Poster # 307

Role of High Mobility Group AT-Hook 1 (*HMGA1*) Gene Expression in Reactivation of Latent HIV

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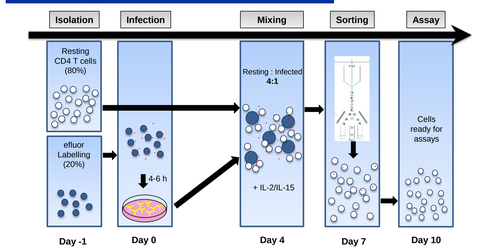
Introduction

- Histone deacetylase inhibitors (HDACis) are latency reversing agents that promote a transcriptionally favorable environment for integrated HIV provirus.
- HDACi suberoylanilide hydroxamic acid (SAHA) induced expression of HIV RNA in patients on suppressive cART, but failed to reduce the size of the latent reservoir.
- SAHA exhibited mixed effects on host genes and protein expression, some of which appear to be inhibitory for HIV reactivation, including up-regulation of transcriptional repressors or down-regulation of activators (1).
- High Mobility Group AT-Hook 1 gene (*HMGA1*) was upregulated by SAHA both at the RNA and protein level (1).
- *HMGA1* encodes a host factor that potentially promotes viral latency by competing with Tat for TAR binding (2).

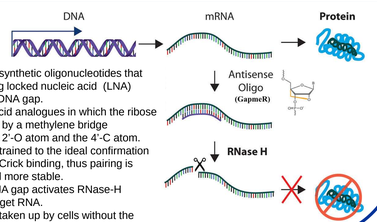
Objective: we aimed to test two hypotheses:
1) the degree of *HMGA1* upregulation by SAHA negatively correlates with levels of HIV reactivation
2) knocking down of *HMGA1* improves the ability of SAHA to reactivate latent HIV.

Figure 1. Methods

Human primary CD4 T cell *in vitro* model of HIV latency



GapmeR (Exiqon, Inc.) knockdown mechanism



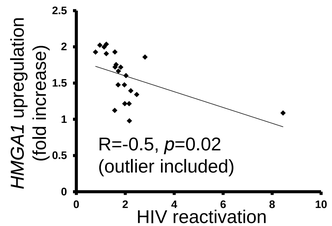
- GapmeRs are synthetic oligonucleotides that contain flanking locked nucleic acid (LNA) regions and a DNA gap.
- LNA: nucleic acid analogues in which the ribose ring is "locked" by a methylene bridge connecting the 2'-O atom and the 4'-C atom.
- Pairing is constrained to the ideal confirmation of the Watson-Crick binding, thus pairing is more rapid and more stable.
- The central DNA gap activates RNase-H cleavage of target RNA.
- GapmeRs are taken up by cells without the need for transfection.

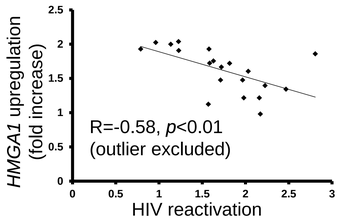
(1) White et al., 2015. *Antiviral Res.* 123:78-85. PMID: 26343910
(2) Eilebrecht et al., 2013. *RNA Biol.* 10(3): 436-444. PMID: 23392246

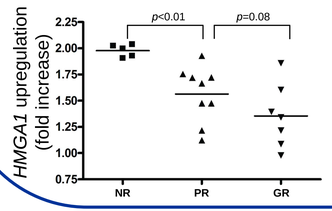
Figure 2. The degree of *HMGA1* upregulation by SAHA negatively correlates with levels of HIV reactivation.

Experiment 1: correlation of *HMGA1* upregulation by SAHA with levels of HIV reactivation

CD4 T cells *in vitro* model of HIV latency N=21 → Treat cells with SAHA (1-1.25 uM) or solvent DMSO for 24 h → Measure expression of HIV multiply spliced (MS : tat/rev) RNA and *HMGA1* by droplet digital PCR → Correlate (Pearson R) HIV reactivation (fold change SAHA/DMSO) with *HMGA1* upregulation (fold change SAHA/DMSO)







Fold increase of MS RNA (SAHA/DMSO)
NR (non-responder):<1.5
PR (poor responder):1.5 – 2.0
GR (good responder):>2.0
Significance was determined using a *t*-test

Conclusions

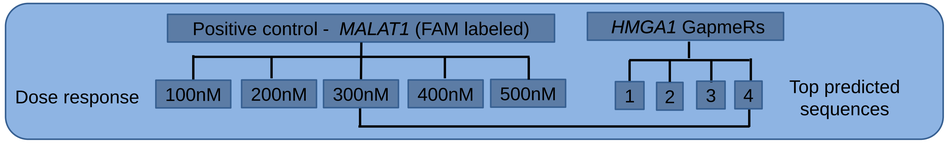
- The degree of *HMGA1* upregulation by SAHA negatively correlates with level of HIV reactivation.
- GapmeRs can be used in primary CD4 T cells to induce gene expression knockdowns. This approach is advantageous when working with *in vitro* primary cell models of HIV latency, as conventional transfection procedures result in high levels of cell death.
- Even a modest knockdown of *HMGA1* expression using GapmeRs resulted in an increase of reactivation of HIV with SAHA.

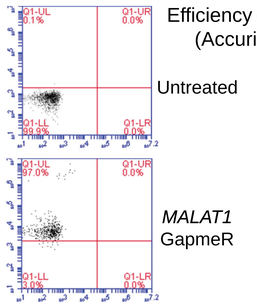
Future Work

- Further optimize knockdowns of *HMGA1*.
- Perform knockdown experiment using *in vitro* latency model in multiple donors.
- Perform time course experiment to determine the timing of *HMGA1* upregulation relative to HIV reactivation.

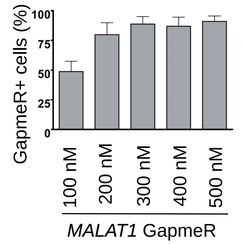
Figure 3. Primary CD4 T cells efficiently take up GapmeRs, resulting in knockdown of target genes.

Experiment 2: assessment of gene knockdowns using GapmeR technology

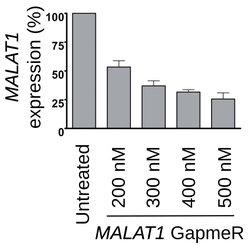




Efficiency of GapmeR uptake (Accuri flow cytometer)



Efficiency of knockdowns (droplet digital PCR)



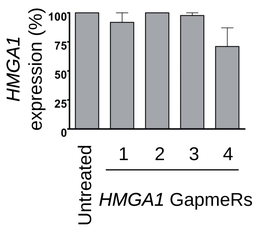
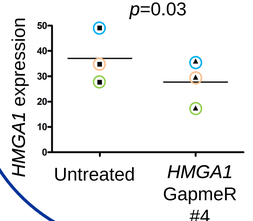


Figure 4. Knockdown of *HMGA1* improves the ability of SAHA to reactivate latent HIV in primary CD4 T cells.

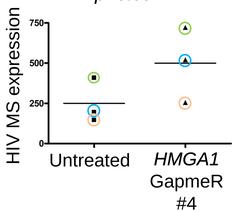
Experiment 3: measuring HIV activation following *HMGA1* knockdown and SAHA treatment

CD4 T cells *in vitro* model of HIV latency, N=3 → *HMGA1* knockdown: treat cells with GapmeR #4 for 3 days → Treat cells with SAHA (1 uM) or solvent DMSO for 24 h → Measure expression of HIV multiply spliced (MS) RNA and *HMGA1* by droplet digital PCR

Average reduction in *HMGA1* expression (day 3): 33%, *p*=0.03



Average increase in HIV reactivation (day 4): 210%, *p*=0.09



- *HMGA1* copies from ddPCR were normalized to host *RPL27*, multiply spliced (MS) RNA was calculated as average copies/cell.
- Background expression (in DMSO-treated cells) was subtracted from expression in SAHA-treated cells.
- Significance was determined using a *t*-test
- Circle colors indicate samples from the same blood donor.