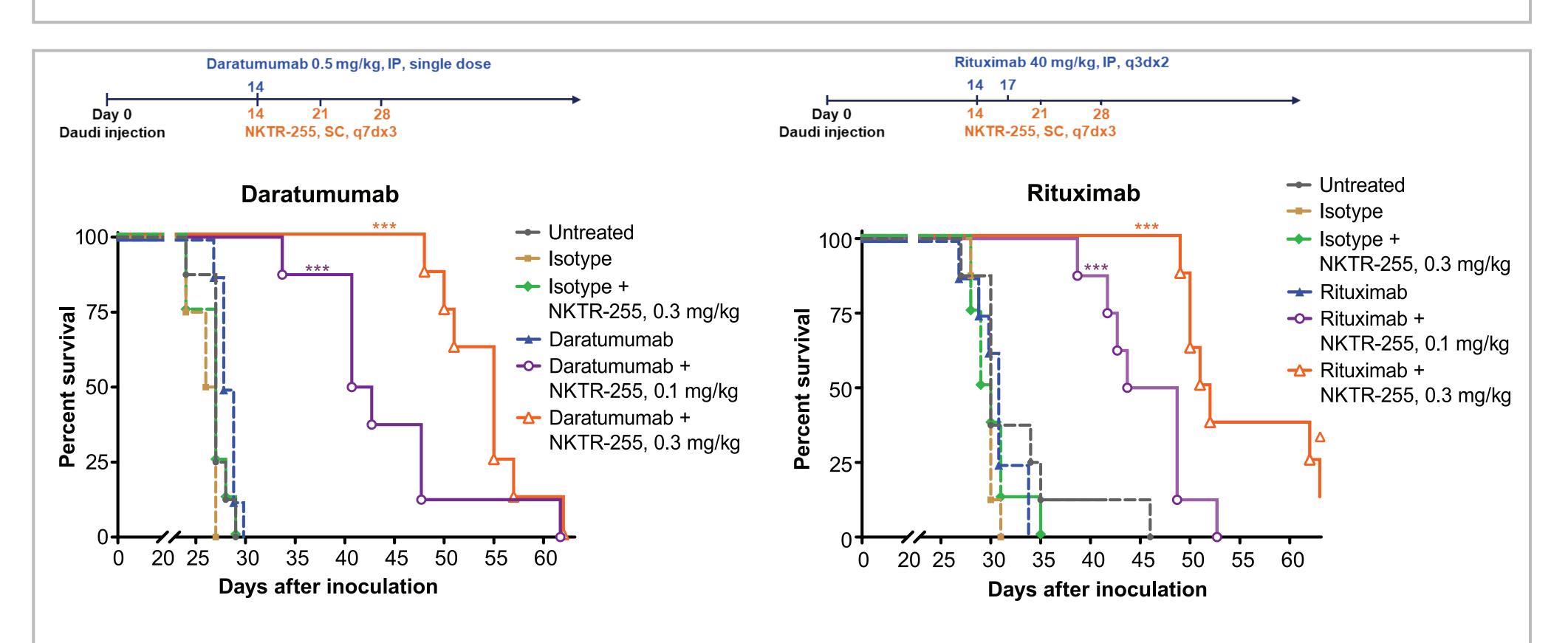


Figure 6. NKTR-255 enhanced therapeutic efficacy of the tumor-targeted antibodies with ADCC mechanisms in the Daudi B-cell lymphoma model

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).



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.

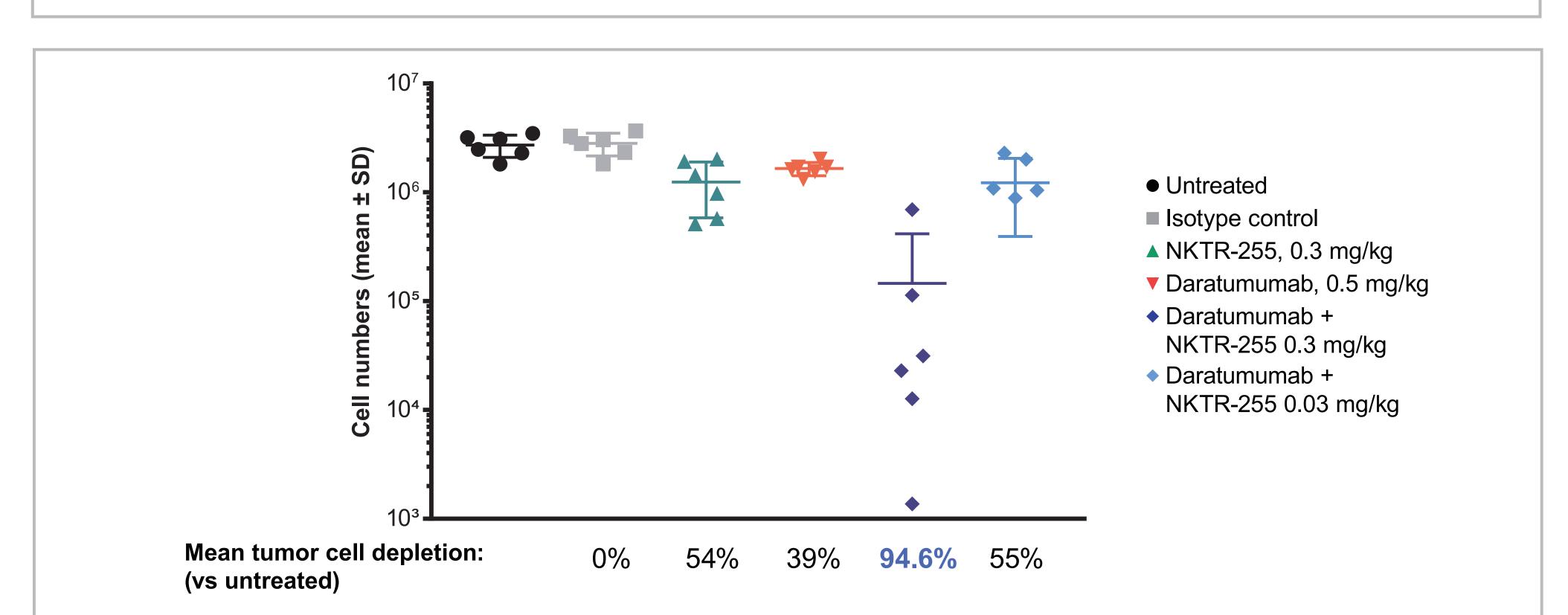


Figure 8. NKTR-255 and daratumumab synergized to deplete bone marrow resident tumor cells.

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

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

