A Polymer-Associated Human IL-15 (NKTR-255) with Optimized Biological Activity and Unique Mechanisms of Action on CD8 T Cells and NK Cells

Tanya O Robinson[1], Shweta M Hegde[1], Achintyan Gangadharan[1], Takahiro Miyazaki[2] and Kimberly S Schluns[1]

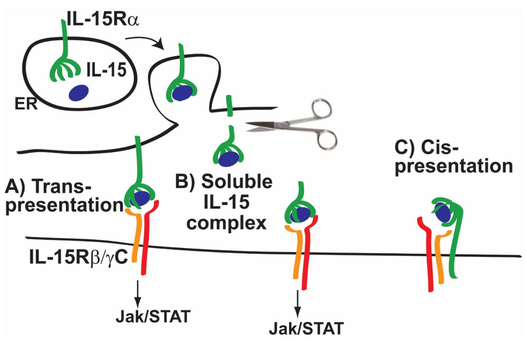
[1]Department of Immunology, University of Texas MD Anderson Cancer Center, Houston, TX, USA,
[2]Nektar Therapeutics, San Francisco, CA, USA

Abstract

IL-15 has anti-tumor activity but with limited efficacy due to its unfavorable pharmacokinetic properties and tolerability. Nektar Therapeutics has developed a polymer-conjugated human IL-15 (NKTR-255) that exhibits a prolonged in vivo half-life and enhanced potency, which is currently being examined as a potential cancer immuno-therapeutic agent. Since responses by IL-15 can be mediated by transpresentation via the IL-15Rα, as soluble IL-15/IL-15Rα complexes, or by cis-presentation, we investigated the role of IL-15Rα in driving NKTR-255 responses by naïve and memory CD8 T cells and NK cells in mice. The effects of NKTR-255 were examined by the adoptive transfer of CFSE-labeled naïve ovalbumin-specific CD8 T cells (OT-I) or established memory OT-I T cells followed by systemic administration of NKTR-255. To assess responses by central and effector memory T cell subsets, sorted CD44^{hi} memory phenotype CD8 T cells were transferred into wild-type (Wt) recipients followed by NKTR-255 treatment. Additionally, NK cell responses to NKTR-255 were analyzed in IL-15Rα bone marrow (BM) chimeras by BrdU incorporation. Naïve CD8 OT-I T cells transferred into Wt and IL-15Rα$^{-/-}$ mice proliferated at similar levels and acquired a central memory phenotype in response to NKTR-255. Interestingly, naive IL-15Rα$^{-/-}$ OT-I T cells had a deficient response to NKTR-255 but not to rhIL-15 or soluble IL-15 complexes. Additionally, proliferation by memory IL-15Rα$^{-/-}$ OT-I T cells in response to NKTR-255 was partially impaired compared to Wt OT-I cells. Sorted memory CD8 T cells maintained their proportion of CD62L+ and - subsets after NKTR-255-stimulated proliferation. Since IL-15Rα expression is essential for NK cell development, BM chimeras were generated with either IL-15Rα$^{-/-}$ or Wt BM in Wt recipients. In this model system, similar levels of BrdU were incorporated in IL-15Rα$^{-/-}$ and Wt NK cells after treatment with NKTR-255. These findings suggest naive CD8 T cells are critically dependent on cis-presentation of NKTR-255, while memory CD8 T cells are only partially dependent. For both naive or memory CD8 T cells, transpresentation of NKTR-255 was not required. In contrast to CD8 T cells, NK cell responses to NKTR-255 are not dependent on cis-presentation. Overall, these findings highlight the potential of polymerized IL-15 to modify IL-15Rα dependency leading to different mechanisms of action on CD8 T cells and NK cells and unique therapeutic effects.

Background

Endogenous IL-15 Expression and Mechanisms of Action



- IL-15 stimulates the proliferation and cytotoxic functions of CD8 T cells and NK cells leading to enhanced anti-tumor responses [1,2,3]
- Endogenous IL-15 responses by lymphocytes do not require self expression of IL-15Rα but rather require transpresentation [1,4,5]
- The efficacy of rIL-15 as a cancer immunotherapeutic agent has been limited due to its short *in vivo* half-life [6]
- Nektar Therapeutics has developed a polymer-associated human IL-15 (NKTR-255) that exhibits a sustained pharmacodynamics and enhanced *in vivo* responses.
- NKTR-255 binds both mouse and human IL-15Rα.

Objective

Investigate the role of IL-15Rα in driving NKTR-255 responses by naïve and memory CD8 T cells and NK cells in mice.

References

1) Stonier, S. and Schluns, K.S. 2010. *Immunology Letters.* 127:85-92
2) Patidar, M. et al. 2016. *Cytokine and Growth Factor Reviews.* 31:49-59
3) Van den Bergh, J. et al. 2017. *Pharmacology and Therapeutics.* 170:73-79
4) Burkett P.R. et al. 2003. *PNAS* 100:4724-29,
5) Schluns, K.S. et al. 2004 *Blood* 103:988-994
6) Robinson, T. and Schluns. K. 2017. *Immunology Letters.* 190:159-168

Results

Figure 1

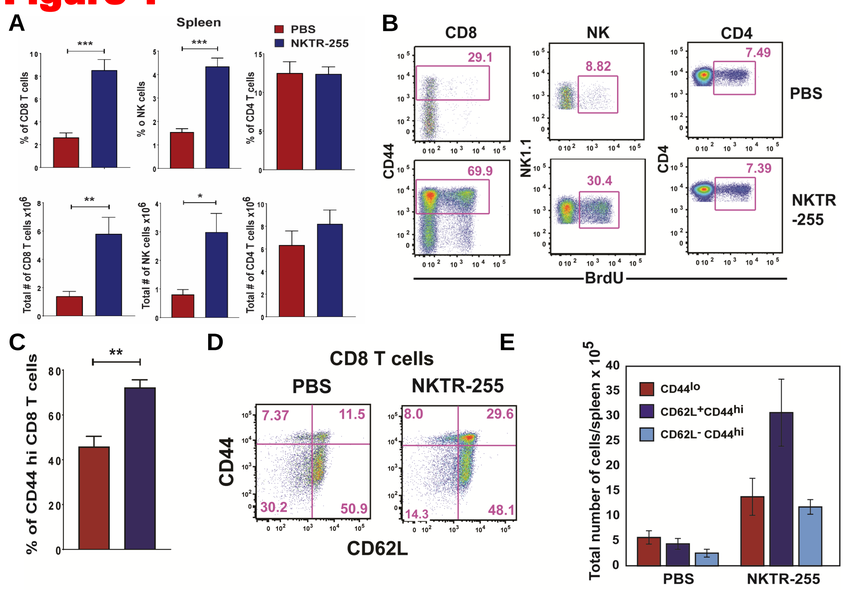


Fig 1. NKTR-255 increases CD8 T cells and NK cells. C57BL/6 mice (n = 5/group) were treated with NKTR-255 (0.03 mg/kg, intraperitoneal (i.p.). Both NKTR-255 and PBS treated were given BrdU (2mg, i.p.) every 2 days. Five days after treatment, splenocytes were harvested and analyzed by flow cytometry. (A) Graphs show mean frequency and total numbers of CD44^{hi}CD8 T cells, NK cells, and CD4 T cells. Errors bars represent SEM. (B) Representative flow cytometry plots showing BrdU incorporation by CD8 T cells, NK cells, and CD4 T cells. (C) Average frequency of CD44^{hi}CD8 T cells. D) Representative plots showing CD62L and CD44 expression by CD8 T cells. E) Total number of CD8 T cell subsets in spleen. *p<0.05, **p<0.01, and ***p<0.001 as determined using a Student's *t*-test.

Figure 2

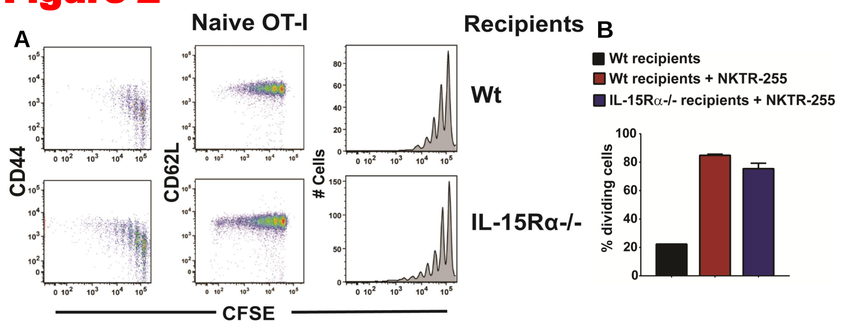


Fig 2. Naïve CD8 T cells proliferate and become central memory T cells in response to NKTR-255, independent of IL-15Rα expression by the recipient. CFSE-labeled CD45.1$^+$ naïve ovalbumin-specific TCR Transgenic CD8 T cells (OT-I) were transferred into CD45.2$^+$ wild-type (Wt) or IL-15Rα$^{-/-}$ mice (n = 3/group). One day post-transfer, mice were treated with NKTR-255 (0.03 mg/kg, i.p.). Five days later, CFSE dilution of donor CD45.1$^+$ donor T cells in spleens was analyzed by flow cytometry.

Figure 3

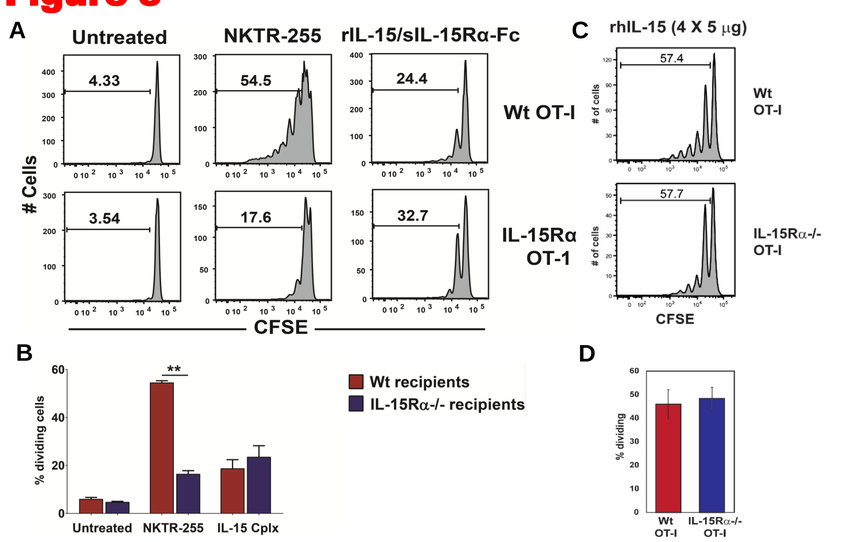


Fig 3. Expression of IL-15Rα by naive CD8 T cells is important for optimal responses to NKTR-255, but not sIL-15 complexes or rhIL-15. CD45.1$^+$ Wt OT-I CD8 T cells and CD45.1$^+$/CD45.2$^+$ IL-15Rα$^{-/-}$ OT-I CD8 T cells were mixed at 1:1 ratio, CFSE-labeled, and transferred into CD45.2+ Wt mice (n = 3/group). One day later, mice were treated i.p. with NKTR-255 (0.03 mg/kg) or rIL-15/sIL-15-Rα-Fc (0.5 μg/3 μg). Eight days post-treatment, CFSE dilution of donor cells in peripheral LNs and spleens were analyzed by flow cytometry. A) Representative histograms of CFSE intensity by indicated donor OT-I T cells from pLN. Similar results were observed in OT-I T cells from spleen (data not shown). (B) Bar graph shows average frequency of dividing cells among indicated OT-I T cell population ± SEM. ***p<0.001. C,D) CFSE of Wt and naïve IL-15Rα-/- OT-I 10 days after 4 treatments of rhIL-15 (5μg) delivered by i.p. injection every 2 days.

Figure 4

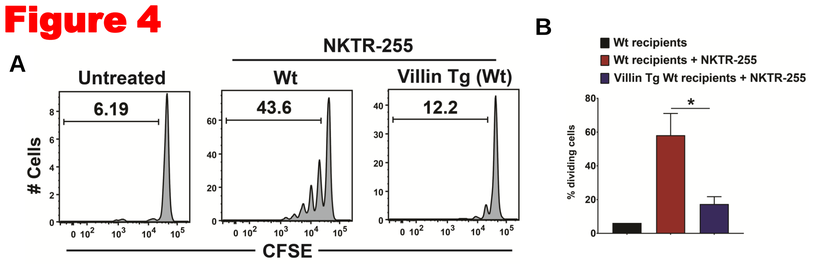


Fig 4. Soluble IL-15Rα inhibits responses by NKTR-255. CFSE-labeled CD45.1$^+$ OT-I CD8 T cells were transferred into CD45.2$^+$ Wt or Villin Tg (Wt) mice (n = 3/group). One day post-transfer, mice were treated with NKTR-255 (0.03 mg/kg, i.p.). Six days later, splenocytes were harvested. A) Histograms show CFSE dilution in CD45.1$^+$ donor T cells. (B) Bar graph shows mean ± SEM. *p<0.05.

Figure 5

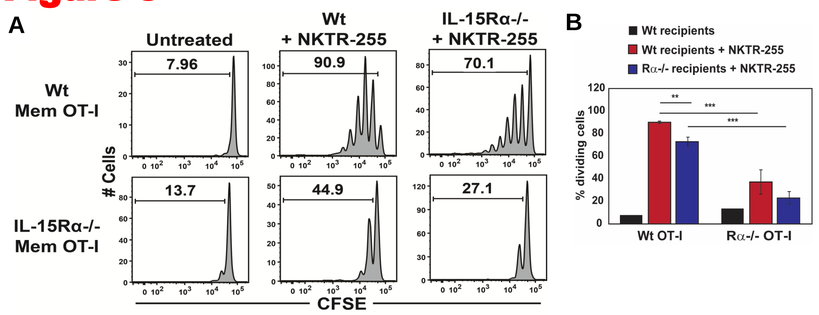


Fig 5. IL-15Rα-/- memory CD8 T cells have impaired responses to NKTR-255. CFSE-labeled CD8 T cells containing Wt or IL-15Rα$^{-/-}$ memory OT-I CD8 T cells (CD45.1$^+$) were transferred into CD45.2$^+$ Wt or IL-15Rα$^{-/-}$ mice (n = 3/group). One day later, mice were treated with NKTR-255 (0.03 mg/kg) i.p. Six days post-treatment, CFSE dilution in CD45.1$^+$ donor OT-I T cells in splenocytes was analyzed. A) Histograms show CFSE dilution in CD45.1$^+$ donor T cells. (B) Bar graph shows mean ± SEM. *p<0.05.

Figure 6

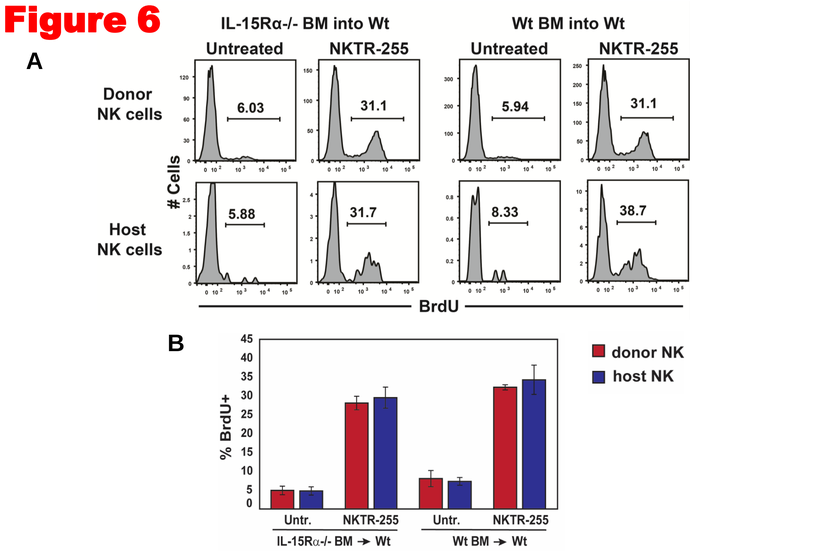


Fig 6. NK cells do not require IL-15Rα$^{-/-}$ to respond to NKTR-255. BM chimeras were generated by transferring CD45.2$^+$ bone marrow (BM) IL-15Rα$^{-/-}$ or Wt cells into lethally irradiated CD45.1$^+$ Wt mice (1000 Rads). After 2 months, mice were treated with NKTR-255 (0.03 mg/kg, i.p.). Both treated and untreated mice were given BrdU (2 mg, i.p.) every 2 days. Five days post-NKTR-255 treatment, BrdU incorporation was analyzed in CD45.2$^+$ and CD45.1$^+$ host NK cell splenocytes by flow cytometry (n = 3/group).

Working Hypothesis

	NK cells	Memory CD8 T cells	Naive CD8 T cells	CD4 T cells
IL-2/15Rβ	++++	+++	++	+
IL-15Rα	++	++++	+++	+

For NKTR-255 responses, the dependence for self expression of IL-15Rα inversely correlates with IL-2/15Rβ levels. We speculate that memory CD8 T cells respond most strongly to NKTR-255 because these cells express both high levels of IL-2/15Rβ and IL-15Rα, while NK cells express high IL-2/15Rβ but not has much IL-15Rα.

Acknowledgements

This study was supported by Nektar Therapeutics, San Francisco, CA, USA.