Review

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PrEParing for the unexpected: mechanisms and management of HIV pre-exposure prophylaxis failure

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Pre-exposure prophylaxis (PrEP) for HIV is a proven and effective tool for preventing HIV. However, there are instances where individuals taking PrEP have contracted HIV infection. Most of these cases are due to nonadherence to the drug, while other cases of apparent PrEP failure are due to unrecognized HIV infection at baseline. Importantly, there are also now at least three well-documented cases of PrEP failing despite adequate adherence; these are cases of PrEP 'breakthrough'. This article outlines the potential mechanisms of PrEP failure, as well as how to identify and manage these patients. Finally, we provide a perspective on the future of PrEP as a key tool in preventing HIV worldwide.

First draft submitted: 7 May 2018; Accepted for publication: 29 June 2018; Published online: 7 September 2018

Keywords: HIV • pre-exposure prophylaxis (PrEP) • prophylaxis failure

Pre-exposure prophylaxis for HIV (known as PrEP), is a new paradigm in HIV prevention. PrEP has been shown to be highly effective at preventing HIV when individuals are able to access and adhere to the drug, and is generally safe [1]. As the use of PrEP becomes more common worldwide, an important concern that has arisen is that of 'PrEP failure'. PrEP failure is when an individual becomes infected with HIV despite taking PrEP, and can arise due to several mechanisms. As more cases of PrEP failure arise, understanding the causes of PrEP failure, and how to prevent and manage these cases, will be essential. In this review paper, we summarize the evidence for the use of PrEP to prevent HIV, describe instances of PrEP failure in research and clinical settings, summarize the currently understood mechanisms of PrEP failure, and outline approaches to recognizing and managing these cases.

PrEP as an effective tool for HIV prevention

PrEP with two antiretroviral drugs, tenofovir disoproxil fumarate and emtricitabine (TDF/FTC), is a proven new tool for HIV prevention. PrEP is either taken once a day, or episodically (see exact dosing regimen below) around times of high-risk exposures, in order to decrease the chance of contracting HIV. Numerous studies have now investigated the efficacy of PrEP in various populations, including heterosexual populations, gay, bisexual and other men who have sex with men (gbMSM), trans women who have sex with men, and individuals who use injection drugs [2–13]. A summary of the main efficacy studies conducted to date are presented here.

The Partners PrEP, TDF2, VOICE and FEM-PrEP studies investigated heterosexual women and men at mostly African sites, and some sites in the USA. The Partners PrEP study assessed 4747 HIV-1 serodiscordant heterosexual couples in Kenya and Uganda, divided into three arms: TDF (1584 participants), TDF/FTC (1579 participants) and placebo (1584 participants) [2]. There were 82 new HIV-1 infections in total during the follow-up period. This translated to a relative risk reduction in incidence of HIV of 67% with TDF (95% CI: 44–81; $p < 0.001$) and 75% with TDF/FTC (95% CI: 55–87; $p < 0.001$). There was no significant difference between the two active regimens ($p = 0.23$). Among those with detectable drug levels in blood, the relative risk reduction improved to 90%. In the TDF2 trial, 1219 heterosexual men and women in Botswana were randomized to TDF/FTC

or placebo [3]. However, the study was concluded early due to low retention and logistical issues. In a modified intention-to-treat analysis that included 33 participants who became infected on the study (9 in TDF/FTC arm, 24 in placebo), efficacy of TDF/FTC was 62.2% (95% CI: 21.5–83.4; p = 0.03). The VOICE trial was a randomized placebo-controlled trial with 5029 heterosexual females in South Africa, Zimbabwe and Uganda, divided into four groups: TDF, TDF/FTC, 1% TDF vaginal gel and placebo [4]. There were 312 new infections on the study, but there HIV incidence was not significantly different in any of the arms. Similarly, the FEM-PrEP trial randomized 2120 women in Kenya, South African and Tanzania to TDF/FTC or placebo, and observed no difference in HIV incidence between arms [5]. However, a major challenge in both studies was maintaining adherence to the interventions, which likely accounts for the negative findings.

Studies that investigated PrEP efficacy in gbMSM and trans women include iPrEx (the only study to explicitly include trans women who have sex with men), IPERGAY (the only study to investigate an intermittent PrEP regimen) and the PROUD study. In iPrEx, 2499 gbMSM and trans women who have sex with men were randomly assigned to TDF/FTC or placebo [6]. Overall, the intervention arm showed a 44% reduction in HIV incidence (95% CI: 15–63; p = 0.005). When participants had detectable drug concentrations, the reduction improved to 92%. IPERGAY was a double-blind, randomized controlled trial which studied intermittent dosing of PrEP in gbMSM in France and Canada [7]. Participants were randomized to a regimen of two doses of TDF/FTC (300 mg/200 mg per pill) or placebo 2–24 h prior to sexual activity, one dose 24 h after and one dose 48 h after. There was a relative risk reduction in HIV-1 acquisition of 86% (95% CI: 40–98; p = 0.002) in the TDF/FTC arm. Participants took a median of 15 pills per month. Sustained efficacy was observed in the IPERGAY Open Label Extension, where the relative reduction in HIV incidence with on-demand PrEP compared with the placebo arm of the randomized phase was 97% (95% CI: 81–100), and the median number of pills used was 18 per month interquartile range (IQR 11–25) [8]. In the PROUD study, 544 gbMSM were randomized to either start daily TDF/FTC immediately, or defer using PrEP for 1 year. The trial was discontinued early due to a dramatic relative risk reduction for HIV acquisition of 86% (90% CI: 64–96; p = 0.0001) in the immediate versus deferred group [9].

In the Bangkok Tenofovir study, 2413 participants who use injection drugs were divided into two arms: 1204 to daily dosing of tenofovir, and 1209 to placebo [10]. There were 17 new infections in the tenofovir group, and 33 in the placebo group, translating to a relative risk reduction of 48.9% (95% CI: 9.6–72.2; p = 0·01). In those with detectable tenofovir concentrations in their blood, the risk reduction improved to 74%.

Together, this evidence base has clearly demonstrated that when taken appropriately, PrEP is a safe and effective tool for HIV prevention. Efforts have thus increasingly turned to scaling up the rollout of PrEP among at-risk populations, and real-world experience has corroborated the excellent effectiveness of this intervention. For instance, the open-label PROUD demonstration project showed that PrEP reduced HIV incidence by 86% (90% CI: 64–96; p = 0.0001) among gbMSM attending sexual health clinics in England [9]. In an observational study in a clinical setting providing PrEP in San Francisco (Kaiser Permanente Medical Centre), there were no new HIV diagnoses during 388 person-years of follow-up [11]. In the US Demonstration Project, 437 gbMSM and trans women from two sexually transmitted infection (STI) clinics initiated and were retained on PrEP, and there were only two new HIV infections during the study (0.43 infections per 100 person-years [95% CI: 0.05–1.54]) [12]. The individuals who developed infections had tenofovir diphosphate (TFV-DP) levels consistent with two or fewer doses per week. In an ongoing implementation study in New South Wales, Australia, gbMSM deemed high risk are being rapidly recruited to initiate PrEP at over 20 clinics across the state [13]. As of September 2017, there was only one confirmed case of seroconversion among study participants. HIV diagnoses among gbMSM in the state have declined by 35% less than a year after the start of recruitment, and early HIV infections in gbMSM have declined 44%. The potential for PrEP to aid in curbing HIV epidemics worldwide is considerable and promising.

PrEP failure

Since the advent of PrEP, there have been a number of documented cases of it failing, in both research and clinical settings. Occasionally, PrEP can appear to have failed in individuals who initiate the medication in the context of unrecognized HIV infection at baseline [2,6,14]. However, the vast majority of cases of PrEP failure are simply due to nonadherence to the drug. Of greatest concern, there have now been at least three well-documented cases of TDF/FTC-based PrEP failure where adherence appears to have been adequate, and the individual was confirmed to be HIV negative prior to starting PrEP, suggesting true PrEP 'breakthrough'. Breakthrough infections have also been seen in the context of TDF alone. Distinguishing between these scenarios can be challenging because robust adherence data are often not available in clinical practice, and because the finding of HIV drug resistance in a PrEP

Table 1. HIV-1 mutations associated with altered tenofovir and emtricitabine activity.

Mutation	Selected by	Effect	Notes
M184I/V	FTC, 3TC	Reduces susceptibility to FTC and 3TC >100-fold Increases susceptibility to TDF, AZT, d4T Slows development of resistance to TDF, AZT, d4T	M184I tends to emerge before M184V (as it is from a more common HIV-1 nucleotide substitution) but M184V usually outcompetes it within weeks of viral replication
K65R	TDF, ABC, d4T, ddI, 3TC (rarely)	Reduces 3TC and FTC susceptibility five- to tenfold Reduces TDF, ABC, ddI susceptibility about twofold Reduces d4T susceptibility about 1.5-fold	Rarely occurs with TAMs due to bidirectional antagonism
Thymidine analog mutations – Type 1: M41L, L210W, T215Y – Type 2: D67N, K70R, T215F, K219 Q/E	AZT and d4T	Negative impact on virologic response to TDF, ABC, ddI (Type 1 >Type 2)	Development of M184V blunts effect of TAMs on TDF, AZT and d4T, and reduces susceptibility to ABC and ddI
K70E/G/Q/T/N/S	TDF, ABC, d4T	Reduces susceptibility to TDF, ABC, d4T Causes low-level resistance to FTC, 3TC	Uncommon K70Q/T/N/S are less likely to have aforementioned effects
Q151M complex		Low level resistance: TDF, FTC, 3TC High level resistance: AZT, d4T, ddI, ABC Intermediate-level resistance (if in combination with two or more accessory mutations)	Accessory mutations: A62V, V75I, F77L, F116Y
Beta3-Beta4 insertions		Intermediate-level resistance to FTC, 3TC High-level resistance to remaining NRTIs	Usually occur in combination with multiple TAMs T69 insertions: greatest reduction in TDF susceptibility

3TC: Lamivudine; ABC: Aabacavir; AZT: Zidovudine; d4T: Stavudine; ddI: Didanosine; FTC: Emtricitabine; NRTI: Nucleoside reverse transcriptase inhibitor; TAM: Thymidine analog mutation; TDF: Tenofovir disoproxil fumarate.
Adapted with permission from [16].

user can be either the cause or the result of the infection, as described further below. Here, we provide an overview of these scenarios, summarize the proposed mechanisms to explain them, and describe the lessons that each one offers for how PrEP should best be delivered.

Unidentified baseline infection

It is critical to conduct a thorough assessment for HIV infection prior to initiating PrEP because of the risk of developing HIV drug resistance. This risk exists because established HIV infection must typically be treated with at least three antiretroviral drugs in combination. The use of only two nucleoside reverse transcriptase inhibitors such as TDF and FTC is generally inadequate to achieve full virologic suppression, and rapidly selects for resistance mutations (a summary of mutations that can potentially affect the activity of TDF and FTC can be found in Table 1). Early studies on HIV monotherapy and dual therapy show why this is important. For example, in a human study on emtricitabine monotherapy, M184V mutations developed in 8 of 38 evaluable participants within 15 days of therapy [15].

In comparison, monotherapy with TDF was assessed in two human studies and both showed that no mutations developed after 28 days of exposure (at which point the study was concluded) as shown by bulk sequencing [17,18]. However, animal studies show discrepant findings. In a study of 12 macaques with established simian/HIV (SHIV) infection who were treated with daily tenofovir monotherapy, K70E mutations were detectable after a median of 2 weeks. K65R mutations were detectable at low levels as early as 2 weeks, but were only detected by bulk sequencing at 8 weeks (range: 4–20 weeks) [19].

Studies of zidovudine/lamivudine (ZDV/3TC) dual therapy suggest that M184 mutations also appear to emerge readily, while protecting against the emergence of resistance to zidovudine [20–22]. Whether M184 mutations analogously protect against the emergence of K65R mutations is not clearly known from clinical data, because TDF/FTC became available in an era when dual therapy was no longer considered appropriate.

Given the ease and speed at which mutations develop in both individuals taking monotherapy and dual-therapy regimens, ruling out HIV infection prior to initiating PrEP is essential. Indeed, the original randomized trials of PrEP demonstrated that the risk of developing HIV drug resistance was greatest among those who started PrEP with unrecognized baseline infection, rather than those who acquired HIV during follow-up, as summarized in Table 2. Guidelines thus provide recommendations on how to confirm the absence of HIV infection prior to starting PrEP. A thorough exposure history is important in all cases, to determine the timing of the most recent high-risk exposure. In high-resource settings, individuals being considered for starting PrEP should also have their

Table 2. Summary of HIV infections, resistance and suspected breakthrough cases in major pre-exposure prophylaxis trials.

Study	Infected on study		Unrecognized baseline infection		Possible breakthrough cases
	Infected	Resistant	Infected	Resistant	
iPrEX [6]	100 (36 on TDF/FTC, 64 on placebo)	None	10 (2 on TDF/FTC, 8 on placebo)	2 on TDF/FTC (M184V/I) 1 on placebo (M184V)	
Partners PrEP [2]	103 (21 on TDF/FTC, 30 on TDF, 52 on placebo)	3 on TDF/FTC (2 M184I/V, 1 M184I/V + K65R) 1 on TDF (M184I/V) 2 on placebo (M184I/V)	18 (4 on TDF/FTC, 8 on TDF, 6 on placebo)	2 on TDF/FTC (M184V) 1 on TDF (K65R/K70E)	3 participants in TDF/FTC arm with low-frequency M184V/I or K65R mutations. However, 2 had undetectable TDF levels at first sign of infection. Third case had detectable TDF throughout the trial and M184V mutation in 1.9% of virus variants 1 month after infection; however, cannot exclude intermittent PrEP taking
TDF2 [3]	33 (9 on TDF/FTC, 24 on placebo)	1 on placebo (K65R at low levels)	3 (1 on TDF/FTC, 2 on placebo)	1 on TDF/FTC (K65R, M184V, A62V)	
FEM-PrEP [5]	68 (33 on TDF/FTC, 35 on placebo)	4 on TDF/FTC (M184I and M1841V) 1 on placebo (M184I/V)	5 (1 on TDF/FTC, 4 on placebo)	None	3 women on TDF/FTC had FTC resistance, but baseline infection cannot be ruled-out
VOICE [4]	312 (52 on TDF, 61 on TDF/FTC, 61 on TFV gel, 130 placebo)	1 on TDF/FTC (M184I/V)	22	2 on TDF/FTC (M184V)	
iPrEx OLE [25]	41 (28 on TDF/FTC, 13 not)	1 on TDF/FTC (M184V)			
PROUD [9]	23 (3 immediate, 20 deferred)	0 on TDF/FTC	3 (2 immediate, 1 deferred)	2 on TDF/FTC (M184V/I)	
IPERGAY [7]	16 (2 on TDF/FTC, 14 on placebo)	None	3 (details unavailable)	Unknown	

FTC: Emtricitabine; PrEP: Pre-exposure prophylaxis; TDF: Tenofovir disoproxil fumarate.

HIV-negative status documented using a fourth-generation assay [23]. This ELISA assay detects both HIV antibodies (IgG and IgM, which typically take a few weeks after infection to appear in the plasma), and the HIV p24 antigen (which is detectable in the plasma earlier, beginning at 3–4 weeks and lasting until 5–6 weeks after infection). For this reason, the window period for this test (i.e., the period of time after infection during which the absence of detectable antibody or antigen may lead to a false-negative result) is a median (interquartile range) of 18 (16, 24) days, but may reach up to 42 days [24]. This is a considerable improvement over the window period seen with third-generation tests, which have window periods of 22 (19–25) days.

Because the window period limits the sensitivity of all antibody-based testing during early infection, clinical assessment for signs and symptoms of acute HIV infection is also recommended at each appointment, prior to starting or continuing PrEP. Findings may include: fever, weight loss, anorexia, fatigue, gastrointestinal upset, rash, headache, lymphadenopathy, pharyngitis, myalgias or arthralgias, aseptic meningitis, oral ulcers and leukopenia [26–28]. When acute HIV infection is strongly suspected, an HIV RNA nucleic acid amplification test (NAAT) test should be done, if available. The window period for this test is approximately 7–10 days [29]. Alternatively, a fourth-generation assay can be repeated after 7–21 days. Initiation of PrEP should be deferred until such testing confirms the absence of HIV infection. In cases of a recent possible exposure to HIV, HIV testing should be repeated before initiating PrEP, in order to take into account these window periods.

PrEP failure due to nonadherence

There is general agreement that the vast majority of cases of PrEP failure are simply due to poor adherence to the drug, as evidenced by the low PrEP drug levels seen in seroconverters within the main PrEP efficacy trials (Table 2) [14,23,30]. For instance, in the iPrEx trial, a subgroup analysis among those randomized to active drug showed that 8% of those who developed HIV infection had detectable levels of study drug in either plasma or peripheral blood mononuclear cells (PBMCs), compared with 54% of individuals who did not develop infections and were considered to be 'on treatment' (taking drug on >50% of days) [6]. In the Partners PrEP trial, 29 individuals from the TDF or TDF/FTC groups who became infected had drug levels measured; 31% had detectable TDF levels in plasma taken at the seroconversion visit, compared with 82% of 902 plasma samples taken from a random

subgroup of 198 participants who did not acquire HIV [2]. Detectable levels of drug were associated with a reduction in the relative risk of acquiring HIV of 86% for the TDF group, and 90% for the TDF/FTC group. In the TDF2 study, of the four individuals in the TDF/FTC group who developed HIV, half had detectable drug levels in plasma sampled before and closest to their estimated seroconversion date [3]. In contrast, among 69 participants matched by sample date and who did not seroconvert, 80% had detectable levels of TDF, and 81% had detectable levels of FTC.

Because the amount of time required for drug exposure to produce *de novo* drug resistance is known, finding such mutations when a much shorter time interval has elapsed since the last documented negative HIV test may argue against nonadherence as the mechanism of PrEP failure. In contrast, finding wild-type virus despite a lengthier period of potential drug exposure is more consistent with PrEP nonadherence. Obtaining HIV genotyping as soon as possible in PrEP patients with newly diagnosed HIV infection may thus be helpful in uncovering the cause.

Data on episodic use of PrEP as a prevention strategy, as well as pharmacokinetic studies of FTC and TDF, provide further insight into the relationship between adherence and PrEP efficacy. Through triangulation of clinical data from the iPrEX trial and open-label extension with pharmacokinetic data from the STRAND study, it has been possible to correlate the number of daily doses of PrEP taken per week with various levels of protection from HIV in gbMSM. In an early model, the reduction in HIV acquisition among gbMSM was estimated at 76% if two doses were taken per week, 96% for four doses per week and 99% for seven doses per week [31]. TFV-DP levels of 16 fmol per million PBMCs were associated with a 90% reduction in HIV acquisition compared with placebo [31]. More recently, models have been developed based on TFV-DP levels in dried blood spots (DBS), which provide a more reliable marker of long-term PrEP adherence [32]. In individuals with DBS TFV-DP concentrations consistent with two or fewer tablets per week, HIV incidence was 2.3 infections per 100 person-years. For two to three tablets, incidence was 0.6 per 100 person-years, and for four tablets or more, there were no infections per 100 person-years ($p < 0.001$) [25]. Therefore, daily dosing of PrEP may not be necessary to achieve high levels of protection from HIV, at least among gbMSM, but poor adherence appears to significantly reduce the efficacy of PrEP.

Pharmacokinetic studies have also assessed how quickly FTC and TDF metabolites accumulate and reach steady-state concentrations in various tissues, which informs clinical recommendations about how many daily doses should be taken before protection is likely. Two studies have shown that steady-state concentrations for FTC in PBMCs are reached after either 2 or 3 days [33,34]. In contrast, TFV-DP steady-state concentrations in PBMCs may be reached between 3 and 11 days [33,35]. In rectal tissue specifically, steady-state concentrations were achieved after 5 days [33]. In addition, TFV-DP tends to concentrate in rectal tissue up to ten-times more than in PBMCs, seminal cells and cervical tissues [33]; this finding may underlie the high efficacy observed in gbMSM, in whom rectal exposure remains the primary route of HIV acquisition.

Of relevance to episodic PrEP use, a substudy of the ANRS IPERGAY trial assessed pharmacokinetic parameters after a single dose of two TDF/FTC tablets (i.e., 600 and 400 mg, respectively) in 12 men [35]. Emtricitabine was detectable in rectal biopsies 30 min after ingestion and was detectable up to 24 h later, at mean concentrations comparable to steady-state concentrations in people with HIV on long-term TDF/FTC-based antiretroviral therapy. Tenofovir was not detected in rectal biopsies until 24 h postingestion, and the levels were lower than those on long-term TDF/FTC therapy. In plasma, the time to maximum concentration was 1 h, and 2 h for tenofovir and emtricitabine, respectively. The maximum concentrations were high, at 401 and 2868 μg/l, respectively.

Taken together, these data have led most experts to recommend that daily PrEP be taken for at least 1 week before users consider themselves to be 'protected'. However, it should be noted that we still do not conclusively know what drug levels for either FTC or TDF confer full protection from HIV, and the true values could be less than those achieved at steady state.

Both FTC and TDF have been shown to be detectable in the female genital tract (FGT) within 2–4 h, and reach a peak concentration by 6 h [36]. TDF metabolites have been reported to concentrate at levels 10–100-times higher in rectal mucosa than in the FGT [34,37]. In contrast, FTC metabolites may concentrate up to 140-times higher in FGT than in rectal mucosa. Pharmacokinetic models have shown that after three daily doses of TDF/FTC, ≥98% of individuals achieve mucosal tissue protection [34]. However, to protect the FGT, pharmacokinetic models project that at least six of seven doses per week would be required, whereas only two of seven doses per week would be required to protect colorectal tissue. This modeling data are consistent with the Partners PrEP trial which showed that with high levels of adherence to TDF/FTC, there was a 92% risk reduction (95% CI: 19–99%) for women [2]. Therefore, while PrEP appears to have high efficacy for protecting the FGT from HIV when taken almost daily, low

adherence to PrEP may affect FGT protection more significantly than colorectal protection. In addition, current data suggest that intermittent dosing, such as used in IPERGAY, would be insufficient to protect the FGT.

PrEP breakthrough due to infection with multidrug-resistant HIV

Of the first three well-documented cases of individuals contracting HIV while fully adherent to PrEP, two are believed to be due to infection with PrEP-resistant virus. Prior to the first case being reported in 2016 [38], the possibility of this scenario had already been demonstrated in macaque studies, in which six macaques received weekly doses of TDF/FTC 3 days prior to, and 2 h following, rectal exposure with SHIV containing the K65R mutation [39]. During a 28-week period in which viral doses were gradually escalated, the temporal risk of infection was reduced 5.2-fold (95% CI: 1.2–22.2) by TDF/FTC ($p = 0.0280$). However, four of six macaques contracted SHIV by the end of the study. Another study in macaques investigated the efficacy of TDF vaginal gel at protecting against vaginally transmitted SHIV with K65R mutation [40]. In the control group, four of six macaques developed infection after a median of five exposures. In the intervention group, which used 1% TDF gel 30 min prior to twice-weekly vaginal exposure to SHIV, five of six macaques remained uninfected after 20 exposures. Both studies showed that vaginal TDF and oral TDF/FTC may provide some protection against PrEP-resistant virus, but that breakthrough is possible.

The first human case is a 43-year-old man who had been on daily PrEP with TDF/FTC for 24 months and reported having condomless, receptive anal sex with multiple men during the 2–6 weeks preceding his infection [41]. The patient reported 100% adherence, which was supported by pharmacy records and measurement of TFV-DP levels in DBS. On day 0, when the patient was first tested for HIV and was antibody screen reactive, p24 antigen reactive and western blot nonreactive, tenofovir concentrations were 152 ng per milliliter (as measured by liquid chromatographic-tandem mass spectrometry of plasma samples), consistent with recent administration of TDF. Dried blood spots obtained on day 24 showed a TFV-DP level of 2297 fmol per punch, which is consistent with long-term adherence (steady-state concentrations of TFV-DP after 8 weeks of daily TDF/FTC administration are typically 1560 ± 468 fmol per punch) [32].

Genotypic and phenotypic testing revealed an M184V mutation, which compromises the activity of FTC, and several thymidine analog mutations (TAMs) and revertant substitutions including 41L, 69D, 70R and 215E, which decrease TDF susceptibility [42]. Given the short duration of TDF/FTC exposure between documentation of his infection and collection of the plasma sample that was genotyped, and the mechanism of tenofovir resistance being related to TAMs, which are primarily selected for by thymidine analogs rather than TDF, the authors concluded that this virus was transmitted with the mutations already present, rather than being selected for due to the use of PrEP [42]. Because the patient did not wish to stop his PrEP medication, his therapy was empirically expanded to include darunavir, ritonavir and raltegravir, and ultimately tailored to darunavir/cobicistat, rilpivirine and dolutegravir when the results of resistance testing came back.

In the second case, a 26-year-old male in New York City developed HIV infection after starting PrEP [43]. He was in a serodiscordant relationship with another male, and had two instances of insertive, condomless anal sex with different partners approximately 1 and 3 months prior to developing HIV. TFV-DP levels were measured approximately 1 month after the first positive HIV test, revealing a DBS concentration of 1478 fmol per punch, and a concentration in a hair sample of 0.0448 ng/mg. Both levels are consistent with adequate adherence before and following the first documented signs of seroconversion. Genotypic and phenotypic analysis revealed both the K65R and M184V mutations, conferring resistance to TDF and FTC, respectively (see Table 1). They also found mutations to nonnucleoside reverse transcriptase inhibitors (NNRTI), specifically K103S, E138Q and Y188L. The virus was confirmed to be different to the virus infecting his partner, and so transmission from his partner was ruled out. The conclusion was that a multidrug-resistant virus was transmitted as NNRTI resistance would be unlikely to have developed under the selection pressure of TDF/FTC. However, because sampling was not done at the time of infection, it remains possible that the virus had existing NNRTI resistance, and developed NRTI mutations under selective pressure from TDF/FTC alone. In this case, treatment was intensified with dolutegravir after confirmatory tests confirmed HIV infection.

These cases raise questions about the prevalence of circulating 'PrEP-resistant' virus in areas where PrEP is being scaled up, and demonstrate that different resistance pathways in the HIV-1 reverse transcriptase can produce PrEP failure. The mechanism of reduced TDF susceptibility in the first case was the presence of multiple TAMs, conferring a 1.3-fold decrease in susceptibility, as opposed to the K65R mutation seen in Case 2, which confers a 2-4-fold decrease [44]. The authors of the first case noted that in British Columbia, Canada, the frequency of

Table 3. Summary of proposed mechanisms of pre-exposure prophylaxis breakthrough.

Mechanism	Explanation
Transmission of multidrug-resistant HIV	Transmission of PrEP-resistant HIV from an infected partner, causing PrEP to be ineffective. Likelihood depends on the local epidemiology of resistance mutations in circulating virus
High burden of viral inoculum	A high volume of viral inoculum, or frequency of inoculation, which is able to overcome the local protective effects of PrEP
Disruption and inflammation at the mucosal barrier	Repetitive mucosal injury from intercourse, as well as concomitant STIs, are independent predictors of HIV transmission and could overwhelm the efficacy of PrEP
Variable pharmacokinetics of PrEP in mucosal tissues	Unknown factors could produce inter- and intraindividual variability in the concentration of tenofovir and emtricitabine in the rectal and genital tract, which may alter the efficacy of PrEP locally
Tenofovir monotherapy	Breakthrough may occur due to inadequate tenofovir levels and/or variable pharmacokinetics of tenofovir, without an additional agent to provide protection
Parenteral transmission	The evidence base regarding the efficacy of PrEP for preventing HIV transmission related to injection drug use is limited to a single randomized trial of TDF versus placebo, in which it was not possible to determine whether efficacy resulted from preventing sexual versus IDU-related transmission [10]

IDU: Injection drug use; PrEP: Pre-exposure prophylaxis; STI: Sexually transmitted infection; TDF: Tenofovir disoproxil fumarate.

resistance (score ≥30 using the Stanford Drug Resistance database algorithm) to FTC, TDF or both in 2014/2015 was 1.7, 0.004 and 0.001%, respectively of all 9643 patients followed during this time period. In surveillance of genotyping data from King County, Washington, intermediate to high levels of resistance to TDF/FTC (also using the Stanford Drug Resistance database) were found in 6% of available sequences, but viremia with such sequences was estimated at 0.4–0.6% of all people with HIV in the county [45]. In addition, a 2012 review revealed tenofovir resistance in only 0.4% of 19,823 reverse transcriptase (RT) sequences from treatment-naive HIV-1-infected individuals worldwide, but having more than or equal to three TAMs was the most common mechanism (0.27% prevalence), followed by K65R (0.1%) and K70E (0.015%) [46]. A more recent study observed a high frequency of K65R/N or K70E/G/Q-mediated tenofovir resistance at first-line treatment failure, ranging from 20% in western Europe to 60% in west/central Africa, but TAMs were not considered in this analysis [47]. As PrEP rollout expands worldwide, continued surveillance of the mutations that may affect PrEP efficacy is needed.

PrEP breakthrough due to other mechanisms

The third case of PrEP breakthrough involves a 50-year-old male who was part of the Amsterdam PrEP project [48]. He had been on a daily TDF/FTC PrEP regimen for 8 months prior to testing positive for HIV on a fourth-generation HIV test, and reported mostly receptive condomless anal sex with roughly 12–75 sexual partners per month during the 3 months prior to his infection. In addition, he reported using a range of substances during sexual activity, including injection drugs, but reported using clean equipment each time.

At month 8, he was tested for HIV after presenting with symptoms ultimately due to an *Escherichia coli* urinary tract infection, and anal lymphogranuloma venereum infection. Serology tests could not initially confirm the diagnosis, but PrEP was stopped due to a high suspicion for HIV. After 3 weeks, he had detectable HIV RNA in his blood. In contrast to the above two cases, resistance testing revealed wild-type HIV-1. Tenofovir concentrations in his DBS sample were 2234 fmol/punch, indicating adherence was likely adequate. After the HIV infection was confirmed at this time, he was started on HIV therapy with TDF, FTC, darunavir/ritonavir and dolutegravir.

While the exact cause of PrEP failure in this instance is unknown, several factors may have contributed. A summary of the currently proposed mechanisms of PrEP breakthrough can be found in Table 3. It seems most likely that rectal mucosal injury due to the amount and frequency of condomless receptive anal sex, rectal lymphogranuloma venereum infection (itself associated with both prevalent and incident HIV, and a potentially high burden of viral inoculum related to multiple partners [49]) may have overwhelmed the effect of PrEP, and led to repeated, localized infection in the rectum. In addition, injection drug use as a source of infection could not be ruled out either; the Bangkok Tenofovir study shows us that while PrEP may be effective in people using injection drugs, it is unclear whether PrEP protected against sexual or injection drug exposure.

Variable pharmacokinetics of TDF and FTC in rectal mucosa could also have contributed. That plasma viremia was only detectable after the cessation of PrEP may support this notion, if infection was initially confined to the mucosa while the medications were present, and then spread systemically after they were stopped. However, documentation of wild-type virus and the patients' sigmoid biopsies being negative for HIV at the time of infection argue against this theory.

An additional proposed mechanism of PrEP breakthrough with wild-type virus is viral sequestration. Such a mechanism has been proposed by the authors of a case report of HIV postexposure prophylaxis (PEP) failure, in which a 53-year-old female treated with PEP after a needlestick injury developed HIV [50]. She initiated PEP within 2 h of exposure and completed a 4-week course, with a 4-day interruption at days 22–25. Six weeks after the completion of PEP, she developed symptoms of acute HIV infection, and subsequently was diagnosed with HIV. Single-genome sequencing of plasma viral RNA ruled out infection with drug-resistant virus. The authors of this case report hypothesized that virus was sequestered by dendritic cells, making PEP ineffective at fully eradicating the HIV. Once PEP was ceased, the sequestered virus was able to replicate, resulting in acute HIV infection. While this mechanism is yet to be supported by further evidence or other cases, this phenomenon may become relevant for individuals who discontinue PrEP.

Failure of TDF alone as PrEP

TDF alone has been studied as a regimen for PrEP in several studies, and may have cost advantages over TDF/FTC in resource-limited settings. Although the continuation phase of the Partners PrEP trial found that the efficacy of TDF was not statistically significantly different from that of TDF/FTC, the point estimate for this comparison favoured dual therapy (hazard ratio = 0.67; 95% CI: 0.39–1.17). There have been two cases of breakthrough with wild-type HIV in individuals using TDF alone (300 mg once daily) for Hepatitis B therapy [51]. At the time of diagnosis, both individuals had consistently undetectable levels of hepatitis B DNA for ≥3 years, and tenofovir plasma levels were therapeutic for both hepatitis B and HIV, suggesting that transmission was not due to poor adherence.

A 2008 study of macaques challenged weekly with rectal SHIV predicted that dual-agent PrEP regimens would be more efficacious than single agent regimens [52]. Macaques were divided into four groups that received the following: subcutaneous human-equivalent dose of FTC, oral human equivalent dose of TDF/FTC, subcutaneous human equivalent dose of TDF/FTC and intermittent subcutaneous human dose equivalent TDF/FTC (2 h before and 24 h after exposure to virus). There were six new infections in a 14-week period; four among macaques on FTC monotherapy and two among macaques on oral TDF/FTC. The relative risk reduction of HIV infection in the first two groups (where infections occurred) was, respectively, 3.8 and 7.8-fold lower than untreated macaques ($p = 0.02$ and $p = 0.008$, respectively). The remaining groups did not have new infections. This data supports the use of a dual-regimen over monotherapy, in addition to providing support that intermittent PrEP usage can be an effective prevention strategy. The WHO recommends TDF alone as an alternate PrEP regimen for resource-limited settings [53]. However, dual-therapy with TDF/FTC is preferred in high- and middle-income countries [23,53–56].

Recognizing PrEP failure

As PrEP becomes more widely utilized, it is essential that providers know when to suspect PrEP failure, and be able to confirm it. As discussed above, confirming the absence of infection prior to initiating PrEP is critical, and should include both laboratory (e.g., a fourth-generation ELISA test) and clinical (screening for signs and symptoms of acute HIV infection) methods [23,53–56]. Current guidelines recommend that repeat assessments occur every 3 months and that HIV negativity be documented prior to renewing prescriptions [23,53–56]. Recently reported cases in which drug-resistant HIV was detected after refill prescriptions were provided without requiring HIV testing demonstrate the importance of regular HIV testing [57].

Importantly, it is unclear whether being on PrEP alters the operating characteristics of current HIV diagnostic tests. In one study among adults with acute HIV infection initiating antiretroviral therapy, HIV-specific antibody production was often reduced or halted altogether, with baseline fourth-generation testing being nonreactive in 18% of participants. Of those nonreactive to the fourth-generation assay, 76% were Fiebig stage I and the remainder were Fiebig stage II and III [58]. In addition, in this study, fourth-generation HIV tests were more sensitive than third-generation tests at detecting acute HIV infection prior to starting antiretrovirals (ARVs), but third-generation tests were more sensitive once therapy was initiated. For individuals at Fiebig stages I and II, for example, the third-generation assay was nonreactive in only 7% of cases at 24 weeks of therapy, while the fourth generation was nonreactive in 29% of individuals at this time. As PrEP rollout expands, it will be critical to gather detailed clinical and serial HIV testing data from the rare individuals who do acquire HIV despite PrEP, to better understand how its use alters the performance of existing diagnostic tools.

Another critical aspect of reducing the risk of PrEP failure is to continually assess and counsel patients about medication adherence. Adherence may decrease over time, and there are several reports of PrEP patients who

unfortunately seroconvert after their adherence patterns change from regular to inconsistent dosing [59]. For example, at a clinic in Montreal, Canada, which follows several hundred PrEP patients, HIV incidence was 3.9/100 person years among patients who stopped PrEP [60]. The reasons for stopping varied widely, and included economic, adverse drug effects, and changes in relationships.

Strategies to support adherence should be multifaceted, and can include interventions such as ensuring proper education and counselling about HIV, PrEP and side effects; screening and support for comorbid conditions, such as substance use and depression; self-efficacy-based interventions (such as smartphone apps); and external reminders (text messages, support groups).

Management of suspected PrEP failure

There are two potential approaches to managing suspected cases of PrEP failure. The first option is to stop PrEP immediately and retest the patient after the test 'window period' has elapsed (up to approximately 42 days after exposure for a fourth-generation test, or up to 10 days after exposure if an HIV viral load or HIV RNA NAAT is used) to confirm the diagnosis. Advantages of this approach are that it minimizes the risk of resistance if HIV is present, by minimizing selection pressure on the virus, and that it is feasible for non-HIV specialists to implement. Disadvantages of this approach are that if the patient has a true infection, the virus will have time to replicate, and the patient will be more likely to transmit virus to partners if continuing to engage in high-risk activity [61].

The second option is to intensify the patient's drug regimen, by empirically adding antiretroviral drugs to which the patient's potential HIV infection is likely to be susceptible. A recommended approach would involve adding a potent integrase strand transfer inhibitor such as dolutegravir, given the low rates of circulating integrase strand transfer inhibitor resistance in most global settings, as well as the effectiveness of these agents at rapidly reducing viral load [62–64]; together with ritonavir- or cobicistat-boosted darunavir, given the potency of this protease inhibitor against drug-resistant HIV [23,65–67]. Benefits of this approach include the potential to limit the size of the viral reservoir at its earliest stage, high likelihood of preventing the emergence of resistance and ability to reduce the chance of onward transmission due to virologic suppression [61]. However, initiating therapy early may make it challenging to confirm the diagnosis, and may be less feasible in some settings since nonspecialists may not be comfortable prescribing these drugs, and due to the need for immediate access to drug insurance.

Conclusion

As PrEP use becomes more widespread, awareness and understanding of PrEP failure will become important. Most cases of PrEP failure are due to unrecognized baseline infections, and low levels of adherence to PrEP. There have been three documented cases where PrEP failure is not explained by these causes; in these cases, several mechanisms have been proposed, including the transmission of 'PrEP-resistant' HIV. There are several guidelines that outline approaches to detecting and managing cases of PrEP failure.

Future perspective

As research into the field of PrEP failure evolves, there are several areas of high importance. First, a deeper understanding regarding the pharmacokinetics of PrEP drugs in varying tissues is needed, along with the implications this has on PrEP efficacy and intermittent dosing regimens for different populations. Second, continued surveillance will be needed regarding the resistance profile of circulating viruses in different geographic settings, since the prevalence of PrEP-resistant virus will dictate the risk of PrEP breakthrough. Third, investigation of the influence of PrEP on the operating characteristics of HIV tests during acute infection will be essential to helping clinicians monitor and manage patients with suspected PrEP failure. Finally, as more reports of PrEP breakthrough arise, elucidating the underlying mechanisms will be crucial to managing and preventing further cases.

Although daily and on-demand oral TDF/FTC remain the only proven PrEP options at present, clinical trials are currently investigating the efficacy of oral tenofovir alafenamide/emtricitabine or TAF/FTC (in the Gilead-sponsored DISCOVER trial) and of injectable cabotegravir or cabotegravir (CAB; HIV Prevention Trials Network protocol 083) as PrEP. In general, the potential mechanisms of failure for these agents if they become approved for a PrEP indication in the future would likely be based on the same principles: inadequate adherence could lead to incident HIV infections, PrEP-resistant virus could lead to breakthrough infections, and variable tissue pharmacokinetics and virus sequestration could jeopardize PrEP efficacy under unique circumstances. A major advantage of injectable PrEP is that it obviates the need for daily (or on demand) oral medication adherence, although the long terminal half-life of such agents implies that engagement in care will be critical to ensure that

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missed doses do not lead to incident infections as drug levels fade. Transitioning those who discontinue injectable PrEP onto short-term oral agents with a shorter half-life may be required to cover the 'pharmacokinetic tail' associated with such depot medications. While the mutations conferring resistance to TAF/FTC are the same as for TDF/FTC, since TAF is simply a different prodrug of the active moiety TFV-DP, a potential advantage of cabotegravir-based PrEP is the unique resistance profile of this agent [68] and low prevalence of integrase inhibitor resistance circulating in most global settings [65–67]. Ongoing surveillance of relevant HIV mutations, and further study into the tissue distributions of TAF and CAB will be needed if these agents become available as PrEP in the future.

Executive summary

Pre-exposure prophylaxis as an effective tool for HIV prevention

- Pre-exposure prophylaxis (PrEP) with two antiretroviral drugs, tenofovir disoproxil fumarate and emtricitabine (TDF/FTC), is a safe and efficacious tool for HIV prevention.

PrEP failure

- PrEP failure is mostly due to drug nonadherence, but there are now at least three documented cases of PrEP failing when adherence was adequate.
- Ruling out baseline HIV infection prior to initiating PrEP is essential, to help prevent the emergence of antiretroviral drug resistance in HIV.

PrEP failure due to nonadherence

- Nonadherence to PrEP explains the majority of cases of PrEP failure.
- In some cases, it may be challenging to differentiate failure due to nonadherence versus viral resistance if resistance testing is not done.

PrEP breakthrough due to infection with drug-resistant HIV

- There are at least two well-documented cases of PrEP breakthrough due to infection with 'PrEP-resistant HIV'.
- In the first case, PrEP resistance was attributed to the M184V mutation in reverse transcriptase, along with several thymidine analog mutations and revertant substitutions including 41L, 69D, 70R and 215E.
- In the second case, PrEP resistance was attributed to the K65R and M184V mutations in reverse transcriptase.

PrEP breakthrough due to other mechanisms

- At least one well-documented case of PrEP failure with wild-type HIV-1 has been documented, in which the mechanism for failure is unclear.
- Proposed mechanisms include: high burden of viral inoculum, disruption and inflammation of the mucosal barrier, variable pharmacokinetics of PrEP in mucosal tissue and potential parenteral transmission.

Failure of TDF alone as PrEP

- There are at least two well-documented cases of individuals taking TDF for Hepatitis B therapy who acquired HIV despite good drug adherence.
- TDF alone is an effective PrEP regimen, but TDF/FTC is recommended over TDF alone when it is available to avoid PrEP failure.

Recognizing PrEP failure

- Frequent follow-up and HIV testing every 3 months is essential for recognizing incident HIV infections among PrEP users.
- It is unclear to what extent being on PrEP may affect the window period of HIV tests.
- Adherence to PrEP is a key component of preventing PrEP failure, and is a crucial area where health providers can intervene.

Management of suspected PrEP failure

- There are currently two main approaches to managing suspected PrEP failure.
- The first option involves stopping PrEP immediately and re-testing after the window period to confirm HIV infection.
- The second option is to intensify a patient's drug regimen by empirically adding antiretroviral drugs to which the patient's potential HIV infection is likely to be susceptible.

Conclusion & future perspective

- As PrEP use worldwide becomes more common, being able to identify and manage cases of PrEP failure is essential.
- Further research should investigate the pharmacokinetics of PrEP drugs in varying tissues, the epidemiology of PrEP-related resistance mutations in different geographic regions, and how HIV testing evolves under the influence of PrEP.
- New PrEP agents are being developed which may offer advantages related to pharmacokinetic properties, potential for drug nonadherence, and viral drug resistance.

Financial & competing interests disclosure

Dr Tan is supported by a New Investigator Award from the Canadian Institutes of Health Research and Ontario HIV Treatment Network, and has received grants from Gilead Sciences Inc. and ViiV Healthcare. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

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