Restoring Innate and Adaptive Immune Repertoire in Multiple Myeloma for Therapeutic Application

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BACKGROUND

Despite advances and improvements in survival, majority of multiple myeloma (MM) patients ultimately relapse. Extensive analysis on the properties of tumor cells has provided interesting insights into disease biology allowing for the identification of novel targets and development of related therapeutics. However, microenvironmental influences, especially the immune microenvironment, are key to drive the disease and impact outcome.

In addition to humoral immunodeficiency, the immunosuppressive microenvironment observed in MM includes a dysfunction in the adaptive immune system with an increase in immunosuppresive cells (Tregs or myeloid-derived supressor cells). This is accompanied by a significant impairment of innate immunity, specifically a progressive decline in natural killer (NK) cells function (low expression of activating receptors and high expression of certain inhibitory receptors). These factors allow tumor immune escape and ultimately myeloma cell growth.

OBJECTIVES

NKTR-255 is a polymer-conjugated human IL-15 that retains binding affinity to the alpha subunit of IL-15 receptor and exhibits reduced clearance to thereby provide a sustained pharmacodynamics response. Our aim in this study was to evaluate the role of NKTR-255 to overcome some of the immune dysfunction observed in MM.

MATERIAL & METHODS

We have evaluated the impact of NKTR-255 and IL-15 on effector immune cell populations from peripheral blood of healthy donors (HD) and MM patients at different stages of disease. NK cells were isolated by negative immunomagnetic selection to perform a specific flow assesment of their effector functions after treatment with NKTR-255 or IL-15 and their cytokine release pattern was evaluated using ELISA techniques.

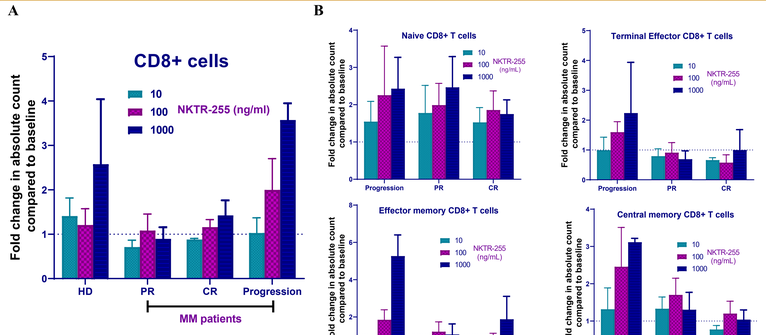
SUMMARY

Treatment with NKTR-255 rescued the immune effector cell decline observed in MM patients, promoting *ex vivo* the survival and expansion of effector memory and central memory CD8+ T cells, and to a lesser extent NK cells, in PBMCs from HD and MM patients in a dose-dependent manner. Interestingly, the natural killer T (NKT) cells (a heterogeneous group of T cells that shares properties of both T and NK cells with an important role in MM), were also increased in number by NKTR-255 with an enhancement of NKG2D expression.

NKTR-255 showed a significant role in the improvement of NK cell effector functions, reverting the inhibitory status of NK cells from MM patients through the increase of NKG2D and other activating receptors that are essential for tumor cell recognition and killing. This resulted in a greater degranulation potential of NK cells after tumor exposure, a higher release of pro-inflammatory cytokines and, consequently, significantly improved susceptibility of MM cell lines to NK cell direct action in a dose-dependent manner in cytotoxicity assays. Antibody-dependent cellular cytotoxicity (ADCC) of NK cells was also enhanced by NKTR-255, showing synergy with anti-myeloma monoclonal antibodies, such as Daratumumab or Elotuzumab. Importantly, we did not observe any direct effect of IL-15 or NKTR-255 on growth and viability of MM cells.

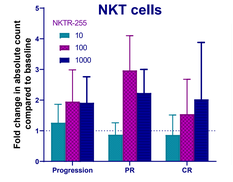
RESULTS

1. NKTR-255 enhances *ex vivo* expansion of memory CD8+ T cell subsets from PBMC of MM patients



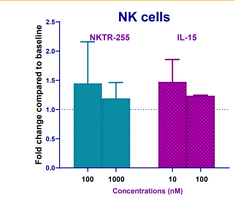
CD8+ expansion by NKTR-255 in MM. PBMC retrieved from peripheral blood (PB) of healthy donors (HD) (n=3) or MM patients from different stage of the disease (n=10) were incubated for 5 days with increasing doses of NKTR-255. Absolute count of selected immune populations after treatment was determined by flow cytometry using SpheroTech Accucount beads and compared to absolute count at baseline.

2. NKTR-255 induces growth of NKT cells in MM patients



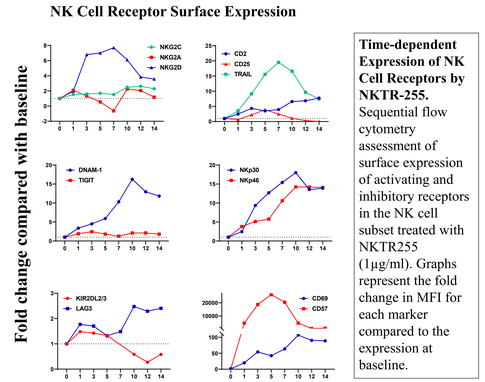
NKT cells growth induced by NKTR-255 treatment. CD3+CD56+CD16 + NKT cell proliferation upon 5 days of treatment with NKTR-255.

3. NKTR-255 expands MM NK cells

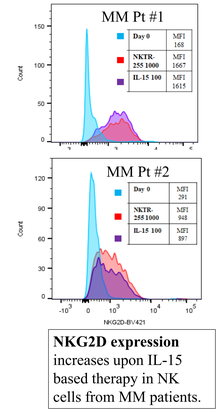


NK cell proliferation in PBMC from 10 MM patients after 5 days of treatment with NKTR-255 or IL-15.

4. Activation of NK cells derived from MM patients by NKTR-255

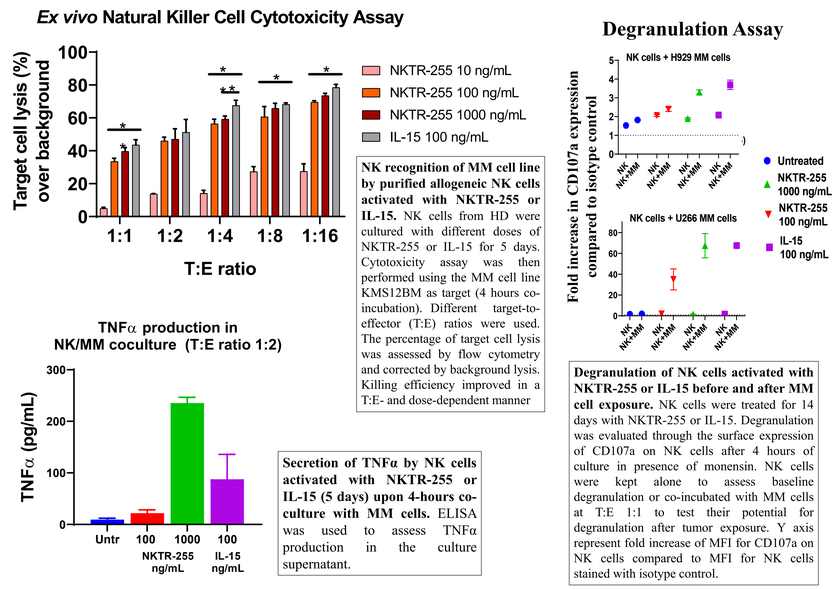


Time-dependent Expression of NK Cell Receptors by NKTR-255. Sequential flow cytometry assessment of surface expression of activating and inhibitory receptors in the NK cell subset treated with NKTR255 (1μg/ml). Graphs represent the fold change in MFI for each marker compared to the expression at baseline.



NKG2D expression increases upon IL-15 based therapy in NK cells from MM patients.

5. NKTR-255 enhances antitumor activity of NK cells against MM *ex vivo*

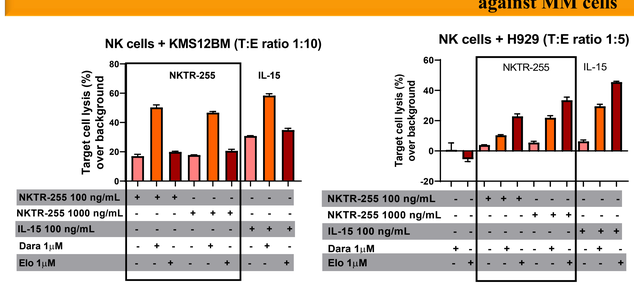


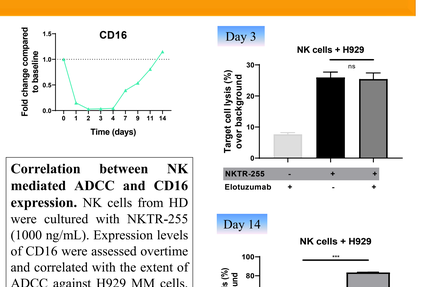
NK recognition of MM cell line by purified allogeneic NK cells activated with NKTR-255 or IL-15. NK cells from HD were cultured with different doses of NKTR-255 or IL-15 for 5 days. Cytotoxicity assay was then performed using the MM cell line KMS12BM as target (4 hours co-incubation). Different target-to-effector (T:E) ratios were used. The percentage of target cell lysis was assessed by flow cytometry and corrected by background lysis. Killing efficiency improved in a T:E- and dose-dependent manner

Secretion of TNFα by NK cells activated with NKTR-255 or IL-15 (5 days) upon 4-hours co-culture with MM cells. ELISA was used to assess TNFα production in the culture supernatant.

Degranulation of NK cells activated with NKTR-255 or IL-15 before and after MM cell exposure. NK cells were treated for 14 days with NKTR-255 or IL-15. Degranulation was evaluated through the surface expression of CD107a on NK cells after 4 hours of culture in presence of monensin. NK cells were kept alone to assess baseline degranulation or co-incubated with MM cells at T:E 1:1 to test their potential for degranulation after tumor exposure. Y axis represent fold increase of MFI for CD107a on NK cells compared to MFI for NK cells stained with isotype control.

6. NKTR-255 augments NK antibody-dependent cellular cytotoxicity (ADCC) against MM cells

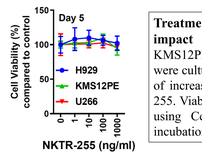




NK mediated ADCC against MM cells. NK cells from peripheral blood of MM patients were cultured with different doses of NKTR-255 or IL-15. After 14 days of treatment, stimulated NK cells were co-incubated for 4 hours with untreated MM cells or MM cells pre-incubated for 30 minutes with Elotuzumab (anti-CS1) or Daratumumab (anti CD38) antibodies. Killing efficiency was assessed by flow cytometry.

Correlation between NK mediated ADCC and CD16 expression. NK cells from HD were cultured with NKTR-255 (1000 ng/mL). Expression levels of CD16 were assessed overtime and correlated with the extent of ADCC against H929 MM cells. Significant ADCC was observed with NK cells treated for 14 days with NKTR-255 and this correlated with increased expression level of CD16.

7. Absence of direct effect of NKTR-255 or IL-15 on MM growth



Treatment with NKTR-255 does not impact MM cell viability. H929, KMS12PE and U266 MM cell lines were cultured in the absence or presence of increasing concentrations of NKTR-255. Viability of MM cells was assessed using CellTiter-Glo after 5 days of incubation.

CONCLUSIONS

Taken together, our data suggest a significant impact of NKTR-255 on the activation of effector cell function to efficiently target MM cells. This study has important translational implications and highlights the importance of restoring the balance in innate and adaptive immunity in MM.

COI

Miyazaki T. & Madakamutil L.: *Nektar Therapeutics*: Employment, Equity Ownership. **Munshi N.**: *Amgen, Celgene; Abbvie; Adaptive; Janssen; Takeda; Oncopep*: Consultancy.