Characterization and comparison of NKTR-255, a polymer-conjugated IL-15, and IL-15 superagonists

Takahiro Miyazaki, Mekhala Maiti, Sara Sheibani, Olga Zemska, Saul Kivimäe, Marlene Hennessy, Phi Quach, Ping Zhang, Thomas Chang, Loui Madakamutil, Jonathan Zalevsky

Nektar Therapeutics, San Francisco, CA

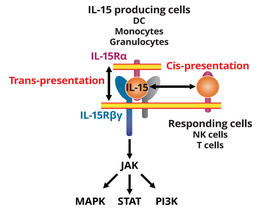
NEKTAR

Poster # P622

Introduction

IL-15 is a cytokine that activates and provides survival benefit to NK and CD8 T cells and has great potential for the treatment of cancer. Exploiting the therapeutic value of native IL-15 has been challenging due to its unfavorable pharmacokinetic properties and poor tolerability. Several IL-15-based proteins aimed to overcome these problems are currently in development. NKTR-255 is a polymer-conjugated IL-15 that is designed to retain a binding affinity to IL-15 receptor alpha (IL-15Rα). We investigate the pharmacological properties of NKTR-255 and IL-15 superagonists, IL-15/IL-15Rα complex molecules[1], to explore differentiations between the IL-15Rα dependent and independent cytokines, respectively.

IL-15-Mediated Signaling Through Cis- and Trans-Presentation[2]



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/IL15Rβγ complex can induce JAK-STAT signaling, increasing survival and proliferation. This process is crucial for the proper support of IL-15 biology.[3]

Results

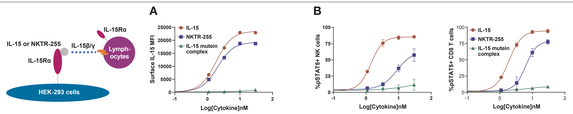
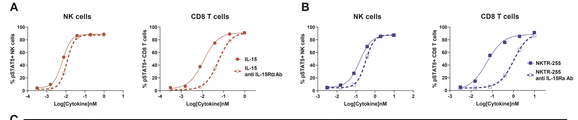


Figure 1. IL-15 and NKTR-255 bound to surface IL-15Rα on the receptor-overexpressing HEK-293 cells and the surface-bound cytokines induced phosphorylation of STAT5 in human NK and CD8 T cells

IL-15Rα overexpressing HEK-293 cells were incubated with rhIL-15, NKTR-255 or IL-15 mutein complex for 30 minutes. Surface binding of the cytokines was measured by flow cytometry (A). HEK-293 cells pre-incubated with rhIL-15, NKTR-255 or IL-15 mutein complex were co-cultured with human PBMC for 20 minutes and the pSTAT5+ population within CD56+ NK and CD8+ T cells was measured by flow cytometry (B).



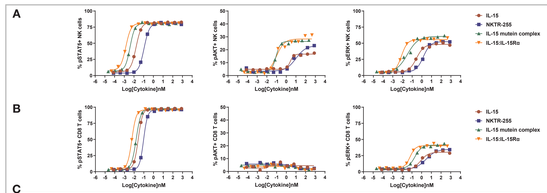
C

Human PBMC	IL-15			NKTR-255		
	EC_{50} nM		EC_{50} ratio (+Ab/-Ab)	EC_{50} nM		EC_{50} ratio (+Ab/-Ab)
	-Ab	+Ab		-Ab	+Ab	
NK cells	0.007	0.011	1.6	0.16	0.32	2.0
CD8 T cells	0.011	0.057	5.2	0.055	1.03	18.7

Figure 2. IL-15Rα was necessary for optimal IL-2Rβγ engagement of IL-15 and NKTR-255 on NK and CD8 T cells

Human PBMCs were pre-incubated with a neutralizing antibody against IL-15Rα for 15 minutes and then stimulated with rhIL-15 (A) or NKTR-255 (B) for 20 minutes. The pSTAT5+ population within CD56+ NK and CD8+ T cells was measured by flow cytometry. EC_{50} values were calculated from concentration-response curves (C).

Results



C

Human whole blood N = 6		EC_{50} nM (mean ± SD)						EC_{50} ratio to pSTAT5			
		NK cells			CD8 T cells			NK cells		CD8 T cells	
		pSTAT5	pAKT	pERK	pSTAT5	pAKT	pERK	pAKT	pERK	pAKT	pERK
IL-15Rα dependent cytokines	IL-15	0.025 ± 0.010	1.3 ± 1.3	0.76 ± 0.60	0.021 ± 0.0059	ND*	1.6 ± 1.4	44	27	ND	100
	NKTR-255	0.15 ± 0.051	7.7 ± 3.7	4.4 ± 3.0	0.083 ± 0.024	ND	2.3 ± 0.71	48	28	ND	63
IL-15 superagonists	IL-15 mutein complex	0.0050 ± 0.0019	0.11 ± 0.12	0.051 ± 0.024	0.027 ± 0.014	ND	0.23 ± 0.074	19	10	ND	10
	IL-15:IL-15Rα	0.0046 ± 0.0033	0.12 ± 0.19	0.051 ± 0.052	0.018 ± 0.010	ND	0.18 ± 0.079	21	9.6	ND	12

*: not determined

Figure 3. NKTR-255 maintained similar signaling profile as IL-15, in contrast to IL-15 superagonists

Human whole blood was stimulated with rhIL-15, NKTR-255, IL-15 mutein complex or IL-15:IL-15Rα for 20 minutes. Induction of phosphorylated STAT5, AKT and ERK1/2 in CD56+ NK cells (A) or CD8+ T cells (B) was measured by flow cytometry. EC_{50} values were calculated from concentration-response curves (C).

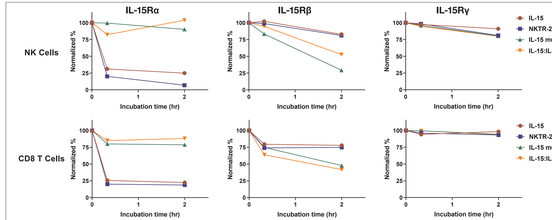
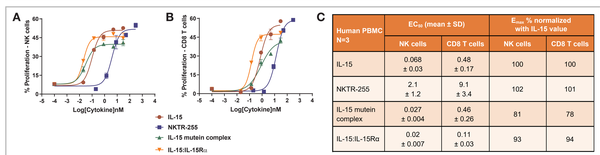


Figure 4. IL-15 and NKTR-255 reduced the surface expression of IL-15Rα on NK and CD8 T cells whereas IL-15 superagonists reduced IL-2Rβ expression

Human whole blood was incubated with rhIL-15 (2.5 nM), NKTR-255 (23 nM), IL-15 mutein complex (0.25 nM) or IL-15:IL-15Rα (0.5 nM) for 20 minutes or 2 hours. Surface expression of IL-15Rα, IL-2Rβ and IL-2Rγ in CD56+ NK and CD8+ T cells was measured by flow cytometry. Each MFI value was normalized with the MFI value of the cytokine-untreated sample.



C

Human PBMC N=3	EC_{50} (mean ± SD)		E_{max} % normalized with IL-15 value	
	NK cells	CD8 T cells	NK cells	CD8 T cells
IL-15	0.068 ± 0.03	0.48 ± 0.17	100	100
NKTR-255	2.1 ± 1.2	9.1 ± 3.4	102	101
IL-15 mutein complex	0.027 ± 0.004	0.46 ± 0.26	81	78
IL-15:IL-15Rα	0.02 ± 0.007	0.11 ± 0.03	93	94

Figure 5. IL-15 and NKTR-255 induced proliferation of human NK and CD8 T cells similarly to IL-15 superagonists

Human PBMCs were labeled with CFSE and cultured for 5 days in the presence of rhIL-15, NKTR-255, IL-15 mutein protein or IL-15:IL15Rα. Proliferation of CD56+ NK cells (A) or CD8+ T cells (B) was assessed by flow cytometry.

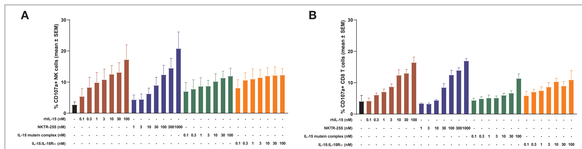


Figure 6. IL-15 and NKTR-255 induced higher CD107a+ (degranulation marker) NK and CD8 T cells as compared to IL-15 superagonists

Human PBMCs from 3 healthy donors were cultured overnight in the presence of rhIL-15, NKTR-255, IL-15 mutein complex or IL-15:IL-15Rα. Surface CD107a on CD56+ NK cells (A) or CD8+ T cells (B) was measured by flow cytometry.

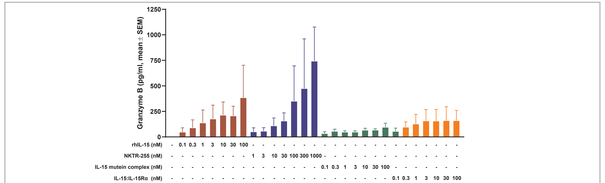


Figure 7. IL-15 and NKTR-255 induced higher maximal Granzyme B secretion as compared to IL-15 superagonists

Human PBMCs from 3 healthy donors were cultured overnight in the presence of rhIL-15, NKTR-255, IL-15 mutein complex or IL-15:IL-15Rα. Secreted Granzyme B protein was quantified in cell-free supernatants by ELISA.

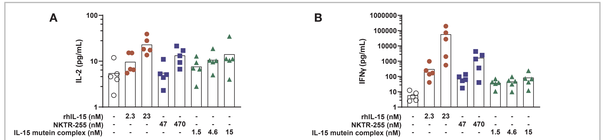


Figure 8. IL-15 and NKTR-255 showed higher maximal induction of IFNγ production than IL-15 superagonist

Human PBMCs from 5 healthy donors were stimulated with rhIL-15, NKTR-255, IL-15 mutein complex. After 48 hours secreted IL-2 (A) or IFNγ (B) was quantified in cell-free supernatants by a multiplex MSD assay.

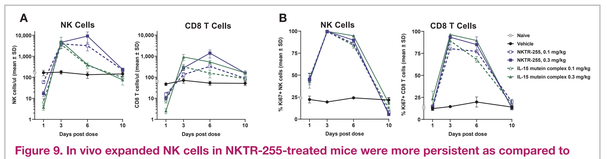


Figure 9. In vivo expanded NK cells in NKTR-255-treated mice were more persistent as compared to IL-15 superagonist

Balb/c mice (N=4/group) received a single IV dose of 0.1 or 0.3 mg/kg of NKTR-255 or IL-15 mutein complex. Blood samples were collected at day 1, 3, 6 and 10 following dosing to assess absolute number (A) or Ki67+ population (B) in NKp46+ NK cells and CD8+ T cells by flow cytometry.

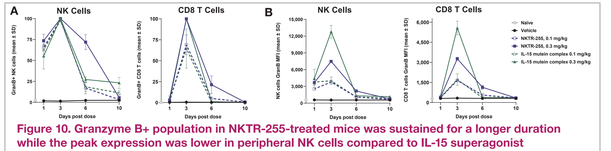


Figure 10. Granzyme B+ population in NKTR-255-treated mice was sustained for a longer duration while the peak expression was lower in peripheral NK cells compared to IL-15 superagonist

Balb/c mice (N=4/group) received a single IV dose of 0.1 or 0.3 mg/kg of NKTR-255 or IL-15 mutein complex. Blood samples were collected at day 1, 3, 6 and 10 following dosing to assess Granzyme B+ population (A) or Granzyme B expression (B) in NKp46+ NK and CD8+ T cells by flow cytometry.

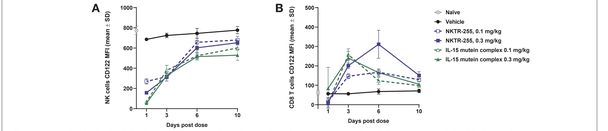


Figure 11. NKTR-255 induced less reduction of or longer sustained increase of IL-2Rβ surface expression relative to IL-15 superagonist on NK cells or CD8+ T cells, respectively

Balb/c mice (N=4/group) received a single IV dose of 0.1 or 0.3 mg/kg of NKTR-255 or IL-15 mutein complex. Blood samples were collected at day 1, 3, 6 and 10 following dosing to assess IL-2Rβ (CD122) expression on peripheral NKp46+ NK (A) or CD8+ T cells (B) by flow cytometry.

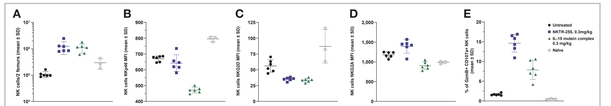


Figure 12. NKTR-255 increased Granzyme B+ CD107a+ intra bone marrow NK cells in a Daudi B lymphoma model as compared to IL-15 superagonist

SCID mice (N=6/group) inoculated with Daudi cells were treated with two doses of NKTR-255 or IL-15 mutein complex (0.3 mg/kg IV, 14 and 21 days after inoculation). The bone marrow from both femurs was collected 3 days after the second treatment to assess absolute number (A), NK receptor expression: NKp46 (B), NKG2D (C), NKG2A (D) or Granzyme B+ CD107a+ population (E) in NKp46+ NK cells.

Conclusions

- NKTR-255 is an IL-15Rα dependent cytokine similar to IL-15
  - NKTR-255 binds surface IL-15Rα and the surface-bound NKTR-255 mediates signaling
  - IL-15Rα is necessary for optimal IL-2Rβγ engagement of IL-15 and NKTR-255
- NKTR-255 has similar in vitro pharmacological properties to IL-15 and has different properties when compared to IL-15 superagonists
  - Signaling profile
  - IL-2Rβ internalization
  - Degranulation and Granzyme B/IFNγ secretion
- In vivo expanded NK cells in NKTR-255-treated mice may have properties more favorable for anti-tumor therapy when compared to expanded NK cells in IL-15 superagonist-treated mice
  - Prolonged survival
  - Substantial NK cytotoxic function

References

1. Rubinstein et al. *Proc Natl Acad Sci USA*. 2006;103:9166-71.
2. Marcais et al. *Front Immunol*. 2013;4:450.
3. Stonier and Schluns. *Immunol Lett*. 2010;127:85-92.

Presented at Society for Immunotherapy of Cancer 2019 Annual Meeting