Restoring NK Cell Activities in Multiple Myeloma with IL-15 Receptor Agonist NKTR-255

Conflict of Interest

- Nothing to disclose
Background

- Multiple myeloma (MM) is characterized by an **immunosuppressive microenvironment** that enables tumor development through the activation of cells with a suppressive effect, disruption of antigen presentation and dysregulation of proliferation and functionality of effector cells.

- Natural Killer (NK) cells play a major role in anti-tumor surveillance hindering tumor growth through their potent cytotoxic properties. Nevertheless, MM cells can also induce an **inhibition of NK cell effector functions**.

- The **restoration of NK cell anti-tumor activity** represents a key goal for new immunotherapeutic approaches.

- Among these strategies, **cytokines** could be a potential therapeutic resource due to their capability to control the proliferation of the different immune subpopulations and increase the anti-tumor cytotoxicity.
The Challenge to Therapeutic Use of IL-15

- IL-15 and IL-2 belong to the same cytokine family, yet important differences exist.
- IL-15 promotes proliferation and cytotoxicity of NK cells, NKT cells, γ/δ T cells or memory CD8+ T cells, enhancing innate and adaptive immunity against MM cells in pre-clinical studies.¹⁴

- Previous efforts to harness IL-15 biology have been limited.
- IL-15 displays rapid clearance from plasma and in vivo signaling is short-lived.
- Sharp exposure levels cause adverse effects before demonstrating efficacy benefits.

¹Weng et al. Oncoimmunology, 2016
²Wagner et al. J Clin Invest, 2017
³Tognarelli et al. Front Immunol, 2018
⁴Xu et al. Cancer Res, 2013

Unpublished data provided by Nektar Therapeutics
NKTR-255: an IL-15-based Therapeutic for Immuno-Oncology

NKTR-255 is a novel immunotherapeutic agent consisting of polymer-engineered (PEG) IL-15 designed to optimally engage all three IL-15 receptors (IL-15R) accessing the full spectrum of IL-15 biology.

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 IL-15Rα.

PEGylation significantly improved IL-15 pharmacokinetic profile, enhanced plasma exposure and reduced total clearance across species on single dose.

Unpublished data provided by Nektar Therapeutics

Kirk et al. Abstract #342, SITC 2016, Maryland (USA)
Major Aims of the Study

- Evaluate changes in the expression profile of inhibitory and activating markers on NK cells after treatment with NKTR-255.

- Test the *ex vivo* enhancement of NK cell effector functions (degranulation, cytokine release, direct cytotoxicity or ADCC) to target MM cells following stimulation with NKTR-255.

- Explore the potential of NKTR-255 alone or combined with anti-CD38 antibodies to limit the growth of MM cells in an immunocompetent humanized murine model of MM.

- Analyze the *in vivo* effect of NKTR-255 alone or combined with anti-CD38 antibodies on the immune cell compartment.
NKTR-255 Shifts the Balance of Stimulatory Receptors vs Inhibitory Receptors on NK Cells from MM Patients

Follow-up of receptor surface expression of NK cells from MM after administration of NKTR-255

[Graph showing fold change in MFI compared with baseline for Activating and Inhibitory receptors over time (days)]
NKTR-255 Increases *Ex Vivo* Expression of Stimulatory Receptors and Activation Markers on NK Cells

Follow-up of receptor surface expression of NK cells from MM after administration of NKTR-255
NKTR-255 Tilts the Balance Towards a More Activated Phenotype on NK Cells and Promotes Expansion of Activated NK Cells

Variation of NKG2D+ NK cell number over baseline after 5 days of incubation with NKTR-255 in PBMC from 9 MM patients

Tracking of NKG2D expression (MFI) on NK cells along 14 days of incubation with NKTR-255 at 1000 ng/mL
MM Patient Derived NK Cells Show Improved Degranulation and Cytokine Production in Response to Tumor Targets After Treatment with NKTR-255

Collection of PBMCs from MM patients
Incubation with NKTR-255 x 7 days
Isolation of NK cells
Stimulated NK cells +/- MM cells
4 hours culture
Preparation for flow cytometry and analysis of CD107a expression/isotype control

Degranulation assay

- NK
- NK+U266
- NKTR-255
- rhIL-15

compared to isotype control

Dose (µg/ml)

0 0.1 1 0.1 0 0.1 1 0.1

NKTR-255 rhIL-15 NKTR-255 rhIL-15

compared to isotype control

Dose (µg/ml)

0 0.1 1 0.1 0 0.1 1 0.1

NKTR-255 rhIL-15 NKTR-255 rhIL-15
MM Patient Derived NK Cells Show Improved Degranulation and Cytokine Production in Response to Tumor Targets After Treatment with NKTR-255

Collection of PBMCs from MM patients

Incubation with NKTR-255 x 7 days

Isolation of NK cells

Stimulated NK cells +/- MM cells

16 hours culture

Collection of supernatants

ELISA test to measure concentration of specific cytokines (IFNγ or TNFα)

Interferon γ release assay
MM Patient Derived NK Cells Show Improved Degranulation and Cytokine Production in Response to Tumor Targets After Treatment with NKTR-255

Collection of PBMCs from MM patients
Incubation with NKTR-255 x 7 days
Isolation of NK cells
Stimulated NK cells +/- MM cells
16 hours culture
Collection of supernatants
ELISA test to measure concentration of specific cytokines (IFNγ or TNFα)

Interferon γ release assay

TNF α release assay
Dose and T:E Ratio-Dependent Increase in NK Cytotoxicity After Administration of NKTR-255

Collection of PBMCs from MM patients
Incubation with NKTR-255 x 7 days
Isolation of NK cells
Stimulated NK cells + CTV-stained MM cells at selected T:E ratios
4 hours co-culture
Staining with a viability dye, preparation for flow cytometry and assessment of target cell lysis

Target cell lysis (%) over background

- 0.01 µg/ml NKTR-255
- 0.1 µg/ml NKTR-255
- 1 µg/ml NKTR-255
- 0.1 µg/ml rhIL-15

T: KMS12BM
E: HD NK

T:E ratio

1:1 1:2 1:4 1:8 1:16
NKTR-255 Enhances Anti-Tumor Responses of Human NK Cells Against MM Cell Targets

Assessment of NK cytotoxicity against MM cells after 4-hour co-incubation of NK and MM cells

- **U266 (T:E ratio 1:8)**
- **H929 (T:E ratio 1:5)**
- **KMS12BM (T:E ratio 1:2)**
NKTR-255 Enhances Anti-Tumor Responses of Human NK Cells Against MM Cell Targets

Assessment of NK cytotoxicity against MM cells after 4-hour co-incubation of NK and MM cells.
NKTR-255 Increases Daratumumab or Elotuzumab-Mediated Antibody-Dependent Cellular Cytotoxicity (ADCC)

Assessment of NK ADCC after 4-hour co-incubation of NK and Elo/Dara pre-treated MM cells
No Direct Effect of NKTR-255 or Recombinant Human IL-15 on Growth and Viability of MM Cells

Viability assessment of 5 MM cell lines after 10 days of incubation with maximal doses of NKTR-255/IL-15
A Humanized Mouse MM Model Was Employed for the *In Vivo* Studies

Day 0:
- Tumor volume >50 mm³

5 days for acclimation to the environment

- Human CD34+ hematopoietic stem cells
- Subcutaneous engraftment with 5x10⁶ NCI-H929 cells

Start of treatment:
- Randomization according to tumor volume, humanization rate and cord blood donor

- Daily monitoring of unexpected signs of distress
- Body weight monitored three times weekly
- Tumor volume monitored three times weekly

End-point: tumor volume >1500 mm³
Collection of blood, spleen and tumor tissue

All animal experiments were approved by the local ethic committee and ethically conducted complying with the US Public Health Service Policy on Human Care and Use of Laboratory Animals
NKTR-255 Enhances the Anti-MM Activity of Daratumumab in the Humanized Mouse Model of MM

When tumors reached an average volume of 50 mm$^3$, mice were randomized (n=5 per cohort) to receive:

- Vehicle
- Daratumumab 5 mg/kg weekly
- NKTR-255 0.3 mg/kg weekly
- Daratumumab 5 mg/kg + NKTR-255 0.3 mg/kg weekly

Tumor volume was monitored three times a week (mean ± SEM). Each group was compared to the vehicle.

While both daratumumab and NKTR-255 treatment delayed tumor growth as single agents (35.4% and 29.6%, respectively), the combination further increased (66.4%) inhibition of tumor growth.
NKTR-255 Improves Immune Status Following Anti-CD38 Treatment

Analysis by flow cytometry of immune cell populations in peripheral blood from mice at the end of the study.

Analysis by flow cytometry of CD38+ immune cell populations in tumor tissue from mice at the end of the study.
Conclusions

1) The induction of an activated profile in NK cells by NKTR-255 results in an effective enhancement of their anti-myeloma effector functions (direct cytotoxicity, degranulation, cytokine release, aDCC) in ex vivo assays.

2) In vivo studies confirmed superiority of the combination of daratumumab and NKTR-255 compared to single agents in controlling MM growth.

3) NKTR-255 improves the immune cell compartment both in the tumor tissue and in blood following anti-CD38 treatment.

4) NKTR-255 is an attractive novel immunotherapeutic approach for clinical evaluation in multiple myeloma.

5) NKTR-255 is being currently explored in patients with relapsed/refractory hematologic malignancies (NCT04136756)
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