

# Pre-Exposure Prophylaxis and Antiretroviral Resistance: HIV Prevention at a Cost?

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Pre-exposure prophylaxis (PrEP), the use of antiretrovirals (ARVs) by human immunodeficiency virus (HIV)–uninfected individuals to prevent acquisition of the virus during high-risk sexual encounters, enjoyed its first 2 major successes with the Centre for the AIDS Programme of Research in South Africa (CAPRISA) 004 and the Pre-Exposure Prophylaxis Initiative (iPrEx). These successes were buoyed by additional positive results from the TDF2 and Partners PrEP trials. Although no seroconverters in either arm of CAPRISA developed resistance to tenofovir, 2 participants in iPrEx with undetected, seronegative acute HIV infection were randomized to receive daily oral tenofovir-emtricitabine and resistance to emtricitabine was later discovered in both men. A similar case in the TDF2 study resulted in resistance to both ARVs. These cases prompted us to examine existing literature on the nature of resistance mutations elicited by ARVs used for PrEP. Here, we discuss the impact of signature mutations selected by PrEP, how rapidly these emerge with daily ARV exposure, and the individual-level and public health consequences of ARV resistance.

As we enter the fourth decade of the human immunodeficiency virus (HIV) infection pandemic, [1] 4 randomized, controlled trials have delivered the first tangible successes in the science of pre-exposure prophylaxis (PrEP). In 2010, the Centre for the AIDS Programme of Research in South Africa (CAPRISA) 004 demonstrated a 39% reduction in HIV transmission among heterosexual South African women who used a tenofovir-based vaginal gel before and after sex [2]. Several months later came findings from the Pre-Exposure Prophylaxis Initiative (iPrEx), a study of daily oral tenofovir-emtricitabine among transgendered women and men who have sex with men, which showed a 44% decrease in incident HIV infections compared with placebo [3]. In both studies, increased adherence to

study medication was associated with even greater protection [2, 3].

At the 2011 International AIDS Society Conference in Rome, Italy, positive results were reported from 2 additional studies of PrEP among heterosexual individuals. The TDF2 study showed 63% protective efficacy of daily tenofovir-emtricitabine among sexually active men and women [4], whereas the Partners PrEP trial of serodiscordant couples demonstrated 62% and 73% reductions in transmission for daily oral tenofovir and tenofovir-emtricitabine, respectively [5].

Lost in the excitement over these promising results is any significant discussion about the potential for antiretroviral (ARV) resistance to develop among persons who are administered PrEP. Among the 38 women in CAPRISA randomized to receive tenofovir gel who became HIV-infected, no tenofovir-associated resistance mutations were detected using population (bulk) sequencing [2]. Deep sequencing of samples from the seroconverters of CAPRISA to detect low-frequency resistant variants is currently underway (S. A. Karim, written communication, April 2011). Analyses of resistance are also ongoing for Partners PrEP [5].

Resistance in iPrEx and the TDF2 trial is a different story. In iPrEx, seroconversion occurred in 38

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participants on the tenofovir-emtricitabine arm, including 2 men who had seronegative acute HIV infection at the time of randomization; both of these men went on to receive active drug. By week 4, both had evidence of resistance to emtricitabine, although only 1 of the 2 was confirmed to have developed the mutation as a result of study drug exposure [6]. For the other participant, it remains unclear whether the resistance mutation was transmitted (primary) or acquired. In the TDF2 study, 1 participant with unrecognized acute HIV infection initiated tenofovir-emtricitabine and developed resistance to both ARV agents [4].

These cases of resistance, emerging when PrEP functionally became incompletely suppressive ARV therapy, provide a useful starting point for inquiries into the consequences of PrEP in terms of drug resistance and its implications for public health. Here, we address several critical questions facing clinicians, public health practitioners, and policy-makers by examining existing literature on resistance to the ARVs utilized for PrEP.

## WHICH MUTATIONS ARE SELECTED BY THE COMPONENTS OF PRE-EXPOSURE PROPHYLAXIS?

### Emtricitabine

Signature mutations for emtricitabine occur at codon 184. Single-nucleotide alterations mediate amino acid changes from methionine to isoleucine (M184I) or valine (M184V) [7, 8], resulting in extremely high-level resistance to both emtricitabine and its congener, lamivudine. Although it is perhaps counterintuitive, trials from the early 1990s onward have demonstrated benefits in viral load suppression when lamivudine is kept in regimens following the emergence of the M184V mutation [9, 10]; given the similarities between lamivudine and emtricitabine, the same effect is assumed to occur with emtricitabine. Three principal hypotheses have been proposed to explain why lamivudine and emtricitabine remain useful despite the presence of M184V. First, continued drug exposure maintains the selection pressure eliciting M184V, and viruses carrying this particular mutation demonstrate markedly decreased replicative capacity [11, 12]. Second, a combination of in vitro and in vivo data suggests continued direct antiretroviral activity despite this mutation [13, 14]. Finally, increased susceptibility to other nucleoside reverse transcriptase inhibitors (NRTIs)—especially zidovudine and tenofovir—is induced by the presence of M184V [15].

### Tenofovir Disoproxil Fumarate

Mutations at 2 specific codons of reverse transcriptase impact the efficacy of tenofovir: K65R and K70E/G. The K65R mutation involves a transition at the second nucleotide position of codon 65, which changes lysine to arginine [16]. This alteration causes an intermediate level of resistance to tenofovir, abacavir, didanosine, lamivudine, and emtricitabine [17]. Paradoxically, K65R *increases* susceptibility to zidovudine [18]. Molecular biological and clinical

cohort evidence strongly suggests K65R is more likely to develop among subtype C strains exposed to certain incompletely suppressive ARV regimens [16, 19, 20], including those that contain tenofovir, stavudine, and didanosine. This observation may be important for the future of PrEP in the developing world, given that subtype C accounted for nearly one-half of all infections globally between 2004 and 2007—especially in India and sub-Saharan Africa [21].

Similar to K65R, single-nucleotide alterations in codon 70 result in an amino acid shift from lysine (K) to glutamate (E), with a second purine-to-purine transition required to yield glycine (K70G) [22]. By itself, K70E/G moderately reduces susceptibility to tenofovir, didanosine, and abacavir and has slight negative effects on lamivudine and emtricitabine activity.

## HOW RAPIDLY DO EMTRICITABINE- AND TENOFOVIR-ASSOCIATED RESISTANCE MUTATIONS DEVELOP?

Although the primary mutations selected by emtricitabine and tenofovir are mediated by single-nucleotide changes, we know from a variety of monotherapy and dual-therapy data that emtricitabine and lamivudine select for M184V much more rapidly than tenofovir does for either K65R or K70E/G. Data from animal models provide further insight into the consequences of incompletely suppressive single- or dual-agent regimens given for extended periods.

### Monotherapy Studies

Within 15 days, single-agent emtricitabine exposure causes M184V to develop in ~1 in 5 recipients [23]. Earlier work on lamivudine demonstrated similar results, with a dramatic increase in the proportion of patients who received lamivudine monotherapy harboring M184V, from 20% at 2 weeks to 80% at 4 weeks. Within 12 weeks, all 20 participants in the study had the mutation [24].

In sharp contrast to this rapid selection of M184V, 2 separate studies of tenofovir monotherapy in humans demonstrate that the drug can be administered for up to 28 days without evidence of resistance by bulk sequencing [25, 26]. However, among a group of 12 macaques with established simian/human immunodeficiency virus (SHIV) infection treated with daily single-agent tenofovir, K70E became detectable by real-time polymerase chain reaction after a median of 2 weeks. K65R arose at low levels between 2 and 12 weeks, and a median of 8 weeks of tenofovir exposure was required before K65R became detectable on bulk sequencing (range, 4–20 weeks) [27]. This suggests that human studies of 28-day tenofovir monotherapy ended prior to the point at which resistance emerges.

### Dual-therapy Studies

The most important data on the evolution of resistance under dual therapy come from NUCA 3001, a randomized trial from

the mid-1990s comparing monotherapy with zidovudine or lamivudine to dual therapy with both agents [28]. M184V developed within 12 weeks in the majority of participants initiating any lamivudine-containing regimen. After 1 year of treatment, M184V was detected in 87% of patients receiving dual therapy, compared with 100% of those receiving lamivudine monotherapy. Importantly, only 32% of dual-NRTI recipients developed mutations conferring zidovudine resistance over the same period—a significant lag compared with the zidovudine monotherapy arm, in which 61% of participants had resistance at 1 year [29].

This trend toward postponed emergence of zidovudine-associated mutations in the presence of M184V was seen in a similar study, NUCB 3001, which compared zidovudine plus lamivudine to zidovudine alone. Among dual-therapy recipients, 95% developed M184V by week 8. When resistance analyses were performed at the completion of 24 weeks of follow-up, just 25% of patients receiving dual therapy had developed mutations at zidovudine resistance codons, in contrast to 69% of those receiving zidovudine monotherapy ( $P = .006$ ) [11].

If M184V similarly delays emergence of tenofovir-associated mutations, it could have important ramifications for managing individuals infected despite PrEP. Preserving susceptibility to tenofovir would allow its future use in combination ARV therapy. Although *in vivo* data are lacking, the crippled replicative capacity of K65R + M184V double mutants *in vitro* supports the hypothesis that M184V may be “protective” against the acquisition of K65R, at least in non-subtype C isolates [30, 31].

#### **Additional Animal Data**

In a study of PrEP among macaques rectally challenged with SHIV, 6 of 12 animals receiving ARVs developed breakthrough infection and continued receiving the medications to which they were originally assigned. Four infections occurred among macaques that received emtricitabine monotherapy, whereas 2 animals that received tenofovir-emtricitabine became infected [32]. In all instances, the virus that established infection was wild-type. Two of the 6 animals developed mutations in their viruses: 1 animal with M184I in the tenofovir-emtricitabine arm 3 weeks after infection and 1 animal with M184V in the emtricitabine-only arm 10 weeks after infection. Importantly, however, the macaques that developed M184I/V mutations were the 2 with the highest peak viremias, suggesting that selection of resistant mutants may be potentiated by high viral replication [32]—a hallmark of acute HIV infection [33–35]. In light of this, it is noteworthy that the presence of ARVs during early infection may blunt peak viremia in human hosts. A case report of failed non-occupational post-exposure prophylaxis describes seroconversion without any acute retroviral symptoms and a peak viremia of just 647 copies/mL, 11 days after stopping the planned 28-day regimen of tenofovir-emtricitabine. The virus establishing

infection was genotypically and phenotypically pan-susceptible to available ARVs [36].

We can use these data to construct a rough timeline of events from the date of infection in an individual receiving tenofovir-emtricitabine-based PrEP to the emergence of resistance under continued ARV exposure. With daily use, one can expect M184V to appear between 2 and 4 weeks, with a majority of recipients harboring the mutation by the 8-week mark. K65R will likely follow. If there is in reality no significant protective effect from M184V against the accumulation of other NRTI mutations, then one could see K65R evolving as early as the fourth week of treatment. For individuals intermittently adherent to PrEP, the timeline for emergence of mutations may be very different.

### **WHAT DO PRE-EXPOSURE PROPHYLAXIS FAILURES TELL US ABOUT THE EVOLUTION OF ANTIRETROVIRAL RESISTANCE?**

Nonadherence seems to be the main reason for the PrEP failures observed in CAPRISA and iPrEx. Quite simply, one must actually use the ARVs for them to prevent infection. Although it is reasonable to assume that the same is true for TDF2 and Partners PrEP, the reasons for the lack of any efficacy of daily tenofovir-emtricitabine among heterosexual women in the FEM-PrEP study, which was halted in early 2011, remain unclear [37].

Thirty-four of the 36 participants who seroconverted during the course of iPrEx had samples available for analytical pharmacology, but only 3 had detectable study drug in their plasma or peripheral mononuclear cell samples at the time of HIV infection diagnosis [6]. In CAPRISA, tenofovir was detected in genital tract secretions of just 36% of seroconverters, compared with 83% of HIV-uninfected women [38]. Of course, these measurements only function as surrogates for adherence behavior, because determinations of ARV levels are not made at the time of transmission.

The development of resistance could also be viewed as a surrogate for adherence. Consider HIV-infected patients receiving combination ARV therapy. With very poor adherence, they are essentially protected from acquiring resistance mutations; insufficient systemic concentrations of drug are present to exert any meaningful selection pressure on the virus. Among highly adherent patients, suppression of viral replication eliminates opportunities for mutations to develop and propagate. Those patients in the middle of the adherence spectrum are at greatest risk for resistance.

PrEP presents a more complicated problem. For both topically and orally administered PrEP, poorly adherent recipients are unlikely to develop resistance but are more likely to become HIV-infected; as adherence improves, the risk of HIV acquisition is reduced. However, with increasing exposure to partially

suppressive ARVs comes an increasing risk of selecting resistance mutations. Thus, the *absence* of detectable resistance among PrEP recipients who became HIV-infected during iPrEx and CAPRISA can yield some insight into the minimum level of adherence needed to select for resistance. Because of the poor systemic absorption of tenofovir after intravaginal application [39], women in CAPRISA may have been functionally protected from developing resistance. Two of the 3 iPrEx participants with detectable drug levels reported at least 50% adherence, providing additional circumstantial evidence that the level of adherence required to select for NRTI mutations (detectable by bulk sequencing) is closer to that of protease inhibitors (~85%) than to that of nonnucleoside reverse transcriptase inhibitors (NNRTIs; ~10%) [40].

### **WHAT ARE THE CONSEQUENCES OF PRE-EXPOSURE PROPHYLAXIS FAILURE, IN TERMS OF ANTIRETROVIRAL RESISTANCE?**

The consequences of PrEP failure can be viewed at both individual and population levels. Consider a hypothetical PrEP recipient with modest adherence who becomes HIV-infected during a high-risk sexual encounter. Understandably concerned about the event, this person begins taking the ARVs as originally prescribed. For this individual and other, similar individuals, all initial preferred regimen options are potentially compromised. Currently, the World Health Organization recommends pairing zidovudine or tenofovir with lamivudine or emtricitabine as the dual-NRTI backbone of efavirenz- or nevirapine-based first-line ARV regimens [41]. M184V markedly reduces susceptibility to both lamivudine and emtricitabine, enhances the activity of zidovudine and tenofovir, and leaves efavirenz or nevirapine as the only other fully active agent in the regimen. Unfortunately, these 2 NNRTIs have low genetic barriers to resistance; single mutations can cause cross-resistance, rendering both agents inactive [17]. Second-line regimens involve swapping out the NNRTI for a boosted protease inhibitor and interchanging zidovudine and tenofovir, depending on which agent was used in the initial regimen [41]. Thus, M184V by itself can significantly increase the risk of failure for NNRTI-based first-line regimens that are the standard of care globally, and it can impact the efficacy of second-line regimens in resource-limited settings as well.

On a population level, the principal concerns include the potential for PrEP to influence the prevalence of resistance and limited access in the developing world to ARVs beyond second-line regimens. Transmitted drug resistance, the primary acquisition of a strain of HIV that is already resistant to at least 1 ARV, ranges in prevalence from 10% to 15% in the United States [42] and Europe [43], although signature mutations for tenofovir and emtricitabine consistently occur at very low frequencies among treatment-naïve individuals. M184V is detected in 1%–1.5% of

pretreatment samples; K65R is seen in <0.5% [42, 43]. Mathematical modeling studies have examined different PrEP efficacies and the downstream effects on the prevalence of resistance, with mixed results [44]. Generally, modeling shows that use among undiagnosed, HIV-infected persons (as in our hypothetical example) raises the prevalence of acquired drug resistance, whereas risk compensation among PrEP users could act to negate its overall effect on reducing HIV infection incidence [45]. Partners of individuals who develop resistant HIV infection from exposure to PrEP agents may themselves be partially protected from infection, owing to reduced transmission efficiency of viruses with resistance mutations—especially M184V [46].

### **HOW SHOULD THESE RESISTANCE DATA GUIDE OUR MANAGEMENT OF PRE-EXPOSURE PROPHYLAXIS AS WE MOVE FORWARD?**

What iPrEx, CAPRISA, Partners PrEP, and TDF2 have demonstrated is not the effectiveness of PrEP but rather its efficacy within structured clinical trials settings. Oral contraceptives offer a useful analogy: with perfect daily use, 3 out of 1,000 women will become pregnant, whereas under typical usage conditions, 90 out of 1000 actually become pregnant [47]. It is reasonable to believe that PrEP will exhibit a similar disparity between efficacy and effectiveness when brought into mainstream use. In practice, PrEP recipients will not have the benefit of study monitors and frequent reminders to get HIV testing. It is precisely that scenario—partially suppressive ARVs administered over long periods without close supervision—that heightens the risk of resistance. If oral tenofovir-emtricitabine is ineffective in certain subpopulations for reasons other than adherence (as suggested by the investigators of FEM-PrEP) [37], the risk of resistance will be present even in the absence of any benefit from daily ARV prophylaxis.

We also must be cognizant of the role that frequent HIV testing will play in the long-term success of PrEP and in containment of resistance should breakthrough infections occur. Our timeline for resistance mutation emergence suggests that testing should be at least every month and preferably should use a sensitive assay capable of detecting early HIV infection (eg, a fourth-generation antibody/antigen assay or nucleic acid amplification testing). However, given 2009 statistics showing that only 19% of Americans aged 18–64 years received an HIV test in the past 12 months [48], testing on so frequent a schedule does not seem plausible. Identifying strategies to pair frequent testing with PrEP administration should be a research priority, as we move forward.

Infectious diseases consultants are confronted each day with the consequences of poor antimicrobial stewardship, and until now, this responsibility has remained exclusively with medical providers. However, PrEP changes the equation. For the first

time, prescribers will be asking patients to become responsible stewards and counting on them to manage these medications appropriately. In light of the individual and public health consequences of PrEP failure, this is a heavy burden to impose on persons with varying degrees of biomedical understanding. It is therefore incumbent upon providers to scrutinize candidates for PrEP and make decisions about the appropriateness of these medications on a case-by-case basis. Prescription of PrEP indiscriminately will place in jeopardy the long-term utility of tenofovir and emtricitabine as first-line agents. Providers must act cautiously and wisely as PrEP becomes a reality.

## SUMMARY

Recent successes with the use of ARVs for HIV infection prophylaxis have understandably sparked excitement among providers and prevention scientists. This must be tempered with clear-eyed assessments about potential long-term consequences of resistance despite benefits of PrEP in the short term. Mutations that impact the efficacy of first-line ARVs can develop in as little as 2 weeks of daily PrEP administered to HIV-infected persons, whether the infection was undetected at baseline or acquired while receiving PrEP. If the prevention of new infections is offset by an increase in resistance and a loss of principal treatment options, then perhaps the costs of PrEP outweigh its advantages.

PrEP holds promise as a tool for prevention, but it may not be the best option for every person at risk for acquiring HIV. As evaluations of PrEP continue, a principal goal must be to determine a profile of patients who are most likely to benefit from the intervention—and most likely to comply with a regimented care plan incorporating both administration of PrEP and frequent HIV testing. Furthermore, studies are needed to determine how best to optimize adherence among PrEP recipients and how to intervene if the drugs are not taken properly.

None of the challenges posed by PrEP are insurmountable, but as we move forward with this newest part of our armamentarium, we must act carefully and responsibly—and require that recipients of PrEP do the same.

## Notes

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