The Dose Response Curve Slope Quantifies Lenacapavir's *in vivo* Inhibition of HIV-1

547

Anthony D. Abeyta-Lopez[1,2], Branden Ngo[2], Moonki Chae[2], Qi Tifany Chu[2], Jun Lai[2], Emily Fray[2], Janet D. Siliciano[2], Robert F. Siliciano[1,2,3]
[1]Department of Physiology, Pharmacology, and Therapeutics, and [2]Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA,
[3]Howard Hughes Medical Institute, Baltimore, Maryland, USA

BACKGROUND

Lenacapavir (LEN) is a first-in-class long-acting HIV-1 capsid (CA) inhibitor used in prevention and treatment[1,2]. LEN acts at both early and late stages of the HIV-1 life cycle. It binds CA, altering capsid structure to prevent target cell infection. LEN also binds the CA domain of the Gag precursor protein, blocking assembly and release[1-3]. We studied LEN pharmacodynamics at both stages using a model[4] that incorporates both the IC_{50} and slope (Hill coefficient) of the dose-response curve (Fig. 1). Results were expressed in terms of inhibitory potential[4] *(IP)*, the number of logs by which LEN reduces single round infection events (Fig.1D)

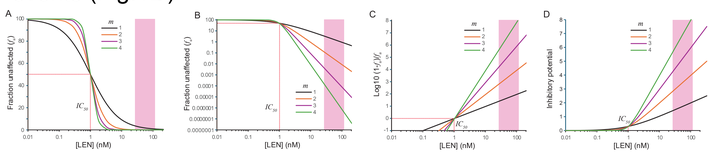


Fig.1 ***Contribution of Hill coefficient (m) to viral inhibition.*** (**A**) Standard dose-response curves showing the fraction of infection events unaffected by the drug (f_u) as a function of drug concentration for drugs with the same IC_{50} (red dotted line) and different values of the slope parameter (*m*, Hill coefficient), an indicator of cooperativity in drug action. A curve with an *m* value of 1 (expected for no cooperativity) is shown in black. Clinical concentrations (pink shaded area) are typically well above the IC_{50}. (**B**) Curves from A plotted on a logarithmic Y axis revealing that *m* has a large effect on inhibition. (**C**) Median effect plots ($log((1- f_u)/ f_u)$ vs *log* drug concentration) linearize the dose-response curves. (**D**) Inhibitory potential (log ($1/f_u$)) is the number of logs by which the drug reduces single round infection events. It is strongly influenced by the slope of the dose-response curve[4].

METHODS

We used a single round infectivity assay with LEN included during infection of target cells, during virus production, or at both stages (Fig. 2).

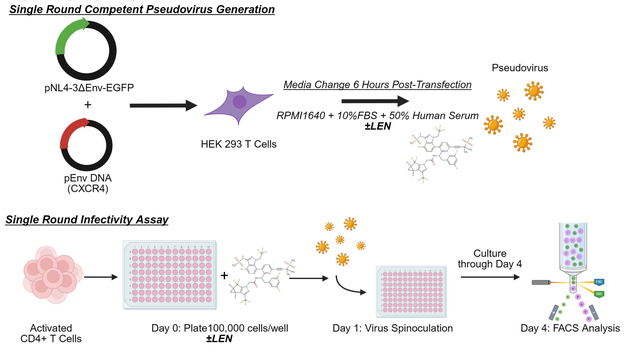


Fig. 2. ***Single round infectivity assay..***

RESULTS

- LEN affects both the infection of new target cells and virion production occurring with producer cells, with a one-log lower IC_{50} for inhibiting infection when compared to virion production (Fig. 3A,B)
- Median-effect plots of both mechanisms demonstrate slope values >1, indicating positive cooperativity in inhibition with greater cooperativity observed during the production stage.

An upward inflection in Lenacapavir's inhibition of HIV-1 virion production demonstrates that an increase in binding cooperativity results in greater antiretroviral efficacy.

- A single line, though, cannot define the median-effect plot of LEN's inhibition of virion production due to an upward inflection occurring in the clinical concentration range. This is better represented by a segmented linear model displaying a significantly higher slope within the clinical concentration range (m = 3.674, Fig. 3H)
- This inflection is also observed when both mechanisms are combined (Fig. 3F), illustrating the dominant effect LEN's inhibition of viral production has on total HIV-1 inhibition.
- This phenomenon suggests that, although the IC_{50} value for virion production is greater, the steeper slope observed in inhibition of virion production shows LEN has a larger impact on inhibition through this mechanism.
- To compare the inhibition produced by LEN at each stage, we used the metric inhibitor potential (*IP*):

$$IP=log_{10}(1/f_u)= log_{10}(1+(C/IC_{50})^m)$$

where *C* is the LEN concentration[3].

- At the lower end of the clinical concentration range, LEN produced more inhibition of the infection stage.

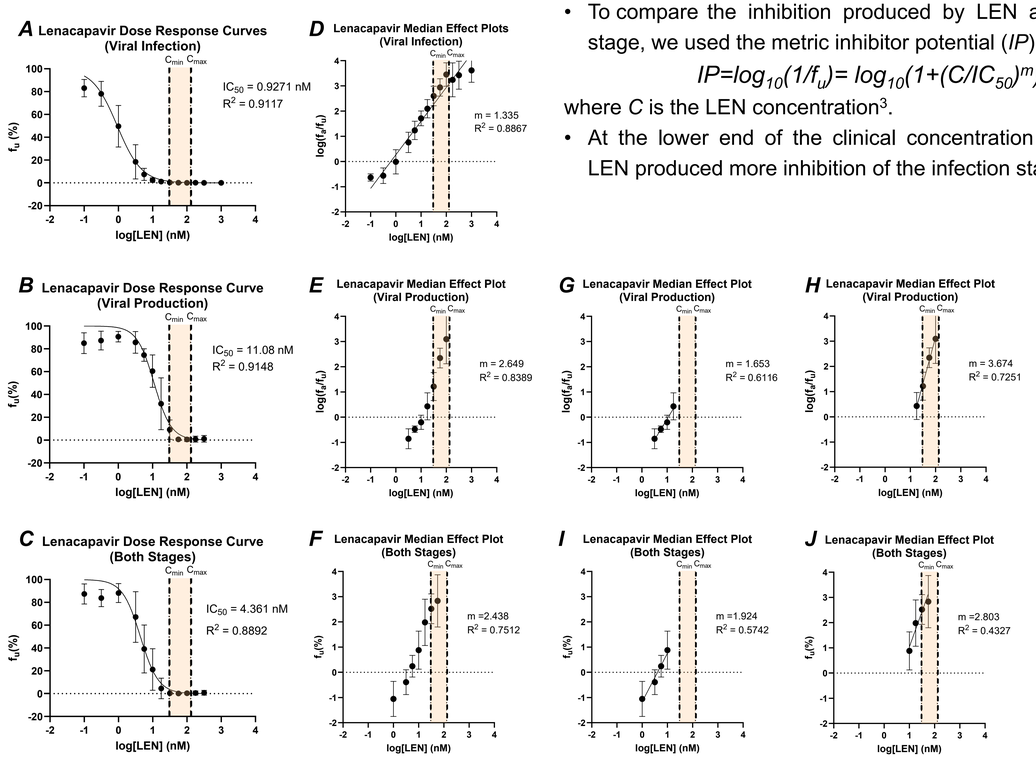


Fig.3. ***Effect of LEN at different stages of the HIV life cycle.*** (**A-C**) Standard dose-response curves for inhibition of infection (**A**), virus production (**B**) and both stages (**C**).. (**D-F**) Median effect plots for inhibition of infection (**D**), virus production (**E**) and both stages (**F**). Segmented median effects plots for inhibition of virus production (**G,H**) and both stages (**I,J**).

- However, due to the upwardly infected dose-response curve for virus production, the effects of LEN were of similar magnitude for both stages at the higher end of the clinical concentration range. (Fig. 4A).
- The combined effects of LEN at both stages can be analyzed using class models for drug synergy[5] (Fig. 4B).

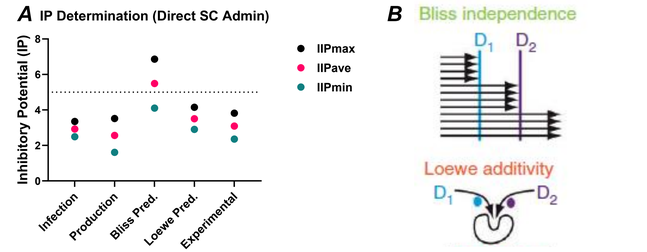


Fig. 4. ***Combined effects of LEN at the infection and virus production stages of the viral life cycle.*** (**A**) Inhibitory potential for LEN at the maximum, average, and minimal plasma concentration for the infection stage, the production stage, the combined effect predicted by the Bliss and Loewe models for drug synergy[5], and the experimentally measured combined effect. Effective ART regimens for the treatment of established infection have combined IP values >5 (dotted line). (B) Models for synergy. The Bliss model assumes that 2 drugs act independently while the Loewe model assumes that drugs compete for the same binding site.

- The combined effects of LEN acting at both stages of the viral life cycle matched those predicted by the Loewe model, as expected based on a common binding site.
- The level of inhibition achieved did not exceed the 5 log threshold associated with successful combination therapy for established HIV infection[5].

CONCLUSIONS

- **Lenacapavir displays different pharmacodynamics at both stages and in combination with high cooperativity occurring throughout.**
- **An upward inflection occurs in LEN's inhibition of virion production, resulting in increased antiviral activity at clinical concentrations.**
- **By IP analyses, LEN's overall inhibition of HIV-1 is not sufficient to suppress established HIV-1 infection, suggesting it should not be used as monotherapy with the current dosing regimen.**

References: 1: Yant et al, Nat Med 2019. 2: Link et al, Nature 2020. 3: Kelly et al, NEJM 2024. 4: Shen et al, Nat Med 2008. 5: Jilek et al, Nat Med 2012.

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PLAIN LANGUAGE SUMMARY

Here we quantify the inhibition that lenacapavir can achieve through both of its mechanisms of action independently and in combination. We describe a unique phenomenon in lenacapavir's inhibition of virion production where, after reaching a set concentration, small changes in drug concentration confer greater inhibition of viral replication.