Evaluation of HIV-1 latency reversing agents by a modified virus growth assay (VOA)

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Abstract

Background. Inhibitors of histone deacetylases (HDACis) reactivate HIV-1 replication in *in vitro* models of HIV latency. However, the recent study by Bullen *et al*. has underscored the difficulty to reactivate HIV-1 latency in primary resting memory CD4 T cells isolated from aviremic long-term treated HIV-1 infected patients (Bullen *et al*., Nature Medicine 2014). We hypothesized that mimicking the modalities of administration of HDACis in patients *in vivo*, and in particular, the continuous exposure of primary resting memory CD4 T cells to HDACis in culture would result in efficient reactivation of HIV-1 replication.

Methods. Resting memory CD4 T cells were isolated from 10 aviremic (<20 HIV-1 RNA copies/mL) long-term treated (>2 years) HIV-1 infected subjects and cultured either in the presence of CD3/CD28 (positive control) or continuous exposure to clinically relevant concentrations of HDACis *i.e.* SAHA, romidepsin, belinostat, panobinostat and givinostat throughout the culture period of 14 days. HIV-1 RNA and P24 protein were measured at the end of the culture period using validated diagnostic tests.

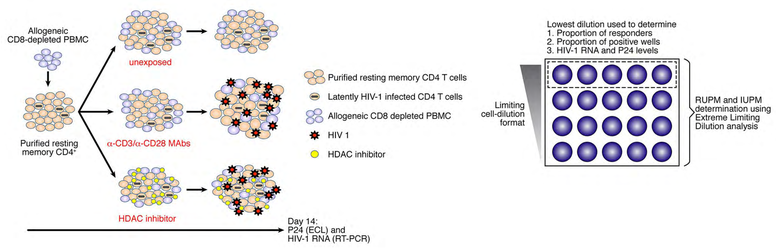
Results. Using the modified VOA, all the HDACis tested (SAHA, romidepsin, panobinostat, belinostat and givinostat) induced reactivation of HIV-1 replication in primary resting memory CD4 T cells isolated from aviremic long-term treated HIV-1 infected patients as indicated by the high levels of HIV-1 RNA and P24 production measured in culture supernatants. Of note, HIV-1 reactivated in the cell cultures was also infectious as demonstrated by the transmission of HIV-1 infection *in vitro* to CD8-depleted blood mononuclear cells from healthy subjects. Interestingly, givinostat was more potent than the other HDACis in reversing HIV-1 latency *in vitro* on the basis of the percentage of patients reactivating HIV and the proportion of HIV positive wells. Finally, the combined treatment of anti-CD3/anti-CD28 MAbs plus givinostat did not result in a significant increase of HIV-1 replication, suggesting that both HDACis and TCR signals may target similar populations of latently HIV-1 infected CD4 T cells.

Conclusions. These results support further investigation and development of HDACis in the armamentarium of the therapeutic agents to achieve HIV functional cure.

Study group at enrollment

Subject ID	Age	Sex	Duration of HIV infection (years)	CD4 T cell Count (cells/µl)	Viral Load (HIV-1 RNA copies/ml)	Time on HAART (years)
01	47	M	5	760	<20	5
05	41	F	9	1370	<20	6
06	51	M	6	391	<20	6
07	36	M	6	760	<20	3
08	41	M	15	323	<20	6
10	49	M	8	543	<20	8
12	29	F	8	473	<20	2
14	37	M	6	1267	<20	2
16	49	M	25	549	<20	2
18	45	M	3	376	<20	2

Figure 1: Schematic representation of the experimental strategy used



Results

Figure 2: HDACi efficiently reactivate HIV-1 replication from latently infected resting memory CD4 T cells isolated from long-term treated HIV-1 infected subjects

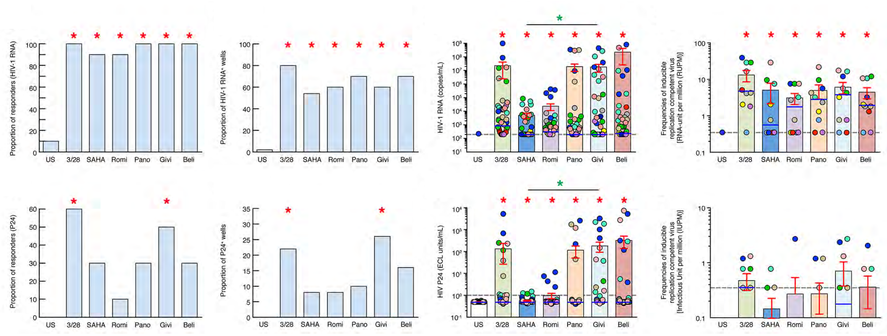


Figure 3: P24 production induced following HDACi treatments is strongly associated with HIV-1 RNA levels

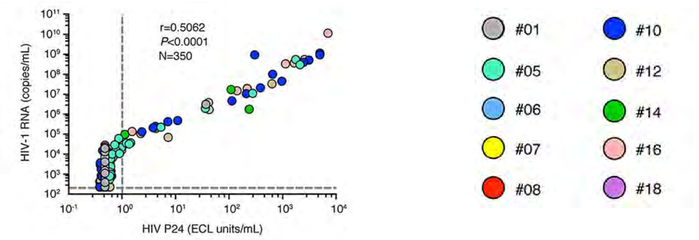


Figure 4: Evaluation of the infectivity of HIV-1 reactivated by HDACis treatments

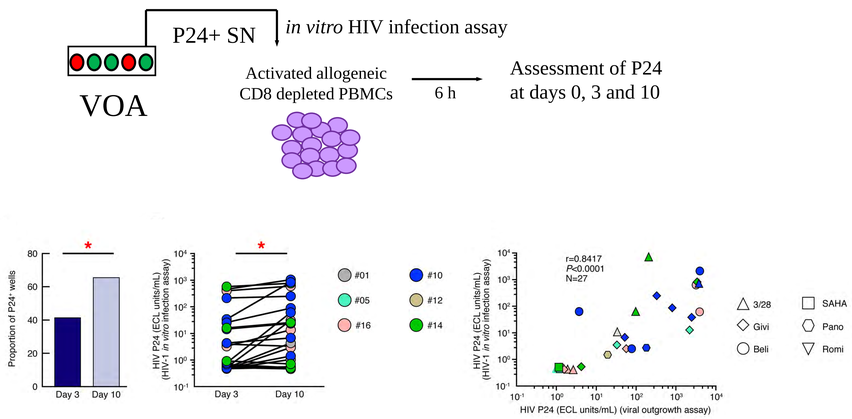


Figure 5: Lack of synergistic effect between givinostat treatment and TCR stimulation on the reactivation of HIV-1 replication

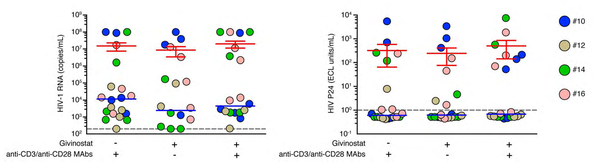


Figure 6: Level of expression of activation markers and proliferation capacity of purified resting memory CD4 T cells exposed to allogeneic CD8-depleted mononuclear cells

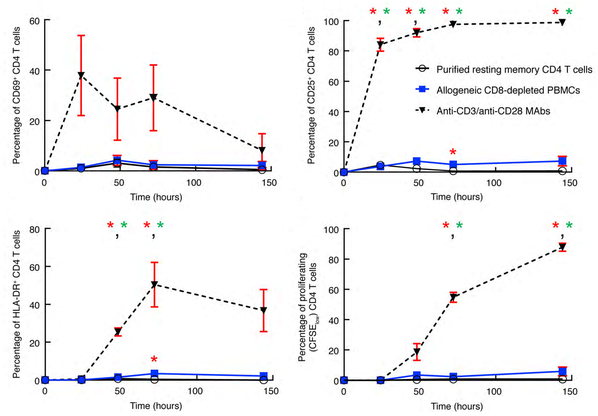
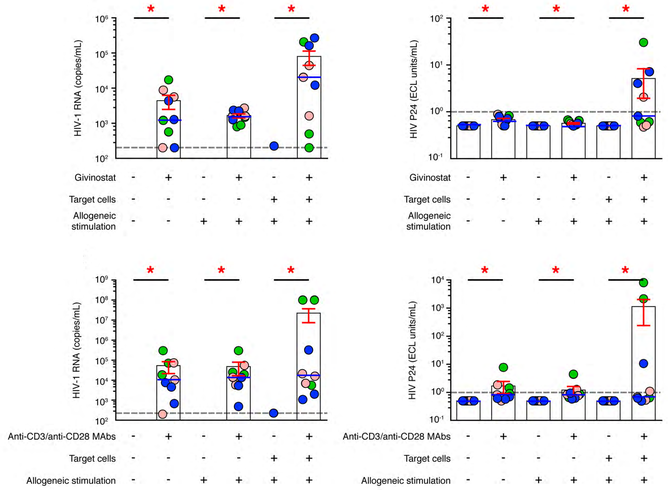


Figure 7: Contribution of allogeneic CD8-depleted PBMCs as HIV-1 targets and/or as provider of additional signals



Conclusions

In the present study, we demonstrated that

1. All HDACis tested (at clinically relevant doses) are potent inducers of replication competent and infectious HIV-1 in resting memory CD4 T cells of HIV-1 infected long-term ART treated patients using the modified VOA,
2. Givinostat is the most effective reactivator of HIV-1 replication as compared to the other HDACis *in vitro*,
3. P24 production induced following HDACi treatments was strongly associated with HIV-1 RNA levels,
4. Both HDACis and TCR signals may target similar populations of latently HIV-1 infected resting memory CD4 T-cells,
5. Allogeneic CD8-depleted PBMCs used in the present experimental strategy mainly served as HIV-1 targets and modestly contributed to provide additional signals.