HIV-1 Drug Resistance in Pre-exposure Prophylaxis Trials

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Contents

Abstract

When used with other proven strategies for prevention of HIV-1 acquisition, oral and topical preexposure prophylaxis (PrEP) has been shown to be effective in multiple randomized, placebo-controlled clinical trials throughout the world. Preexposure prophylaxis trials have included over 20,000 men and women at

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risk for HIV infection through sexual or intravenous exposure. A consistent finding is that drug exposure is essential for PrEP efficacy. In PrEP users with breakthrough infection, selection of drug-resistant virus is a possible outcome, presenting a unique sequence of events and outcomes compared with therapeutic use of antiretroviral drugs. Study findings have indicated that drug resistance selected by PrEP occurs rarely, except in cases where PrEP is initiated in very early infection, prior to seroconversion, and detectable only with nucleic acid tests. In this review, we discuss the factors associated with PrEP which may contribute to drug resistance and summarize the frequency and characteristics of HIV-1 drug resistance reported to date from global clinical trials. A theoretical framework of the causes and consequences of drug resistance in PrEP is considered as a basis of the real-life outcomes and challenges in implementing PrEP.

Introduction

The concept of chemoprophylaxis, or using antimicrobial agents in uninfected humans to prevent infection, is a widely used and successful strategy for prevention of infection with endemic microbes such as malaria (Breman and Brandling-Bennett 2011). One of the great successes in HIV prevention is providing antiretroviral therapy (ART) perinatally to infected pregnant women to block mother-to-child transmission (MTCT) (Connor et al. 1994). Now with the availability of a large number of potent antiviral drugs, coupled with reduced toxicities and convenient dosing formulations (Gandhi and Gandhi 2014), the benefits of using oral or topical ART as preexposure prophylaxis (PrEP) may outweigh risks of prolonged drug exposure in healthy, uninfected people. As a result, using preexposure prophylaxis (PrEP) to prevent HIV infection in at-risk individuals has moved from the conceptual realm (Youle and Wainberg 2003a, b) to phase II/III safety and efficacy trials and now to initial implementation in demonstration projects and clinical practice.

However, as was revealed in early single-dose treatment strategies for MTCT prevention, suboptimal exposure to ART can result in PrEP failure and selection for drug-resistant variants in the infected infants (Arrive et al. 2007; Eshleman and Jackson 2002; Eshleman et al. 2001; Johnson et al. 2005; Micek et al. 2010). Due to the incidence and nature of drug resistance in this setting, concern over the use of PrEP for sexual transmission has been raised (Cohen and Baden 2012; Hurt et al. 2011; Liu et al. 2006). While the clinical impact and treatment options of viruses harboring drug-resistance mutations acquired by suboptimal ART initiated after established infection are well known and may be relevant to breakthrough infections during PrEP, the potential impact of PrEP-selected drug resistance at the population level is less clear. The benefits (infections averted) versus risks (drug resistance) with PrEP use have been modeled with significantly differing outcomes and interpretations based on input variables and assumptions (Abbas et al. 2011;

Baggaley et al. 2011; Dolling et al. 2012). In addition, in antiretroviral drug-experienced populations, the prevalence of circulating strains with drug resistance to PrEP agent(s) may impair the efficacy of PrEP. Now, with accumulating results from initial global randomized PrEP efficacy trials, the benefits and risks of PrEP use for HIV acquisition can undergo evidence-based assessment, allowing an in-depth understanding of the nature and frequency of PrEP-associated drug resistance, a critical step toward optimizing its use as a prevention strategy in all at-risk populations.

PrEP Efficacy for Prevention of HIV Sexual Transmission: Summary of Results from Randomized Controlled Trials

There are now multiple reports from randomized double-blind placebo-controlled clinical trials spanning four continents and over 20,000 individuals testing the safety and efficacy of oral and topical PrEP coupled with other proven prevention strategies [reviewed in (Baeten and Celum 2013; Celum and Baeten 2012)]. Topical PrEP as 1 % tenofovir (TVF) vaginal gel used pre- and postcoitally was first shown to be effective in preventing HIV transmission by sexual exposure in African women (Abdool Karim et al. 2010). Daily oral dosing of tenofovir disoproxil fumarate (TDF, the oral prodrug of TFV) used alone or co-formulated with emtricitabine (FTC) proved efficacious in preventing sexual transmission in men who have sex with men and transgender women (MSM/TGW) from South America, South Africa, Thailand, and the United States in the iPrEx study (Grant et al. 2010), in serodiscordant African male and female partners in the Partners PrEP study (Baeten et al. 2012), and in African men and women in the TDF2 study (Thigpen et al. 2012). Finally, the Bangkok tenofovir study demonstrated that daily oral TDF dosing was associated with a 48.9 % reduction in HIV infections in injecting drug users randomized to taking TDF compared with placebo (Choopanya et al. 2013). A consistent finding from these studies showing a reduction in HIV-1 acquisition ranging from 42 % to 73 % in participants randomized to the PrEP arms is that PrEP efficacy is directly associated with drug exposure. In nested, case-control studies within each of these trials, the overall relative infection risk reduction further increased to over 90 % in participants with measurable plasma or cellular drug levels.

Not all PrEP trials with similar designs have shown reduced infections in the active drug arms when compared to placebo. The FEM-PrEP study, which enrolled African women, was stopped early due to futility where a similar infection frequency occurred in participants randomized to oral FTC/TDF and placebo (Van Damme et al. 2012). And in the multi-arm VOICE trial, a statistically indistinguishable number of infections occurred in women randomized to either 1 % TFV vaginal gel, daily oral TDF or FTC/TDF, or placebo (Marrazzo et al. 2013). The basis of differences in efficacy outcomes between these two studies and those demonstrating protection against HIV acquisition is an active area on investigation. One key factor is product adherence, determined directly by antiretroviral drug level measurements in the blood plasma and cells. Overall, women randomized to the active arms in the

FEM-PrEP and VOICE trials had insufficient product use to measure efficacy. While it is evident that distinct PrEP modalities will need to be tailored to particular at-risk populations and their circumstances contributing to HIV-1 transmission and that tenofovir-based PrEP regimens can effectively block HIV acquisition when used regularly and in combination with other prevention methods, a setting of incomplete adherence coupled with exposure risk potentially increases the chances of infection and selection for PrEP-associated resistance.

Selection and Expansion of Drug-Resistant HIV in Response to Suboptimal Antiretroviral Therapy

There are now nearly three decades of experience with therapeutic antiretroviral agents designed to target multiple stages in the HIV-1 life cycle (Arts and Hazuda 2012). Regular use of combination therapy can provide durable virologic suppression within an individual to levels below that detected by standard clinical viral load assays and can have a favorable impact in lowering the community viral load or the aggregate level within a defined geographical region (Das et al. 2010; Montaner et al. 2010). But although the available arsenal of antiretroviral drugs shows continued improvement in potency, pharmacodynamics, formulation, and toxicities, the generation and selection of drug-resistant variants continue to be a barrier to durable suppression, especially in developing countries with limited regimen choice and lack of regular virologic monitoring (Hamers et al. 2013; Sigaloff et al. 2011).

In individuals with existing infection and ongoing viral replication, sustained use of non-suppressive therapy or intermittent use of suppressive therapy will quickly promote selection and expansion of drug-resistant variants. Even during subsequent virologic suppression following a regimen change, drug-resistant variants remain archived in target cells as proviral DNA, potentially limiting future therapeutic options. Distinct outcomes of ART use following established infection within an individual are shown schematically in Fig. 1a–c to highlight the dynamic makeup of quasispecies that may arise during treatment failure. The same ART used as PrEP can also select for drug-resistant variants when breakthrough infections occur or when PrEP is initiated with unrecognized infection (Fig. 1d–f), underscoring the importance of careful virologic monitoring before and during PrEP use. The outcomes schematized in Fig. 1 have been observed in many of the PrEP trials where regular serologic testing was performed.

The development of resistance to any particular drug is driven by the high error rate in HIV-1 reverse transcriptase. With a mutation frequency of approximately 4×10^{-5} per target base per replication cycle and a nearly 10 kb genome size, there is roughly one mutation produced per replication cycle (Mansky 1996; Mansky and Temin 1995). Coupled with an estimated 10^{10} virions produced per day (Ho et al. 1995; Wei et al. 1995; Perelson et al. 1996), the fixation of a new, randomly generated mutation under targeted selection can be rapid, as notably illustrated by M184V selection after suboptimal lamivudine monotherapy (Wainberg et al. 1995; Larder et al. 1995; Schuurman et al. 1995). While mutations conferring reduced
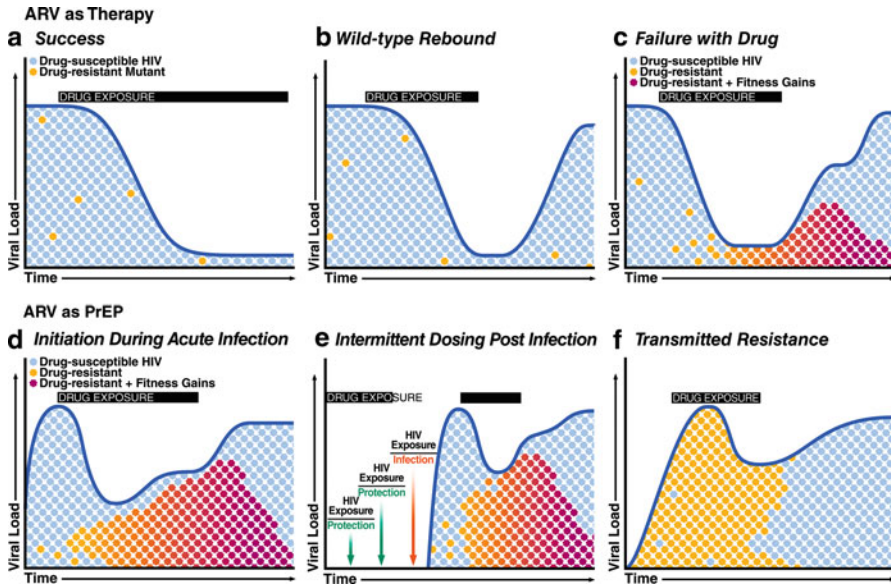
Fig. 1 Antiretroviral (ARV) drug exposure and the emergence of drug-resistant variants when used as therapy versus PrEP. The frequency of emergent drug-resistant variants and relative abundance within the viral quasispecies during distinct treatment modalities are influenced by factors such as the genetic barrier in establishing the codon(s) conferring resistance, the drug activity within a given target of viral replication, and the replication capacity in a particular environment (fitness). The *top panel* (**a**–**c**) is a schematic of possible outcomes in which ARVs are administered therapeutically, after incident infection. (a) Through random mutation, drug-resistant variants are generated sporadically in individuals with ongoing replication but remain at residual levels in settings of successful therapeutic ARV. (**b**) Treatment interruption leads to rapid virologic rebound of the more highly fit, wild-type species. (**c**) Drug-resistant variants may be selected in settings of non-suppressive therapy where continued exposure with ongoing replication may select for increasingly more fit viruses (*darker symbols*). If drug exposure is removed, residual archived wild-type virus will typically outgrow the drug-resistant species and predominate. The *lower panel* (**d**–**f**) shows possible outcomes when ARVs are inadvertently administered as PrEP in a setting of unrecognized infection. (**d**) When administered as PrEP in a setting of unrecognized infection, inadvertent postexposure initiation of ARV may be ineffective for durable suppression, selecting for minor variant drug-resistant species that may expand and evolve with fitness gains due to continued exposure. Following treatment interruption, archived wild-type virus outgrows. (**e**) Intermittent dosing with temporal lapses of protective drug exposure risks selection and outgrowth of drug-resistant variants during periods of continued ARV exposure. (**f**) Acquisition of transmitted or primary drug resistance to PrEP regimens will result in PrEP failure. Reversion by back mutation to wild-type, drug-susceptible virus can occur after discontinuation of PrEP, followed by eventual outgrowth of drug-susceptible variants if gains in fitness occur. Residual drug-resistant variants remain archived as proviruses in the cellular reservoir and may influence future treatment outcomes

susceptibility to ART often have impaired fitness in the absence of selection compared with wild-type, drug-susceptible strains, continued replication under selection can further select for additional compensatory mutations conferring fitness gains in the host's viral population (Condra et al. 1995; Cote et al. 2001; Gatanaga et al. 2002; Molla et al. 1996; Zhang et al. 1997), altered tropism, and virulence

(Coffin 1995; Kuritzkes 1996; Milich et al. 1993; Nijhuis et al. 2001) as shown in Fig. 1c. Following transmission of monophyletic or a limited number of polyphyletic founder viruses (Keele et al. 2008), the rapid expansion, high mutation and recombination frequencies (Onafuwa-Nuga and Telesnitsky 2009), multiple host, and therapeutic selection pressures can collectively promote the creation of complex viral quasispecies within an individual. Sensitive diagnostic assays that can quantify drug-resistant variants present at a minor proportion of the population within an individual have revealed mutations conferring drug resistance in ART-naïve individuals at residual levels (≈1 %) within the viral quasispecies (Johnson et al. 2007, 2008; Liu et al. 2011; Metzner et al. 2011; Simen et al. 2009; Havlir et al. 1996). Preexisting low-level or minor variant drug resistance in treatment-naïve individuals can affect treatment outcomes, especially with particular NRTI- and NNRTI-selected mutations (Simen et al. 2009; Johnson et al. 2008; Havlir et al. 1996; Metzner et al. 2009; Li et al. 2011).

An alternative source of drug-resistant HIV-1 is that transmitted from a treatment-experienced partner, also known as primary resistance. Individual mutations conferring drug resistance can be detected in upwards of 20 % of the circulating strains in geographical areas that have access to ARV, changing with regional exposure levels and predominate treatment regimens over time (Chaix et al. 2009; Grant et al. 2002; Hamers et al. 2011; Jain et al. 2010; Little et al. 2002; Yerly et al. 2007; Wheeler et al. 2010). Such levels of circulating resistance within a population have driven national treatment guidelines to include baseline, pretreatment genotyping. How transmitted resistance might impact PrEP efficacy is an area of interest, especially where ARV included in PrEP regimens are also a component of first- and second-line therapies. Interestingly, numerous outcome predictions based on modeling the impact of the spread of drug resistance result in disparate scenarios [reviewed in (Baggaley et al. 2011)].

When assessing the role of PrEP agents in contributing to the selection and expansion of drug-resistant viruses, it is important to consider drug resistance in the context of drug exposure within the infection window to aid in differentiating transmitted from acquired (drug-selected) resistance. While transmitted resistance can be unequivocally confirmed by phylogenetic mapping of the source and index virus within the partnership, the presence of drug-resistance mutations associated with any particular PrEP regimen in the absence of drug exposure is highly likely to originate from transmitted strain(s) and not selected de novo by PrEP. As the frequency, nature, and origin (e.g., whether PrEP selected or transmitted) of drug-resistance findings accumulate from randomized clinical trials and demonstration projects, the impact of circulating resistance on PrEP efficacy can be directly assessed.

Detecting Drug Resistance in PrEP Studies

HIV-1 drug resistance in clinical practice is primarily measured and interpreted through two distinct but complementary approaches: (1) genotype testing, which includes direct sequencing of the HIV-1 drug target reading frames, usually *pol*, and

Table 1 Drug resistance assays used in phase II/III PrEP trials

	Genotype	Phenotype	Ultrasensitive
CAPRISA 004	In-house	None	AS-PCR
iPrEx	TRUGENE	PhenoSense	AS-PCR UDS[a]
Partners PrEP	ViroSeq, In-house	None	UDS
TDF2	In-house	None	AS-PCR
FEM-PrEP	TRUGENE	PhenoSense	AS-PCR UDS
Bangkok tenofovir study	TRUGENE	None	None

[a]*UDS* Ultradeep sequencing

(2) phenotypic susceptibility testing, which involves determining the concentration of a given compound necessary to inhibit viral growth in vitro [reviewed in (Tang and Shafer 2012)]. Both approaches measure the bulk population of viruses within an individual and, as a result, are insensitive to viral species carrying drug-resistance mutations below a given threshold (e.g., $\approx$20 % for population genotyping). As a research tool, multiple methods have been used to detect and quantify minor variant drug resistance within a population but below that detected by clinical tests, including allele-specific PCR-based assays that differentiate single-base changes conferring resistance, probe-based ligation assays, clonal sequencing, and highly parallel ultradeep sequencing [reviewed in (Gianella and Richman 2010)]. Ideally, genotype, phenotype, and ultrasensitive detection methods would be used together for monitoring drug resistance in PrEP failures as each approach can provide unique insights into the extent and nature of drug resistance. However, due to the high clinical diagnostic value, global accessibility, standardization of interpretation, and relatively low cost, drug-resistance genotyping is the primary diagnostic tool for drug-resistance monitoring in PrEP clinical trials (Table 1).

Designing PrEP Regimens to Minimize Drug Resistance

When designing effective regimens for PrEP, a number of factors are taken into consideration (Anderson et al. 2011; Derdelinckx et al. 2006; Fernandez-Montero et al. 2012; Garcia-Lerma et al. 2008; Amico 2012). Ideally, these include selecting compounds that target pre-integration events in the viral life cycle, demonstrate high antiviral activity and extended half-life in target tissues, exhibit synergies in activity and mutation impact if used in combination, and posses a high genetic barrier to resistance, which is the combined components that contribute to the generation of the specified resistance mutation and maintenance of the viral species in the population (Luber 2005). For these reasons, coupled with relatively favorable toxicity profiles, flexible formulations, efficacy in preventing transmission in nonhuman primate models under conditions that mimic sexual transmission in humans (Garcia-Lerma et al. 2008; Van Rompay et al. 2006; Subbarao et al. 2006; Radzio et al. 2012), and extensive history of therapeutic use, two nucleoside/nucleotide reverse transcriptase inhibitors (NRTI) have been used in the completed clinical trials to date.
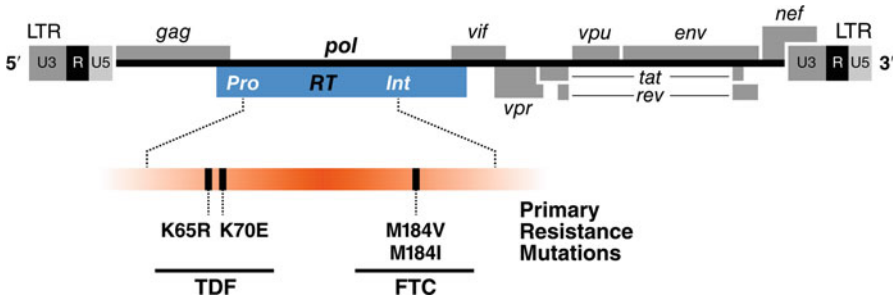
Fig. 2 **Drug-resistance mutations in HIV-1 reverse transcriptase selected by FTC/TDF PrEP.**
Two primary drug-resistance mutations in HIV-1 reverse transcriptase are selected by each of
tenofovir (TFV/TDF) and emtricitabine (FTC). TFV-associated codon changes are K65R (Lys to
Arg) and K70E (Lys to Glu) and FTC-associated codon changes are M184I or V (Met to Ile or Val).
Each mutation confers reduced susceptibility in vitro and in vivo. A single-base nucleotide change
in RT codon A62V (Ala to Val) or S68G (Ser to Gly) does not directly confer changes in
susceptibility to TFV but is a compensatory mutation associated with TFV exposure and partially
restores viral replication capacity impairment conferred by K65R

Tenofovir (TFV), formulated as either a 1 % topical vaginal gel or as the orally
available prodrug tenofovir disoproxil fumarate (TDF), has been administered as
PrEP alone or together with emtricitabine (FTC). The co-formulated FTC/TDF oral
pill TRUVADA™ is cleared by the US Food and Drug Administration for use as
prevention in uninfected adults at high risk of HIV acquisition through sexual
exposure. Both compounds act at pre-integration steps by terminating the nascent
DNA chains in RNA-dependent and DNA-dependent DNA synthesis during the
viral life cycle (Arts and Hazuda 2012).

The viral mutations associated with reduced susceptibility to TFV/TDF and
related drugs are K65R and K70E (Margot et al. 2006a; Miller et al. 1999; Wainberg
et al. 1999; Gallant et al. 2004) and to FTC are M184V and M184I (M184V/I)
(Margot et al. 2006b), where the first amino acid listed for a given codon in RT
represents the wild-type, drug-susceptible form and the second represents the
mutant, drug-resistant form (Fig. 2). Additional RT mutations A62V and S68G
associated with TDF exposure are considered compensatory mutations that improve
viral replication capacity of poorly fit K65R mutants (Margot et al. 2006b;
Svarovskaia et al. 2008). Although K65R and M184V/I are generated by a single-
base substitution and thus may arise frequently in the course of HIV replication, viral
species with these mutations demonstrate significantly reduced replication capacity
and fitness in vitro and in vivo in the absence of selection (Yerly et al. 2007; Wheeler
et al. 2010; Margot et al. 2006a; Petrella and Wainberg 2002; Miller et al. 2002;
White et al. 2002; Frankel et al. 2007) thus conferring a relatively high barrier to
resistance. Additionally, the presence of M184V causes increased sensitivity to TDF
(Miller et al. 1999; Whitcomb et al. 2003; Deval et al. 2004), a synergy that is often
taken advantage of in clinical practice (Wainberg and Gotte 2000). Finally, these
ARVs provide a strong pharmacological barrier for sexual transmission.
Emtricitabine concentrations are significantly higher in vaginal secretions compared

to that measured in blood after single oral dosing, while TDF-DP (the active intracellular form) is up to $100\times$ higher in the colorectal mucosa compared with vaginal and cervical tissues following a single dose (Anderson et al. 2011; Kwara et al. 2008; Patterson et al. 2011).

Genotypic, Phenotypic, and Minor Variant Drug Resistance in PrEP Trials

To date there are six completed phase III, randomized, placebo-controlled PrEP trials reporting drug-resistance results. The overall study design for monitoring HIV-1 infection status and drug resistance was similar across studies. HIV status at screening, entry, and post-randomization visits was assessed by serologic monitoring. Blood plasma, cells, or other tissue samples were typically collected and archived for retrospective measurements of HIV-1 nucleic acid and/or drug levels and, when collected at sufficient frequency, were used to establish the infection window and drug exposure levels. Upon receiving a positive rapid test result post-randomization, study drug was discontinued and confirmatory serotesting and/or RNA testing performed. In the iPrEx, Partners PrEP and TDF2 studies, participants with unrecognized, acute infection (RNA positive, seronegative) at entry were retrospectively identified. In confirmed seropositives, blood plasma collected at or proximal to the initial seropositive visit was tested for drug resistance by standard genotyping. In some studies, additional diagnostics were performed including drug-resistance phenotype and allele-specific PCR and/or deep sequencing for ultrasensitive detection of minor variant drug resistance (Table 1). In the iPrEx and FEM-PrEP studies, longitudinal sampling and testing was performed to monitor drug resistance over time in participants with FTC/TDF-associated resistance at seroconversion and randomized to the active drug arm (Grant et al. in press; Liegler et al. 2014).

The drug-resistance mutations and frequencies reported from the CAPRISA 004 (Abdool Karim et al. 2010; Wei et al. 2014), the iPrEx (Grant et al. 2010; Liegler et al. 2014), Partners PrEP (Baeten et al. 2012; Lehman et al. in press), TDF2 (Thigpen et al. 2012), FEM-PrEP (Van Damme et al. 2012; Grant et al. in press), and the Bangkok tenofovir (Choopanya et al. 2013) trials are summarized in Table 2, categorized by participants' timing of infection (pre-randomization vs incident) and randomization arm. Overall, in participants with incident (on-study post-randomization) infection, the frequency of TFV/TDF- or FTC-associated drug resistance was low, including those randomized to the PrEP arms with measurable drug levels near the infection window. Of the 142 seroconverters with incident infections and in the PrEP arms of the CAPRISA 004, iPrEx, Partners PrEP, TDF2, and Bangkok tenofovir studies, none showed genotypic or phenotypic drug resistance associated with the PrEP regimens used at or near the seroconversion visit. In contrast, four of 33 (12 %) women on the oral FTC/TDF arm in the FEM-PrEP study showed genotypic and phenotypic resistance to FTC (M184V/I) at the seroconversion visit (Van Damme et al. 2012; Grant et al. in press). Tenofovir resistance was not observed, and two showed phenotypic hypersusceptibility to this drug. Two of

Table 2 Genotypic drug-resistance testing results reported from PrEP trials

Study	Subjects randomized	Agent	Infected at entry				Incident infection			
			Study drug		Placebo		Study drug		Placebo	
			Resist/Tot Tested	PrEP-associated mutations	Resist/Tot Tested	PrEP-associated mutations	Resist/Tot Tested	PrEP-associated mutations	Resist/Tot Tested	PrEP-associated mutations
CAPRISA 004	1,085 women	1 % TFV gel coitally dependent use	0/1	none	0/9[a]	none	0/38	none	0/60	none
iPrEx	2,499 MSM and transgender women	FTC/TDF daily oral use	2/2	M184V selected[b] M184I unknown[c]	1/8	M184V transmitted	0/48	none	0/83	none
Partners PrEP	4,758 male/female couples	TDF daily oral use	1/5	K65R selected[d]	0/6	none	0/15	none	0/51	none
		FTC/TDF daily oral use	1/3	M184V selected[e]			0/12	none		
TDF2	1,219 men and women	FTC/TDF daily oral use	1/1	K65R M184V A62V selected[f]	0/2	none	0/9	none	0/24	none
FEM-PrEP	2,120 women	FTC/TDF daily oral use	0/1	none	0/1	none	4/33	1 ea M184I selected[g] 3 ea M184V 2 transmitted 1 selected[h]	1/35	M184V transmitted
Bangkok tenofovir study	2,413 men and women (IVDU)	TDF daily oral use	none	none	0/2	none	0/15	none	0/32	none

[a]Two participants randomized to placebo were deemed ineligible for study.
[b]Mutation not found at enrollment. FTC-selected resistance
[c]Unable to genotype sample (VL = 48 cps/mL). Presence of mutation at enrollment unknown
[d]Mutation not found at enrollment. TDF-selected resistance
[e]Mutation not found at enrollment. FTC-selected resistance
[f]Mutations not found at enrollment. FTC- and TDF-selected resistance
[g]FTC detected in plasma during infection window
[h]FTC detected in plasma during infection window

these participants, 1 with M184I and 1 with M184V, had moderate- and high-study drug levels at seroconversion, respectively, implicating selection by the PrEP regimen. However, seroconversion occurred within 4 (M184V) and 8 (M184I) weeks of study entry, leaving open the possibility that infection was incubating prior to PrEP initiation – a situation with increased frequencies of emergent drug resistance.

In PrEP studies reporting ultrasensitive testing for minor variant drug resistance in seroconverters performed by AS-PCR and/or deep sequencing, background mutation frequencies (that observed in WT viruses in the absence of drug selection) were established for each individual assay and were typically ≤1 %. While minor variant drug resistance was observed above background levels from seroconverters in both placebo and active drug arms, examples seen in subjects randomized to the PrEP arms and therefore potentially PrEP selected are highlighted here.

In CAPRISA 004 (Wei et al. 2014) and TDF2 (Thigpen et al. 2012), AS-PCR measurements in blood plasma and vaginal swabs (CAPRISA 004) near the seroconversion visit showed no evidence of minor variant resistance to TFV. Seven of 27 (26 %) women in the CAPRISA 004 TFV gel arm had measurable TFV in vaginal fluids. However the majority had insignificant or undetectable TFV levels indicating the absence of drug selection pressure.

In the iPrEx and FEM-PrEP studies, minor variant DR in blood plasma from participants randomized to the FTC/TDF arms was observed, however infrequent and at very low proportions within the population measured by AS-PCR and 454 deep sequencing (Grant et al. in press; Liegler et al. 2011). In iPrEx, one seroconverter's virus had M184I detected at 0.53 % of the plasma viral population by AS-PCR but below background by 454 sequencing This subject had detectable but low drug levels in blood plasma and cells, opening the possibility of selection by PrEP but without significant outgrowth within the population. Similarly, one FEM-PrEP seroconverter showed M184I at 0.66 % of the population but at background levels by 454 sequencing. Study drug was not detected in this woman near the seroconversion window, suggesting spurious detection of drug-resistance mutations near the background cutoff level, rather than PrEP-selected resistance.

Blood plasma samples at the seroconversion and proximal follow-up visits from subjects in the Partners PrEP study (oral FTC/TDF, TDF alone, placebo) were analyzed for minor variant drug resistance by 454 deep sequencing (Lehman et al. in press). Of those in the oral FTC/TDF arm, a virus from 1 subject showed M184V at 16 % of the viral population (SC visit), decreasing to 1.7 % 4 weeks later, without detectable study drug. Viruses from two other participants with detectable drug showed minor variant resistance mutations: 1 with M184V at 1.9 % from the post-seroconversion visit and another with M184V (at 7.7 %), M184I (at 5.4 %), and K65R (at 1.2 %) in the seroconversion visit sample. This rare example of K65R in incident infections may reflect the significantly impaired fitness or replication capacity conferred by K65R, especially when in combination with M184V (Miller et al. 1999, 2002; Margot et al. 2006b; Petrella and Wainberg 2002; White et al. 2002; Frankel et al. 2007), and/or insufficient drug exposure, as PrEP was discontinued at the first evidence of seroconversion. In the oral TDF arm, 1 of 30 participants showed M184I at a low level (2.5 %), a mutation that is not selected

by TDF. This mutation was detected as a minor variant in the placebo arm of Partners PrEP, FEM-PrEP, and iPrEx participants, possibly maintained at low levels by APOBEC3G-induced G-to-A hypermutation (Neogi et al. 2013).

Development of Elevated Drug-Resistance Frequencies When Initiating PrEP During Acute, Seronegative Infection

A striking finding from these studies is the relatively high frequency of PrEP-associated drug-resistance mutations seen in subjects who inadvertently initiated PrEP with unrecognized (RNA positive, seronegative) infection at randomization when compared to that seen in study participants with incident infections. Of the 13 participants with unknown acute infection initiating PrEP at randomization, five in the combined iPrEx, Partners PrEP, and TDF2 studies showed genotypic resistance to FTC (M184V) or TDF (K65R) at their initial seroconversion visit. In four of these participants, the virus at enrollment did not carry these resistance mutations, favoring selection by PrEP regimens during the initial 4 weeks of use. The additional subject from the iPrEx study with M184I had a low viral load at entry that was insufficient for a genotype (48 copies/mL), so it cannot be unequivocally determined whether the mutation conferring resistance to FTC was transmitted or selected. Of note, one subject from the TDF2 study developed multidrug resistance in a stepwise manner over time where M184V was detected at the first visit after study entry, followed by additional TDF-associated mutations K65R and A62V at the second visit 6 months later. Although enrollment of participants with unrecognized acute infection who went on to develop PrEP-selected resistance was rare among all in PrEP studies, the relative frequency of generating resistance in this subset of participants was high (5/13, 38 %) and possibly avoidable with HIV-1 RNA testing prior to PrEP initiation and delaying PrEP in those with symptoms consistent with acute viral infections.

In the absence of continued selection by PrEP, the FTC-associated resistance in blood plasma virions declines to residual levels over time, through outgrowth of the more highly fit WT variant generated through back mutation, or presents at very low levels under PrEP selection. Participants in both the iPrEx and FEM-PrEP trials with FTC resistance mutations M184V/I were followed longitudinally after stopping PrEP for up to nearly 18 months, and blood plasma samples were assayed for the relative proportion of coexisting drug-resistant and susceptible variants by sensitive allele-specific PCR and deep sequencing assays (Grant et al. in press; Liegler et al. 2012, 2014) and unpublished data). In all cases analyzed from both the placebo and control arms (n = 7), the drug-resistant variants proportionally decreased from 95 % to 100 % at seroconversion to residual levels (<0.5 %) in the blood plasma over time. Although most demonstrated a more prolonged time course for complete reversion (median 9 months), one participant showed complete reversion and overgrowth at the RT codon 184 from Ile to Met within 4 weeks of discontinuing study drug. These results are consistent with the time course of transmitted M184V

reversion over time in ARV-naïve subjects (Liegler et al. 2011; Jain et al. 2011) and highlight the value of baseline resistance testing as early in infection as possible.

Distinct Patterns of Drug Resistance in PrEP: What Is Driving It?

The frequencies and nature of PrEP-associated drug resistance fall into distinct patterns that are likely outcomes of multiple diverse factors including the temporal sequela of exposure to drug and infectious virus, the pharmacodynamics of the individual compounds and formulations in diverse anatomical target sites under changing physiologic states, and the genetic barrier to resistance specific for each PrEP regimen and other factors. The interplay between the drug activity, viral resistance barriers, and how these factors might affect the relative risk of infection and frequency of resistance is schematized in Fig. 3. The distinct scenarios diagramed in panels A to D reflect various outcomes noted with use of chemoprophylaxis and HIV infection. Panel A represents effective PrEP where infection occurs only with very low drug exposure and where the overall genetic barrier to resistance is sufficiently high to prevent its emergence. This scenario reflects WT
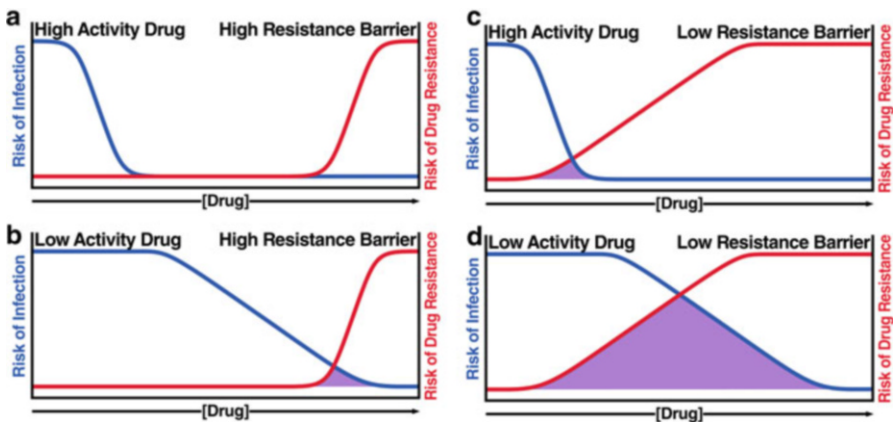

Fig. 3 Schematic of the interplay between pharmacologic and virologic factors that influence the risk of infection and drug resistance in a PrEP setting. Panels **a to d** represent theoretical schematics of the relative frequency of generating drug-resistant HIV-1 (*shaded area* at curve intersections) in settings of breakthrough infection during PrEP use. The relative risk of infection (*blue line*) is plotted against the relative risk of emergent drug resistance (*red line*) with increasing drug concentration at the anatomical and subcellular target of entry. (**a**) In a setting of high drug activity and a high barrier to resistance, the infection window occurs with insufficient drug levels to select for resistance. (**b–c**) Drug resistance can occur, although infrequently with either low drug activity or a low barrier to resistance where drug levels are suboptimal, allowing viral replication, but sufficiently high to select for drug resistance. (**d**). Increased frequency of drug resistance may occur in a setting of both low drug activity and low resistance barrier, such as that resulting from single-dose nevirapine treatment given to pregnant women to prior to delivery to prevent mother-to-child transmission

infection seen in the majority of seroconverters in PrEP studies with low or undetectable drug levels. Panels B and C may reflect the infrequent cases of resistance seen in PrEP where local concentrations of the drug may be insufficient to block infection and/or create a sufficient barrier to resistance. Distinct tissue-specific pharmacodynamics for FTC and TFV may uncouple the combined synergy in target tissues such as the cervicovaginal or colorectal mucosa (Thompson et al. 2013), leading to the observed predominance of FTC-selected mutations M184V/I in FTC/TDF oral PrEP. Finally, panel D represents settings where drug resistance is high, such as that observed with limited dosing monotherapy for prevention of MTCT. A deeper understanding of the factors that influence ARV activity and emergence of resistance in target tissues of viral entry and dissemination is critical for designing more effective PrEP regimens, formulations, and dosing strategies.

Progress Toward Next-Generation PrEP

Favorable results from initial randomized placebo-controlled PrEP efficacy trials and the US Food and Drug Administration's first label approval for an antiretroviral compound to be used as chemoprophylaxis for prevention of sexual HIV-1 transmission have led to demonstration projects worldwide where PrEP efficacy is tested in open-label, clinical settings. Ongoing demonstration projects include daily oral FTC/TDF PrEP [reviewed in (Baeten et al. 2013)], allowing direct comparisons to the PrEP efficacy trials. Comprehensive monitoring for drug resistance and drug exposure in seroconverters from these studies should yield additional insights into the overall impact of PrEP use and drug resistance. There is, however, room for overall improvement in strategies for optimizing PrEP and monitoring virologic, behavioral, toxicity, and other outcomes.

Additional compounds and formulations with improved penetration in target tissues, innovative dosing and delivery strategies, and additional viral targets are needed to further increase PrEP efficacy with expanded use while maintaining low toxicity and high genetic resistance barriers [reviewed in (Abraham and Gulick 2012)]. The ÉCLAIR study is a phase IIa safety and tolerability study evaluating the injectable long-acting investigational integrase inhibitor GSK-744 LA in uninfected men. Promising results were reported using a long-lasting nanoparticle formulation of the HIV-1 integrase inhibitor dolutegravir, with successful protection against rectal SHIV challenges (Andrews et al. 2013). Other long-lasting nanoparticle ARV formulations intended for periodic injections and targeting multiple HIV-1 pol enzymes are in various stages of investigation in small animal models measuring pharmacokinetic profiles in target tissues and cells (Puligujja et al. 2013; Martin et al. 2013).

There are multiple ongoing trials testing oral tenofovir-based PrEP dosing strategies and drug combinations to reduce pill burden and minimize overall drug exposure but maintain effective exposure for situational risk. Intermittent PrEP (pre- and postexposure) use has been shown to be efficacious in reducing SHIV

infection through rectal exposure in macaques (Garcia-Lerma et al. 2010). In this study of multiple dosing strategies, none of the breakthrough infections showed evidence of drug resistance. The ANRS IPERGAY study includes MSM and "on demand" oral FTC/TDF, taken at the time of sexual exposure. The HIV Prevention Trials Network (HPTN) 067 study ADAPT, enrolling MSM/TGW and women who have sex with men (WSM), is a behavioral study with a 1:1:1 randomization of three arms using oral FTC/TDF with either daily dosing, time-driven dosing, or event-driven dosing. The NEXT-PrEP (HPTN 069/ACTG 5305) study is a four-arm phase II safety and tolerability trial investigating combinations of oral daily FTC, TDF, and the HIV-1 entry inhibitor maraviroc (MVC). Drug concentration measurements in all study participants and drug resistance testing in seroconverters in these various studies will aid in determining the oral dosing formulation and timing needed to prevent infection while minimizing exposure for reduced toxicity. This relationship was estimated using drug level measurements in blood and levels of protection from HIV acquisition in the iPrEx trial combined with defined intermittent and daily dosing strategies in the STRAND study (Anderson et al. 2012). While this serves as an important basis for determining the most effective and least harmful dosing strategy, further evaluations within these and other trials are necessary to further optimize the next-generation PrEP for diverse user needs.

Conclusions

The proven efficacy of PrEP in preventing HIV acquisition in clinical trial settings is one of the celebrated successes in HIV prevention research and brings cautious optimism for continued success with more widespread use. One clear message from PrEP trials is that successful PrEP requires drug uptake. The risk of infection increases with suboptimal PrEP use, as does the potential drug resistance. Despite a range of efficacies and adherence levels reported, drug resistance selected by PrEP was largely seen in subjects initiating PrEP during acute, unrecognized infection. Monitoring for acute viral symptomatology and the presence of HIV nucleic acids may be useful diagnostic tools at PrEP initiation. Additionally, using combination regimens and drug formulations with increased potency at PrEP initiation may minimize this occurrence. In incident infections, the occurrence of drug resistance, even as minor variants, was infrequent in participants with measurable drug levels indicating exposure. However, there are limitations in interpreting these findings – in all PrEP trials, study drug was discontinued at the first evidence of infection, thus limiting drug exposure that may generate resistance with longer duration. Guidelines for PrEP use in clinical practice indicate monitoring for infection with PrEP at a minimum of every 12 weeks (Centers for Disease Control and Prevention 2011, 2012), less frequent than the monthly monitoring in clinical trials.

Continued rigorous assessment of drug resistance in breakthrough infections while using PrEP is necessary with expanded use in clinical settings and as other compounds, formulations, dosing strategies, and novel drugs are tested and implemented.

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