American Society *of* Hematology

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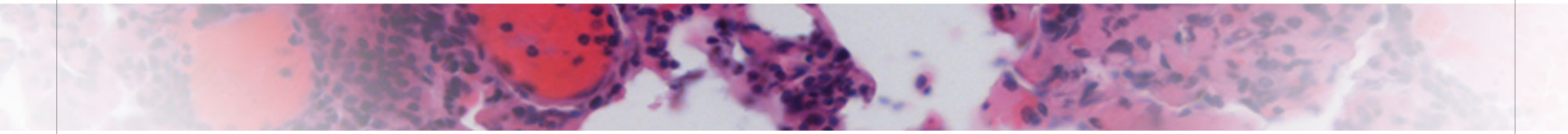

Restoring NK Cell Activities in Multiple Myeloma with IL-15 Receptor Agonist NKTR-255

Rafael Alonso Fernández, Laetitia Pierre-Louis, Yan Xu, Shidai Mu, Joaquín Martínez-López, Takahiro Miyazaki, Rao Prabhala, Kenneth C Anderson, Loui Madakamutil, Nikhil C Munshi and Mariateresa Fulciniti

Conflict of Interest

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- Nothing to disclose

Background

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- 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

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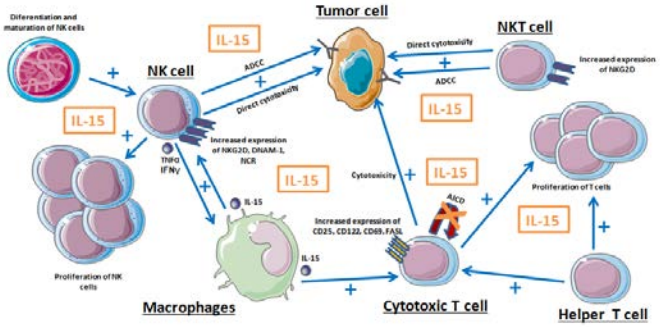


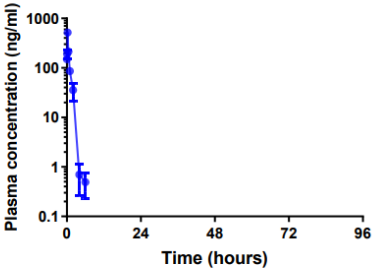
- IL-15 and IL-2 belong to the same cytokine family, yet important differences exist.

- 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.[1-4]

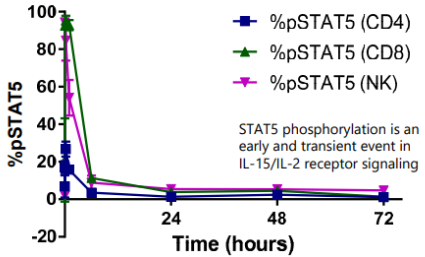
- Previous efforts to harness IL-15 biology have been limited.

- IL-15 displays rapid clearance from plasma and *in vivo* signaling is short-lived.

- Sharp exposure levels cause adverse effects before demonstrating efficacy benefits.



Mouse PK: IL-15 0.5mpk i.p., serum assayed by ELISA



Mouse PD: IL-15 0.3mpk i.p., whole blood stained for leukocyte surface markers and pSTAT5, measured by flow cytometry

Unpublished data provided by Nektar Therapeutics

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

NKTR-255: an IL-15-based Therapeutic for Immuno-Oncology

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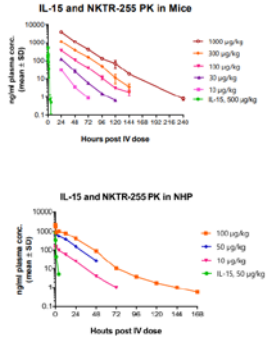
NKTR-255 is a novel immunotherapeutic agent consisting of **polymer-engineered** (PEG) **IL-15** designed to optimally engage all three IL-15 receptors (IL-15R) accessing the full spectrum of IL-15 biology.

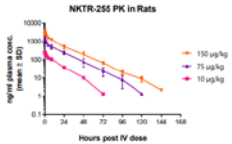
Design goals:

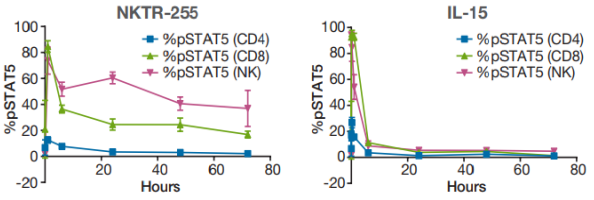
✓ Improve PK and PD to sustain IL-15 activity and achieve large pharmacodynamic effect without need for daily dosing.

✓ Retain binding to IL-15Rα to maintain full spectrum of IL-15 biology.

✓No mutagenesis or complex to IL-15Rα.







Kirk et al. Abstract #342, SITC 2016, Maryland (USA)

Unpublished data provided by Nektar Therapeutics

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

Major Aims of the Study

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- 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

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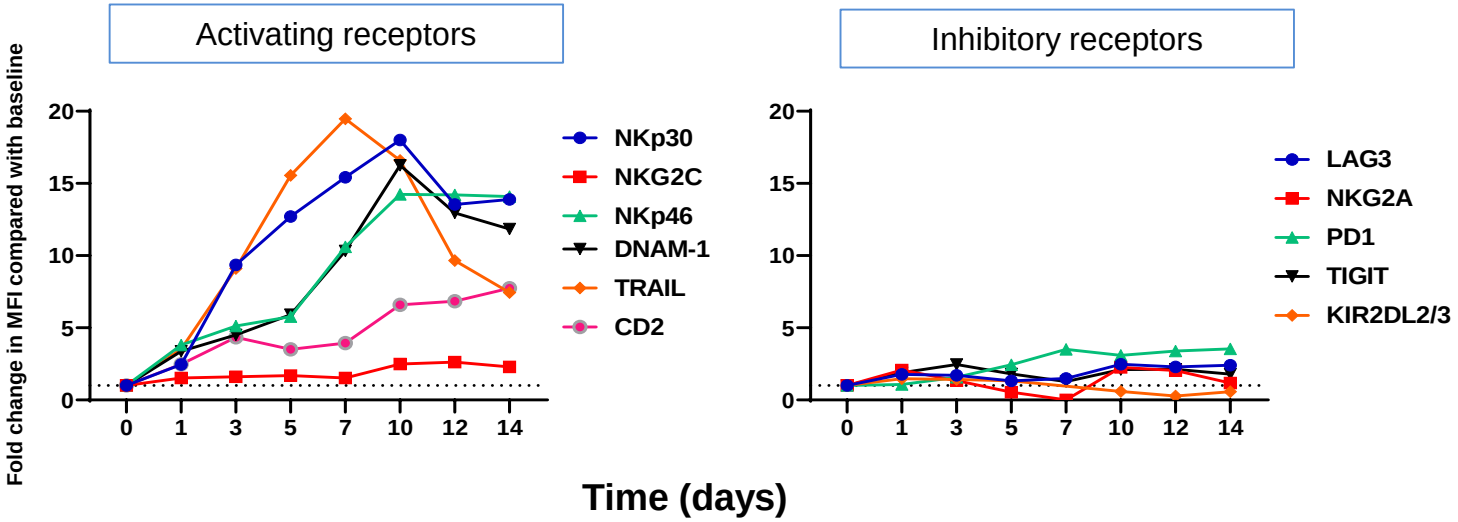
Follow-up of receptor surface expression of NK cells from MM after administration of NKTR-255



NKTR-255 Increases *Ex Vivo* Expression of Stimulatory Receptors and Activation Markers on NK Cells

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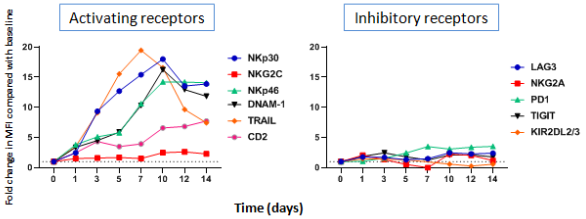
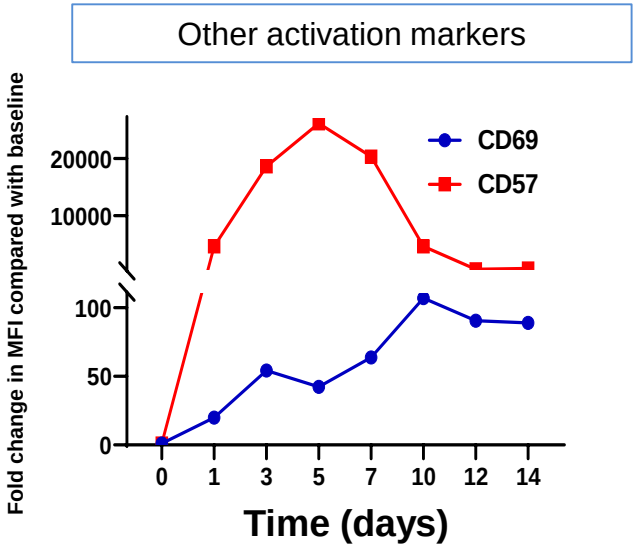
Follow-up of receptor surface expression of NK cells from MM after administration of NKTR-255





NKTR-255 Tilts the Balance Towards a More Activated Phenotype on NK Cells and Promotes Expansion of Activated NK Cells

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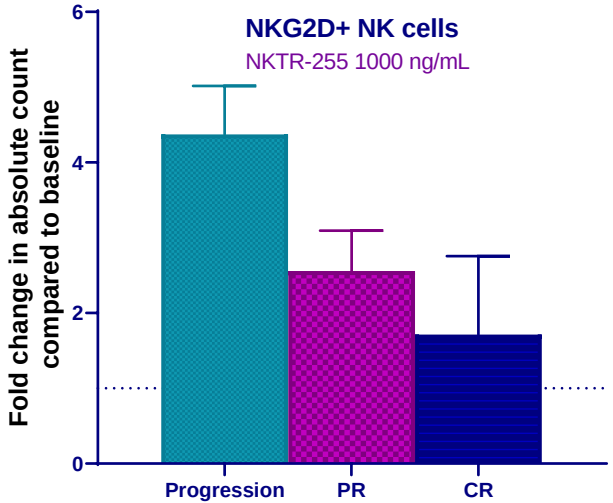
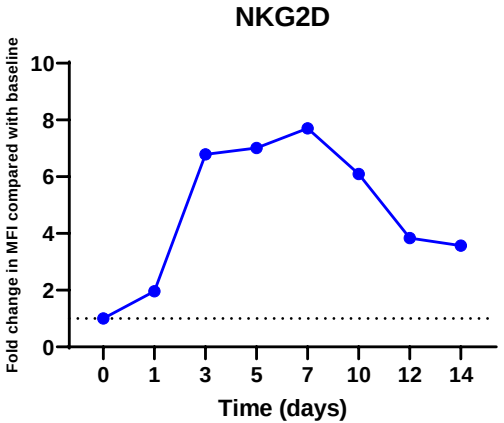
Variation of NKG2D+ NK cell number over baseline after 5 days of incubation with NKTR-255 in PBMC from 9 MM patients



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

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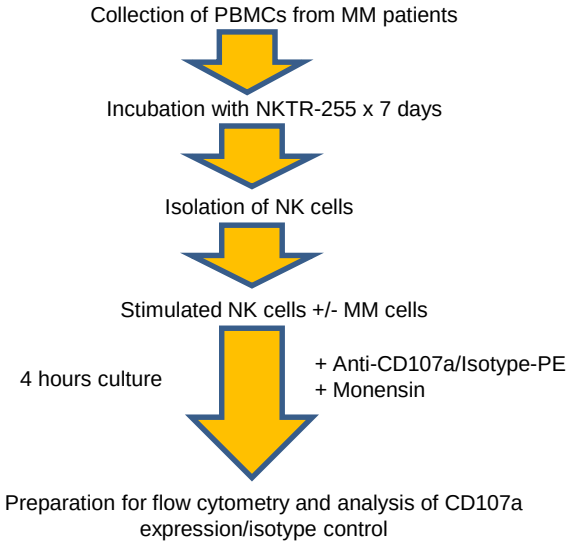
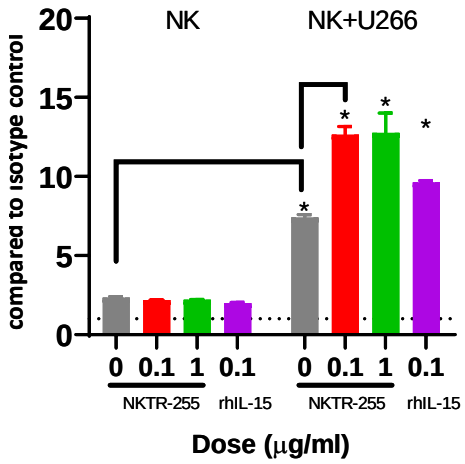
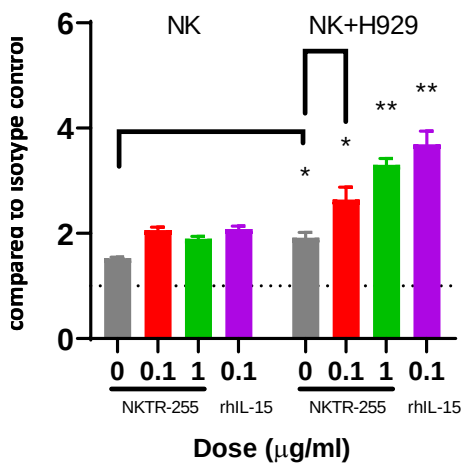


Degranulation assay





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

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Collection of PBMCs from MM patients

Incubation with NKTR-255 x 7 days

Isolation of NK cells

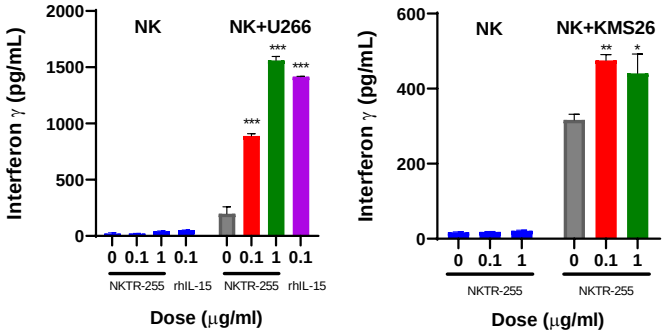
Stimulated NK cells +/- MM cells

16 hours culture

Collection of supernatants

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

Interferon γ release assay



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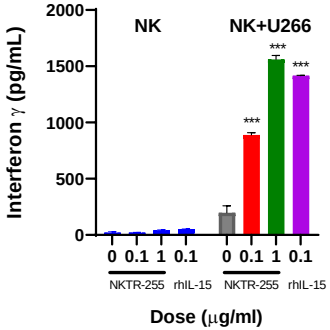

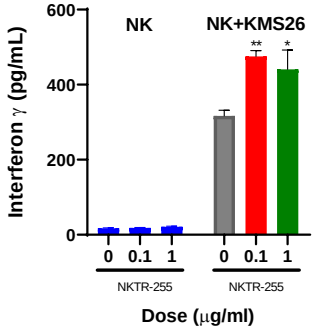

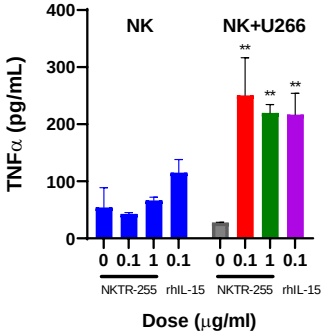

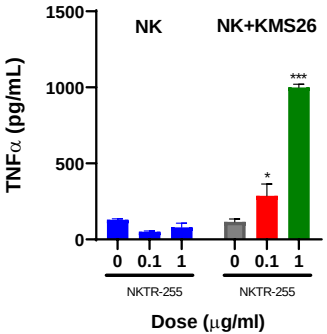

TNF α release assay

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

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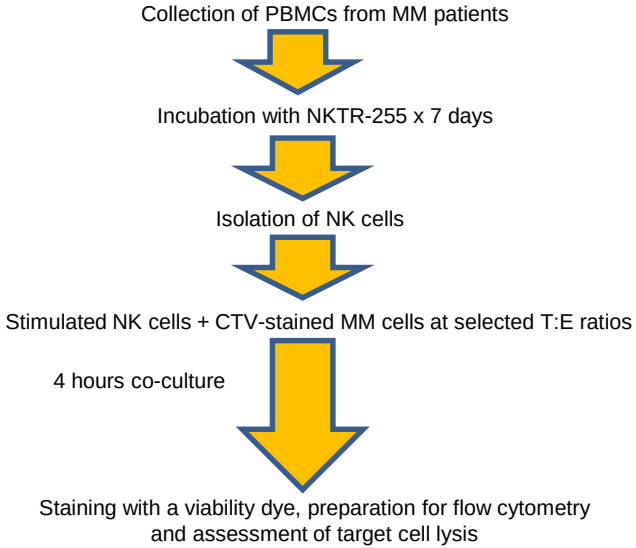

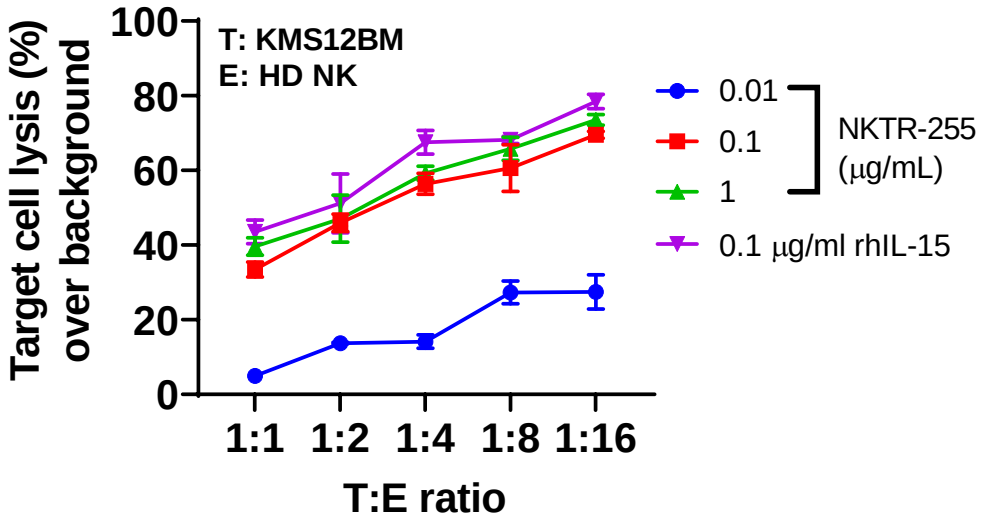

NKTR-255 Enhances Anti-Tumor Responses of Human NK Cells Against MM Cell Targets

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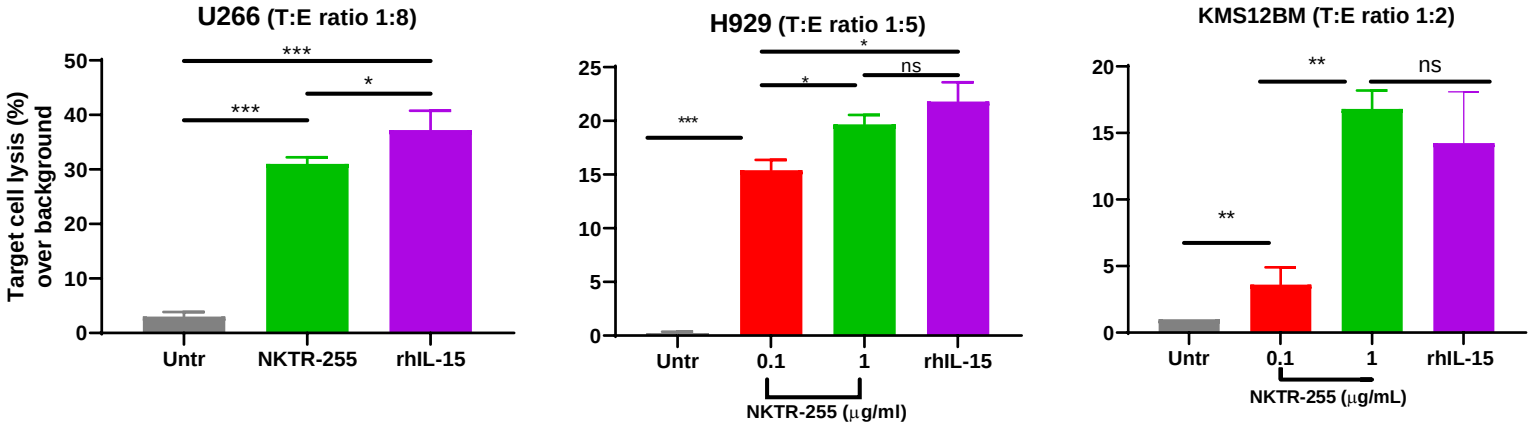
Assessment of NK cytotoxicity against MM cells after 4-hour co-incubation of NK and MM cells



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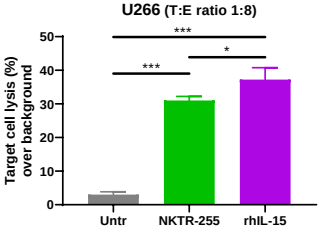
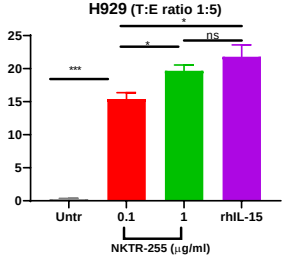
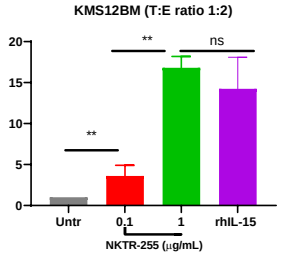
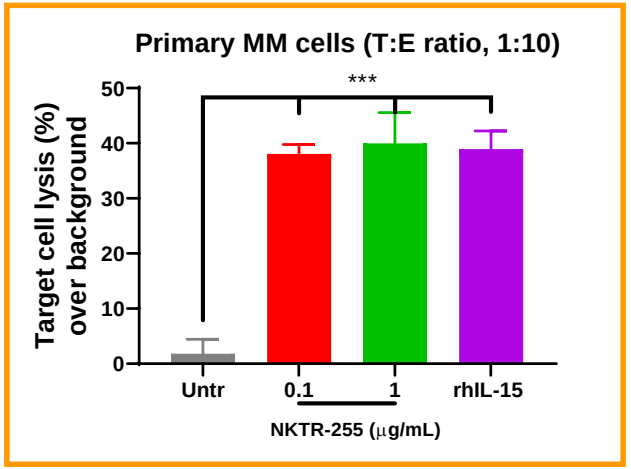
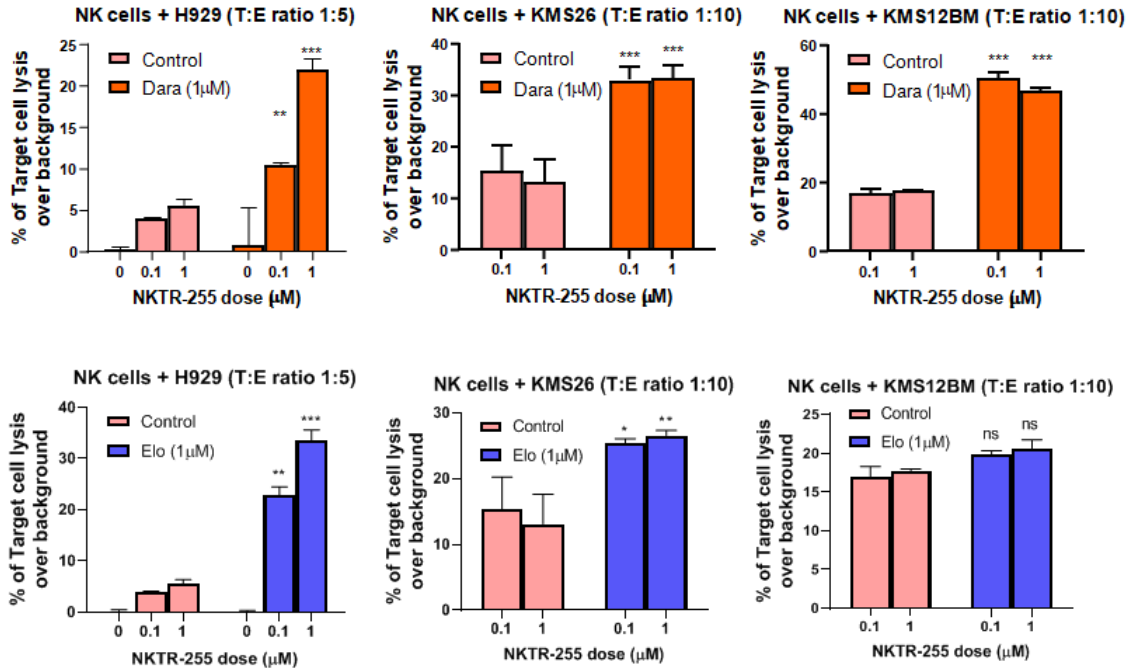








NKTR-255 Increases Daratumumab or Elotuzumab-Mediated Antibody-Dependent Cellular Cytotoxicity (ADCC)



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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

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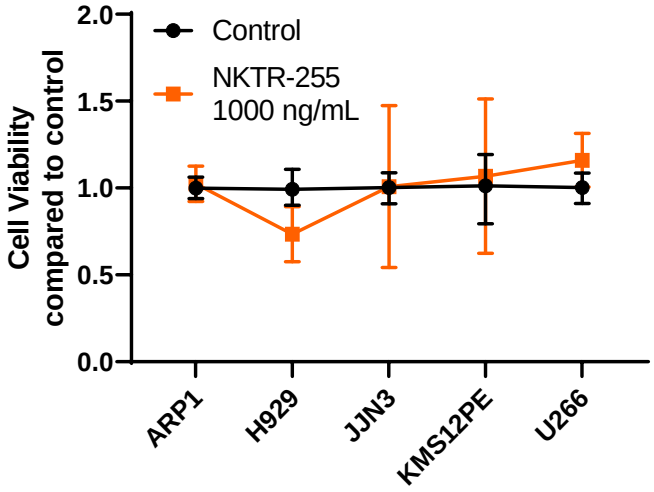
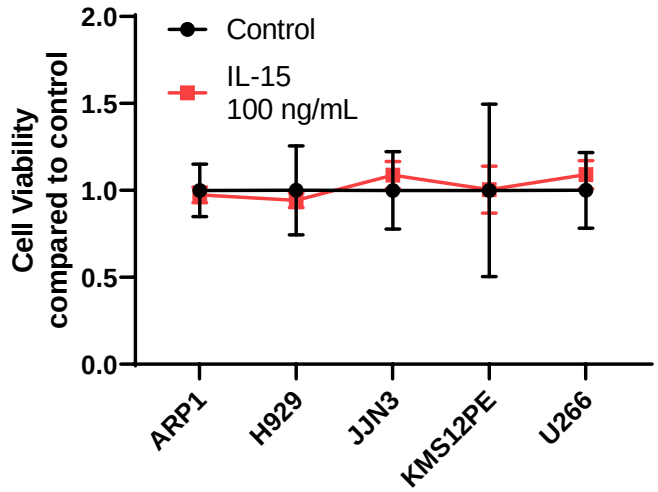
Viability assessment of 5 MM cell lines after 10 days of incubation with maximal doses of NKTR-255/IL-15





A Humanized Mouse MM Model Was Employed for the *In Vivo* Studies

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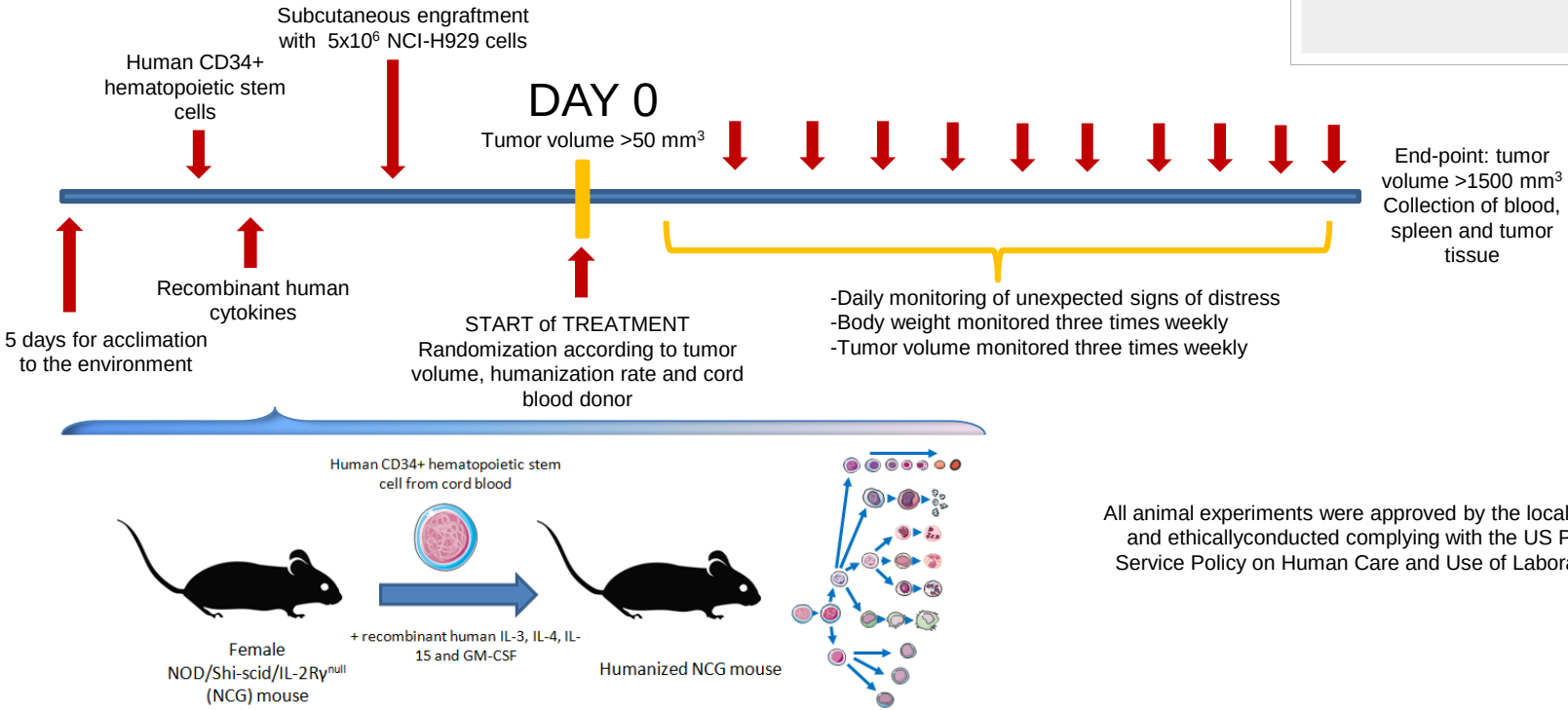

All animal experiments were approved by the local ethic comittee and ethicallyconducted complying with the US Public Health Service Policy on Human Care and Use of Laboratory Animals

NKTR-255 Enhances the Anti-MM Activity of Daratumumab in the Humanized Mouse Model of MM

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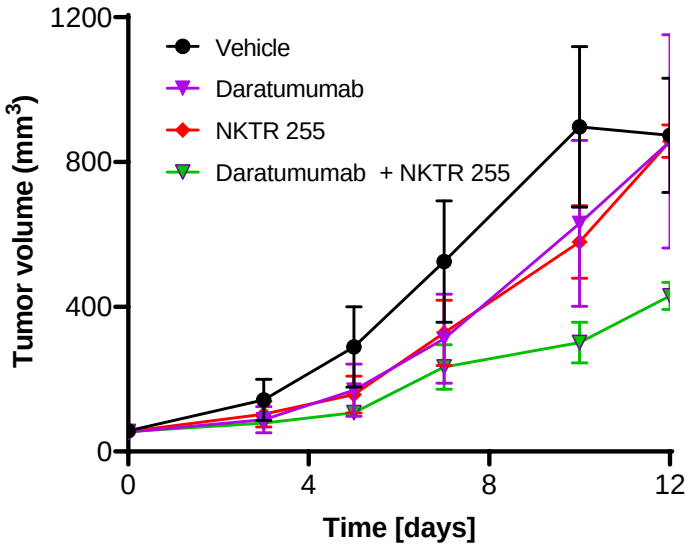

- When tumors reached an average volume of 50 mm^3, mice were randomized (n=5 per cohort) to receive:

-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

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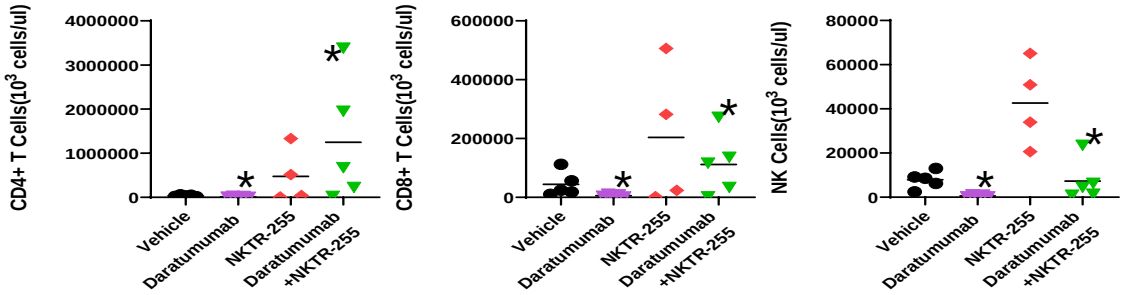
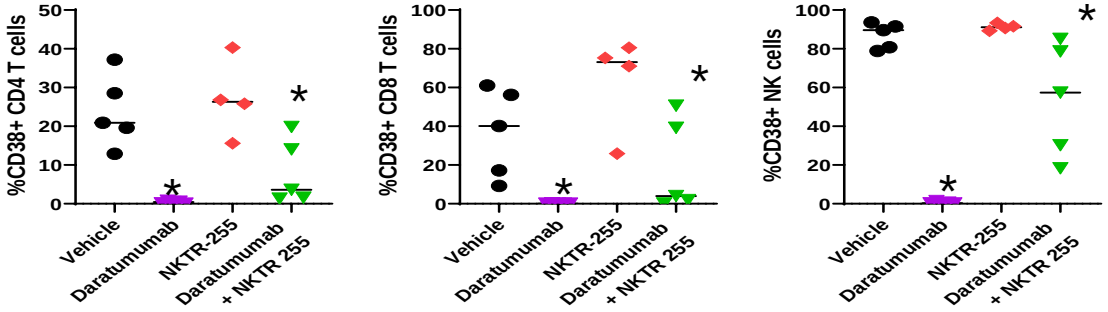
Analysis by flow cytometry of immune cell populations in peripheral blood from mice at the end of the study



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



Conclusions

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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)

Acknowledgements

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Almudena García-Ortiz
Jessica Encinas
Elena Maroto Martín
José María Sánchez-Pina
Clara Cuéllar

All lab and clinical team

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Sociedad Española de Hematología y Hemoterapia
Fundación Española de Hematología y Hemoterapia

NEKTAR

Takahiro Miyazaki

All NEKTAR team

Loui Madakamutil