MODULATION OF IRF7-DRIVEN TRANSCRIPTION AS A STRATEGY TO CONTROL HIV-1 LATENCY

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BACKGROUND

The persistence of a latent viral reservoir represents a major barrier shared by current HIV cure strategies. Emerging evidence suggests that modulation of innate immunity could impact viral latency and contribute to the clearing of HIV reservoir. Our previous data indicated that IRF7 expression correlates with HIV latency reversal. Here, we investigate the role of IRF7 in HIV-1 transcription and latency, with the aim of identifying novel therapeutic agents useful in HIV eradication.

ACKNOWLEDGEMENTS

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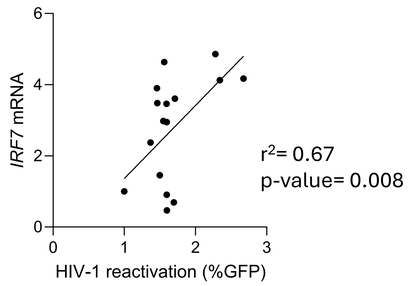
- **IRF7 controls latent HIV-1 transcription and plays a role in both HIV-1 reactivation or blockade**
- **Modulation of IRF7 expression through pharmacological agents might represent an asset to current HIV-1 cure strategies**

MATERIALS AND METHODS

Latency was evaluated by non-clonal models drug-treated, alone or in combination with known LRAs. qPCR and WB were used to assess gene and protein expression. IRF7 loss and gain-of-function models were developed by siRNA or plasmid overexpression. IRF7 role in HIV-1 transcription was evaluated by measuring LTR-driven transactivation in TZM-bl cells and co-immunoprecipitation. Immunophenotyping of *ex vivo* treated CD4+ T-cells from ART-suppressed PLWH was performed by flow cytometry and latency reactivation/promoting activity was measured by qPCR in cell supernatant. Statistical significance for *in vitro* and *ex vivo* experiments was calculated using appropriate t test in Graphpad Prism (v9.3.0). All experiments were performed in at least three independent replicates and mean ± SD values are shown. $*p<0.05$; $**p<0.01$; $***p<0.001$.

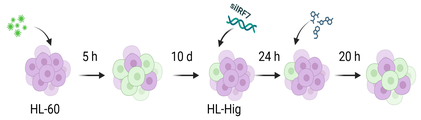
RESULTS

Latency reversal capacity of different LRAs correlates with IRF7 mRNA expression levels

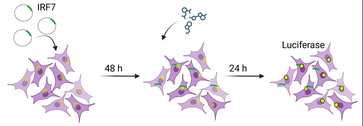


Pearson correlation plot of HIV latency reversal activity versus *IRF7* gene expression in a non-clonal model of latency.

Modulation of the expression levels of IRF7 alters latency reversal capacity of LRAs



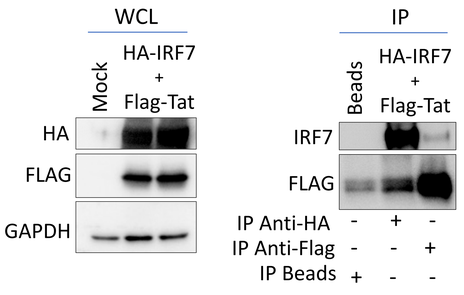
siRNA downregulation in HL60 cells latently infected with a non replicative VSV HIV-1-GFP+ virus (HL-Hig) and treated with LRAs for 20h.

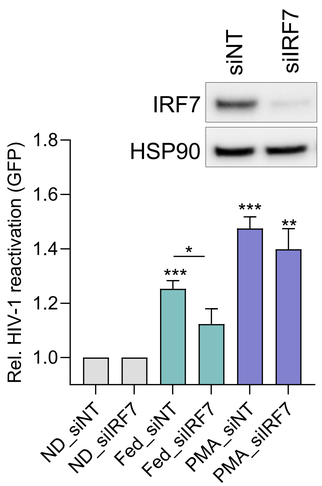


IRF7 plasmid overexpression in TZM-bl cells expressing luciferase under the control of HIV1-LTR and treated with LRAs for 24h.

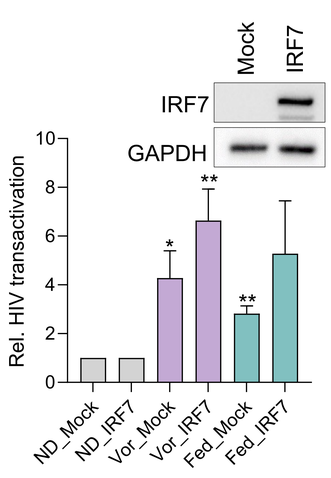
IRF7 participates in HIV-1 transcription control

Physical interaction of HIV-1 Tat protein and IRF7 analyzed by co-immunoprecipitation in 293T transiently co-transfected with IRF7-HA tagged and HIV Tat-Flag tagged expression plasmids.



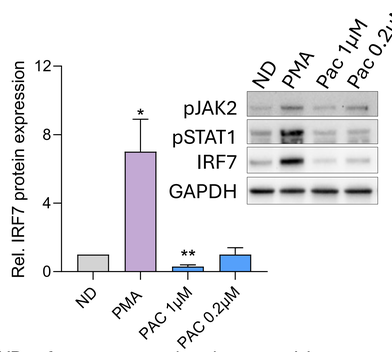


IRF7 protein downregulation confirmed by WB 24h post siRNA transfection. Viral reactivation measured as the proportion of GFP+ cells post incubation with PMA 0.1µg/mL or fedratinib 5 µM. ND: no drug

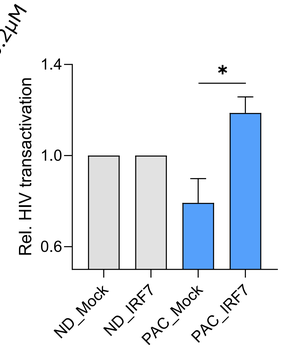


Overexpression of IRF7 confirmed by WB. HIV-1 Tat transactivation assay in Mock or IRF7 transfected TZM-bl cells treated or not with fedratinib or vorinostat (5 µM).

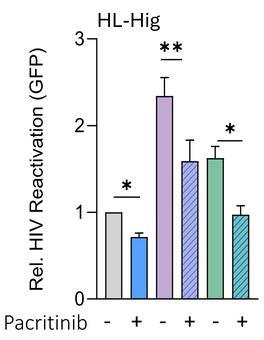
Modulation of IRF7 expression by pacritinib correlates with its LPA capacity and counteracts reactivation capacity of LRAs



WB of representative immunoblots of HL60 cells treated with PMA 0.1µg/mL and a dose response of pacritinib. Bar plots represent the mean quantification of bands obtained by densitometry analysis of 3 independent experiments.

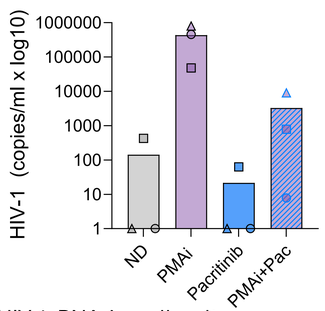


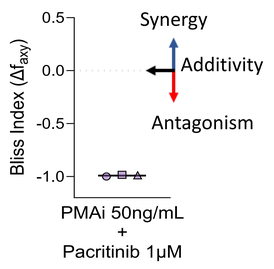
Pacritinib (1µM) activity measured by HIV-1 Tat transactivation assay after IRF7 overexpression in TZM-bl cells, mock or transiently transfected with an IRF7 expresión plasmid.



Pacritinib significantly blocked HIV-1 reactivation both, alone (1µM) or in combination with PMA 0.1µg/mL or vorinostat 5 µM in non-clonal models of HIV-1 latency, HL-Hig (HL60-derived) and J-Hig (Jurkat-derived)

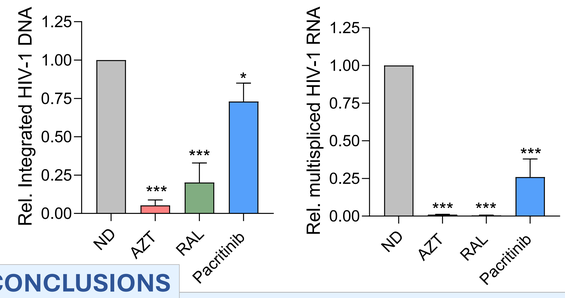
Pacritinib blocks reactivation *ex vivo* in CD4+ T cells of PLWH





HIV-1 RNA in cell culture supernatant of isolated CD4+ T cell from ART suppressed PLWH treated with treated with pacritinib alone or in combination with PMA+ionomycin and calculated Bliss independence index score for the combination

Pacritinib reduces the presence of multispliced HIV-1 transcripts in primary CD4+T cells



CONCLUSIONS

Our results demonstrate the key role of IRF7 in HIV-1 transcription and latency. Moreover, we have identified novel therapeutic agents that may impact the HIV reservoir representing a promising step towards HIV eradication.