Entinostat is a histone deacetylase (HDAC) inhibitor selective for class 1 HDACs and activates HIV production from latently infected primary T-cells

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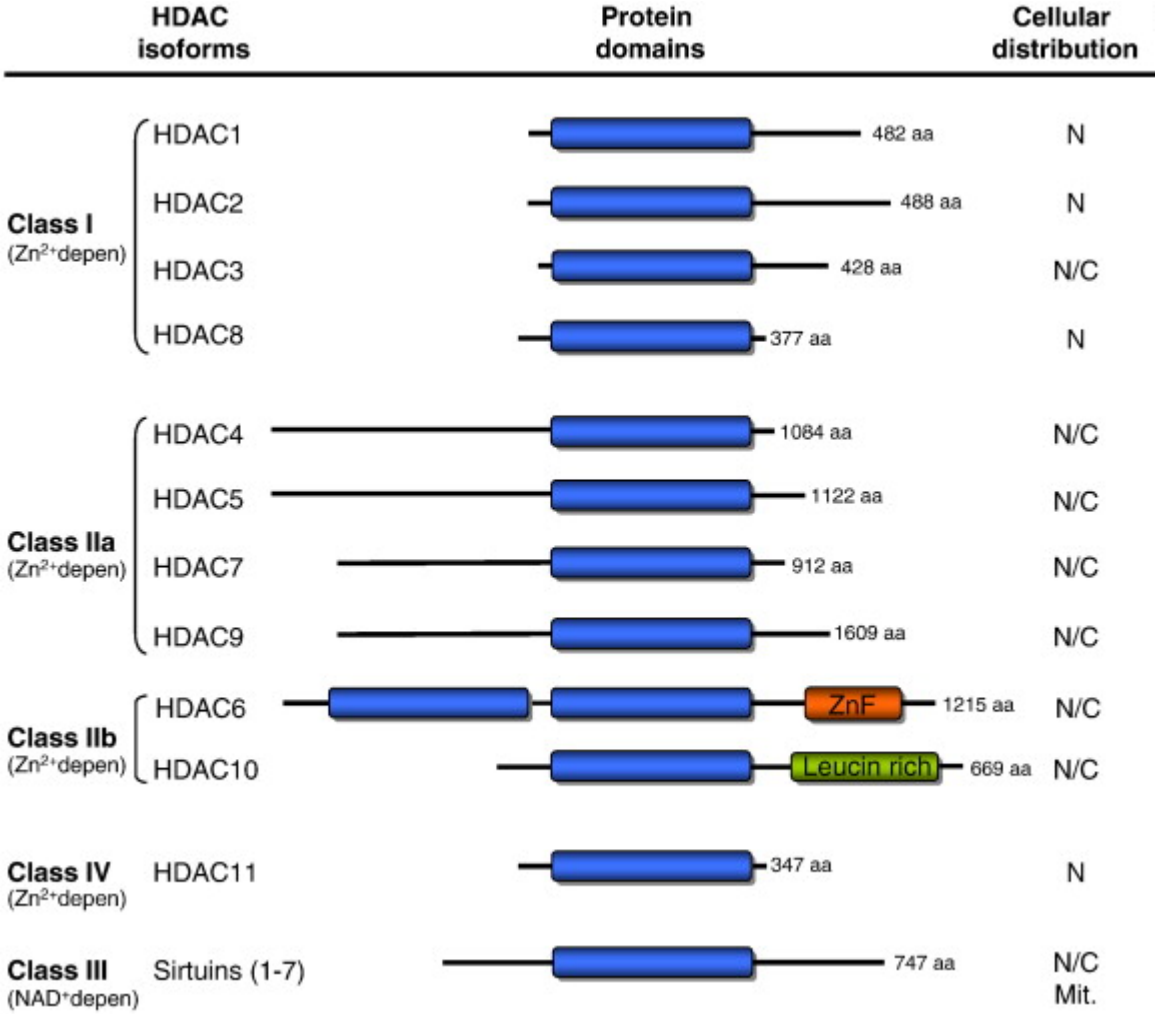
Background

- Two recent clinical trials have shown that the HDACi vorinostat can increase HIV transcription in vivo
  - Archin Nature 2012; Elliot CROI 2013
- Important to identify newer compounds that are more potent, less toxic and perhaps more selective which could move rapidly into clinical trials
- In latently infected cell lines HDAC1, 2 and 3 are major HDACs involved in maintenance of latency
  - Archin AIDS 2009; Huber J Biol Chem 2011
- Inhibitors of Class I but not Class II HDACs increased HIV production from latency in patient derived cells ex vivo
  - Archin AIDS 2009; Huber J Biol Chem 2011; Keedy J Virol 2009

Histone Deacetylases



Marcotullia, BBR Reviews on Cancer, 2011

Aim

To determine the relative potency and toxicity of a panel of HDACi in latently infected primary CD4 T-cells and latently infected cell lines

HDACi used in this study

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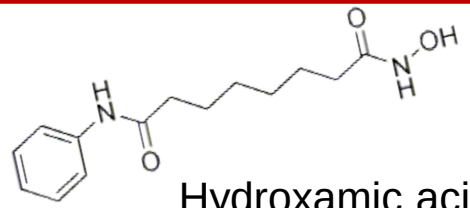
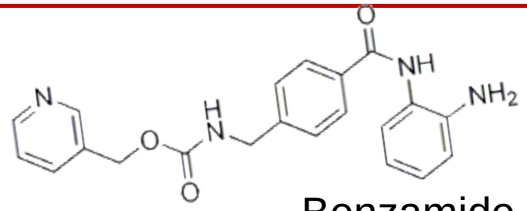
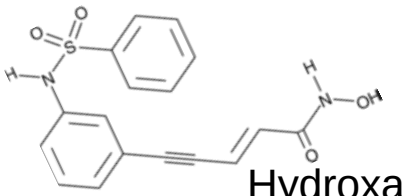
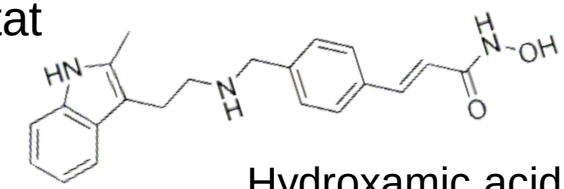
HDACi	Class	Activity in vitro	Activity in vivo
Vorinostat  Hydroxamic acid	Pan	• Cell lines • Ex-vivo T-cells • Primary model	Yes (Archin Nature 2012; Elliot CROI 2013)
Entinostat  Benzamide	Class I	• Cell lines	
MCT-3 (Ox analogue)  Hydroxamic acid	Class I	• Cell lines	Research only
Panobinostat  Hydroxamic acid	Pan	• Cell lines • Ex-vivo T-cells • Primary model	Ongoing trial

Entinostat

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- Class I selective, HDAC1 and HDAC3
  - Khan et al Biochem J 2008; Tatamiya et al AACR meeting 2004
- In 23 Phase I and II trials for conditions including myeloid and lymphocytic leukaemia; non-small cell lung cancer; breast and colorectal cancer
  - clinicaltrials.gov
- Increased histone acetylation and ERK protein translation in tumor tissue
  - Pili et al Br J Cancer 2012; Witta et al J Clin Oncol 2012
- May suppress T-regulatory cell function
  - Shen et al Nature 2012
- Well tolerated and Ames test negative

Latency models used in this study

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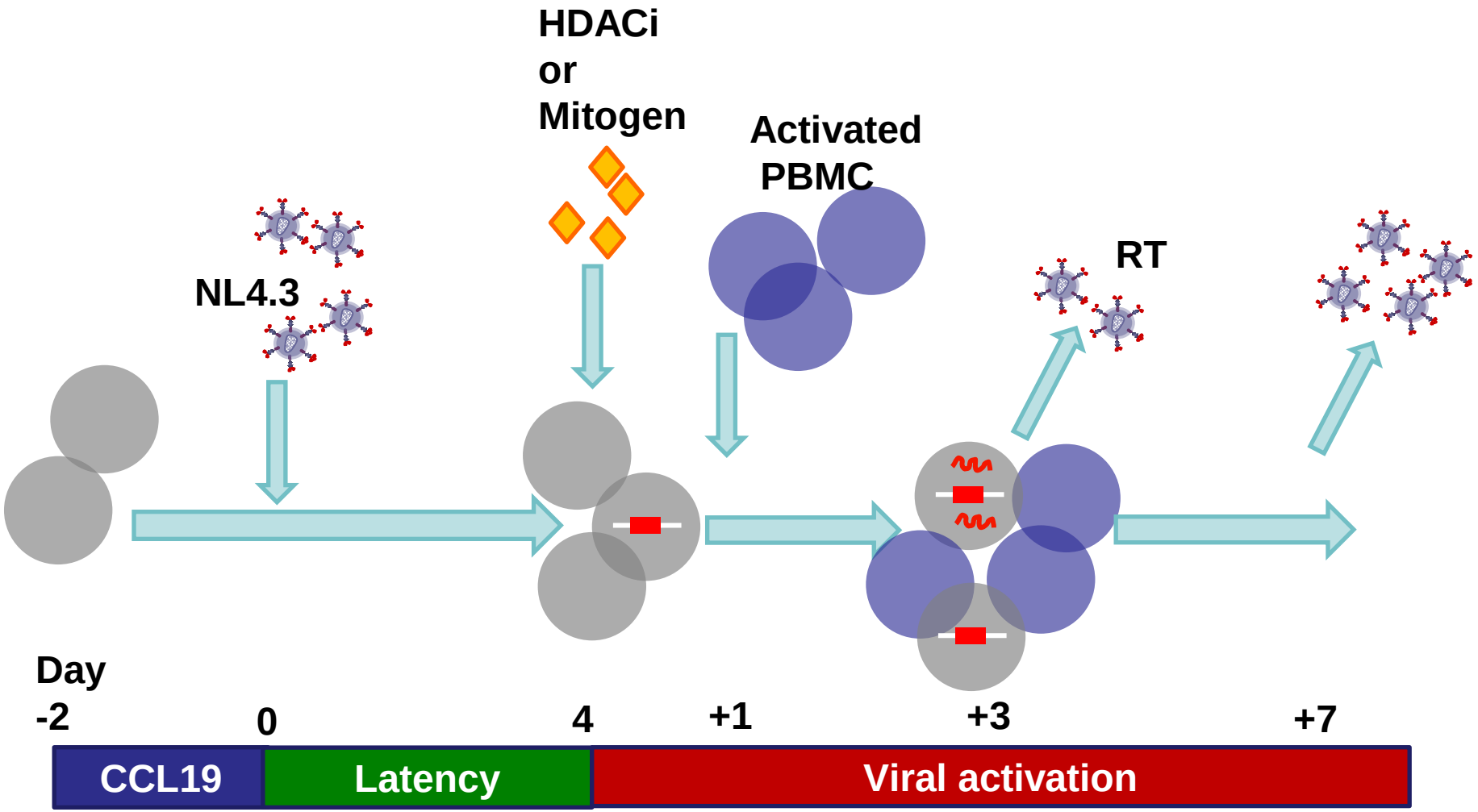
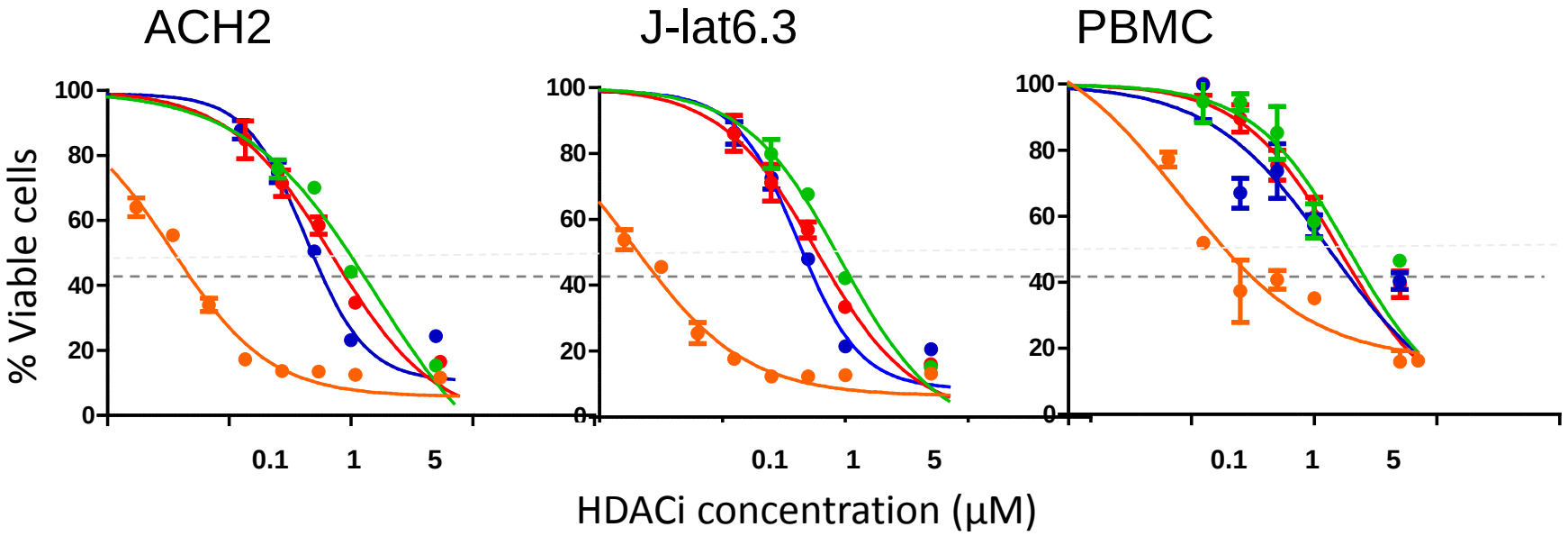
- ACH2 cells
  - Clonal CD4 T-cell line (derived from CEM cells)
  - Single integrated copy of HIV
- J-lat 6.3 clone
  - Clonal CD4 T-cell line (derived from Jurkat cells)
  - Single integrated copy of HIV (full length minus env, nef; + GFP)
- Primary CD4 T-cell model of chemokine induced latency
  - Resting CD4 T-cells
  - Random HIV integration sites

Resting CD4 T-cell latency model



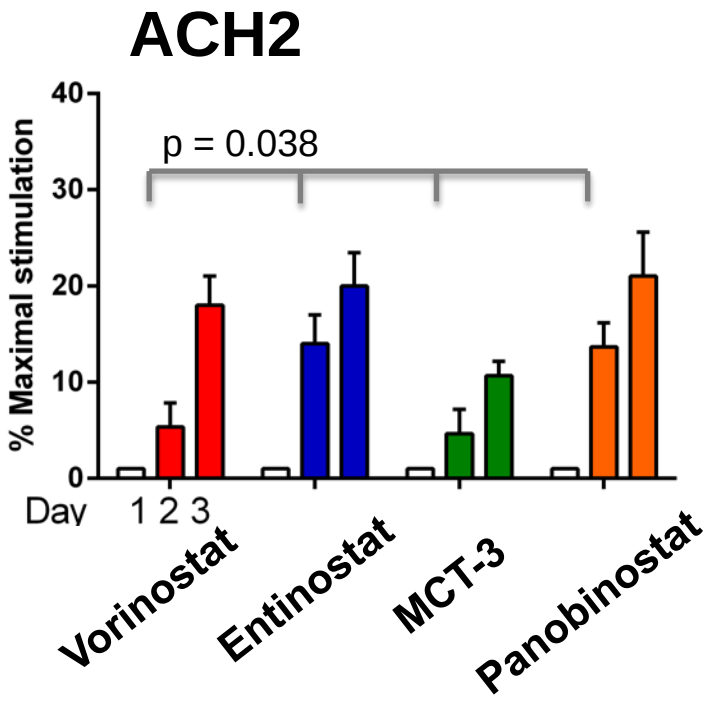
Saleh, Blood 2007; Cameron, PNAS 2010; Saleh, Retrovirology 2011

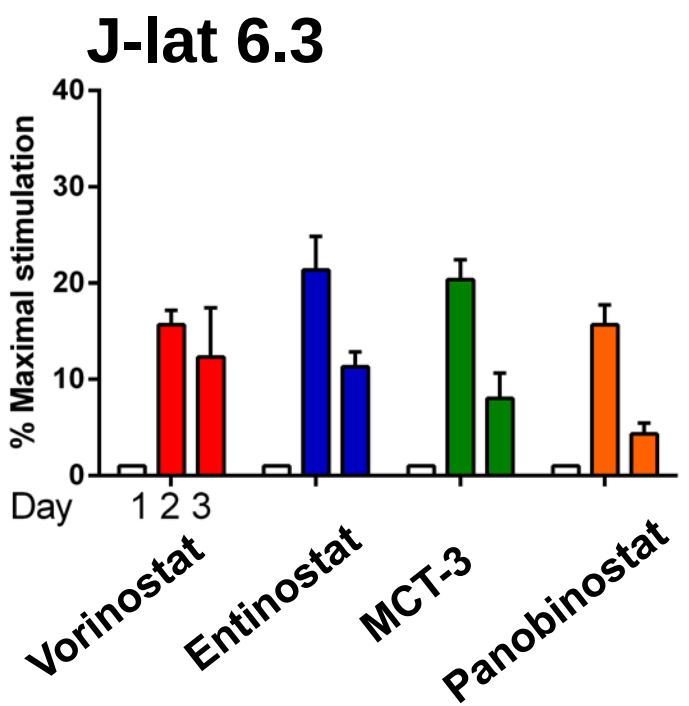
Toxicity observed in all cells used



Vorinostat	0.42µM	0.41µM	1.6µM
Entinostat	0.65µM	0.59µM	1.3µM
MCT-3	1.58µM	0.93µM	1.7µM
Panobinostat	0.07µM	0.04µM	0.09µM

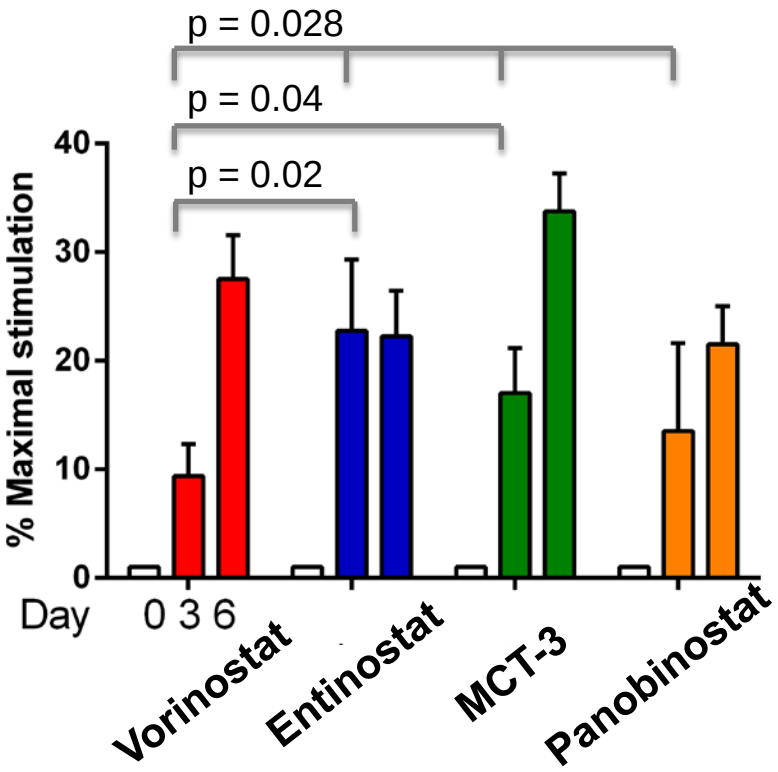
All HDACi activated virus production in latently infected cell lines





Mean ± SD (n=3)

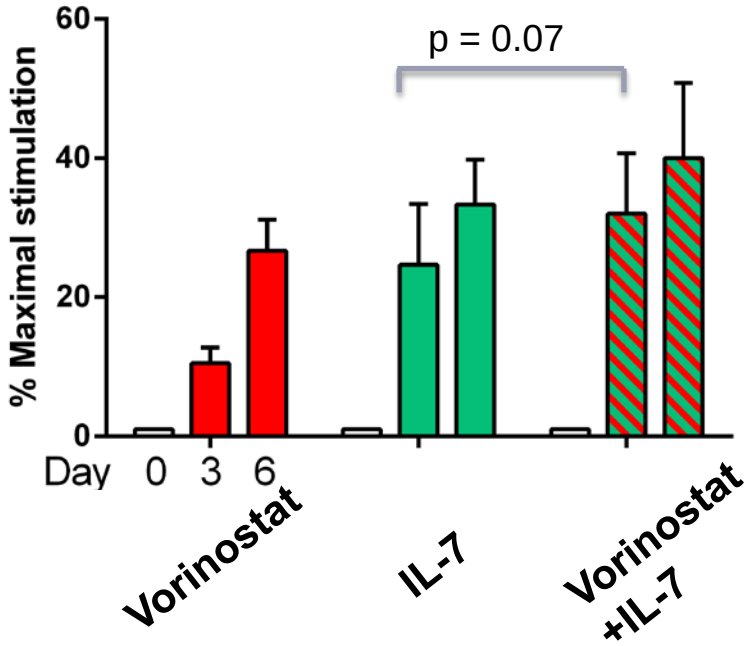
Entinostat activates HIV production in latently infected primary CD4 T-cells



Overall differences by Kruskal-Wallis
Differences between each HDACi and Vorinostat (paired t-test)

Mean ± SD (n=4)

No synergism with vorinostat and IL-7



Mean ± SD (n=3)

No change in HDAC expression with CCL19 treatment

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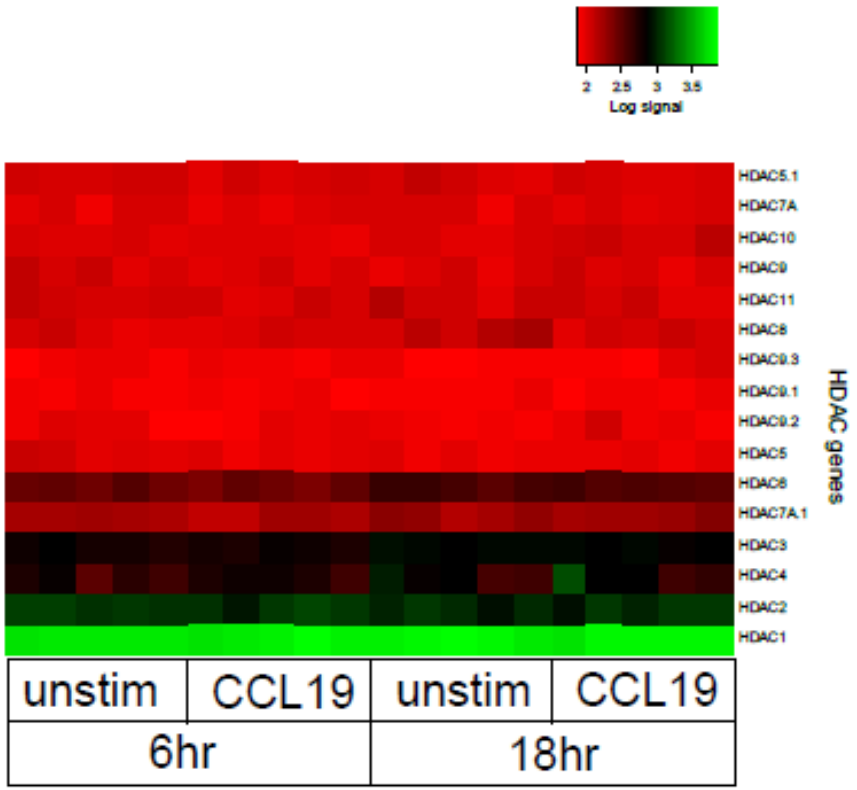
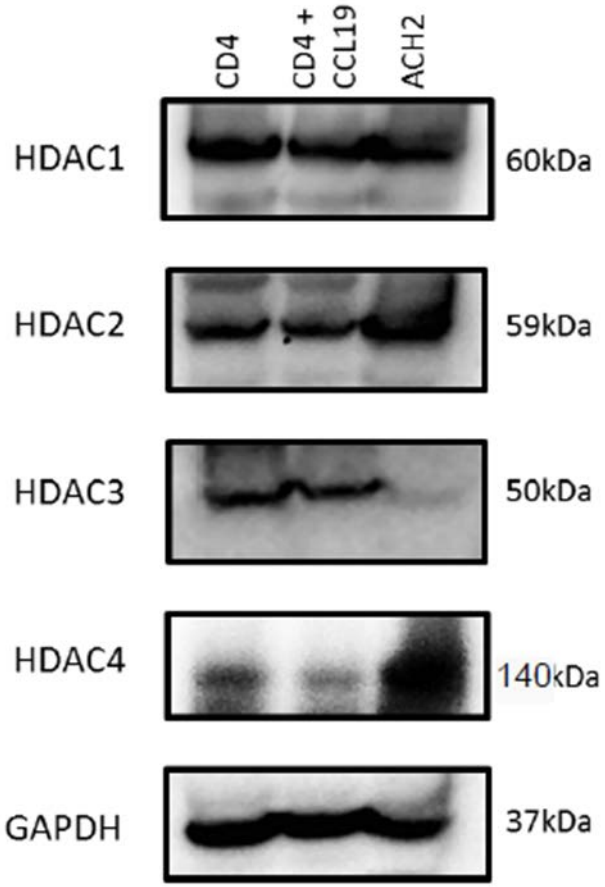
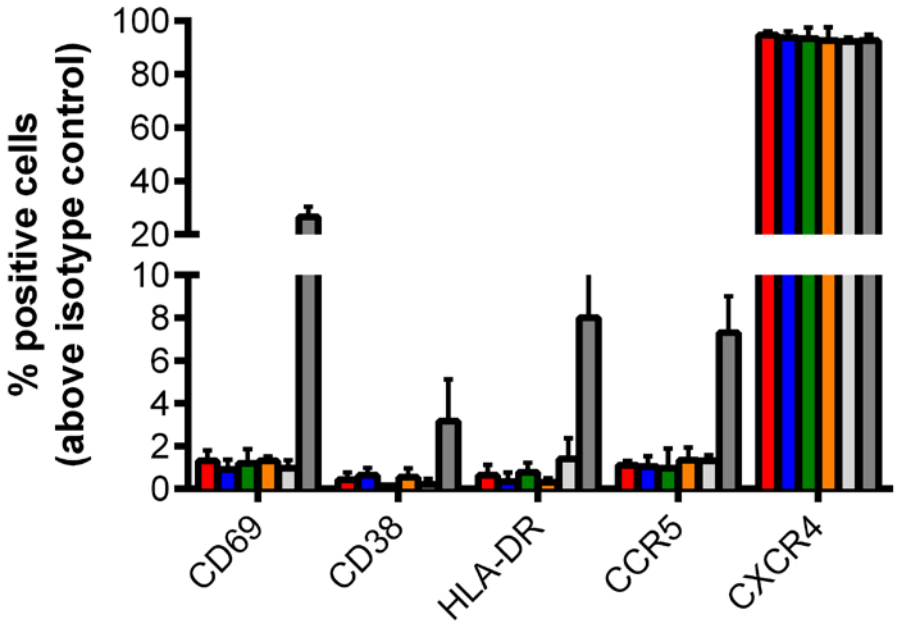




No change in activation markers following HDACi



Mean ± SD (n=4)

Chromatin immunoprecipitation (ChIP) analysis of latently infected T-cells

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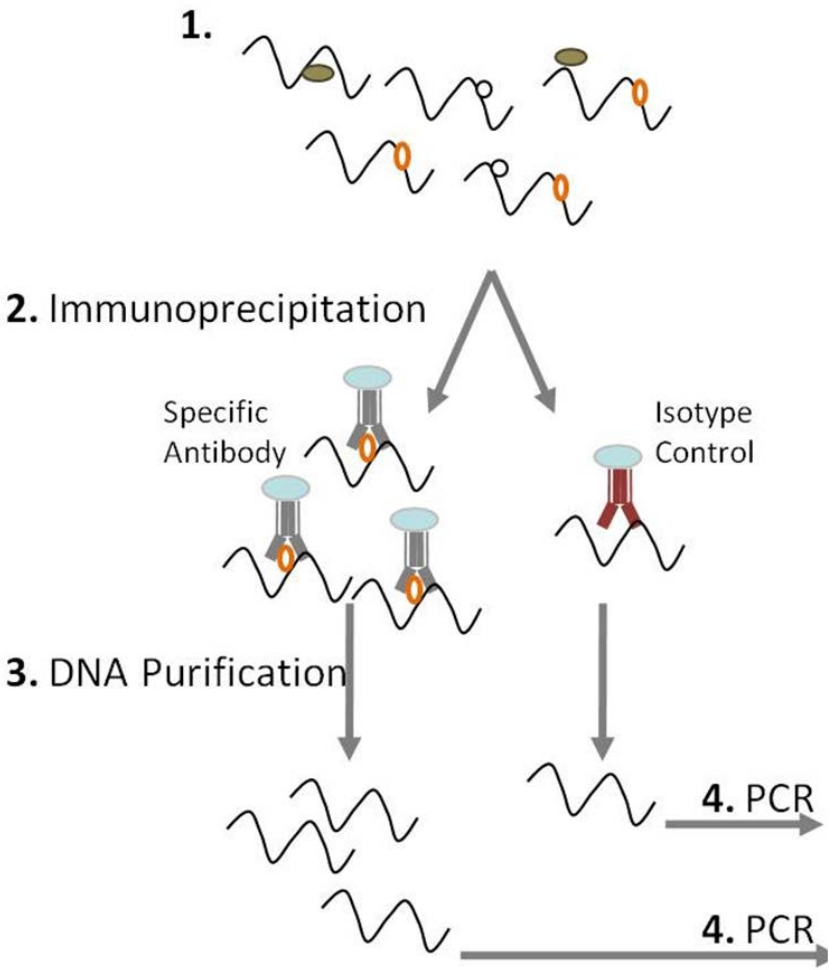

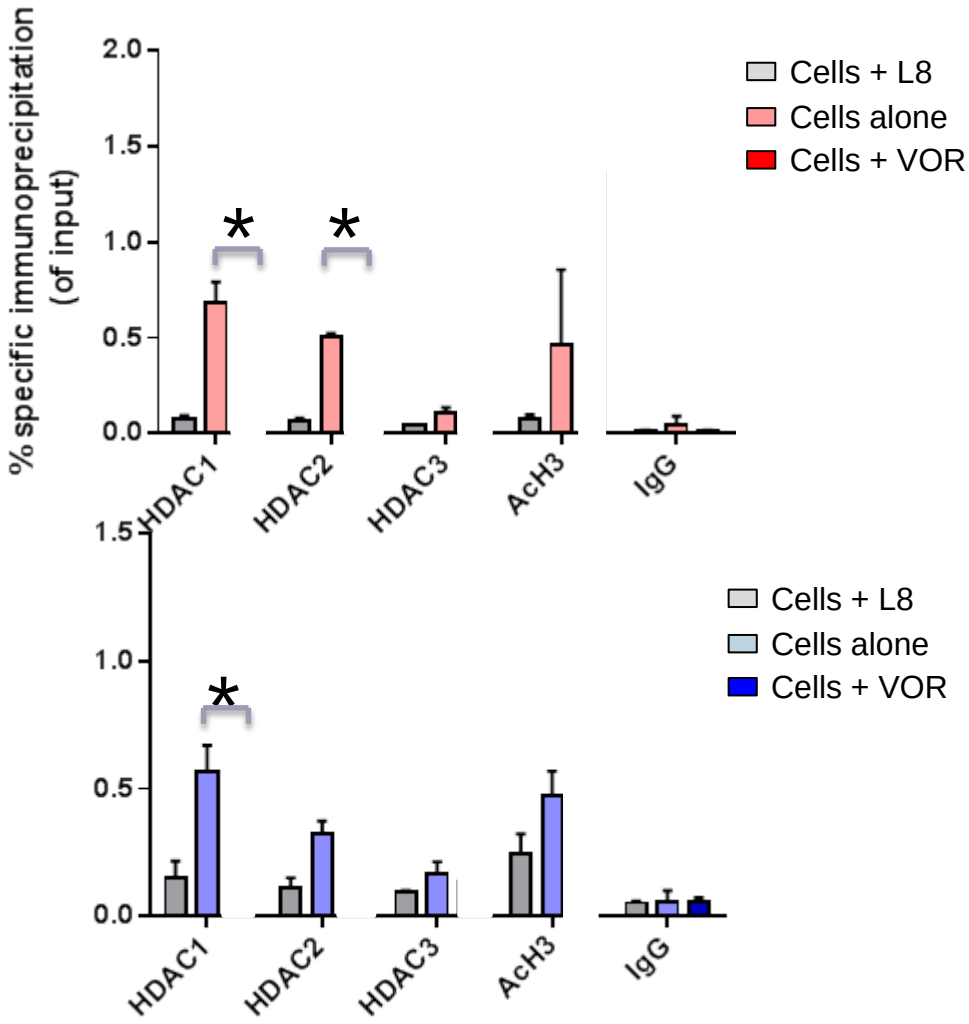

Mean ± SD (n=3) * $p<0.05$

Limitations of study

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- Assay was semi-quantitative
- Only in vitro models of latency were tested not patient derived cells
- No assessment of cell death as a result of induction of viral production
- Viral production alone in vitro may not ultimately indicate efficacy in depletion of latently infected cells in vivo

Conclusions

- Variation in toxicity and potency of HDACi between latently infected cell lines and latently infected CCL19-treated CD4 T-cells
- Entinostat, which is selective for Class I HDACi, induced significant viral production in latently infected CCL19-treated CD4 T-cells
- Based on toxicity profile and activity in vitro, entinostat should be considered for future clinical trials

Acknowledgements

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Medical Research. Practical Action.

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Stronger Together Against HIV