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HIV PrEP Trials: The Road to Success

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Summary

The global HIV epidemic cannot be controlled by current treatment or prevention strategies. Pre-exposure prophylaxis (PrEP) using antiretrovirals is a promising approach to curbing the spread of HIV transmission. Recently, four clinical trials demonstrated favorable results when antiretroviral PrEP was administered topically or orally. However, two additional trials were unable to demonstrate a benefit, indicating that further study is required to define the populations and conditions under which PrEP will be effective. Adherence is highly correlated with protection, yet the exact level of adherence required is unknown. Future studies may require increased drug exposure testing and more objective methods to monitor adherence in real-time. Although the development of drug resistance in the PrEP trials has been low, it remains a concern, as therapeutic options could be compromised for those who seroconvert while on PrEP.

Keywords

HIV; prevention; adherence; tenofovir; prophylaxis

Introduction

The HIV epidemic remains a significant global burden. In 2010 there were an estimated 2.7 million new infections worldwide [101]. In low to mid income countries, the rate of new infections is outpacing the rate of initiation of antiretroviral therapy [101]. Therefore, treatment alone will not stem the HIV epidemic, and effective prevention strategies must be implemented to reduce morbidity and mortality. Although interventions such as counseling, condoms, and circumcision, have been modestly successful in reducing the spread of HIV, pre-exposure prophylaxis (PrEP) approaches are also needed to reduce the overall spread of HIV infection.

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PrEP may include topical or systemic interventions that are administered in a scheduled or exposure-driven manner to individuals at high risk of acquiring HIV infection. This may include men who have sex with men (MSM), individuals with infected partners (serodiscordant couples), sex workers, intravenous drug users, or individuals who live in HIV endemic areas. Several products, both topical and systemic, with and without active antiretrovirals, have been considered for use in PrEP. To date, tenofovir (TFV) and emtricitabine (FTC) are the only agents that have demonstrated effectiveness in clinical populations. TFV and FTC are nucleotide/nucleoside analog reverse transcriptase inhibitors, chosen initially to be studied for PrEP due to their efficacy in animal models[1–4], safety profiles, and favorable pharmacokinetic profiles (long half-lives of active metabolites)[5, 6]. Randomized clinical trials have resulted in mixed results with efficacy ranging from 0–75% [7–11] (Table 1). This paper will summarize the findings from clinical antiretroviral-based PrEP trials, identify current gaps and unanswered questions in the prevention field, and review planned studies to assist in the proper implementation of PrEP. Since TFV vaginal gel and TFV and FTC (either dosed orally as single agents or as Truvada ®, a fixed dose combination oral tablet containing both FTC and tenofovir disoproxil fumurate (TDF)) are the only compounds that have completed Phase III trials, this manuscript focuses on data regarding efficacy of these compounds and their utility for PrEP. Other agents that have yet to complete large scale efficacy studies are also reviewed as the next generation of PrEP candidates.

Clinical Trials

Topical Pre-Exposure Prophylaxis: Successes

CAPRISA 004 was the first successful PrEP trial using an antiretroviral to protect against HIV infection. It provided proof of concept for the interventional efficacy of TFV formulated as a topical gel [7]. CAPRISA 004 was a double-blind, placebo controlled Phase II study of 4mL of coitally-applied 1% TFV vaginal gel in 889 HIV negative women at an urban and rural South African site. The prefilled applicator was to be instilled into the vagina up to 12 hours before and after sex for a maximum of 2 applications in a 24 hour period. This was referred to as “BAT 24” dosing (**B**efore and **A**fter, no more than **T**wo doses in **24** hours). The women returned for monthly visits in which they brought back used and unused applicators, and underwent HIV testing, safety assessments, and counseling. After 30 months of follow-up, the study concluded that TFV 1% gel was 39% effective at preventing HIV infection. Efficacy was calculated to be 54% in women who were >80% adherent, based on returned used applicator counts. A subsequent analysis demonstrated that a vaginal luminal TFV concentration ≥ 1,000 ng/mL was predictive of protection against HIV infection, whereas luminal TFV concentrations <1,000 ng/mL had rates of infection similar to placebo [12] It would be expected that consistent dosing using the BAT 24 dosing scheme should have maintained the luminal TFV concentrations >1,000 ng/mL based on findings from a previous Phase I study of vaginally applied TFV1% gel, that found median CVF concentrations of TFV 24 hours after applying the dose, to be 100,000 ng/mL and median vaginal tissue concentrations to be 7,000 ng/mL [13].

Topical Pre-Exposure Prophylaxis: Setbacks

In the early 2000s, six topical surfactants, polyanions, and general antimicrobials (COL-1492, Cellulose sulfate, SAVVY, Carraguard, BufferGel, and PRO2000 Gel) were investigated for prevention of HIV. Although many showed promise in preclinical and Phase I studies, Phase II and III studies showed them to be either ineffective or permissive for HIV infection[14–19]

In contrast to the CAPRISA 004 results discussed above, the Microbicide Trials Network VOICE study (MTN 003) stopped a daily 1% TFV gel arm early due to futility in November 2011 [102]. VOICE is a Phase II 5-arm study in 5,000 women designed to compare the HIV PrEP efficacy of daily vaginal dosing of 1% TFV gel or a placebo gel, daily oral dosing of TDF 300mg, daily oral TDF 300mg combined with daily oral FTC 200mg, or an oral placebo tablet. This surprising result may be explained either by lack of adherence to the daily gel regimen, or by an unforeseen increase in genital tract mucosa permissiveness to HIV with frequent gel dosing. Full study results are expected in early 2013 [102].

Topical Pre-Exposure Prophylaxis: Ongoing Studies of Tenofovir Gel

At the time of this writing (November 2012), a number of studies are ongoing to further assess the utility of 1% TFV gel (Table 2). FACTS 001 is designed to confirm the effectiveness identified in CAPRISA 004 with a BAT24 regimen. It is enrolling 2200 women between 18–30 years of age at 9 study sites in South Africa. The first subject was enrolled in October 2011 and results are expected in 2014 [103]. Also, as a follow-up to CAPRISA 004, CAPRISA 008 is an open label study enrolling 700 HIV uninfected women who previously participated in CAPRISA 004 in order to collect additional safety data and implement a clinical care model for the provision of 1% TFV gel through family planning services [104].

Additional studies involving 1% TFV gel aim to test the safety of topical gel use in pregnant and breastfeeding women [105] and the interaction potential with other vaginally used products including antifungal creams, antimicrobial creams, and contraceptive rings [106]. Since a rectal study of the 1% tenofovir vaginal gel showed it to be neither safe nor acceptable for rectal use [20]. MTN 007 evaluated a reformulated gel (containing, containing less glycerin),, for rectal use in 65 HIV negative men and women. When applied rectally once daily for one week, this new product was found to be safe and acceptable for rectal use [107]. A Phase II study (MTN 017) is planned to further assess safety and adherence of this rectal-specific gel when used both daily and before and after sex in 186 men who have sex with men and transgendered women [108]. The study participants will be using the tenofovir rectal gel in combination with daily oral TDF/FTC for a total of eight weeks.

Oral Pre-Exposure Prophylaxis: Successes

Two Phase II studies have evaluated the safety of daily TDF 300 mg as PrEP. In 2007, FHI360 performed a 12-month study in 936 HIV-negative women from Ghana, Cameroon, and Nigeria who were at high risk for HIV infection. No significant differences were found between the TDF and placebo arms in clinical or laboratory safety outcomes [21]. No patients in the TDF group and 2 in the placebo group experienced ≥ grade 3 elevations in liver enzymes. No patients in either group experienced ≥ grade 3 elevations in kidney or bone toxicity markers. Overall efficacy was not evaluated as 2 study sites were stopped early, which resulted in low statistical power to make this comparison. CDC 4323, a 2-year, 4-arm study evaluated clinical and behavioral safety of daily TDF 300mg in 400 HIV-negative MSM in Atlanta, San Francisco, and Boston. To evaluate whether daily pill use had an effect on behavioral risk in MSM, 2 arms began daily TDF or placebo immediately, and 2 arms delayed daily TDF or placebo for 9 months. Sexual risk behavior did not change significantly in any arm during the trial [22]. A 1.1% (p=0.004), 0.8% (p=0.003), and 0.7% (p=0.11) decrease in bone mineral density (BMD) was associated with TDF use at the femoral neck, total hip, and L2–L4 spine respectively, although no increase in fracture risk was reported [23].

In 2010, the first clinical study to evaluate the efficacy of oral PrEP was published. The iPrEx study was performed in 2499 MSM and transgender women from Peru, Ecuador, South Africa, Brazil, Thailand, and the United States. Study subjects were randomized to receive either a daily placebo or fixed dose combination of TDF 300 mg + FTC 200 mg (Truvada®). In the Truvada arm, there was a 44% (95% CI 15 – 63%) reduction in HIV acquisition compared to placebo [8]. Adverse effects with Truvada that were significantly different from placebo included nausea (2 vs <1% of subjects, p=0.04) and unintentional weight loss (2 vs 1%, p=0.04). In a case-control analysis, detectable drug was found in blood plasma of only 3 of the 34 HIV seroconverters (9%) and in 22 of 43 seronegative matched controls (51%) from the Truvada arm. In comparing the drug concentrations in these subjects to a separate pharmacokinetic study (STRAND) which thoroughly evaluated drug exposure after directly-observed twice weekly, four time weekly, or daily dosing, it was imputed that >50% of the subjects in iPrEX seronegative controls were taking less than 2 doses per week [24]. The degree of protection observed in this study, despite the low level of adherence, may be explained by pharmacokinetic findings by Patterson et al: after a single dose of Truvada, TFV concentrations are up to 100-times greater in colorectal tissue than blood plasma. Although the target concentration for protection has not yet been identified, it's likely that these concentrations are providing a protective effect with sporadic dosing [25].

In 2012, favorable results from the Phase III TDF2 and Partners PrEP studies were published. TDF2 was performed in 1219 heterosexual men and women age 18–39 yrs in Botswana, with subjects receiving daily Truvada or daily placebo tablets. Due to low retention rates (33% of subjects did not complete the study protocol) and the expected inability to meet study power, the study was closed before full enrollment. A significant protective effect of Truvada (62% (95% CI 16–83%) risk reduction) was noted in the final analysis [10]. Nausea, vomiting, and dizziness occurred at a higher rate in the Truvada arm (11.1% (p=<0.001), 4.2% (p=0.008) and 4.1% (p=0.03), respectively), as did a significant decline in BMD (p<0.001 at the hip and lumbar spine; p=0.004 at the forearm). Partners PrEP enrolled 4747 HIV-1 serodiscordant heterosexual couples from Uganda and Kenya who had been in a stable relationship for at least 3 months. The seronegative partner (the male in 62% of the couples) was randomized to receive placebo, TDF, or Truvada daily. A 67% (95% CI 44–81%) relative risk reduction in HIV acquisition was observed in the TDF arm and a 75% (95% CI 55–87%) relative risk reduction was observed in the Truvada arm [9]. Neutropenia was observed more frequently in patients taking active study drug (13% in placebo arm, 15% in the TDF arm (p=0.15), 18% in Truvada arm (p<0.001)). A difference in gastrointestinal side effects and fatigue was observed after 1 month on therapy. These resolved over time, with the exception of diarrhea (1.4% subjects in placebo arm, 1.6% subjects in TDF arm (p=0.21), 1.7% subjects in Truvada arm (p=0.02)). There were no significant differences in kidney or bone markers of toxicity. Detectable drug concentrations were found in 29% of the subjects who seroconverted and in 82% of the samples analyzed from seronegative controls. These results suggest higher overall adherence than was observed in the iPrEX study, possibly due to differences in risk perception for stable couples when individuals know their partners are infected.

Oral Pre-Exposure Prophylaxis: Setbacks

Described above is the success of oral PrEP in three unique populations: MSM, discordantly-infected couples, and high risk heterosexual men and women. However, two additional studies in high risk women evaluating daily TDF with or without FTC were stopped early for futility.

FEM-PrEP was a Phase III placebo-controlled study that was to enroll 2120 HIV negative women ages 18–35 years from Kenya, South Africa, and Tanzania [11]. Subjects were

assigned to receive daily Truvada or placebo for 52 weeks, and HIV acquisition was to be followed over 60 weeks. In April 2011, the independent data safety monitoring board recommended that the study be stopped for futility. Final analysis revealed a relative risk reduction in HIV acquisition in the Truvada arm of 0.94 (95% CI 0.59–1.52). A greater proportion of women in the Truvada arm experienced nausea ($p=0.04$), vomiting ($p<0.001$), and elevated alanine aminotransferase levels ($p=0.03$). A 1.7% rate of drug discontinuation due to hepatic or renal abnormalities was noted. An age, study site, and time of infection matched case-control analysis of TFV plasma concentrations obtained at the HIV detection visit, and the visit prior, demonstrated drug taking behavior in only 20–30% of women in both groups. This level of adherence was considered insufficient to evaluate efficacy. However, this level of adherence may have been similar to iPrEx (with non-seroconverters dosing ≤ 2 times per week). Notably, while high concentrations of TFV have been found in rectal tissues following oral dosing, concentrations in the female genital tract are up to 100 fold lower than in colorectal tissue [25]. Further analysis of drug exposure over time is ongoing to better understand adherence in cases and controls across the entire study enrollment period [26].

The VOICE study has been described above in the context of futility with daily 1% TFV gel. In September 2011, the independent data safety monitoring board recommended that the TDF tablet arm also be terminated early due to futility [109]. It's not clear whether, as with the FEM-PrEP study, this futility is due to poor adherence or other biological factors. The Truvada arm is still active, and the results are expected in early 2013.

To summarize, 3 Phase II/III trials have demonstrated that daily use of TDF with or without FTC can effectively prevent HIV infection. These studies included MSM and heterosexual men and women. Although the numbers are small for statistically powered comparisons, in 2 of the studies, significant difference in protection were not observed based on gender (male vs female) or route of exposure (vaginal vs, rectal vs, penile). In contrast, 2 studies performed exclusively in heterosexual women were stopped, or had arms stopped, for futility. In July 2012 the Food and Drug Administration approved Truvada®, for daily use as pre-exposure prophylaxis in uninfected individuals at high risk of acquiring HIV [110]. With this approval, it is expected that more providers will be initiating PrEP in their high-risk patients. As PrEP becomes more widespread, it is important for providers to understand both the advantages and the current limitations of PrEP. Adherence is known to be a critical predictor of success, but the exact amount of adherence required is unknown. The significant differences in mucosal tissue penetration of TFV and FTC may mitigate poor adherence in MSM and yet require almost perfect adherence in women [25, 27].

Oral Pre-Exposure Prophylaxis: Ongoing Studies of Oral TFV and/or FTC

There are several ongoing clinical efficacy trials (Table 2) that will inform what populations, and under what conditions, oral PrEP should be used clinically. Although the topical TFV and oral TDF arms were stopped early in VOICE (MTN 003), the Truvada arm is ongoing [109]. In light of the FEM-PrEP results, the VOICE results will help establish the utility of daily Truvada in protecting high risk heterosexual women from HIV infection. It will also inform the importance of including FTC in a PrEP regimen, especially if a protective effect is observed in the Truvada.

The Bangkok Tenofovir Study (CDC 4370) is a placebo controlled study in 2400 men and women 20–60 yrs of age and is designed to evaluate the effectiveness of TDF in injection drug users [111]. There are no data for PrEP currently in this population, although intravenous exposure is the most efficient route of HIV transmission. Although it may be difficult to separate out TDF's protective efficacy from exposure due to sexual activity

versus that of intravenous drug use, this study is fully enrolled and results are expected in early 2013.

Two studies are currently evaluating the potential utility of intermittent PrEP. Intermittent PrEP includes any sporadic dosing schedule that differs from once daily dosing. Similar to CAPRISA 004, the hypothesis for intermittent dosing is that comparable protection may be achieved with drug dosing at a time of high risk for HIV acquisition, while pill burden, side effects, and cost may be decreased by not using drug at times of low to no risk. One pilot study determined that adherence to intermittent and/or coitally-dependent regimens may be 28–57% lower than daily dosing [28]. However more quantitative analysis of systemic and/or mucosal drug exposure, and its relationship to protection from HIV infection, is needed to determine the exact level of adherence required for efficacy. Therefore, the ADAPT study (HPTN 067) was designed as a Phase II open label pharmacokinetic (PK) and behavioral study in 540 high risk MSM and heterosexual women in South Africa and Thailand [112]. Once individual PK profiles have been generated from each patient following directly observed weekly dosing for 6 weeks, patients will be randomized to receive either daily Truvada, time-driven Truvada (twice weekly plus post-coital dose), or event driven Truvada (before and after coitus). The primary objective of this study is to determine whether intermittent dosing provides equal drug exposure with fewer pills and fewer side effects. This study will also provide information on acceptability, adherence levels, sexual behavior, and perceptions of different PrEP regimens. ANRS IPERGAY is a Phase III study in 1900 of MSM [113] enrolling in Europe and Canada. This study will compare the effectiveness of Truvada taken only before and after sexual activity compared to a placebo pill over 12–48 months, and will be the first clinical trial to provide efficacy data on intermittent oral PrEP.

Next Generation PrEP

Now that clinical proof of concept data exist for antiretroviral efficacy against HIV acquisition with oral and topical formulations, second generation approaches for PrEP are in development. These include currently used PrEP drugs formulated in new ways (eg vaginal rings, films, and tablets), currently used PrEP drugs combined with newer antiretrovirals (eg TDF combined with maraviroc), or new drugs formulated in novel ways (eg rilpivirine long acting injectable).

Topical PrEP Agents in Development—Vaginal rings are being investigated to deliver topical antiretroviral therapy with less reliance on adherence. Dapivirine, a potent non-nucleoside reverse transcriptase inhibitor (NNRTI) has completed Phase I and II studies for use as a topical gel and is currently in Phase III studies as a vaginal ring [114]. Dapivirine was first conceived as an oral agent, but its physical and chemical properties, also make it a good candidate for topical use [30]. In phase I and II studies, the gel formulation at concentrations up to 0.05% was shown to be safe and acceptable, and to achieve local tissue concentrations above the in vitro IC50 of 0.33ng/mL, while maintaining plasma concentrations <1.1 ng/mL[30–32]. A Phase I study, FAME 02, is planned to assess safety and pharmacokinetics (PK) of both a dapivirine 0.05% gel and a vaginal film, in comparison to a placebo gel and film[33]. The ring formulation of dapivirine, is continuing into Phase III studies. A phase I study of dapivirine 25mg and 200mg rings inserted for 7 days in 25 women found the rings to be safe, and released drug into cervicovaginal fluid at concentrations greater than 1000 times the EC50 (0.3 ng/mL) against HIV-1^{WT} at 4h, 24h, and 7 days after ring insertion [34]. The lowest cervical and vaginal tissue concentrations sampled were above the EC50, and there was minimal systemic exposure. An additional double-blind placebo control study evaluated the safety and PK of the 25mg dapivirine ring in 48 women. A ring was inserted for either 28 days (Group A) or 35 days (Group B) then removed and a second ring was inserted 3 days after removal for another 28 days (Group A)

or 21 days (Group B). A third ring was inserted on day 59 and worn for 24 hours. Dapivirine cervicovaginal fluid concentrations peaked within 24 hours of ring insertion and were maintained well above the IC50 (0.3 ng/mL) at 35 days, with no serious adverse effects [35]. A larger Phase I/II study of dapivirine ring in 280 healthy women, demonstrated a favorable PK profile for monthly use. The rings were inserted every 28 days for 12 weeks and vaginal fluid concentrations remained well above the IC99 (3.3 ng/mL) with >20% of the dapivirine dose released over each 28 day period [36]. Two phase III placebo-controlled studies are currently underway to determine the efficacy of a dapivirine ring against preventing HIV when used monthly. A Study to Prevent Infection with a Ring for Extended use (ASPIRE) is a Microbicide Trials Network (MTN) study at 17 study sites in Africa and plans to enroll 3500 women aged 18–45 and follow them for 12–24 months [114]. The Ring Study, sponsored by the International Partnership for Microbicides (IPM), is enrolling 1650 women in South Africa ages 18–45 who will be followed for 24 months [115]. Results from both Phase III studies are expected in late 2014 or early 2015.

Vaginal rings containing the CCR5 receptor antagonist, maraviroc or a combination of dapivirine and maraviroc have also been developed. A Phase I safety and PK study has recently been completed in 48 women in which a 25mg dapivirine ring, a 100mg maraviroc ring, a combination ring of 100mg maraviroc + 25mg dapivirine, or a placebo ring were each inserted vaginally by 12 women and left inserted for 4 weeks. Safety and acceptability assessments were made throughout the 4 week insertion period and over 24 days afterwards. PK sampling was also performed. Results are expected in early 2013 [116], and will determine if a maraviroc containing ring, or a combination ring containing dapivirine and maraviroc are viable PrEP candidates to move forward into efficacy studies.

Additionally, tenofovir has been developed in a ring formulation. CONRAD 120 is a Phase I study to determine the safety, acceptability, and local PK of a tenofovir-containing ring or a placebo ring inserted for 90 days in 36 women [106]. Vaginal rings containing TFV combined with contraceptive hormones are also in development [107]. An additional novel topical formulation is vaginal dissolving tablets. CONRAD 117 is a planned Phase I study of fast dissolving vaginal tablets containing TFV, FTC, TFV/FTC, or placebo used daily for 7 days in 48 women [106].

Oral PrEP Agents in Development—NEXT-PrEP (HPTN 069) is a Phase II study evaluating the safety and tolerability of the antiretroviral maraviroc (MVC; a CCR5 receptor antagonist), alone or in combination with a second antiretroviral, as an oral PrEP agent [117]. This is the first study to include maraviroc, a drug that works by preventing HIV from entering the target cell, and was designed based on early pharmacokinetic data demonstrating favorable penetration of maraviroc into colorectal and female genital tract tissues. (37,38). The study is being performed in 400 MSM and 200 women in the United States and Puerto Rico. Subjects will be randomized to receive MVC alone, MVC+FTC, MVC+TDF, or TDF+FTC daily for approximately 12 months. The primary objective of this study is to determine the rate of side effects from these regimens as well as tolerability (time to discontinuation). Substudies of this trial include pharmacologic evaluations of drug-drug interactions and the relationship between plasma and mucosal tissue drug concentrations.

Long Acting Injectable PrEP Agents in Development—A number of new drugs are being formulated in novel ways for application to PrEP. Two long acting injectable compounds are currently being developed for monthly to quarterly use as intramuscular injections for the prevention of HIV infection: the NNRTI rilpivirine, and the integrase strand transfer inhibitor (INSTI) GSK 1265744. Rilpivirine has been developed for monthly intramuscular (IM) dosing. A PK study of long acting rilpivirine in 10 women and 6 men showed blood plasma and cervicovaginal fluid (CVF) concentrations detectable for up to 84

days, and vaginal and rectal tissue concentrations detectable for at least 28 days, following a single 600mg IM dose. CVF exposures were 20% greater than blood plasma based on both the AUC_{84d} and Cmax concentrations. Vaginal tissue concentrations were 70%, 75%, and 100% of blood plasma on day 7, 14, and 28 post-dose, respectively. This trend was primarily due to plasma concentrations declining at a faster rate than tissue concentrations. Rectal tissue concentrations were 90% of blood plasma on day 7 and 14 [39]. Additional safety and efficacy studies are needed, but these PK data demonstrate prolonged genital and colorectal concentrations. Plasma and CVF concentrations peaked above the rilpivirine IC90 (94 ng/mL) following a single dose. Multiple dose PK studies will be needed to determine if concentrations will be maintained above this concentration with steady state dosing. A study is planned to collect further safety and acceptability data and to determine PK and ex vivo pharmacodynamics (by exposing tissue biopsies to a viral challenge in culture) in healthy women following single and multiple dosing [118].

GSK1265744 is an INSTI formulated as a long acting injectable with an intended dosing interval of 1–3 months. In a Phase I PK study, GSK1265744 was administered as a single IM injection of 100, 200, 400 or 800 mg, or as a single subcutaneous (SC) abdominal injection of 100, 200, or 400 mg to 8 subjects per dose and route of administration. The drug was well tolerated, had a half-life of 21–50 days, and demonstrated linear PK over the doses given [40]. Plasma exposure at day 10 following an 800mg IM dose were similar to those previously identified with a 30mg oral dose. This is promising, as a 30mg dose had previously demonstrated a potent antiviral effect (>2.5log drop in HIV RNA) when given for 10 days as monotherapy to HIV-infected patients [41]. PK studies are ongoing to quantify genital and colorectal tissue exposure and determine whether they are favorable for a PrEP application.

Targeting Optimal Exposure for Efficacy

In the typical drug development process, a promising drug will move from *in vitro*/animal models to Phase 1 studies where both safety and dose-proportionality (eg the relationship between dose and drug exposure) are characterized, to Phase II trials where small dose ranging efficacy and safety studies are performed, and finally into Phase III trials where a single dose is selected and efficacy and safety are established in a large randomized clinical trial (compared either to placebo or standard of care). Since it is nearly impossible to perform traditional Phase II correlative pharmacokinetic-pharmacodynamic (PK-PD) studies with PrEP (due to low incidence of HIV infection, the sample size would be exceedingly large to study multiple doses and correlate exposure to efficacy), Phase II and III studies were developed based on proof of concept in animal models[1–4], and on the safety of known antiretroviral doses for treatment of HIV. No PK-PD correlates for protection were generated before these studies were initiated. Only recently have there been greater PK-PD investigations for PrEP in animals [42]. Additionally, several Phase I studies have determined the feasibility of generating antiretroviral pharmacology data in healthy volunteer mucosal tissues [25,37,38,43,44]. However, without a surrogate endpoint for efficacy, the target concentrations needed to provide protection from HIV infection remain to be elucidated for all of the PrEP candidates. A recent review of the concentration-response relationship observed in oral PrEP studies demonstrates a relationship between concentrations and protection from HIV acquisition, but the confidence intervals are too large for precise estimates, and these exposures may be more of a surrogate of adherence than a target measure of protection [45].

In humans, typical drug properties thought to influence tissue penetration (eg lipophilicity and protein binding) are not able to accurately predict which antiretrovirals will penetrate into colorectal or genital tract tissue [42]. Therefore the PK profiles in these tissues must be

measured directly. In 2007, Dumond et al reported that the penetration of 11 antiretrovirals in 3 therapeutic drug classes into cervicovaginal secretions ranged from 0.4–411% [43]. Patterson and colleagues measured genital and gastrointestinal tract concentrations of TFV, FTC and their intracellular active metabolites over 14 days following a single oral dose of Truvada [25]. As mentioned above, the exposures for both TFV and its active metabolite were approximately 100 fold greater in colorectal tissue than in the female genital tract: exposures for both FTC and its active metabolite were reversed, being approximately 5-fold greater in the female genital tract than in colorectal tissue. After oral dosing, maraviroc concentrates 2-fold in vaginal tissues and up to 26- fold in colorectal tissues compared to blood plasma, suggesting that this agent has favorable mucosal PK properties for PrEP [37,38].

Results from these PK evaluations support that plasma concentrations cannot serve as a surrogate for tissue exposures without prior exploration of drug distribution into these compartments. The mechanisms behind these differential penetrations are unknown but may partially be due to uptake and efflux drug transporter activity. Drug transporters are membrane proteins responsible for drug trafficking into and out of cells [46]. Although their role has been well characterized in kidney, liver, and intestinal tissues [47], their expression and function in genital and colorectal tissues remains to be explored.

Animal models have defined biologic plausibility for PrEP. However, they have not defined the target mucosal concentrations required for efficacy. PK-PD correlates of protection are currently being investigated in humanized mice and nonhuman primates [48–51]. One additional tool that may assist in bridging the findings from animal models into human studies is the human tissue explant model. In this model, human mucosal tissue from rectal or genital tract compartments is collected and maintained in an *ex-vivo* culture system [52–54]. This model allows for mucosal tissue biopsies to be removed from antiretroviral-dosed healthy volunteers and infected with a laboratory strain of HIV *ex-vivo* to determine whether clinically relevant drug exposure prevents HIV infection. This technique has been used to generate data for TFV gel and the NNRTI UC781 [20, 54]. Dose response curves generated in these tissues may also allow for the generation of E_{max} models which can be used to define a critical target concentration needed for protection that can be explored in clinical trials [21]. However, this model is complex and technically challenging, with often unexplained variability, and more investigations are required before any target concentrations generated can be used to select antiretroviral doses for clinical trials [20].

Optimizing Adherence

Adherence is critically important to the success of any PrEP study, and previous studies have employed a variety of strategies to optimize and measure adherence. Unfortunately, none of the adherence measures employed are optimal, and all have been investigated retrospectively. In CAPRISA 004, women returned all used and unused gel applicators at each monthly study visit. Adherence was approximated by dividing the number of used applicators returned each month by the number of reported sex acts, assuming that two applicators were used per sex act. As stated above, adherence was correlated to increased efficacy (54% efficacy with >80% adherence, 38% efficacy with 50–80% adherence, and 28% efficacy with <50% adherence) [7]. However, genital tract drug exposure was also correlated with efficacy: TFV was detected in the cervicovaginal fluid of 83% of the women who remained uninfected, versus 36% who became infected [55]. Cervicovaginal fluid concentration >1000ng/mL was predictive of protection against HIV infection [12]

Study subject recall and self-report is commonly used in clinical trials but has been shown to significantly overestimate adherence rates. In the iPrEX study of daily oral Truvada in

MSM, a case-control analysis, drug exposure was only detected in 51% of men protected from HIV infection [8], which was imputed as the majority of men using < 2 doses of Truvada per week [24]. However, the self-reported adherence in these men was >85% [8]. Other Truvada PrEP studies have shown a similar relationship between objective measures of drug taking and self-reported adherence. In the FEM-PrEP study, despite 95% of participants reporting that they usually or always took their assigned study drug, and an 88% adherence rate suggested by pill counts, analysis of TFV plasma concentrations revealed that only 20–37% of subjects had taken a dose of Truvada in the 48 hours previous to any study visit [11].

One important factor implicated in adherence is the perceived risk of HIV acquisition in study participants. Most of the women enrolled in FEM-PrEP perceived themselves to have low to no risk of acquiring HIV [11], which may have led to the low levels of adherence to study medication. In contrast, the couples that were part of the Partners PrEP study perceived themselves to be at high risk for acquiring HIV because their stable partner was HIV infected. Qualitative interviews conducted by study participants revealed that participants saw PrEP as an important tool to protect against HIV acquisition while maintaining their relationship, and that the support of the HIV positive partner encouraged adherence [56].

In order to avoid failed clinical trials due to poor adherence, it is important to develop novel tools for real-time adherence monitoring in clinical studies. Plasma drug concentrations measure recent dosing history, but other biomarkers are needed to quantify whether dosing was consistent or inconsistent outside of the 24–72h window prior to the study visit. Antiretroviral hair concentrations have previously been found to be predictive of treatment outcomes in HIV positive patients [57, 58], and was recently evaluated as a marker of TDF drug exposure in 15 healthy adults [59]. Each of the participants was given 300mg of TDF at 2, 4, or 7 doses per week for 6 weeks in a crossover design. Two hundred strands of occipital scalp and pubic hair was cut after each 6 week dosing period and plasma was sampled throughout a 24 hour PK visit at day 28. A log-linear relationship was found between the number of doses per week and TFV hair concentrations with a 65% increase in hair concentration per 2-fold dose increase (95% CI 48–84%; $p<0.0001$) in scalp hair. Pubic hair concentrations were deemed not useful and plasma concentrations were variable depending on when the subject had last dosed. Therefore, occipital hair may be an option for measuring longer-term adherence to a once daily regimen of PrEP.

For the NRTIs TFV and FTC, because their intracellular active metabolites (TFV-DP and FTC-TP) have extremely long half-lives (6 and 2 days, respectively), they may be a useful measure of longer term adherence [5, 6]. TFV-DP and FTC-TP are traditionally measured in peripheral blood mononuclear cells (PBMCs), the processing of which is complex and not feasible at most study sites. However, a simpler alternative has recently been investigated. The FEM-PrEP study stored “Upper Layer Packed Cells” (ULPC) for possible future virologic evaluation. This was the upper portion of the red blood cell pellet formed after centrifugation of whole blood to separate plasma, and contains both red blood cells and PBMCs. Since red blood cells can phosphorylate TFV to a significant degree, and phosphorylate FTC to a modest degree, an investigation exploring the relationship between TFV-DP and FTC-TP in these ULPC samples and PBMCs found a significant correlation for both TFV-DP ($rho=0.64$, $p<0.0001$) and FTC-TP ($rho=0.56$, $p<0.0001$) [27]. These data have led to ongoing pharmacologic analysis of ULPC samples collected in FEM-PrEP. Based on the results of these analyses, this simple sample collection may be a good option for real time monitoring of longer-term TDF/FTC adherence in clinical studies, while utilizing the fractions of blood samples that are usually discarded. While adherence monitoring in clinical studies is important to determining the true effectiveness of a PrEP

strategy, optimal strategies to support adherence to PrEP strategies in clinical settings are yet to be determined.

Preparing for Drug Resistance

The development of antiretroviral resistance is of concern as the use of PrEP becomes more widespread. Particularly in the setting of poor adherence, infection occurring during a window of low drug exposure, followed periods of subtherapeutic treatment with mono or dual therapy, could rapidly lead to drug resistance. Since Atripla® (TDF+FTC+efavirenz (EFV) and other Truvada-containing regimens are first line treatment options for HIV, resistance to these mutations can significantly impact therapeutic options.

To date, there has been a relatively low incidence of developed resistance as a result of PrEP. In iPrEx, no TFV or FTC resistance was detected in subjects who acquired HIV while in the study, likely due to the low overall rates of adherence in those who became infected [8]. However, out of 10 subjects in this study who were infected at the time of enrollment (eg they were seronegative, but had acute HIV infection), two were randomized to the Truvada arm. Both of these patients acquired FTC resistance by week 4 of study treatment. This is in contrast to the placebo arm where only one of the eight patients who were infected at time of enrollment had FTC resistance by week 4 of the study. Likewise in TDF2, the only case of drug resistance occurred in a subject with unidentified acute infection who was enrolled into the active treatment arm and developed resistance mutations to both TFV and FTC [10]. In Partners PrEP, 8 patients on active study drug (TDF or TDF+FTC) were infected at enrollment [9]. Of these, one patient in the TDF group developed resistance to TDF and one patient in the TDF+FTC arm developed resistance to FTC. In FemPrEP, there were 5 cases of FTC resistance mutations; one in the placebo group and four in the Truvada group. At least two of these four resistance mutations are believed to be transmitted resistance [11].

The chances of acquiring resistance due to PrEP interventions must be carefully evaluated in the overall risk:benefit ratio for PrEP [60]. The low proportion of resistance in these studies suggests the majority of patients were either adherent to the degree that they were protected from infection, or their adherence was so low that drug exposures were below that required for selective pressure. The therapeutic window between drug exposure that will select for resistance and that will protect from transmission has not been defined. Although most resistance mutations demonstrate reduced transmission efficiency [61], whether the use of PrEP will lead to increased transmission of drug resistant HIV is currently unknown.

Future Perspectives

PrEP has the potential to soon be a valuable tool in preventing the spread of HIV. However, a wide range of efficacy, (0–75%) has been observed in clinical trials. Due to physiologic and behavioral differences between at-risk populations, optimal PrEP interventions will likely need to be tailored to the population and/or individual. The implementation of long-acting formulations or targeted, exposure-driven dosing strategies may improve overall adherence to PrEP. The generation of robust pharmacokinetic data characterizing the drug exposure in mucosal tissues will be essential for understanding the relationship between dose and efficacy, and designing optimal clinical trials. Additionally, in November 2012, the FDA released a draft guidance for the development of vaginal microbicides [119]. In this guidance, the FDA outlined specific testing that candidates should undergo including 1) the types (laboratory, clinical, resistant, etc.) and titers of viral strains to be tested, 2) the models in which efficacy should be tested (including PBMCs, primary macrophage and dendritic cell cultures as well as cervicovaginal explants, and 3) testing for both local toxicity and systemic exposures. Guidelines on dose selection as well as clinical trial study design are

also included. These and the other recommendations in the document will facilitate the development of future microbicide candidates such that they can be developed most efficiently.

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Executive Summary

Clinical Trials

- The efficacy of PrEP interventions in clinical trials has ranged from 0–75%.
- Drug exposure testing in these trials suggests that adherence plays a major role in the success of PrEP.

Targeting Optimal Exposure for Efficacy

- Drug distribution to mucosal tissues is variable and unpredictable. Higher exposures of TFV in colorectal versus female genital tract tissues may mean that women are more susceptible to infection if non-adherent.
- Target drug exposure required for protection has not been defined for any of the PrEP candidates. Robust testing in animal and *ex-vivo* models is needed to identify pharmacokinetic-pharmacodynamic relationships to optimize dosing in humans and properly design clinical trials.

Optimizing Adherence

- Adherence is a critical predictor of success for any PrEP strategy.
- Current methods relying on study subject reporting are not reflective of true behaviors. Plasma exposure testing is more objective but only reflect recent (eg within 48 hour) dosing.
- Novel tools for real-time adherence monitoring that provide long-term drug exposure information, such as hair samples and red blood cells may help improve adherence rates and clinical trial success.

Preparing for Drug Resistance

- Observed drug resistance in PrEP trials is limited. Currently, the biggest risk for the development of resistant mutations is the inability to identify acute, seronegative infection at the time PrEP is initiated.
- HIV infection must be clearly ruled out before PrEP is initiated in any patient.

The Path To Licensure

- Truvada ® has been approved by the FDA to be used as pre-exposure prophylaxis against HIV infection
- The release of the Draft Guidance on the Development of Vaginal Microbicides will facilitate the development of future vaginal microbicides

Table 1

Completed PrEP Clinical Efficacy Trials

Study	Study Population	Phase	Study Arms		# seroconversions	HR	p-value
			Intervention	# subjects			
CAPRISA 004 [7]	High risk women in South Africa	Phase II	Pre- and Post- Coital 1% tenofovir gel	445	38 infections	0.61	0.017
			Pre- and Post- Coital placebo gel	444	60 infections		
VOICE [102,109]	High risk women in Uganda, South Africa, and Zimbabwe	Phase II	Daily TDF tablet	~1000	Data not yet released	TDF tablet and 1% tenofovir gel arms stopped early for futility. Truvada tablet arm is ongoing	
			Daily Truvada tablet	~1000			
			Daily placebo tablet	~1000			
			Daily 1% tenofovir gel	~1000			
			Daily placebo gel	~1000			
TFV Phase II [21]	High risk women in Ghana, Cameroon, and Nigeria	Phase II	Daily TDF tablet	469	2 infections	0.35	0.24
			Daily placebo tablet	467	6 infections		
iPrEX [8]	MSM and Transgender women in Peru, Ecuador, South Africa, Brazil, Thailand, and the United States	Phase III	Daily Truvada tablet	1251	36 infections	0.56	0.005
			Daily placebo tablet	1248	64 infections		
TDF2 [10]	Heterosexual men and women in Botswana	Phase III	Daily Truvada tablet	610	9 infections	0.38	0.03
			Daily placebo tablet	606	24 infections		
Partners PrEP [9]	Heterosexual serodiscordant couples in Kenya and Uganda	Phase III	Daily TDF tablet	1759	17 infections	0.33	<0.001
			Daily Truvada tablet	1576	13 infections	0.25	<0.001
			Daily placebo tablet	1578	52 infections		
Fem PrEP [11]	Heterosexual women in South Africa, Kenya, and Tanzania	Phase III	Daily Truvada tablet	1024	33 infections	0.94	0.81
			Daily placebo tablet	1032	35 infections		

Table 2

Ongoing PrEP Clinical Trials

Study	Phase	Study Population	Study Interventions	Results Expected
MTN 008 [105]	Phase I	90 Pregnant and 15 breastfeeding women in the US	1% tenofovir gel used daily	2013
CONRAD 118 [106]	Phase I	54 Women in the US	1% tenofovir gel with antifungal cream, antimicrobial gel, or contraceptive ring	TBD
MTN 013/IPM 026 [116]	Phase I	48 Women in the US	Dapivirine vaginal ring, Maraviroc vaginal ring, or Dapivirine/Maraviroc combination ring inserted for 28 days	2013
TMC278-MWRI-01 [118]	Phase I	90 Women in the US	600 or 1200mg of long acting TMC278 (rilpivirine) administered as a single IM dose, multiple dosing groups TBD	End of 2013
CONRAD 120 [106]	Phase I	36 Women in the US	1% tenofovir vaginal ring inserted for 3 months	End of 2012
IPM 014 a/b [115]	Phase I/II	380 Women at multiple study sites in Africa	Dapivirine gel used daily	End of 2012
IPM 020 [115]	Phase I/II	128 Women in the US	Dapivirine gel used daily	End of 2012
MTN 017 [108]	Phase II	186 MSM and transgender women in Peru, South Africa, Thailand, and the US	Tenofovir gel formulated for rectal used daily before and after sex with Truvada by mouth daily	2014
HPTN 067 [112]	Phase II	360 MSM and 180 high risk heterosexual women	Truvada taken daily, pre- and post- intercourse, or twice weekly plus post- intercourse	TBD
HPTN 069 [117]	Phase II	400 MSM and 200 Women	Truvada alone, maraviroc plus Truvada, maraviroc plus emtricitabine, or maraviroc alone	TBD
VOICE [109]	Phase II	2000 High Risk women in Uganda, South Africa, and Zimbabwe	Daily Truvada tablet	2013
FACTS 001 [103]	Phase III	2200 Heterosexual women at 9 sites in Africa	1% tenofovir gel applied 12 h before and after sex	2014
CAPRISA 008 [104]	Phase III	700 Women from CAPRISA 004 in South Africa	1% tenofovir gel applied 12 h before and after sex	2015
ANRS iPERGAY [113]	Phase III	1900 MSM in Europe	Truvada taken before and after intercourse	2016
MTN 020 (ASPIRE) [114]	Phase III	3476 Women at multiple study sites in Africa	Dapivirine vaginal ring inserted every 4 weeks	2014–2015
IPM 027[115]	Phase III	1650 Women in Africa	Dapivirine vaginal ring inserted every 4 weeks	2015