TIGIT/CD155 axis blockade boosts T-cell immunity and HIV-specific degranulation in PLWH on ART

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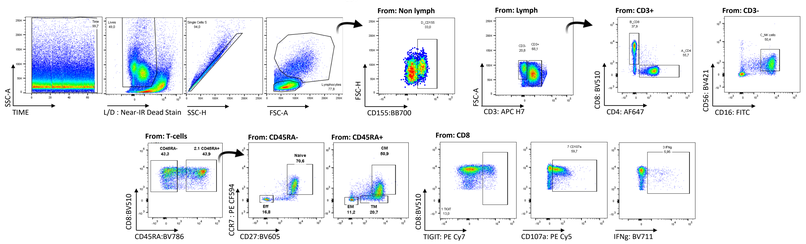
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BACKGROUND

Immune interventions such as immune checkpoint blockade are critical to attaining the HIV cure. To date, these strategies focus on a narrow set of targets using conventional monoclonal blocking antibodies. Although several studies have indicated the potential of targeting TIGIT inhibitory pathway in people living with HIV (PLWH), no focus has been paid to its ligand PVR/CD155. Here, we asses the targeting of PVR/CD155 in PLWH on ART.

METHODS

- **Protein engineer**: we design two soluble Inhibitory Receptors (sIR) proteins (monomeric sIR1 and Fc-dimeric sIR2) targeting PVR/CD155 with a sIR-Control protein.
- **Protein binding and in vitro activity**: sIRs binding to PVR/CD155 was tested by BIACORE and in a cell binding assay using murine Bw5417 expressing huCD155. In vitro activity was tested in a coculture using Jurkat-TIGIT+ and BW5417:OKT3-huCD155+.
- **In vitro activity in PBMCs from PLWH**: we assessed PVR/CD155 blockade by sIRs in PBMCs from PLWH on ART (n=17). PBMCs were stimulated with sIRs in the absence or presence of HIV-1 Gag peptide pool. After 16h, we analyzed antigen-independent and HIV-specific responses by flow-cytometry combining CD3, CD4, CD8, CD45RA, CCR7, CD16, CD56, PVR/CD155, TIGIT, IFNg, TNF, CD107a, IL-2, and IL-10 markers.

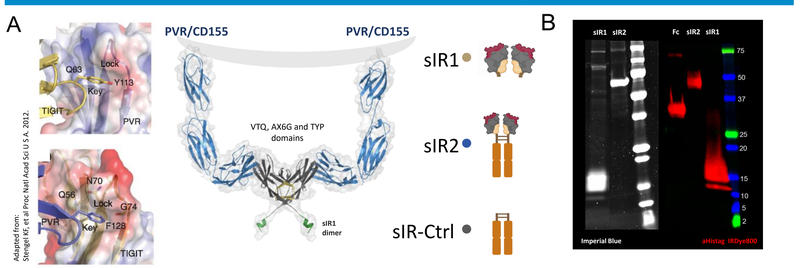


STUDY GROUPS

	PLWH on ART (n= 17)
Sex (male/female)	(15/3)
Age median years (IQR)	51 (44 – 55)
CD4 count median no. cells/µl (IQR)	914 (828 – 989)
CD4 Nadir median (IQR)	272 (227-335)
Viral load	Undetectable
Time on ART median years (IQR)	16.9 (14.6 – 20.3)
Time undetectable median year (IQR)	13.0 (7.9 – 14.3)

Table 1. Clinical details of PLWH on ART (n=17) from which PBMCs were obtained.

IN SILICO DESIGN OF sIR1 AND sIR2 TARGETING PVR/CD155

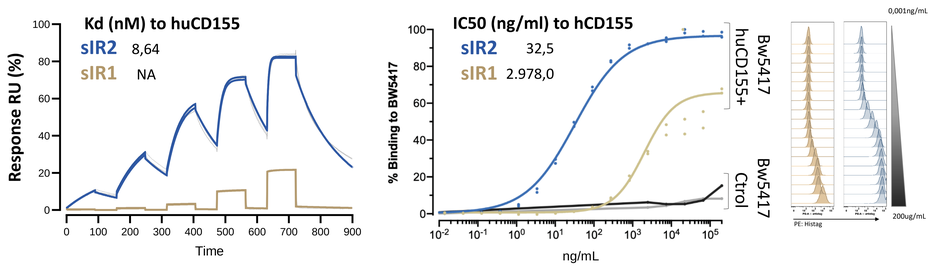


(A) In silico modeling of PVR/CD155 blockade by sIR1 dimer performed by I-TASSER server (left). Schematic representation of the general structure of the sIR1 dimer and sIR2 and sIR-Ctrl proteins (right) (B) SDS-PAGE staining using Imperial Blue (left) and WB detecting his-tag flag in sIRs and sIR-Ctrl proteins (right).

Blockade of PVR/CD155 by sIR2 enhanced T-cell cytokine production and T-cell degranulation in PLWH on ART

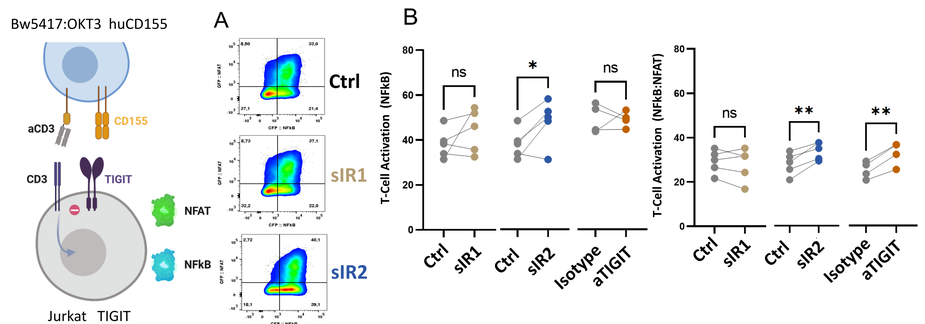
RESULTS

I. sIRs specific bind to PVR/CD155



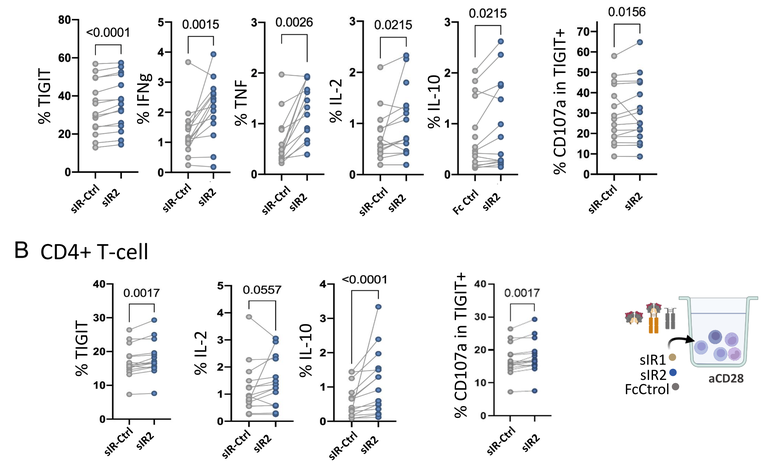
(A) Kinetics of sIR1 and sIR2 interaction to huCD155. BIACORE technique measures the real-time binding association and dissociation rates (Response RU %) using Surface Plasmon Resonance (SPR). Kd for sIR1 and sIR2 was determined by steady-state affinity analysis. (B) Binding assay using murine Bw5417 expressing huCD155. Dose-response is specific for Bw5417 huCD155 cell line compared to Bw5417 parental cell line (left). Representative histograms showing His-tag detection in Bw5417 huCD155 cell line.

II. Blockade of PVR/CD155 with sIR2 increase the co-activation of NFAT::NFkB



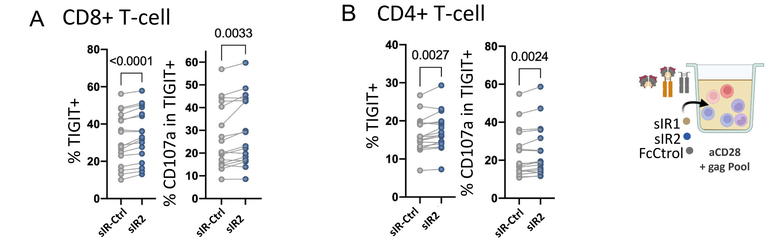
Coculture of NFAT (GFP) and NFkB (CFP) reporter Jurkat overexpressing TIGIT and Bw5147:OKT3 overexpressing hCD155. (A) Representative dot plots showing NFAT::NFkB reporter activation in Jurkat TIGIT+ cells cultured for 6h with Bw5147 hCD155 and sIRs. (B) Blockade of PVR/CD155 - TIGIT pathway by sIR2 induced activation of NFkB and co-activation of NFkB::NFAT while blockade using a monoclonal anti-TIGIT (right panel) only increase co-activation of CFP::NFkB activation.

III. Antigen-independent T-cell responses are modulated by sIRs



Pair-wise comparison of antigen-independent response in CD8 and CD4 T-cells by sIR2 and and sIR-Ctrl proteins. (A) sIR2 enhance the frequency of CD8+TIGIT+ cells and stimulated the production of IFNg, TNF, IL-2, IL-10 in CD8+ T-cells and CD107a in CD8+TIGIT+ cells. (B) sIR2 enhance the frequency of CD4+TIGIT+ cells and stimulated the production of IL-2 and IL-10 in CD4+ T-cells and CD107a in CD4+TIGIT+ cells.

IV. HIV-1 specific T-cell degranulation is increased by sIR2



Pair-wise comparison of HIV-1 specific T-cell response in the presence of sIR2 and sIR-Ctrl proteins. (A) sIR2 enhance the frequency of CD8+TIGIT+ and CD4+TIGIT+ cells and stimulated the production of CD107a in CD8+TIGIT+ cells CD4+TIGIT+ cells.

CONCLUSIONS

We have generated sIRs immunomodulatory proteins capable of binding PVR/CD155. Our data demonstrate blockade of PVR/CD155 and enhancement of T-cell cytokine production by sIR2. Moreover, sIR2 increased HIV-specific degranulation in TIGIT+CD4+ and TIGIT+CD8+ T-cells in PLWH on ART. Thus, we propose PVR/CD155 as a novel immunomodulatory target for PLWH on ART.

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