Single cell imaging corroborates the link between HIV integration and transcription

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BACKGROUND

Antiretroviral treatment fails to cure HIV infection since latent provirus resides in long-lived cellular reservoirs, rebounding whenever therapy is discontinued. The molecular mechanisms underlying HIV latency are complex whereby the possible link between the integration site and the transcriptional state of the provirus is poorly understood. HIV integration is targeted towards active chromatin by the direct interaction with a host protein, LEDGF/p75 [1]. Small molecules, referred to as LEDGINs, inhibit the LEDGF/p75-integrase (IN) interaction and shift integration out of active genes resulting in residual provirus that is more latent [2].

METHODS

We now have optimized a branched DNA imaging method for simultaneous detection of viral DNA and RNA in single cells [3]. We investigated how LEDGIN treatment affects the location, transcription and reactivation of the provirus in both cell lines and primary cells. LEDGIN CX014442 was tested and compared to equivalent concentrations of raltegravir (RAL), a clinically approved IN strand transfer inhibitor. After infection of the cells in the presence of the compounds, the cells were kept in culture to allow silencing of the viral gene expression and were reactivated on day 7 (PBMCs) or 10 (SupT1) post infection (**Figure 1**) with or PMA (PBMCs) or TNF-alpha (SupT1).

Figure 1. A single cell-based method to study HIV latency and reactivation

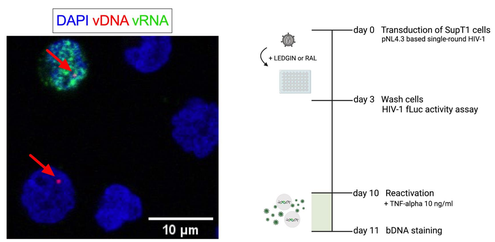
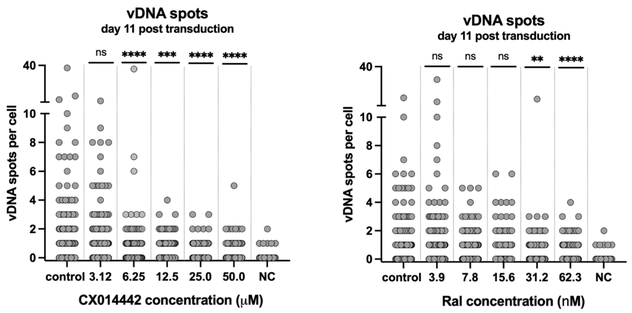


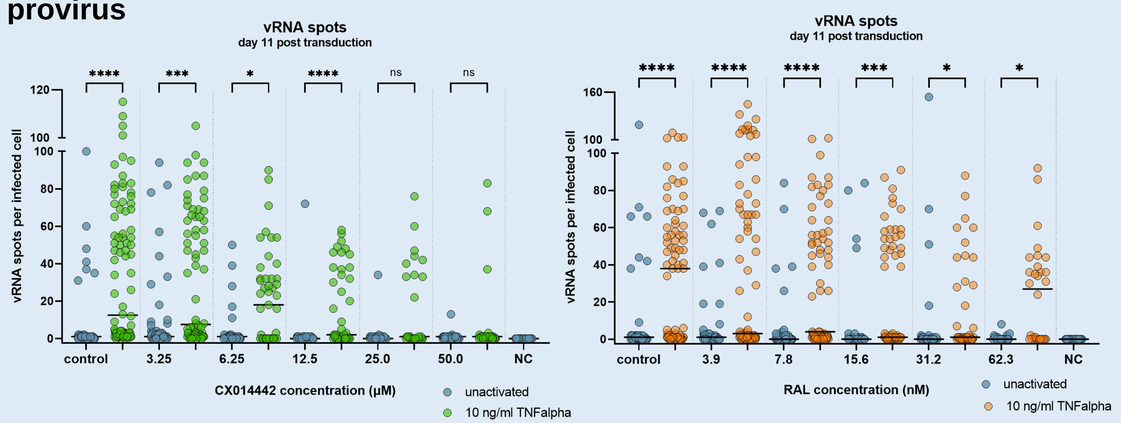
Figure 2. CX014442 and RAL both reduce HIV integration at the single cell level



RESULTS

CX014442 inhibited HIV infection, as shown by a decrease in the number of vDNA spots per cell (**Figure 2**). Furthermore, the 3D nuclear location of the residual provirus after CX014442 treatment was shifted towards the inner nucleus (not shown). CX014442-mediated retargeting hampered both the baseline transcriptional state and transcriptional reactivation of the provirus, as shown by a decrease in the number of vRNA spots per cell (**Figure 3**, upper left panel). CX014442 treatment reduced the vRNA expression per residual copy (**Figure 3**, lower panel). Since RAL failed to reduce HIV transcription and reactivation (**Figure 3**), this effect is not merely the result of reduced infectivity. CX014442-mediated silencing of HIV transcription and reactivation was confirmed in primary PBMCs (**Figure 4**). Paradoxically, at a higher concentration of CX014442 an increase in the number of vDNA spots per cell was observed in primary cells (**Figure 4**). This may reflect a positive selection of deep-latent provirus after CX014442 treatment.

Figure 3. CX014442, but not RAL, reduces transcription and reactivation of the provirus



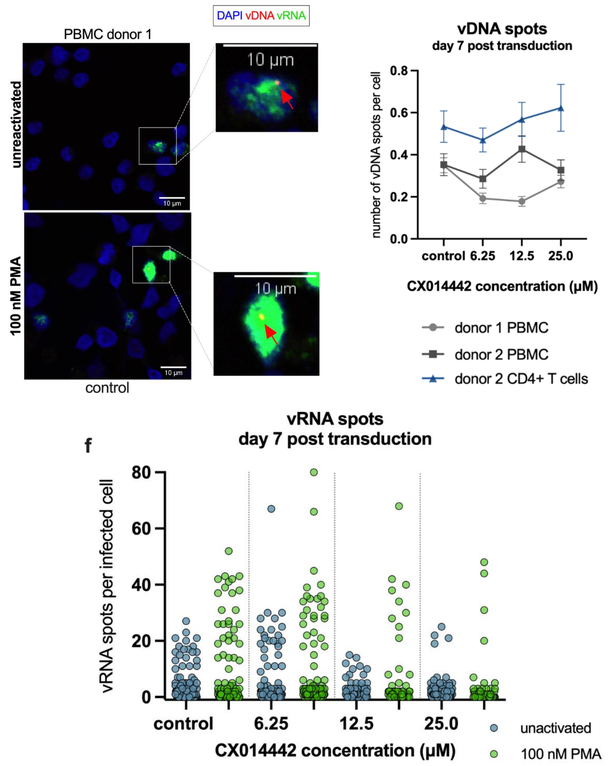
Effect on basal transcription (unactivated cells)

CX014442 (µM)	total$_{vDNA}$	total$_{vRNA}$	vRNA per copy	RAL (nM)	total$_{vDNA}$	total$_{vRNA}$	vRNA per copy
0 (control)	191	463	2.42	0 (control)	110	486	4.42
3.12	106	572	5.40	3.9	95	365	3.84
6.25	59	219	3.71	7.8	64	291	5.55
12.5	51	85	1.67	15.6	66	292	4.29
25.0	31	46	1.48	31.2	54	303	5.61
50.0	21	32	1.52	62.3	21	18	0.75

Effect on reactivation (10 ng/ml TNF-alpha)

CX014442 (µM)	total$_{vDNA}$	total$_{vRNA}$	vRNA per copy	RAL (nM)	total$_{vDNA}$	total$_{vRNA}$	vRNA per copy
0 (control)	106	3078	29.04	0 (control)	81	2700	33.33
3.12	108	2298	21.28	3.9	121	2954	24.41
6.25	66	984	14.91	7.8	60	2135	35.58
12.5	38	701	18.45	15.6	41	1437	35.05
25.0	27	437	16.19	31.2	26	665	25.58
50.0	30	214	7.13	62.3	29	726	25.03

Figure 4. CX014442 treatment in primary cells hampers HIV transcription and reactivation from latency



CONCLUSIONS

In conclusion, retargeting integration with LEDGINs shifted the 3D location, reduced transcription and hampered reactivation of the provirus. These results corroborate the crucial role of the integration site for the transcriptional state of the provirus and support block-and-lock functional cure strategies in which the latent reservoir is permanently silenced after retargeting. Enrichment of vDNA spots over time after LEDGIN treatment point towards a LEDGIN-mediated selection of deep-latent provirus in primary cell cultures. Our *in vitro* findings are consistent with recent studies on the integration profile in elite controllers [4], and patients on long-term ART [5], which suggest host-mediated selection of proviruses with lower transcriptional activity.

REFERENCES

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