HIV

When HIV pays the price: Fitness costs behind lenacapavir resistance

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HIV can take several mutational pathways to become resistant to lenacapavir, each with distinct resistance and fitness profiles (Pennetzdorfer *et al.*, this issue).

INTRODUCTION

Long-acting antiretroviral therapies are transforming HIV prevention and care by delivering antiretroviral therapy (ART) with only intermittent dosing. Lenacapavir (LEN) is the first approved capsid inhibitor and the longest-acting formulation, dosed every 6 months. LEN binds a conserved pocket at the interface of capsid monomers and interferes with multiple steps in the viral life cycle, including nuclear import, integration, and assembly, which helps explain its picomolar potency (*1*). The capsid is an attractive target given that genome-wide diversity analyses have shown that it is highly conserved, consistent with strong structural and functional constraints (*2*). Drugs that target such constrained regions can be highly effective, but they also raise the question of what happens when the virus is forced to escape.

LEN's clinical promise has now been demonstrated across both prevention and treatment. For prevention, the PURPOSE 1 and PURPOSE 2 trials demonstrated that LEN-based pre-exposure prophylaxis (PrEP) can outperform standard oral regimens across multiple populations (*3*, *4*). For treatment, the CAPELLA trial enrolled people with multidrug-resistant HIV and showed that subcutaneous LEN, given with an optimized background regimen, produced rapid and durable viral load declines (*5*). The CALIBRATE study extended this to treatment-naïve individuals and showed that LEN-based regimens can maintain high levels of viral suppression during initial therapy (*6*). Previous in vitro selection experiments had identified candidate LEN resistance mutations (*7*), and these clinical trials confirmed that certain positions were selected during virologic failure. In PURPOSE 2, the two LEN PrEP recipients who acquired HIV infection both had capsid substitutions consistent with LEN resistance (*4*). In CAPELLA, virologic failure was reported in ~31% of participants receiving LEN; of these individuals, 41% had emerging putative LEN resistance mutations (*8*). In CALIBRATE, emergent capsid mutations were reported in approximately two-thirds of LEN recipients with prolonged virologic failure (*6*). However, these findings left many open questions about the impact of single and combination capsid mutations on LEN resistance and replication fitness. In this issue of *Science Translational Medicine*, Pennetzdorfer and colleagues used a panel of clinical and engineered viruses harboring potential LEN resistance mutations from the CAPELLA and CALIBRATE trials to determine their effect on LEN susceptibility and the fitness price that these viruses pay to escape from LEN antiviral activity (*9*).

THREE MAIN RESISTANCE PATHWAYS

Pennetzdorfer *et al.* analyzed 40 clinical isolates harboring putative resistance mutations from 18 individuals with virologic failure on LEN alongside a panel of 44 site-directed mutants (*9*). They tested these variants in three complementary phenotypic systems: a single-cycle Gag-Pro assay, a 5-day multicycle assay measuring cytopathic effects in MT-2 cells, and a 5-day RevLun reporter assay that tracks viral replication by luciferase readout.

Three main LEN resistance pathways emerged (Fig. 1A). The first centered on M66I, which had been the dominant pathway in heavily treatment-experienced participants in CAPELLA. Across assays, M66I alone resulted in large fold changes in the median effective concentration (EC_{50}) value of LEN, confirming it as a high-level resistance pathway (*7*, *9*). The second pathway involved Q67H and K70H/R, which was most commonly seen in the CALIBRATE trial (*6*). Q67H or K70R alone results in only modest levels of LEN resistance; however, when combined, the double mutant yielded medium- to high-level resistance (K70H alone also yielded medium resistance). The third pathway included other resistance-associated mutations, highlighted by N74D, which conferred low-to-medium resistance and was detected in both PURPOSE 2 participants with breakthrough infection despite LEN PrEP (*4*). A recurring pattern across resistance pathways was that the accumulation of additional mutations over time, including combinations of mutations from all three pathways, can lead to progressively increased LEN resistance (Fig. 1B). This stepwise evolution of viral resistance is particularly concerning for LEN because the drug persists for many months after an injection, which markedly prolongs the window during which partially resistant viruses can continue to evolve greater resistance even if no additional LEN doses are given.

THE PRICE OF VIRAL ESCAPE

Given the central role of capsid in uncoating, nuclear import, and assembly, it is not surprising that capsid mutations can come with substantial fitness costs. Pennetzdorfer *et al.* quantified these trade-offs using the single-cycle Gag-Pol system and the multicycle replication readout (*9*). All baseline viruses from CAPELLA and CALIBRATE had replication capacities close to that of wild type. Once LEN resistance emerged, however, the fitness landscape split into distinct trajectories.

Although the M66I mutation resulted in high-level resistance to LEN, it carried extreme fitness cost, reducing replication capacity to below 20% of that of wild-type virus (*9*). In contrast, variants harboring the Q67H and K70R, either alone or in combination, demonstrated only modest fitness

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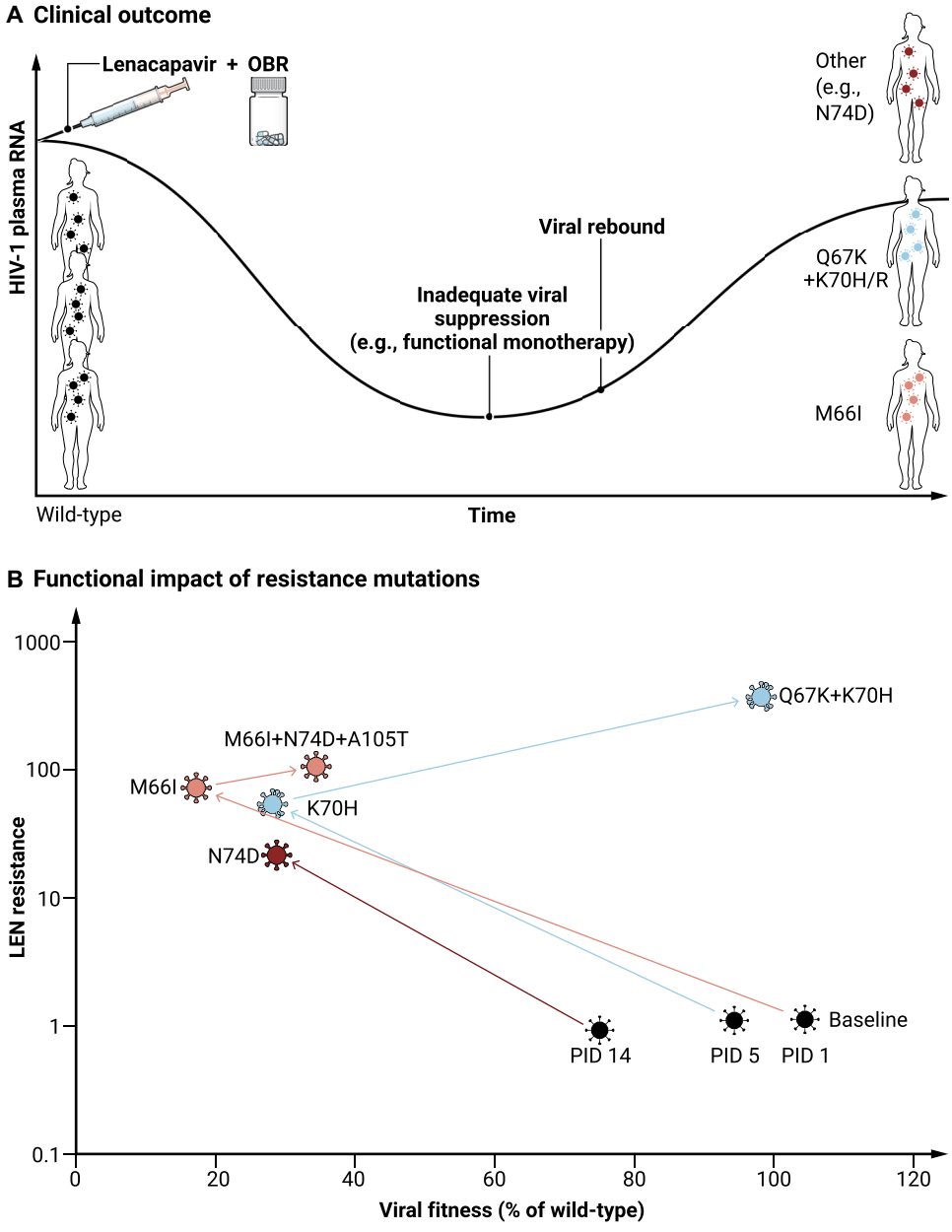

Fig. 1. Clinical evolution and functional trade-offs of lenacapavir resistance. (**A**) After initiation of LEN with an optimized background regimen (OBR), viral load initially declines. In the setting of inadequate viral suppression (e.g., functional monotherapy), selective pressure drives viral rebound. Several resistance pathways can emerge, including those driven by key M66I, Q67H+K70H/R, or N74D mutations. PID, participant ID. (**B**) Levels of LEN resistance and replication capacity of clinical isolates from three representative participants (individuals 1, 5, and 14) are shown using data from table S1 from Pennetzdorfer *et al.* (*9*), each illustrating a distinct resistance pathway. Virions show viral fitness [percent of wild-type replication capacity by the multicycle human PBMC (peripheral blood mononuclear cell) assay] plotted against LEN resistance (EC_{50} fold change relative to wild type by the multicycle RevLun assay), with colors denoting pathways.

costs for most samples (although K70H alone can lead to a substantial fitness cost), with the Q67H+K70R combination resulting in a replication capacity at more than 75% of that of wild type. Other single mutants such as N74D were also found to have a substantial fitness cost (*9*).

The authors found that the accumulation of resistance mutations over time not only increased the level of LEN resistance but also concurrently rescued viral fitness, especially when the initial resistance mutation was associated with substantial fitness costs. In one participant, the M66I mutation was associated with substantial resistance and crippling fitness costs (17% of that of wild-type virus). However, the subsequent accumulation of N74D and A105T mutations led to both an increase in LEN resistance and improvement in viral fitness to 34% of that of wild type (Fig. 1B). This same combination of mutations (M66I, N74D, and A105T) was seen in a second participant who subsequently added the Q67H mutation, resulting in a replication capacity that was restored to around two-thirds of that of wild type. Similar patterns were observed across other

pathways, where early, highly resistant viruses demonstrated poor replication capacity, but continued selection in the presence of LEN allowed them to pick up compensatory changes that both increased resistance and improved fitness (*9*). These data again highlight that LEN resistance is not static but a moving target shaped by the interplay of drug pressure, fitness costs, and compensatory evolution. Structural modeling further supported the authors' phenotypic findings by showing that M66I causes distortion of the LEN binding pocket, whereas Q67H, K70 substitutions, and N74D produce localized changes that weaken binding without the same fitness penalty (*9*).

MOVING FORWARD

What might these findings mean for the use of LEN? First, it is interesting to note that the various LEN trials reported different resistance mutation pathways. The CAPELLA study illustrates how inadequate viral suppression (e.g., in the setting of functional monotherapy with LEN) in those with prior multidrug HIV resistance can drive the selection of high-level LEN resistance, especially that of M66I (*5*, *8*). Although M66I imposes severe fitness costs, it readily emerged in the setting of limited companion antiretroviral drug activity, where any virus that partially evades LEN can gain a decisive selective advantage. In contrast, LEN resistance followed a different pattern (Q67H and K70R) in the CALIBRATE trial of ART-naïve participants, which was consistent with selection for mutations that conferred intermediate resistance but with higher replication capacity (*9*). Last, in PURPOSE 2, the two LEN PrEP breakthrough infections both harbored the N74D mutation (*4*, *9*). Although preliminary, these results suggest the intriguing possibility that the most likely LEN resistance mutations that emerge may depend on the treatment context.

A second implication is that LEN's long-acting pharmacology may potentiate the accumulation of resistance mutations. Even after treatments stop, LEN can persist for months, which can encourage the stepwise accumulation of mutations that further potentiate resistance or improve fitness. These observations support several practical priorities. In treatment settings, avoiding functional monotherapy with LEN is critical. Background regimens should include at least one fully active companion drug whenever possible, and resistance testing should be performed expeditiously for the background regimen during virologic failure where LEN is part of the regimen (*5*). Unfortunately, LEN resistance testing is not currently available outside of research settings. As LEN use expands, establishing regional or national surveillance networks for LEN-associated capsid mutations will become essential. To prevent the accumulation of LEN resistance mutations in the context of virologic failure, discontinuing LEN alone is likely insufficient given the long-acting pharmacology, and efforts should be made to provide alternative regimens for rapid viral suppression. The prevention of capsid resistance emergence is also critical for the next-generation capsid inhibitors.

In prevention, LEN is one of the most promising candidates for long-acting PrEP, especially with once-yearly dosing strategies under study (*10*). The success and durability of LEN for PrEP will depend on maintaining low rates of transmitted LEN resistance in the community. Factors that will shape this include the frequency of LEN-resistant breakthrough infections, which resistance pathways come to dominate during virologic failure, how quickly compensatory mutations accumulate under prolonged drug pressure, and how rapidly wild-type virus reemerges in people with HIV after LEN concentrations fall below selective thresholds. All of these factors will influence which HIV variants are most likely to be transmitted in the community and the continued efficacy of LEN as a critical tool for the prevention of HIV.

As LEN moves into broader use for both PrEP and treatment, these findings highlight the importance of maintaining fully active companion drugs during HIV treatment, improving access to resistance testing and surveillance, and accelerating development of next-generation capsid inhibitors with higher resistance barriers and activity against common LEN resistance mutations. Doing so will help preserve the promise of long-acting therapy while restricting the evolutionary pathways that enable viral escape.

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