TLR8 agonist VTX-2337 enhances NKG2D-mediated cytotoxicity of NK cells

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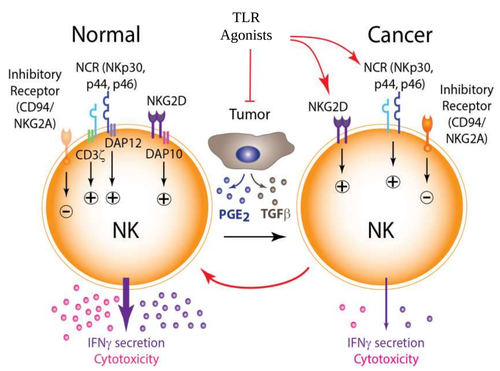
INTRODUCTION

- **NK cells express an array of activating and inhibitory receptors, which facilitate the recognition and lysis of virally infected and transformed cells, but safeguard healthy cells from attack.**
- **NKG2D is an activating receptor expressed on the surface of NK cells that recognizes the ligands MICA/B and ULBP-1 in human, which may be expressed on tumor or virus-infected cells.**
- **While NKG2D-mediated NK cell cytotoxicity plays an important role in tumor immune surveillance, cancer patients may have decreased expression and reduced function of the NKG2D receptor.**
- **We hypothesize that VTX-2337, a selective TLR8 agonist that activates myeloid DC and stimulates the production of pro-inflammatory cytokines including IL-12 and IL-18, may enhance NK cell function through NKG2D.**

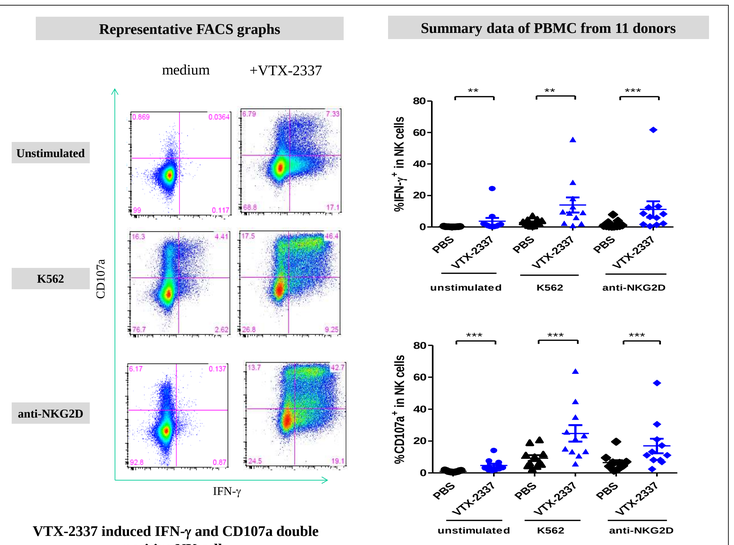


METHODS

- **PBMC collection, treatment, and FACS analysis:** Blood samples were collected from 11 normal donors following informed consent under an IRB approved protocol. PBMCs were isolated by centrifugation using a Ficoll gradient and cultured overnight in RPMI + 10% Human AB serum in the presence or absence of VTX-2337 (500 nM). Following the overnight culture, the PBMC were stimulated with K562 tumor cells, plate-bound anti-NKG2D mAb or left unstimulated for 5 hours. CD107a-PE was added for 5 hours while brefeldin A was included during the last 4 hours of incubation. At the end of the activation, the cells were first stained with fluorophore-conjugated antibodies to surface markers. After subsequent fixation and permeabilization, the cells were stained with anti-IFN-γ-e450. Samples were acquired on FACS Canto II.
- **Cytolytic assays:** K562 cells were labeled with Calcein AM and washed. PBMCs treated with 500 nM VTX-2337 for 48 hours were washed and counted. Labeled K562 cells and PBMCs were mixed at the indicated E:T ratios and incubated for 4 hours at 37ºC. The maximum release of Calcein AM was determined by adding Triton X-100 to the labeled K562 at final concentration of 0.1%. Percentage of NK cell mediated lysis was calculated as (treatment-induced release – spontaneous release) / (maximum release – spontaneous release) X 100%. Anti-NKG2D blocking antibody was added to the assay at the final concentration of 10 ug/mL to assess the involvement of NKG2D in VTX-2337-induced cytolytic activity.

RESULTS

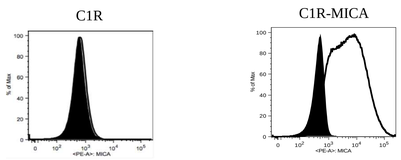
1. VTX-2337 priming stimulates CD107a and IFN-γ expression and enhances NK cell activation mediated through NKG2D.



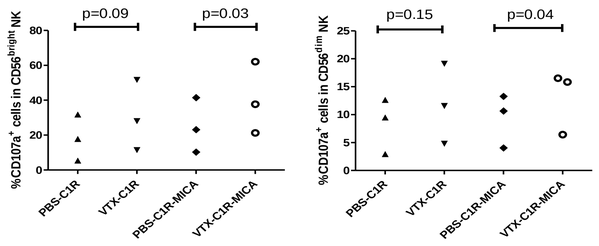
VTX-2337 induced IFN-γ and CD107a double positive NK cells

2. VTX-2337 priming enhances NK cell mediated cytotoxicity of C1R cells that overexpress MICA.

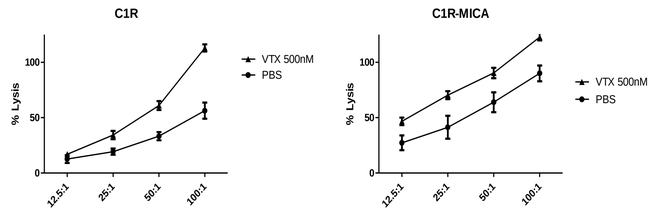
A. Overlay histogram for the expression of MICA in C1R and C1R-MICA cells. Shaded area represent isotype control antibody.



B. VTX-2337 priming enhances C1R-MICA-stimulated CD107a degranulation in NK cells.

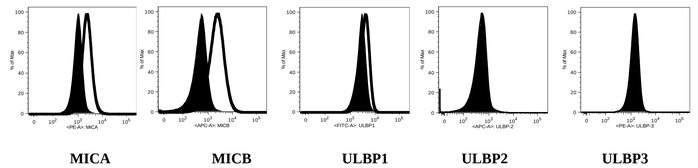


C. VTX-2337 priming of NK cells leads to enhanced lysis of C1R-MICA target cells.

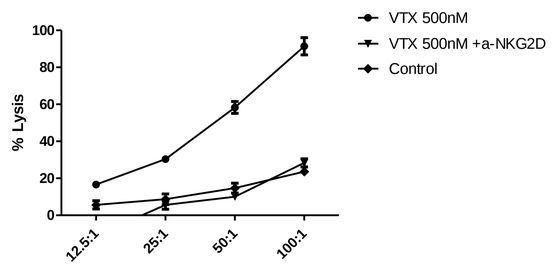


3. K562 cells express NKG2D ligands, MICA/B and ULBP1, while anti-NKG2D mAb inhibits the cytotoxic activity of VTX-2337 primed NK cells.

A. K562 cells express NKG2D ligands.



B. Lysis of K562 cells by VTX-2337 primed NK cells was inhibited by anti-NKG2D mAb.



CONCLUSIONS

- **PBMC from 11 donors stimulated with plate-bound anti-NKG2D antibody with or without prior VTX-2337 treatment showed that VTX-2337 strongly enhanced NKG2D-mediated NK cell activation (IFN-γ and CD107a expression).**
- **Priming with VTX-2337 enhances NK cells responses (CD107a degranulation and cytotoxicity) to C1R target cells that overexpress the NKG2D ligand, MICA.**
- **NK cell cytotoxicity of K562 target cells following VTX-2337 priming is dependent on an interaction between NKG2D and its ligand(s).**

Reference:

1. Mamessier E. et al. Human breast tumor cells induce self-tolerance mechanisms to avoid NKG2D-mediated and DNAM-mediated NK cell recognition. Cancer Res. (2011), 71:6621-32.
2. Lu H. et al. VTX-2337 is a novel TLR8 agonist that activates NK cells and augments ADCC. Clin Cancer Res. (2012), 18:499-509

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