IMPACT of HIV LATENCY REVERSING AGENTS on NATURAL KILLER CELLS

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Background

Current strategies towards **HIV eradication** require unmasking of the HIV latent reservoir using latency reversing agents (**LRAs**), followed by an adequate **immune response** to clear the reactivated infected cells.

Thus, an assessment of the impact of LRAs on immune function is needed.

This has initially been addressed for **CTLs** (*Jones, PLOS Pathogens, 2014*), finding that some histone deacetylase inhibitors (HDACis) may impair CTL effector function.

Here, we evaluate the effect of LRAs on natural killer cell (**NKs**) function.

Methods

- Five LRAs were evaluated at 3 different concentrations, using an intermediate concentration that reflect *in vivo* exposure upon clinical use ("physiological").

		Low concentration	Physiological concentration	High concentration
HDACis	**SAHA**	--	**335 nM**	670 nM
	Romidepsin	5 nM	**10 nM**	100 nM
PKC agonists	**Panobinostat**	5 nM	**20 nM**	100 nM
	Prostratin	100 nM	**1000 nM**	10000 nM
	Ingenol	10 nM	**100 nM**	1000 nM

- NKs were isolated by negative selection from HIV-negative or positive patients and exposed for 24h to the LRAs.

LRA exposure impact on **NK functionality** was evaluated

NK Viability

Assessed by **AnnexinV-7AAD** staining

Cytotoxicity

Evaluated through **degranulation** upon culture with K562 cells, measuring the proportion of **CD107a$^+$** cells within the CD56$^+$ population.

Antiviral Activity

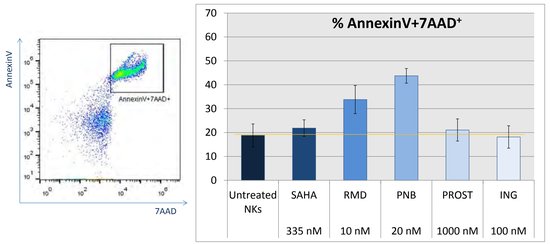
Measured in a **viral inhibition assay** using autologous CD4$^+$T cells infected with JR-CSF. After 7 days of culture, HIV p24 was quantified by ELISA.

NK Receptor Expression

Expression of a panel of **NK activating receptors** was assessed: CD16, NKG2D, NKp30, NKp44, NKp46 and DNAM-1

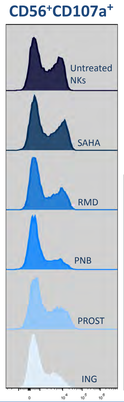
Results

Romidepsin and Panobinostat can be toxic to NKs

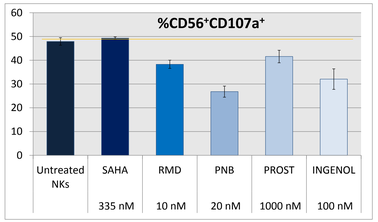


AnnexinV-7AAD binding showed that 24h exposure to a physiologic concentration of RMD or PNB caused toxicity to NKs. Lower doses of these drugs (5 nM) also caused some toxicity. SAHA, PROST and ING did not significantly increase NK cell death.

Panobinostat reduces NK cytotoxicity



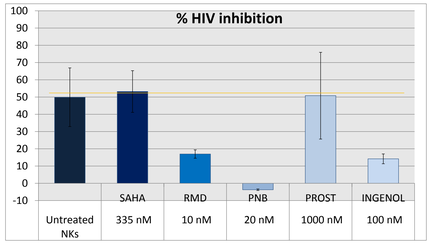
All compounds but SAHA impaired degranulation of NKs to some extent, being more pronounced after exposure to 20 nM of PNB, which caused a decrease in NK degranulation from 48% in untreated NKs to 27%. However, lower dose (5 nM) did not cause a significant decrease in degranulation.



NK cells were cultured with K562 cells and CD107a antibody for 5 hours, and then stained for CD56 expression.

For FACS analysis , cells were gated on the CD56$^+$ population and the proportion of CD107a$^+$ cells was recorded.

Romidepsin, Panobinostat and Ingenol impair antiviral activity of NK cells at physiological concentration



CD4$^+$T cells were infected with JR-CSF and then cultured with autologous NK cells, either treated with LRAs for the previous 24 hours or untreated. After 7 days of culture, p24 was measured by ELISA.

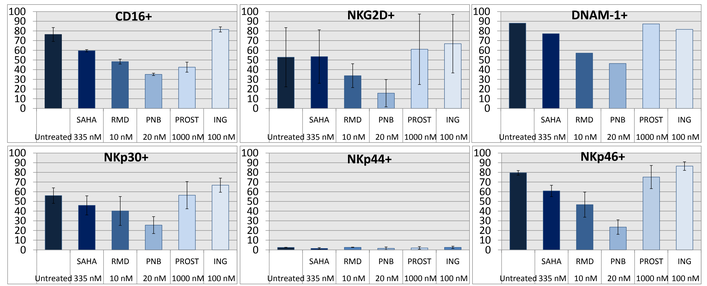
The addition of NKs to infected cells reduced viral production 50%. At physiologic exposures, RMD, PNB and ING reduced the antiviral activity of NKs, while SAHA and PROST did not.

ING at higher concentration (1000 nM) improved NK antiviral activity, showing 84% viral inhibition (graph not shown).

HDACis impact on NK receptor expression

HDACis produced a reduction in NK activating receptor expression, in particular in the case of RMD and PNB, which produced a marked decrease in all of studied receptors. On the contrary, Prostratin and Ingenol did not impact receptor expression.

Gated on CD3$^-$CD56$^+$ population



Conclusions

- Romidepsin and especially Panobinostat impaired viability, cytotoxicity, antiviral activity and receptor expression of NKs at physiological doses.
- Prostratin and Ingenol had a modest effect on NK function.
- SAHA had virtually no effect on NKs at any of the tested concentrations.
- This data suggests that LRAs have a range of impact on NK function, and thus could have effect on viral clearance after reactivation.
- Further studies are needed to model the impact of LRAs on viral clearance, to better design clinical trials.

Acknowledgments

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