Towards a block and lock strategy: LEDGINs hamper the establishment of a reactivation competent HIV reservoir.

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Background

Since HIV integrates into the host genome, it can persist for life in a latent reservoir.

HIV integrase (IN) uses the host co-factor LEDGF/p75 to target integration towards transcription units. LEDGINS, a new class of antivirals, block the IN-LEDGF/p75 interaction and allosterically inhibit IN (early effect). They also cause maturation defects and aberrant progeny virus (late effect)(fig. 1a, see further).

Since a sterilizing cure remains elusive and integration sites may influence viral latency and reactivation, we investigated whether LEDGINS can alter integration site distribution and thus affect the latent reservoir.
Methods

- Integration sites were determined by transducing cells with a lentiviral vector for 3 days in the presence of LEDGINs, then washing them and cultivating them for at least 10 days. Genomic DNA was extracted and integration sites were amplified by linker-mediated PCR and sequenced using 454/Roche pyrosequencing.

- To test reactivation potential, we infected various cell lines (SupT1, MT4 and Jurkat cells) with a single round double-reporter virus (OGH, Chavez et al., 2015, fig. 1d) for 3 days. Different concentrations of LEDGINs were either added during infection with the OGH virus (early effect) or during production of the OGH virus (late effect)(fig. 1b). 8 days post infection (p.i.), cells were reactivated with TNFα or left untreated. Viral reactivation was measured on day 9 via flow-cytometry to detect reporter gene expression.

- Peripheral blood mononuclear cells were isolated from fresh buffy coats using lymphoprep density gradient centrifugation and resting CD4+ T-cells were purified using negative antibody selection. These cells were then activated with IL2 and PHA prior to infection with wild type NL4.3 HIV-1 virus. 3 hours p.i. virus was washed away and different concentrations of LEDGIN or raltegravir (RAL) were added. On day 4, cells were washed and reactivated with PMA and PHA. Virus production was measured in the supernatant by p24 ELISA on day 7. Integrated copies were determined on day 4 via real time nested Alu-LTR PCR and normalized to cell number via CCR5 PCR.
Fig. 1a) LEDGIns affect multiple stages of HIV replication: they block the interaction between LEDGF/p75 and IN and allosterically inhibit integration (early effect). They increase IN multimerisation causing maturation defects and crippled progeny virus (late effect). 1b) To study the early effect, LEDGIns were only added during infection. When studying the late effect LEDGIns were only present during virus production in 293T cells. 1c) Schematic representation of the single round OGH double reporter virus carrying an eGFP driven by the LTR promotor and a constitutive transcriptional unit (EF1α-mKO2) inserted downstream. 1d) Representative dot plot showing different populations on flow-cytometry analysis of SupT1 cells infected with the OGH virus.
Results:
1. LEDGINs during infection reduce infectivity and shift integration out of transcription units (early effect).

Fig. 2a) Dose-response curve showing a decrease in the percentage of mKO2 and eGFP positive cells with increasing LEDGIN concentrations. LEDGINs were added during infection of SupT1 cells with 2 different dilutions of the OGH virus. 2b) Table showing the percentage of lentiviral integration sites relative to specific features, such as integration into the body of genes (Refseq), and transcription start sites (TSS). LEDGINs shift integration out of active genes and transcription units. 2kb and 4kb windows are shown, data obtained from SupT1 cells.
Results:
2. LEDGINs during infection increase the latent fraction and hamper reactivation in cell lines (early effect).

Fig. 3 Dose-response curves showing the effects of LEDGINs added during infection of SupT1 cells with the OGH double reporter virus. 2 different virus dilutions are depicted. 3a) The latent fraction, \((C/(A+B+C)\times 100)\), increases in the presence of LEDGINs in a dose-dependent manner. 3b) Upon stimulation with TNFα, latently infected cells reactivate, increasing the fraction of productively infected cells, \((B/(A+B+C)\times 100)\). LEDGIN treatment hampers this reactivation in a dose-dependent manner.
Results:
3. LEDGINs during production reduce infectivity and result in a reservoir that is more latent and refractory to reactivation (late effect).

Fig. 4 OGH virus was produced in 293T cells in the presence of LEDGINs. Dose-response curves for SupT1 cells infected with 2 different dilutions of these viruses, without any additional LEDGIN treatment, are shown.

4a) LEDGIN treatment decreases the percentage of eGFP and mKO2 positive cells.
4b) LEDGIN treatment increases the latent fraction (C/(A+B+C)) in residually infected cells.
4c) LEDGINs hamper reactivation in a dose-dependent manner as shown by a smaller increase in productive fraction ((B/(A+B+C^100)) upon stimulation with TNFα.
Results:
4. LEDGINs reduce infectivity and hamper reactivation in primary CD4 T-cells.

Fig. 5 Dose-response curves showing the effects of LEDGINs added during infection of primary CD4+ T-cells with wild type NL4.3 HIV-1. Data from 2 representative, independent donors are shown. 5a, b) Both LEDGINs and RAL inhibit integration, determined by qPCR 4 days p.i. The number of copies/cell is expressed relative to the DMSO condition. Absolute integrated copy numbers varied from 1 copy/10,000 cells to 1 copy/100 cells. 5c, d) 4 days p.i. cells were reactivated with PMA and PHA or left untreated. Reactivation is expressed as the fold increase in p24 on day 7 (ratio of p24 levels after reactivation over p24 levels prior to reactivation). Only LEDGIN treatment, and not treatment with raltegravir, reduces reactivation potential.
Conclusion

• LEDGINs are a new class of effective antivirals with multiple mechanisms of action.

• LEDGINs inhibit viral replication and retarget residual integration sites out of transcription units.

• LEDGIN treatment increases the latent fraction of residually infected cells and reduces their reactivation potential (early effect).

• Viruses produced in the presence of LEDGINs are crippled, resulting in lower infectivity, a more latent phenotype of residually infected cells and a reduced reactivation potential (late effect).

• These effects are not present for the classical INSTI raltegravir.

• These data seem to imply that the site of integration affects latency and reactivation potential.
Future outlook

• LEDGINs represent a useful tool to study the effect of the integration site selection on HIV latency in vitro.

• Since current treatment and experimental approaches such as ‘shock and kill’ have not been able to cure HIV so far, different strategies should be investigated. LEDGINs may be part of a new strategy (‘block and lock’), designed to silence the HIV reservoir, rather than eradicate it. The goal of this strategy would be to increase treatment intervals and maybe even obtain a state of ‘HIV remission’.

• LEDGINs might be particularly useful when administered during early infection or as a part of PrEP, but this still needs to be studied in animal models and eventually in clinical trials.

• Further investigation is needed to determine if LEDGINs could also influence the reservoir in chronic infection.
Reference: