215

Lenacapavir Disrupts HIV-1 Core Integrity While Stabilizing the Capsid Lattice

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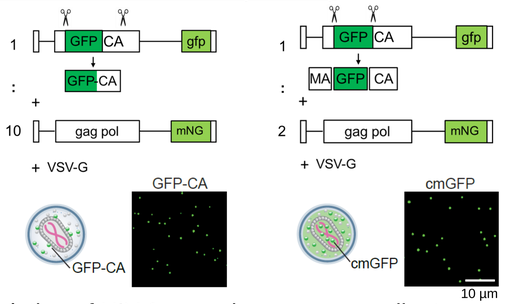
BACKGROUND

Lenacapavir (LEN) is a potent HIV-1 capsid inhibitor (Gilead Sciences) that recently received approval for treatment of multidrug-resistant HIV-1 infection. Structural studies indicate that LEN binds two neighboring capsid subunits and stabilizes the cone-shaped capsid lattice, but its mechanism of viral inhibition and effect on capsids remain elusive. We recently utilized two different approaches to label capsids and demonstrated that intact HIV-1 capsids enter the nucleus and retain their integrity until shortly before integration. Here, we sought to determine the effects of LEN on viral core integrity and kinetics of uncoating by using isolated viral cores and cell-based assays.

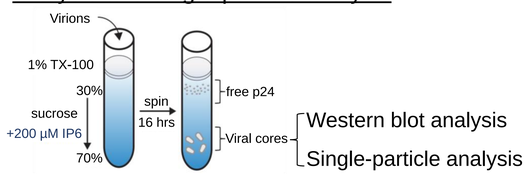
1. We developed capsid labeling strategies to study uncoating and the effect of capsid inhibitors.
   - GFP-CA is incorporated into the capsid lattice and serves as a capsid disassembly marker.
   - Content marker GFP is packaged inside the capsid and serves as a core integrity marker.
2. In vitro, Lenacapavir disrupts core integrity while stabilizing the capsid lattice.
3. In vivo, Lenacapavir disrupts the integrity of nuclear capsids and induces capsid disassembly.
4. The host environment likely plays a role in promoting capsid disassembly upon binding to Lenacapavir.

METHODS

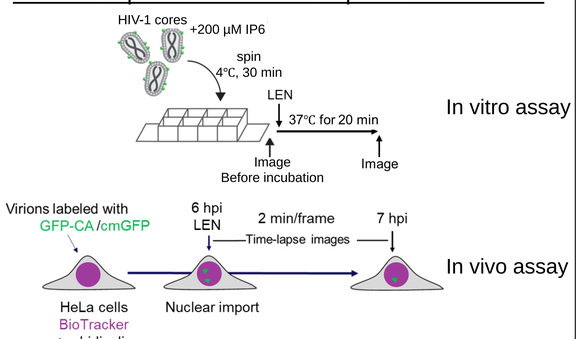
Two different strategies to label HIV-1 virus: GFP-CA and GFP content marker (cmGFP)



Isolation of HIV-1 cores by sucrose gradient fractionation and characterization by Western blot analysis and single-particle analysis

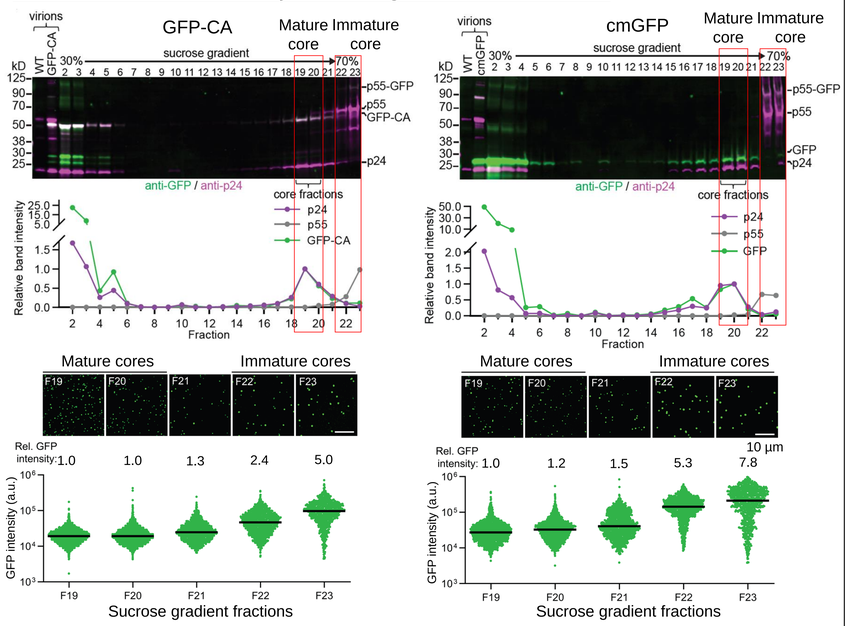


Investigation of the effects of Lenacapavir on isolated capsids and nuclear capsids in HeLa cells

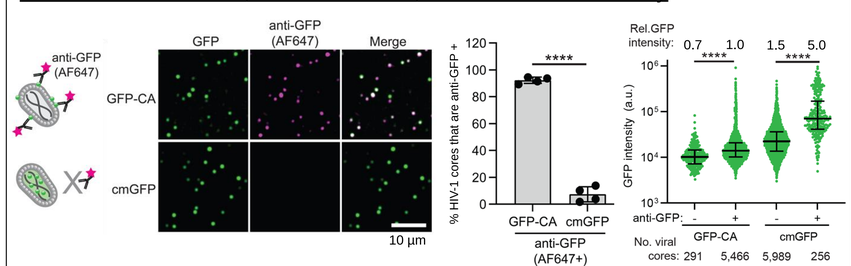


RESULTS

Isolation of HIV-1 cores by sucrose gradient fractionation

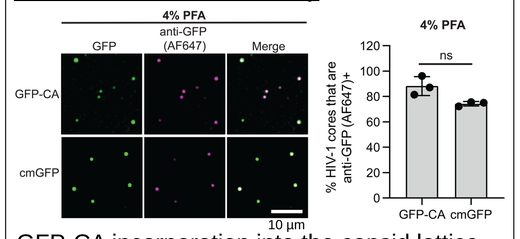


Detection of GFP-CA, but not cmGFP, with anti-GFP antibody

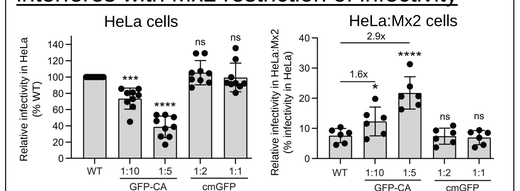


GFP-CA is on the surface of the capsid, whereas cmGFP is on the inside of the capsid.

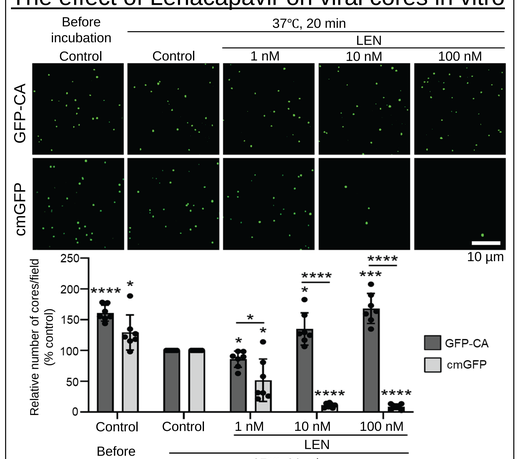
Paraformaldehyde (PFA) fixation results in rupture of cores and increases accessibility of cmGFP to anti-GFP antibody



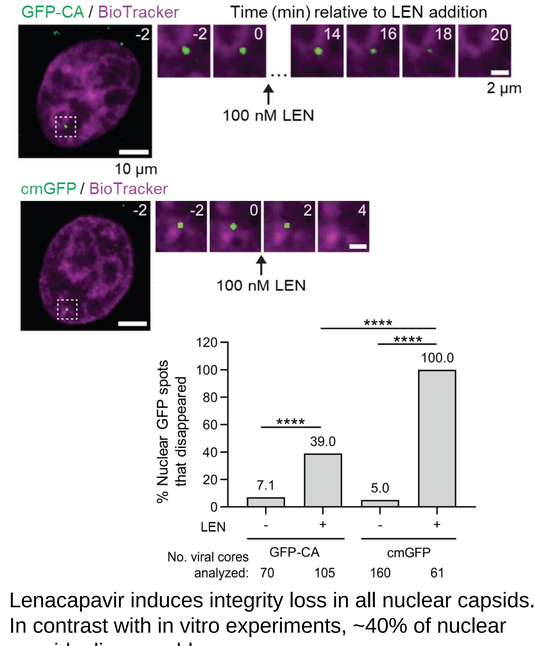
GFP-CA incorporation into the capsid lattice interferes with Mx2 restriction of infectivity



The effect of Lenacapavir on viral cores in vitro

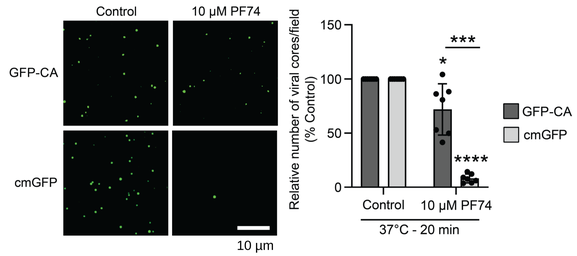


The effect of Lenacapavir on viral cores in vivo



Lenacapavir induces integrity loss in all nuclear capsids. In contrast with in vitro experiments, ~40% of nuclear capsids disassemble.

The effect of PF74 on viral cores in vitro



CONCLUSIONS

Our results show that GFP-CA is an HIV-1 capsid lattice marker and cmGFP is a reporter of core integrity. LEN treatment stabilizes the capsid lattice while disrupting core integrity. These results highlight the importance of retaining an intact capsid until shortly before integration. LEN inhibition's high potency of HIV-1 replication may be explained in part by the disruption of core integrity, rather than inhibition of capsid disassembly.