Identification of Latency Reversing Agents (LRAs) Active in Diverse Primary T-cell Models of HIV Latency

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Abstract

Background

It has been challenging to identify latency reversing agents (LRAs) broadly active across multiple primary cell latency models and patient cells ex vivo. A Jurkat CD4+ T cell model of HIV latency was used to screen the Merck library of 2.9 million compounds. Unlike prior efforts, this screen was conducted with and without a suboptimal concentration of SAHA, a histone deacetylase inhibitor (HDACi), with the goal of sensitizing the latency model to allow the discovery of novel and potentially synergistic LRAs. While 34% of compounds active in the screen acted via known mechanisms of action (HDACis or Farnesyl Transferase (FT) inhibitors) the remaining 66% acted via unknown mechanisms of action (uMOA). Based on the activity of uMOA compounds in different Jurkat T- cell models of HIV latency, 144 compounds were chosen for additional detailed analysis in primary CD4 T-cell models of HIV latency.

Methods

The 144 compounds were analyzed in seven different primary CD4 T-cell models of HIV latency using up to a six-point dose titration, in the presence of suboptimal concentration of panobinostat or vorinostat, tailored to the HDACi responsiveness of each primary cell system. Latency reversal was quantified by changes in reporter expression (eGFP or luciferase), HIV Nef or Tat gene expression, or HIV mRNA, depending model used.

Results

As expected based on previous studies of LRAs in these primary cell models (Spina, PLoS Path 2013) among the 144 selected uMOA compounds, no one tested was active in all models. However, 2 compounds displayed statistically significant activity in six of the seven cell models and 16 compounds exhibited activity in three or more systems. Of note, none of the compounds were overtly toxic at concentrations that exhibited LRA activity. Additionally, no compounds induced CD69 expression in naïve T-cells isolated from healthy volunteers, suggesting their LRA activity was not due to generalized T cell activation

Results

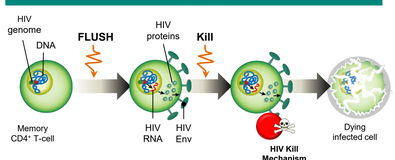


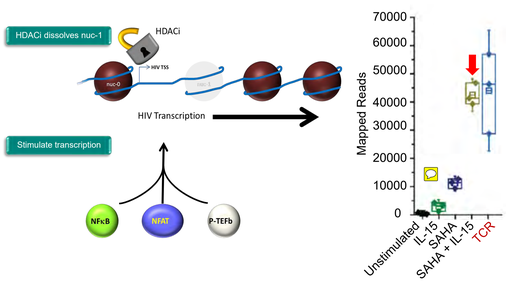
Figure 1. "Flush" and "kill" paradigm.
Examples of "flush" and "kill" agents are shown.

Table 1. Activity of different LRAs in different model systems

		Demonstrated Activity			
LRA class	Example	Jurkat T-cell HIV latency model system	Primary T-cell model system (any)	Patient CD4 T-cells (ex vivo)	BLT mice, NHP, and/or humans
HDACi	Vorinostat	YES	YES	YES	YES
PKC agonist	Bryostatin	YES	YES	YES	Many AEs
Acetaldehyde dehydrogenase inhibitor	Disulfiram	YES	YES	YES	YES
TLR agonist	GS-9620	YES	YES	YES	YES
Bromodomain antagonist	JQ1	YES	YES	YES	NT
Smac mimetic	BIRC-2 antagonist	YES	YES	YES	NT
Estrogen receptor antagonist	Fulvestrant	YES	YES	YES	NT
Proteosome inhibitors	Bortezomib	YES	YES	NT	NT
RIG-I Inducer for RIG-I	Acitretin	YES	YES	NT	NT

NT = Not Tested, AE = Adverse Event

Figure 2. HIV transcription in resting memory T cells is likely restricted at the chromatin and transcription factor levels



Objective: To identify compounds that act synergistically with HDACis to induce HIV transcription

Figure 3. HIV latency synergy screening funnel

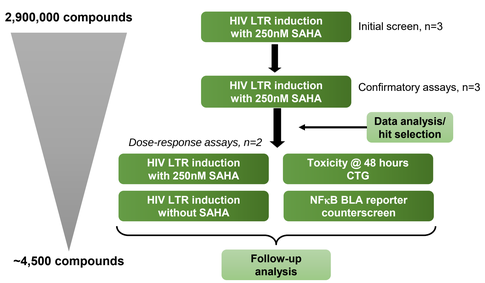
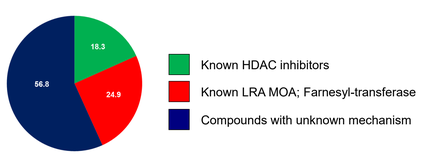


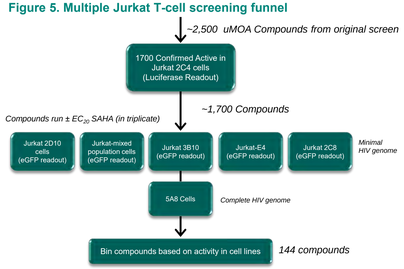
Figure 4. Results of ultrahigh-throughput screen

Of the 4,500 compounds identified in the original screen, ~2,500 had robust activity when tested in 3 separate experiments +/- 250nM vorinostat. Activity was defined as ≥40% of the maximal vorinostat signal. The pie chart indicates the MOAs identified following analysis of historical screening data and structural clustering of the entire hit list. Percentages of each Compound class are indicated.



Results (continued)

Figure 5. Multiple Jurkat T-cell screening funnel



~2,500 uMOA compounds were run through a confirmatory screen in the original screening cell line (2C4 cells). From this screen, ~1,700 compounds with robust (>50% maximal stimulation) were run through an additional 5 cell lines, as indicated. Hits from this screen were run through an additional Jurkat latency model system containing a complete HIV genome. Compounds were then grouped based on activity in these cells and 144 representative compounds selected for screening in Primary T-cell models

Figure 6. Jurkat screening assay

- All Jurkat T-cell lines contain HIV LTR with an eGFP reporter
- Cells selected for analysis based on their differential responses to different LRAs
- All compounds tested +/- EC_{20} of vorinostat

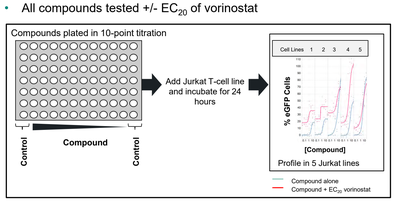
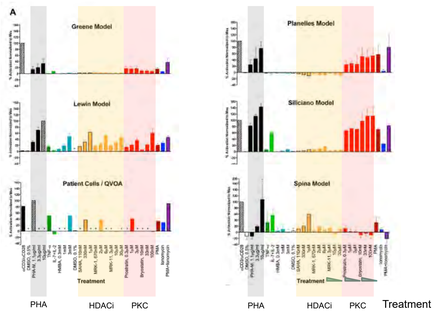


Table 2. Summary of the primary T-cell assay used for screening uMOA compounds

Model/Cell Type	Source of Cells	Cell Cycle Status Upon Infection	Virus/Vector	Readout Upon Reactivation
Greene HLAC	Primary tonsil CD4 T cells	Resting	NL4-3 (WT reporter)	Luciferase (RLU)
Greene PBMC	Primary CD4+ T cells	Resting	NL4-3 (WT reporter)	Luciferase (RLU)
Planelles	Primary naïve CD4+ T cells	Dividing	HIV-1Denv or HIV-1DenvDnefGFP	%eGFP or IC-Gag+ cells
Siliciano	Primary CD4+ T cells	Dividing	NL4-3D6-drEGFP	%eGFP + cells
Spina	Primary CD4+ T cells	Resting	HIV-1 NL4-3 (WT)	TAT mRNA copies
Merck (Karn)	Naïve CD4+ T cells	Dividing	pHR'Nef+CD8a-GFP(+)	% Nef+ cells
Lewin	Primary resting CD4+ cells	Resting	NL4-3 (eGFP reporter)	%eGFP cells

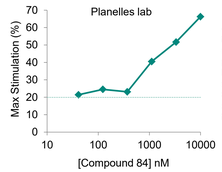
Taken from Spina CA, Anderson J, Archin NM, et al. *PLoS Pathog.* 2013;9(12):e1003834.

Figure 7. Differential activity of LRAs across the primary T-cell assays



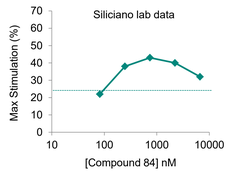
From Spina CA, Anderson J, Archin NM, et al. *PLoS Pathog.* 2013;9(12):e1003834.

Figure 9A. Compound 84: Planelles lab



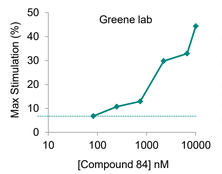
Activity of Compound 84 in the Planelles primary T-cell assay in the presence of 5nM panobinostat for 48 hours. LRA activity is normalized to a percentage of the CD3/CD28 control. The dotted line indicates activity from panobinostat alone.

Figure 9B. Compound 84: Siliciano lab



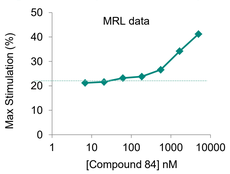
Activity of Compound 84 in the Siliciano primary T-cell assay in the presence of 5nM panobinostat for 24 hours. Activity is normalized to a percentage of a PMA/ ionomycin control. The dotted line indicates activity from panobinostat alone.

Figure 9C. Compound 84: Greene lab PBMC



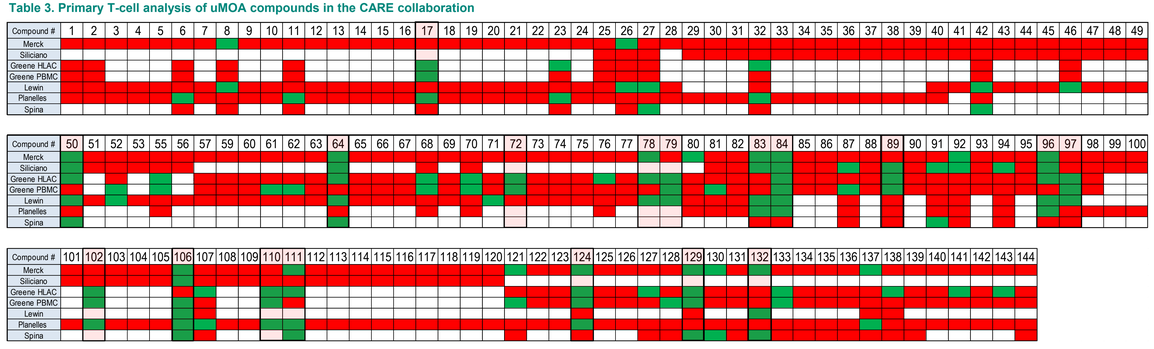
Activity of Compound 84 in the Greene lab primary T-cell assay in the presence of 5nM panobinostat for 48 hours. Activity is normalized to a percentage of a CD3/CD28 control. The dotted line indicates activity from panobinostat alone.

Figure 9D. Compound 84: Merck primary T-cell assay (Karn)



Activity of Compound 84 in the MRL lab (ex-Karn lab) Th17 primary T-cell assay in the presence of 5nM panobinostat for 72 hours. Activity is normalized to a percentage of a CD3/CD28 control. The dotted line indicates activity from panobinostat alone.

Table 3. Primary T-cell analysis of uMOA compounds in the CARE collaboration



Denotes not tested | Denotes active compound | Denotes inactive compound

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Summary/Conclusions

- Synergistic activity of uMOAs is common – Many screening hits of unknown mechanism synergize with known LRAs (in the Jurkat 2C4 cell model)
- Hit compounds from HIV latency screen exhibit differential activity across 5 different Jurkat HIV latency model T cells
- A subset of uMOA compounds are active in different primary T-cell model systems. Consistent with previous data (Spina et al., 2013), the degree of concordance between different assay systems is low
- Among the 144 compounds selected for analysis, 16-18 compounds were active in 3 or more primary T-cell model systems. Further analysis of these compounds in HIV+ patient cells is planned

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