RESISTANCE TO PA-457 (BEVIRIMAT), A NOVEL INHIBITOR OF HIV-1 MATURATION

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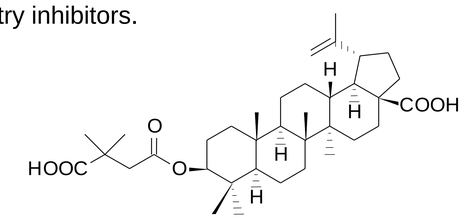
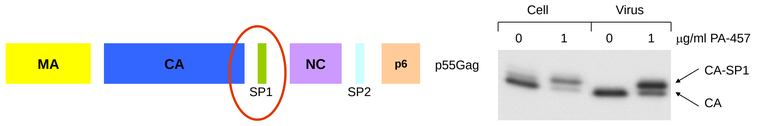
Abstract

PA-457 (bervirimat) potently inhibits both wild-type HIV-1 and isolates resistant to current antiretroviral compounds. It disrupts virus maturation by blocking the cleavage of CA-SP1 to CA. To further investigate the mechanism of action of PA-457, and to anticipate PA-457 resistance that may potentially arise *in vivo*, we have previously isolated and characterized a panel of six PA-457-resistant HIV-1 variants *in vitro*. All the resistance mutations map to the CA-SP1 junction, three at or near the C-terminus of CA (H226Y, L231F, L231M) and three at the 1st and 3rd residues of SP1 (A1V, A3T, A3V). These mutations, with the exception of SP1-A3V and A3T, do not impose a significant fitness defect in Jurkat T-cells; however, all of the residues to which PA-457 resistance maps are highly conserved among HIV-1 isolates, implying that there may be a fitness cost associated with these mutations *in vivo*. To investigate this hypothesis, we are evaluating the replication capacity of PA-457-resistant viral isolates in a range of cells relevant to HIV-1 infection, including macrophages and PBMCs. The stability of mutations conferring PA-457-resistance, and acquisition of secondary mutations during extended passage, are also being investigated. Since it is likely that PA-457 will be used clinically in combination with PR inhibitors, we are examining the impact of mutations that confer resistance to PR inhibitors on the evolution of PA-457 resistance. We have introduced all of our PA457-resistance mutations into a molecular clone bearing substitutions in PR (L10R/M46I/L63P/V82T/I84V) that confer resistance to PR inhibitors and we are characterizing these mutants both in the presence and absence of PA-457. We are also selecting for acquisition of PA-457 resistance using the molecular clone resistant to PR inhibitors. Understanding the evolution of resistance to PA-457 is highly significant as this compound is currently undergoing phase IIb clinical trials in HIV-1 infected patients. The nature of PA-457-resistance arising in the context of both "WT" and mutant PR will elucidate the interplay between mutations in PR and at the CA-SP1 cleavage site and is expected to foreshadow the types of mutations that may arise *in vivo* in treated patients.

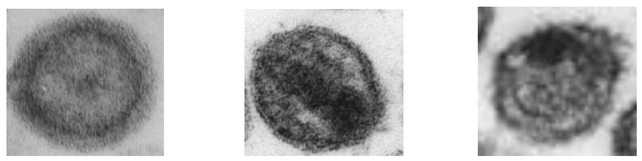
1. PA-457 [3-0-(3',3'-dimethylsuccinyl) betulinic acid] potently inhibits diverse HIV-1 isolates including strains resistant to approved RT, PR, and entry inhibitors.



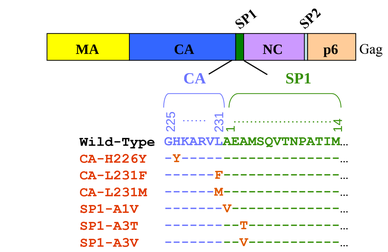
2. PA-457 has a novel mechanism of action. It inhibits a late step in the Gag proteolytic processing cascade, specifically the cleavage of spacer peptide 1 (SP1) from the C-terminus of capsid (CA)[1,2].



3. PA-457 prevents particle maturation and infectivity. Virions from PA-457-treated cells display aberrant acentric cores and an electron-dense crescent inside the viral membrane[1].



4. We have previously isolated a panel of six PA-457-resistant HIV-1 variants in vitro[3].

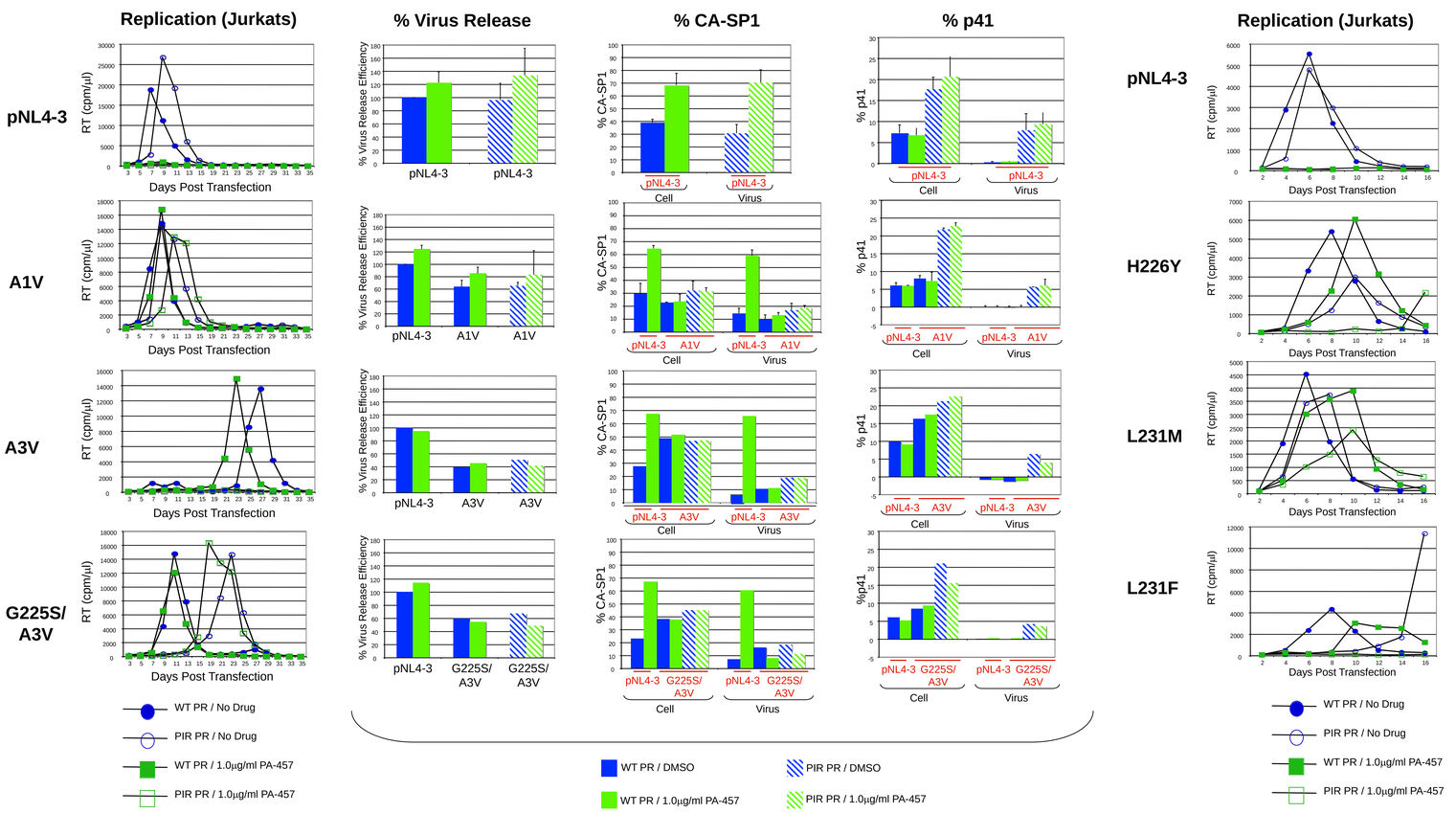


5. Characterization of this panel of PA-457-resistant HIV-1 Isolates showed that[3]:

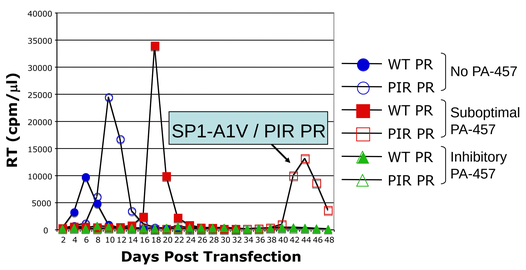
- PA-457 resistance can arise as independent single-amino-acid substitutions
- The majority of these mutations (H226Y, L231F, L231FM, A1V) do not impose a significant replication cost to the virus in Jurkat T-cells.
- Mutations at SP1 residue 3 (A3T, A3V) do impose replication defects; however, the defects can be reversed by acquisition of second-site compensatory mutations (G225S/A3V).
- All residues to which PA-457 resistance maps are highly conserved among HIV-1 isolates, implying that a fitness cost may be associated with changes at these positions *in vivo*.

6. Since it is likely that PA-457 will be used clinically in combination with protease inhibitors, we are examining the impact of protease inhibitor resistance (PIR) mutations on the panel of PA-457-resistant isolates.

7. The PA-457-resistance mutations have been introduced into a molecular clone bearing PIR mutations (L10R/M46I/L63P/V82T/I84V). These mutants have been characterized both in the presence and absence of PA-457.



8. Evolution of PA-457 resistance in the context of PIR mutations.



Summary

- Overall, replication capacity of PA-457-resistant HIV-1 isolates in Jurkat T-cells is not significantly affected by the presence of PIR mutations.
- However some differences were observed; for example, the delay observed with A3V/WT PR is increased in the context of the PIR mutations. The compensatory mutation G225S reversed this delay.
- PIR mutations did not significantly affect CA-SP1 processing.
- PIR mutations did confer some processing defects, as indicated by an increase in p41-Gag levels.
- PIR mutations did not affect virus release efficiency.
- PA-457 resistance in the context of PIR mutations took significantly longer to emerge than in the context of WT PR.
- These results suggest that PA-457 resistance may be less likely to arise in the context of PIR isolates.

References

1. Li et al., PNAS 100:13555-13560., 2003
2. Zhou et al., J. Virol. 78:922-929., 2004
3. Adamson et al., J. Virol. 80:10957-10971., 2006