

OP 1.6

Tyrosine kinase inhibition: the new front in HIV cure efforts

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Background: The HIV-1 latent reservoir persists largely through the natural ability of memory T cells to homeostatically proliferate in response to gc-cytokines IL-7 and IL-15. In addition, HIV-1 also infects cells of the myeloid lineage, a cell type that can penetrate protected tissues including the central nervous system. Dasatinib and related tyrosine kinase inhibitors (TKI) that are FDA-approved for the treatment of chronic myeloid leukemia have several novel activities that can impact HIV-1 infection and the persistence of latent reservoirs. The first activity of dasatinib is the ability to block homeostatic proliferation. A second activity of dasatinib is its ability to block infection by HIV-1 by an unusual mechanism that involves dephosphorylation and activation of SAMHD1, a potent viral restriction factor.

Methods: To investigate the ability of TKIs to inhibit homeostatic proliferation of CD4+ T lymphocytes, we utilized a previously described model of latency based on primary cells, and induced proliferation via incubation with IL-2, IL-7 and IL-15. To study the inhibition of phosphorylation of SAMHD1, we cultured primary monocyte-derived macrophages and incubated them with TKI for 24hrs and then exposed them to HIV-1 Bal. SAMHD1 phosphorylation was evaluated by Western blot and flow cytometry.

Results: We demonstrate that TKIs block the homeostatic proliferation of latently infected T cells and thus prevent maintenance of the latent reservoir, and predict that this will result in accelerated decay over time. In vitro measurements demonstrate that various TKIs exhibit highly different inhibitory potency on lymphocyte proliferation.

We also observed that TKIs phenocopied the documented effects of types I and II IFNs on SAMHD1 dephosphorylation. The signaling pathway downstream of TKI is not associated with modulation of cyclin dependent kinases and is, therefore, different from that following IFN signalling.

The relative potencies of TKI (dasatinib, ponatinib, palbociclic, imatinib) in inhibiting cell proliferation did not correlate with the potencies at inhibiting phosphorylation of SAMHD1. Therefore, we speculate that the tyrosine kinase targets for both activities are likely different.

Conclusions: We conclude that TKI in vitro exhibit two different antiviral activities that are specific for HIV-1 and propose further development of these potentially exciting therapeutics.

PP 1.1

Remodeling of the core leads HIV-1 pre-integration complex into the nucleus of human lymphocytes

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Background: Retroviral replication proceeds through obligate integration of the viral DNA in the nucleus, essential for efficient viral replication. However, to be able to integrate into the host genome, the viral DNA must be led through the nuclear pore complex. Indirect evidences showed that HIV-1 capsid (CA) is the viral determinant for HIV-1 nuclear import. HIV-1 CA has been recently detected in the nucleus of dendritic or macrophage cells after infection but not in primary CD4+T cells, the latter are the main target cells for HIV-1.

Therefore, the morphology of the CA protein associated to the functional PIC during its translocation is completely unknown. Our study aims to elucidate which is the morphology adopted by HIV-1 pre-integration complex (PIC) during the translocation into the nucleus. This step of the HIV-1 life cycle has never been previously elucidated because of the lack of appropriate technologies. Early steps of viral infection can be critical for the establishment of viral latency.

Methods: We applied immune gold labeling and correlative-light electron microscopy (CLEM) to detect the morphology of viral complexes. We also set up a new breakthrough method, called HIV-1 ANCHOR, to track the retrotranscribed DNA by live imaging or by CLEM.

Results: Our study provides a detailed view of the structural remodeling of the viral core prior, during and after HIV-1 nuclear entry. First, we observed that the uncoating is not completed before viral nuclear entry. Multiple CA proteins, forming ‘pearl necklace shapes’, can be translocated with the retrotranscribed HIV-1 DNA inside the nucleus. These Results: indicate that the viral core remodels in partial cores (continue or discontinue structures) during the nuclear passage through the pore. Importantly, our data are the first proof of concept of the possibility to directly target and follow the fate in live cells of HIV-1 DNA during and after nuclear entry.

Conclusions: This study gives a new outlook of how the viral CA remodels to allow the nuclear translocation of HIV-1 PIC. We are now able to live-track the viral DNA in natural HIV-1 target cells. This innovative approach could provide new insights into the mechanisms underlying viral persistence.

PP 1.2

Combination of quadruplex qPCR and next-generation sequencing for qualitative and quantitative analysis of the HIV-1 latent reservoir

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Background: HIV-1 infection requires lifelong therapy with antiretroviral drugs due to the existence of a latent reservoir of transcriptionally inactive integrated proviruses. The goal of HIV-1 cure research is to eliminate or functionally silence this reservoir. To this end, there are numerous ongoing studies to evaluate immunological approaches, including monoclonal antibody therapies. Evaluating the Results: of these studies requires sensitive and specific measures of the reservoir.

Methods: We describe a relatively high-throughput combined quantitative PCR (qPCR) and next-generation sequencing method. Four different qPCR probes covering the packaging signal (PS), group-specific antigen (gag), polymerase (pol), and envelope (env) are combined in a single multiplex reaction to detect the HIV-1 genome in limiting dilution samples followed by sequence verification of individual reactions that are positive for combinations of any two of the four probes (Q4PCR). We compare the new method to quantitative and qualitative viral outgrowth assays (Q²VOAs) and near full-length (NFL) sequencing on paired peripheral blood samples obtained at two time points from the same six individuals enrolled in a clinical trial that involved analytical treatment interruption after infusion of a combination of two broadly neutralizing monoclonal antibodies.

Results: All intact viruses in the six individuals analyzed and 99% of the intact clade B viral sequences in the Los Alamos database were positive for any combination of two of the four probes in the Q4PCR reaction. The advantage of Q4PCR is that the method is relatively rapid, scalable, and both sensitive and specific. Bar coding and next-generation sequencing facilitates the analysis of large numbers of samples simultaneously and enables definitive identification of intact proviruses. In addition, the sequence data provide information on the clonal structure of the latent reservoir and on the nature of the defective proviruses.

Conclusions: The combination of four-probe qPCR and next-generation sequencing is a highly sensitive and specific method for measuring intact proviruses in the HIV-1 latent reservoir.