Improving NK Cell Function in Multiple Myeloma with NKTR-255, a Novel Polymer-Conjugated Human IL-15

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BACKGROUND

Multiple myeloma (MM) is characterized by an immunosuppressive microenvironment that enables tumor development. One of the mechanisms of immune evasion used by MM cells is the inhibition of NK cell effector functions; thus, the restoration of NK cell antitumor activity represents a key goal for new immunotherapeutic approaches, increasing tumor cell recognition, avoiding tumor escape and potentially enhancing the effect of other drugs.

OBJECTIVES

Here we investigate the potential of NKTR-255, a novel polymer-conjugated human IL-15 to engage the IL-15 pathway and overcome the inhibitory status observed in NK cells from MM patients. For this purpose, we have analyzed *ex vivo* and *in vivo* effects of NKTR-255 on phenotypic features, effector functions and cytotoxicity of NK cells against MM cells.

MATERIALS & METHODS

For *ex vivo* analyses, NK cells from MM patients at different stages of disease were isolated by negative immunomagnetic selection. Cytotoxicity against primary MM cells or cells lines and phenotypic changes after incubation with NKTR-255 for 7 days were assessed through an extensive flow cytometry approach, and cytokine-release pattern was evaluated using ELISA.

To assess the effect of NKTR-255 on the NK cell compartment and MM cells in an autologous setting, whole bone marrow (BM) samples from newly diagnosed MM (NDMM) patients were incubated with growing concentrations of NKTR-255 and changes were measured through an automated flow cytometry platform.

In vivo evaluations were conducted employing a fully humanized immunocompetent mouse model subcutaneously engrafted with H929 MM cells.

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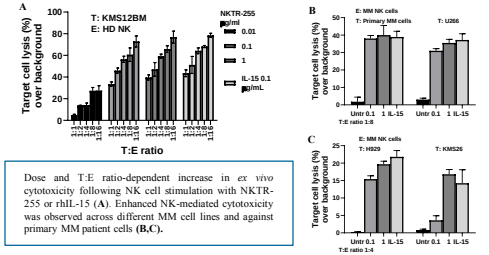
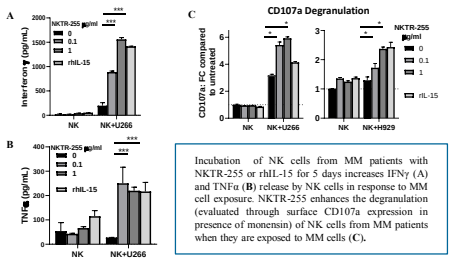
RESULTS

1. NKTR-255 enhances anti-MM responses of human NK cells



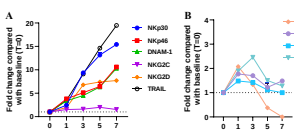
Dose and T:E ratio-dependent increase in *ex vivo* cytotoxicity following NK cell stimulation with NKTR-255 or rhIL-15 (**A**). Enhanced NK-mediated cytotoxicity was observed across different MM cell lines and against primary MM patient cells (**B**,**C**).

2. NKTR-255 boosts effector functions of human NK cells



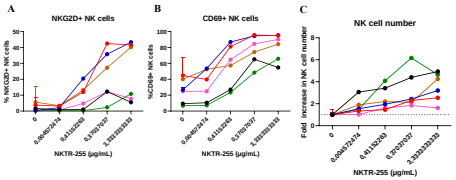
Incubation of NK cells from MM patients with NKTR-255 or rhIL-15 for 5 days increases IFNγ (**A**) and TNFα (**B**) release by NK cells in response to MM cell exposure. NKTR-255 enhances the degranulation (evaluated through surface CD107a expression in presence of monensin) of NK cells from MM patients when they are exposed to MM cells (**C**).

3. Time-dependent activated profile of NK cells induced by NKTR-255

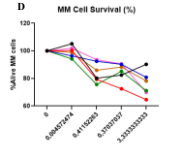


Throughout 7 days of incubation with 1000 ng/mL of NKTR-255 NK cells collected from PH of MM patients show an activated phenotype with significant increase of activatng receptors involved in tumor recognition (**A**) and only a marginal impact on inhibitory receptors (**B**).

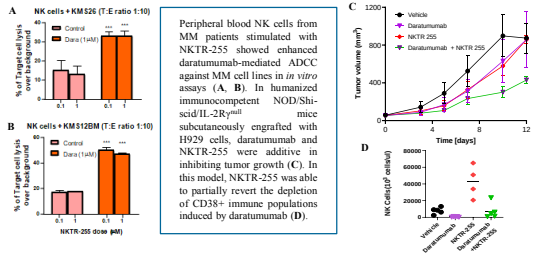
4. NKTR-255 induces activation and growth of bone marrow NK cells, reducing survival of autologous MM cells



Whole bone marrow samples of 6 NDMM patients incubated for 120 hours with 4 doses of NKTR-255 induced a dose-dependent activation profile of NK cells (**A**, **B**), expansion of NK cell populations (**C**), and a reduction of MM cell viability (**D**) as compared to control in an autologous setting using an automated flow cytometry platform for these assessments.



5. NKTR-255 enhances NK ADCC mediated by daratumumab



Peripheral blood NK cells from MM patients stimulated with NKTR-255 showed enhanced daratumumab-mediated ADCC against MM cell lines in *in vitro* assays (**A**, **B**). In humanized immunocompetent NOD/Shi-scid/IL-2Rγ^{null} mice subcutaneously engrafted with H929 cells, daratumumab and NKTR-255 were additive in inhibiting tumor growth (**C**). In this model, NKTR-255 was able to partially revert the depletion of CD38+ immune populations induced by daratumumab (**D**).

SUMMARY

NKTR-255 tilted the balance of NK cells isolated from peripheral blood (PB) mononuclear cells of MM patients towards an activated phenotype, with increased expression of activating receptors (NKG2D, NKp46, NKp30, DNAM-1, CD69, TRAIL) on the surface of treated NK cells. This resulted in an enhanced degranulation, cytokine release and anti-tumor cytotoxicity when the NK cells were exposed to both MM cell lines and primary MM cells. For a more accurate assessment of the effect of NKTR-255 on NK cell activity in an autologous setting in the presence of the BM milieu, we cultured whole BM samples from non-treated newly-diagnosed MM patients with increasing doses of NKTR-255 for 5 days. NK cells experienced a dose-dependent induction of proliferation and activation (as shown by increased expression of CD69 and NKG2D), which translated in a reduced viability of CD138+ MM cells in the presence of NKTR-255.

We further evaluated the *in vivo* effect of NKTR-255 in fully humanized immunocompetent mice subcutaneously engrafted with H929 MM cells. Compared to placebo, weekly injection of mice with NKTR-255 increased the number of circulating NK cells in PB and delayed tumor growth. Finally, we also tested *in vitro* and *in vivo* efficacy of a combination of NKTR-255 with daratumumab. We observed a more efficient antibody-dependent cellular cytotoxicity (ADCC) against MM cells *in vitro* and decreased tumor growth *in vivo*, where NKTR-255 rescued NK cell levels from depletion by daratumumab.

CONCLUSIONS

Taken together, these results support the restoration and expansion of NK cell number and activity in MM with NKTR-255, providing rationale for its clinical use as a novel immunotherapeutic approach in MM alone or in combination with monoclonal antibodies or other immunomodulatory drugs.

COI

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