NKTR-255: Accessing The Immunotherapeutic Potential Of IL-15 for NK Cell Therapies

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The immunity cycle and multiple points of intervention for I-O therapies

1. Release of cancer cell antigens
2. Cancer antigen presentation
3. Priming and activation
4. Trafficking of immune cells to tumor
5. Infiltration of cytotoxic cells into tumors
6. Recognition of cancer cells by immune cells
7. Killing of cancer cells

Source: Oncology Meets Immunology: The Cancer-Immunity Cycle
Chen and Mellman
Immunity, Volume 39, Issue 1, 1 - 10
Nektar’s immuno-oncology strategy to create therapies that cover the immunity cycle

1. Release of cancer cell antigens (cancer cell death)
2. Cancer antigen presentation (dendritic cells/APCs)
3. Priming and activation (APCs & T cells)
4. Trafficking of T cells to tumor (CTLs)
5. Infiltration of T cells into tumors (CTLs, endothelial cells)
6. Recognition of cancer cells by T cells (CTLs, cancer cells)
7. Killing of cancer cells (immune and cancer cells)

NKTR-214 (CD122 Agonist)
- Prime, Proliferate, Activate & Increase Tumor-Infiltrating Lymphocytes (TILs), Increase PD-1 expression

NKTR-262 (TLR Agonist)
- Activate Dendritic Cell Response

NKTR-255 (IL-15)
- Stimulate NK Cells, Sustain Immune Response & Generate T Cell Memory

Therapies need to be accessible as medicines

Target as many steps as possible in the cycle with as few therapies as possible
The potential of IL-15 in immuno-oncology

- IL-15 is a pleiotropic cytokine with roles in innate and adaptive immunity
- Identified by NCI as one of the most promising immuno-oncology agents
- Key role in formation and maintenance of immunological memory
- Essential factor for NK development and homeostasis
- In vitro, IL-15 can reverse tumor-induced NK cell dysfunction
The challenge to therapeutic use of IL-15

- IL-15 displays rapid clearance from plasma
- In vivo signaling activity is similarly short-lived
- Requires daily dosing or multi-day continuous infusion for optimal activity with high Cmax-related toxicity
NKTR-255 – polymer conjugated IL-15

► Design Goals:
  ▪ Improve PK and PD to sustain IL-15 activity and achieve large pharmacodynamic effect without need for daily dosing
  ▪ Retain binding to IL-15Rα to maintain full spectrum of IL-15 biology
  ▪ No mutagenesis or complex to soluble IL-15Rα

► As a result, NKTR-255:
  ▪ Stimulates NK cell activation and proliferation
  ▪ Supports CD8 T-cell survival and memory formation
  ▪ Shows efficacy in various syngeneic tumor models

NKTR-255 is first potential medicine to access the IL-15 pathway by preserving receptor binding to IL-15Rα with antibody-like dosing
NKTR-255 achieves sustained plasma exposure in mice, rats and NHP after single dose

► PEGylation Significantly Improved NKTR-255 PK Profiles:
  ▪ PEGylation significantly enhanced plasma exposure and reduced total clearance
  ▪ Extended plasma exposure across the species on single dose (Mice, Rat and NHP)

► NKTR-255 Half-life ($t_{1/2}$):
  ▪ Mouse: ~14 hrs
  ▪ Rat: ~18 hrs
  ▪ Monkey: ~30 hrs (100µg/kg)

Note: Parent IL-15 was not dosed in the Rat PK study; No apparent gender differences are noted in the Cyno study
Binding to IL-15Rα is required to access the biological functions of IL-15

- Three potential modes of interaction
  - Trans-presentation: IL-15 binds to IL-15Rα on one cell (eg. DC) then signals through Rβγ on a second cell (eg. T-cell)
  - Cis-presentation: soluble IL-15 binds to IL-15Rα and Rβγ on the same cell
  - Binding of soluble complex: soluble IL-15:IL-15Rα heterodimer binds to Rβγ

- Design NKTR-255 to maintain IL-15 biological context
  - IL-15/IL-15Rα therapeutic fusion protein signals in an IL-15Rα independent manner, loses biological context
NKTR-255 retains affinity for IL-15Rα

Many conjugates were screened for ability to bind IL-15Rα
Conjugation chemistry parameters were carefully optimized
NKTR-255 affinity for IL-15Rα is ~10X weaker than IL-15

Affinity measured by BIACore, using IL-15Rα:Fc captured by immobilized anti-Fc
NKTR-255 interaction with β and αβ were also ~8-10X weaker than IL-15
NKTR-255 signaling is mediated via IL-15Rα

NKTR-255 signaling is dependent on IL-15Rα as with IL-15

<table>
<thead>
<tr>
<th>Treatment</th>
<th>IL-15</th>
<th>NKTR-255</th>
<th>IL-15/IL-15Rα complex</th>
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<tbody>
<tr>
<td></td>
<td>WT</td>
<td>KO</td>
<td>WT</td>
</tr>
<tr>
<td>pSTAT5 EC50 (ng/ml)</td>
<td>15.58</td>
<td>347.3</td>
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<tr>
<td>EC50 ratio</td>
<td>22.29</td>
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</table>

NKTR-255 signaling is dependent on IL-15Rα as with IL-15
NKTR-255 drives IL-15-like signaling across species

- In human, NKTR-255 exhibits equal potency across three populations
- NKTR-255 potency to NK cells is preserved across species except for mouse
- NKTR-255 potency to T cells is different depending on species
- NKTR-255 species difference is similar to that for IL-15
- Varying potency across species attributed to differences in IL-15 Rα expression level
Conclusions: NKTR-255 PK and target engagement

► NKTR-255 dramatically improves IL-15 receptor agonist exposure enabling low frequency administration
► NKTR-255 enables sustained IL-15 receptor pathway engagement in NK and T cells
► NKTR-255 retains IL-15Rα binding specificity maintaining IL-15 biological context
Functional characterization of NKTR-255 across immune cell subsets in rodents and non-human primates
NKTR-255 drives IL-15 receptor signaling and proliferation in CD8 T and NK cells in mice

Cell expansion

Ki67+

pSTAT5+
NK cells are the most sensitive in NKTR-255 dose response compared to CD4 and CD8 T cells in vivo in NHPs

NK cell expansion is detected at 0.01 mg/kg dose level

NK cells Ki-67 and pSTAT5 induced at lowest dose level (0.001mg/kg).

Cynomolgus monkeys NKTR-255 IV dose response PD: blood stained for leukocyte surface markers and pSTAT5 + Ki67, measured by flow cytometry
Increased NKTR-255 sensitivity in CD8 T cell memory populations compared to naïve CD8 T cells in vivo in NHPs.
NKTR-255 expands NK cell subpopulations

NK cells at all stages of maturation are highly responsive to NKTR-255

Terminal Effector (CD11b+CD27+)
Most mature
Activation tightly regulated
Higher activation threshold

High Effector (CD11b+CD27+)
High cytokine secretion
Great effector function
Lower activation threshold

Immature (CD11b-CD27-)
Potential to differentiate

Early NK (CD11b-CD27+)
High cytokine secretion

Terminal Effector NK

High Effector NK

Immature NK

Early NK
NKTR-255 increases expression of effector functions in NK cells in mice
NKTR-255 increases levels of cytotoxic enzymes in NK cells in NHPs

NKTR-255 increases protein levels of constitutively expressed cytolytic enzymes Granzyme B and Perforin in NK cells
NKTR-255 enhances murine splenic NK cytotoxicity

NKTR-255

Spleens harvested at Day 1, 4, 6

Effectors cells: NK cells isolated from spleens

Target cells: YAC-1 cells labeled with PKH26

Co-culture at 12.5:1 (E:T ratio) for 4 hr

Label cells with 7-AAD; Analyze lysis of target cells via flow cytometry

A single in vivo dose (0.3 mg/kg) of NKTR-255 sustains NK cell killing activity for at least 6 days ex vivo
In vivo comparison summary of NK vs T cell responses to NKTR-255

- NK cells most sensitive to NKTR-255 stimulation in vivo in mice and NHPs
  - %pSTAT5 and %Ki67 increases measurable at lowest tested dose level (0.001 mg/kg) in NHP
  - Lowest dose level requirement for effective in vivo expansion in NK cells

- NKTR-255 increases intracellular levels of cytotoxic enzymes and prolongs cytotoxic activity of NK cells

- Higher sensitivity to NKTR-255 in CD8 memory T cells relative to naïve CD8 T cells
  - Graded sensitivity within memory subpopulations ($T_{EM} > T_{SCM} > T_{CM} > T_{Naive}$) in proliferative response

- CD4 T cells are the less responsive T cell population to NKTR-255 stimulation in mice and NHPs
NKTR-255 in vivo anti-tumor efficacy
NKTR-255 has potential to enhance anti-cancer immunotherapy via both NK and CD8 cells

- **NK cell biology**
  - Activation balance among human NK subtypes
    - IL-15 is similarly potent to regulatory CD56^{bright} and cytotoxic CD56^{dim/null} NK sub-populations
    - IL-15 pre-activated NK cells show sustained function

- **CD8 cell biology**
  - Supporting memory CD8 T cell longevity and function
    - IL-15 maintains Ag-specific effector CD8 T cells after the contraction phase by promoting their survival and proliferation
NKTR-255 enhances NK cell-dependent anti-tumor efficacy in disseminated CT26 lung metastasis model

Female Balb/c mice (6-8 weeks)  
10⁵ cells CT26 i.v.

NKTR-255 efficacy in disseminated CT26 model is NK cell dependent

NK cell proliferation in the lung

% NKp46 (CD8) (mean ± SEM)

% Ki-67 (NKp46) (mean ± SEM)

Hrs post last dose

NKTR-255 0.3 mg/kg q7dx2, i.v.
NKTR-255 0.1 mg/kg q7dx2, i.v.
NKTR-255 0.3 mg/kg q7dx2, i.v.
Vehicle
NKTR-255

NK cell proliferation

Hrs post last dose

% NKp46 (CD3) (mean ± SEM)

Hrs post last dose

 NKTR-255 0.3 mg/kg

* p<0.05 vs Vehicle, unpaired Student t test

** p<0.0001 vs Vehicle

^ p<0.001 vs Vehicle

*** p<0.001 vs Vehicle

**** p<0.0001 vs Vehicle

(One-way ANOVA, Tukey’s multiple comparison)
A single dose of NKTR-255 provides sustained protection against lung colonization by circulating tumor cells.

1x10^5 CT26 cells inoculated i.v on Day 13 after inoculation

Prophylactic NKTR-255: single dose treatment

Lung nodule count on Day 13 after inoculation

**% Lung Nodules inhibition**

<table>
<thead>
<tr>
<th>% lung nodule inhibition (mean ± SEM, n=12)</th>
<th>0</th>
<th>25</th>
<th>50</th>
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</thead>
<tbody>
<tr>
<td>3 days</td>
<td></td>
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<tr>
<td>6 days</td>
<td>****</td>
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<td>9 days</td>
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- Vehicle
- NKTR-255 0.3 mg/kg; IV

****=p<0.0001 vs respective Vehicle (One-way ANOVA followed by Tukey's multiple comparison test)
NKTR-255 inhibits establishment of spontaneous lung metastasis in 4T1 tumor model

Mean tumor volumes of subcutaneous primary tumors

Number of spontaneous metastatic colonies on Day 14 in lungs

Vehicle, q7dx3
NKTR-255 0.3 mg/kg, q7dx3
NKTR-255 may overcome many limitations of IL-15 as a therapeutic agent

- Improved PK to allow infrequent administration
- Provides sustained IL-15 PD activity from a single dose
- Achieves full breadth of signaling profile characteristic to IL-15

By design, NKTR-255 maintains binding affinity for IL-15Rα

NKTR-255 promotes the proliferation of memory T cells and induces significant NK cell expansion and increased cytotoxic activity

NKTR-255 enhances NK dependent anti-tumor immune responses in vivo

NKTR-255 enables access to the immunotherapeutic potential of the IL-15 pathway by enhancing expansion and activation of both NK and CD8 T cells