ACTIVATION OF HUMAN NK CELLS MODULATES EXPRESSION OF THE INHIBITORY RECEPTOR PVRIG

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Introduction

Poliovirus receptor-related immunoglobulin domain-containing (**PVRIG**) is an immune checkpoint molecule expressed on T and NK cells (1,2). PVRIG inhibits effector cell function upon binding to poliovirus receptor-related 2 (**PVRL2**) (1-3), an adhesion molecule that is overexpressed in some cancers. PVRL2 also binds another inhibitory receptor, T cell immunoreceptor with Ig and ITIM domains (**TIGIT**), as well as the activating receptor DNAX accessory molecule-1 (**DNAM-1**) (4, Figure 1).

This study aimed to investigate the role of PVRIG in regulating human NK cell function.

- Determine whether blocking PVRIG enhances killing of tumour cells by healthy donor PBMCs
- Assess the expression of PVRIG and PVRL2 in primary bone marrow (BM) samples from acute myeloid leukemia (AML) patients
- Assess the expression of PVRIG, TIGIT and DNAM-1 after *in vitro* co-culture of NK cells with activatory stimuli

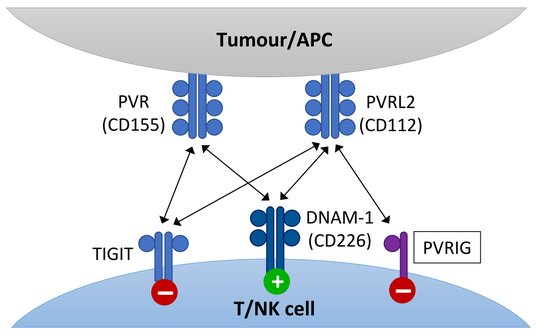

Figure 1. Receptor-ligand interactions in the DNAM-1/TIGIT/PVRIG axis.

PVRIG blockade enhances NK cell killing of tumour cell lines

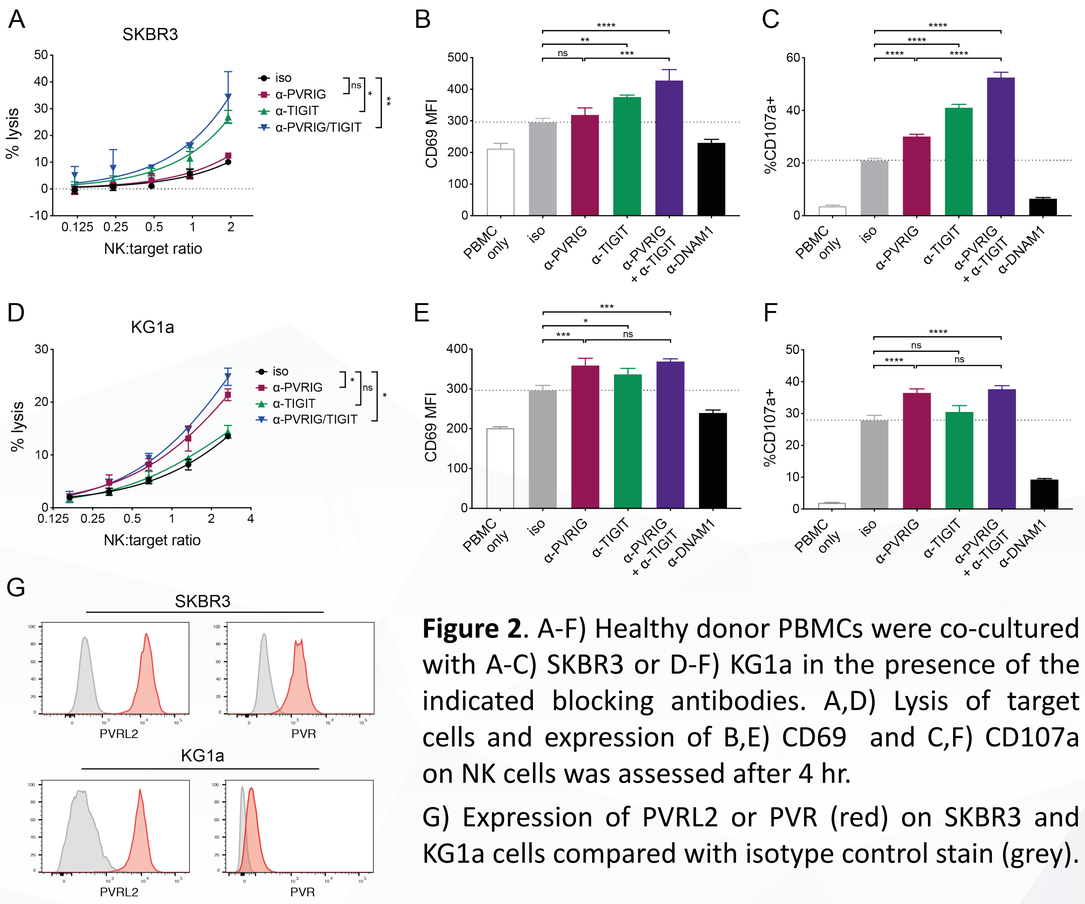

Figure 2. A-F) Healthy donor PBMCs were co-cultured with A-C) SKBR3 or D-F) KG1a in the presence of the indicated blocking antibodies. A,D) Lysis of target cells and expression of B,E) CD69 and C,F) CD107a on NK cells was assessed after 4 hr.

G) Expression of PVRL2 or PVR (red) on SKBR3 and KG1a cells compared with isotype control stain (grey).

PVRIG and PVRL2 are expressed in AML patient bone marrow

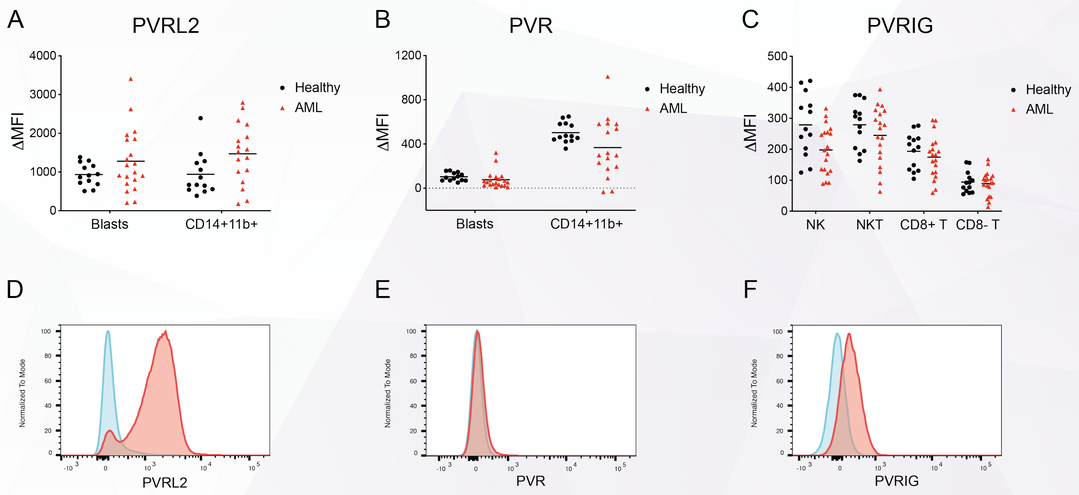

Figure 3. A-C) Expression of A) PVRL2 B) PVR or C) PVRIG on blasts or immune cell types in the bone marrow of AML patients (n = 19-20) or healthy donors (n = 13).

D-F) Representative histograms of D) PVRL2 on AML blasts E) PVR on AML blasts or F) PVRIG on NK cells in the bone marrow of an AML patient. Histograms of test (red) and isotype control stains (blue) are shown.

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PVRIG expression on NK cells is decreased upon activation

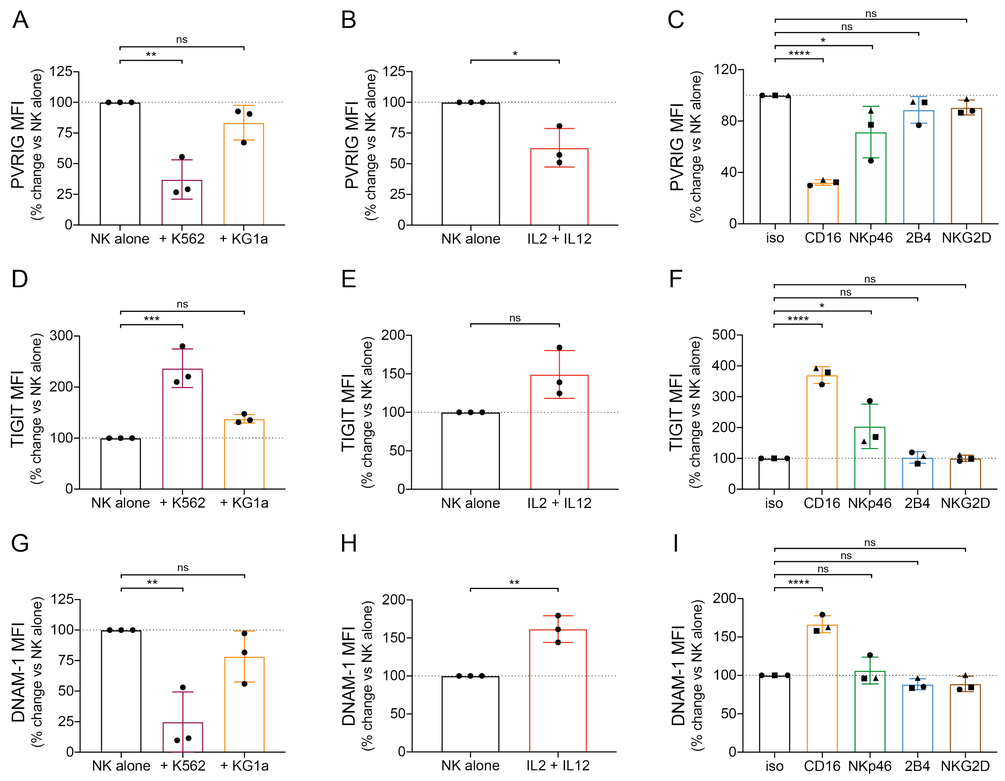

Figure 4. Expression of A-C) PVRIG D-F) TIGIT or G-I) DNAM-1 on isolated NK cells after 24 hr co-culture with tumour cells, or 24 hr stimulation with the indicated cytokines or agonistic antibodies. Percentage change in MFI relative to NK alone is shown.

PVRIG is constitutively recycled from NK cell surface

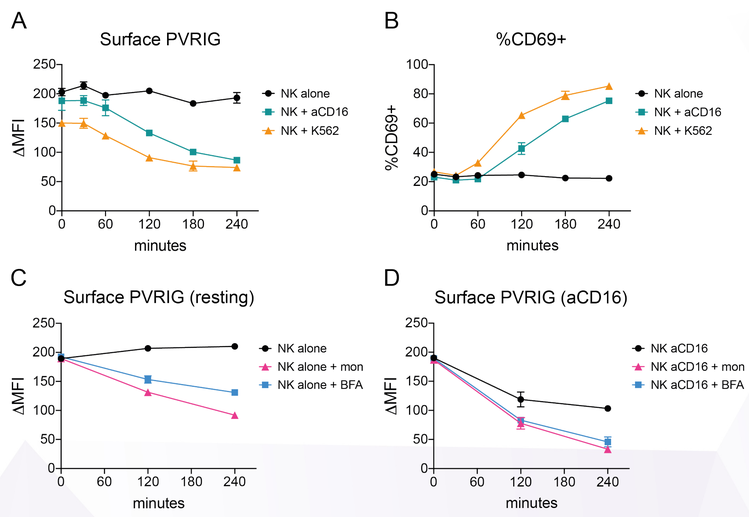

Figure 5. Expression of A,C,D) PVRIG and B) CD69 on isolated NK cells incubated alone, with K562 cells, or with plate-bound α-CD16 antibody at 37ºC for the indicated time points, in the presence or absence of monensin (mon) or brefeldin A (BFA).

Conclusion

- PVRIG blockade enhances killing of PVRL2+ tumour cells by NK cells *in vitro.*
- Recognition of targets or activation of NK cells via cytokines or agonistic receptors modulates PVRIG/TIGIT/DNAM-1 expression, as in Figure 6 below.
- Constitutive recycling of PVRIG suggests that a greater amount of PVRIG is available to be blocked over time than can be observed at a single time point.
- Thus, although NK cells in AML patients do not express higher levels of PVRIG than healthy donors, PVRIG blockade may still be effective, particularly as AML blasts express high levels of PVRL2.

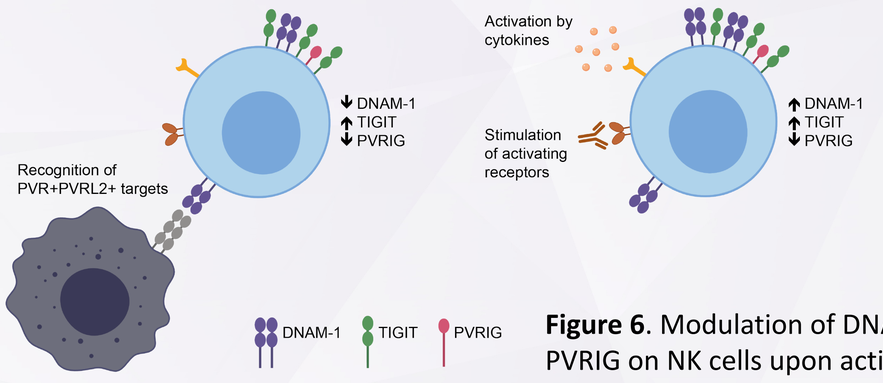

Figure 6. Modulation of DNAM-1/TIGIT/ PVRIG on NK cells upon activation.

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