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HIV Antiretroviral Pre-Exposure Prophylaxis: Development Challenges and Pipeline Promise

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Abstract

The US Food and Drug Administration (FDA) approved oral daily tenofovir/emtricitabine (Truvada) for pre-exposure prophylaxis of human immunodeficiency virus (HIV) infection in 2012 on the basis of two randomized controlled trials (RCTs), one in men who have sex with men (MSM) and another in HIV serodiscordant heterosexual couples. Subsequently, even greater efficacy has been demonstrated in MSM with rapid population-level incidence reductions in some locations. In contrast, studies of antiretroviral pre-exposure prophylaxis (PrEP) in heterosexual women showed only modest or no efficacy, largely attributed to low adherence. The mixed results of antiretroviral-based PrEP bear witness to unique drug development challenges at this complicated intersection of sexual behavior, public health, and drug development. Multiple innovative methods and formulation strategies followed to address unmet medical needs of persons struggling with daily oral PrEP adherence or preference for nonsystemic PrEP options. Clinical pharmacology plays essential roles throughout this PrEP development process, especially in early product development and through pharmacologically informed enhancement and interpretation of clinical trials.

Pre-Exposure Prophylaxis (PrEP) Need and Impact

The global human immunodeficiency virus (HIV) pandemic peaked in the last decade with recent 2% annual declines in HIV incidence in adults, attributed to changes in risk taking behavior, increased HIV testing and treatment, implementation of male circumcision, and pre-exposure prophylaxis (PrEP) with antiretroviral drugs (Figure 1). However, approximately 1.8 million new infections still occur globally each year – 5 thousand daily – 40 thousand in the US (1, 2). This heterogeneous epidemic shows increasing rates of HIV infection in several sub-populations. In the US, for example, black and Hispanic men who have sex with men (MSM) have experienced 4% and 14% increases, respectively, in HIV infection recently, especially in southern states. In contrast, nearly half (43%) of new infections globally occur in eastern and southern Africa where women and girls account for 6 of 10 existing HIV infections. A great unmet medical need persists for HIV prevention methods in diverse populations at risk.

PrEP employs oral, topical, or systemically applied antiretroviral (ARV) drugs before sex or injection drug use to prevent HIV. HIV microbicides are a subset of PrEP strategies applied topically to the vagina or rectum. Providing the receptive sexual partner with control over one's own HIV protection is a key motivation for PrEP. A substantial PrEP research and development effort has been funded by the US and other governments, the Bill and Melinda Gates Foundation, and many other donors. Academic scientists, in close collaboration with and funded almost exclusively by these non-commercial funders, successfully repurposed an oral HIV treatment product for PrEP (fixed dose tenofovir [TFV] disoproxil fumarate [TDF] 300mg/emtricitabine [FTC] 200mg), advanced TDF/FTC implementation in numerous demonstration projects, brought a dapivirine (DPV) intravaginal ring (IVR) to European Medicines Agency (EMA) review, and continue to advance several dozen PrEP products to enable a broader range of product options. While there has been essential collaboration with Big Pharma in ARV PrEP development for more than 15 years, this has not included funding or leadership of any large pivotal clinical trial until current second generation PrEP comparison studies.

In 2011, Gilead submitted to FDA their supplemental NDA package supporting once daily oral TDF/FTC (Truvada™) for HIV prevention which included two randomized controlled trials (RCT) of PrEP efficacy: iPrEx (NIH and Gates funded) and Partners PrEP (NIH funded) (Table 1). TDF (2001), FTC (2003), and the fixed dose TDF/FTC combination (2004) had been approved earlier for HIV treatment. FDA approved the submission in 2012 for adults and for adolescents greater than 35 kg in 2018. Regulatory bodies in at least 25 countries have approved TDF/FTC for use as PrEP. At least 3 generic formulations have been approved by FDA. The package insert recommends TDF/FTC in combination with other safer sex practices in adults and adolescents at “high risk” of HIV infection. Centers for Disease Control and Prevention (CDC) recommended TDF/FTC as PrEP as one prevention option for sexually-active adults and persons who inject drugs (not in the FDA label) at “substantial risk” for HIV acquisition (defined in detail). World Health Organization (WHO) recommends TDF/FTC for persons at “substantial risk” of HIV infection (including persons who inject drugs) defined as groups with >3% HIV incidence.

PrEP uptake in the US remained at very low levels through 2014, but rose substantially through 2018. Demonstration projects describe the successful implementation of TDF/FTC PrEP in many locations globally, largely in communities of MSM and transgender women (TGW). In San Francisco, new infections dropped by 51% from 2012–2016 largely attributed to PrEP implementation (3). In Australia, the Expanded PrEP Implementation in Communities in New South Wales (EPIC-NSW) study recorded only 2 HIV infections in 3,927 person-years (0.05% incidence vs. an historical 2%) in MSM with an associated population level 32% fall in incidence over the period of the 2 year study (4, 5). The estimated number needed to treat (NNT) with PrEP to prevent one HIV infection from sex ranges from 13 to 100 overall among the primary PrEP RCTs (Table 1). For context, the NNT for use of statins or anti-hypertensive medications in persons with unknown heart disease to prevent stroke and MI over 5 years ranges from 67 to 157 (6). Most US insurance companies and government payers (Medicaid, Veterans Affairs, Department of Defense) support PrEP to some degree.

Understanding Heterogeneous PrEP Outcomes

The first RCTs of antiretroviral HIV PrEP concluded within months of each other in early 2010: CAPRISA 004, a study of an on demand TFV vaginal gel in South African women and iPrEx, a study of oral daily TDF/FTC in MSM and TGW (Table 1) (7, 8). These very different strategies and study populations demonstrated modest efficacy, 39% and 44%, respectively. Future trial success of oral TDF/FTC regimens diverged along the lines of study populations: increasingly greater success in studies of MSM, TGW, and serodiscordant couples (9, 10), but modest or no efficacy in several studies of oral and vaginal formulations in heterosexual women (11–15). HIV prevention outcomes tightly aligned with detection of TFV and FTC in blood or cervicovaginal fluid (CVF). Modelling pharmacokinetic (PK) data from within and across PrEP RCTs with pharmacodynamic (PD) seroconversion endpoints indicates a clear TFV concentration-HIV protection response relationships (16, 17). These model results do not take into account the presence of FTC in the PrEP regimens when estimating the TFV EC₉₀, therefore, these TFV EC₉₀ results are only relevant when FTC is also in the dosing regimen as it almost always is in oral dosing studies, but not as commonly in topical studies. Estimating the EC₉₀ for each drug independently, resulting in higher EC₉₀ estimates for TFV-DP, has been done in more recent bottom-up mechanistic modelling and empiric mouse dose-ranging drug combination studies (18, 19). Numerous small PK studies enabled interpretation of very wide concentration heterogeneity across trials as due largely to variable adherence, different PK in vaginal v. rectal tissue, and changes in the vaginal microbiome. Numerous other variables are discussed later.

Adherence vs. PK variation.

Adherence explains most variation among study outcomes, proving so influential in iPrEx and Partners PrEP that FDA emphasized the importance of adherence in the TDF/FTC label revised to add the PrEP indication. Pre- and post-observed dose PK sampling enabled pharmacometric analyses to tease apart relative contribution adherence separate from PK variation (20). Directly observed dosing studies simulated a range of adherence patterns (daily to weekly) in a variety of matrices (plasma, peripheral blood mononuclear cells [PBMC], dried blood spots, and hair) to provide adherence benchmarks for quantitative interpretation of HIV outcomes and adherence covariates (21–23). These benchmarks were also used for ongoing quantitative adherence assessment in several trials to target adherence interventions (24, 25). These tools are widely used to assess adherence in PrEP demonstration projects and clinical practice. Electronic monitoring system (EMS) data from the Partners PrEP trial informed Markov-chain models of dose-taking patterns and participant adherence covariates and is now being integrated with PK and viral dynamic data for a PrEP clinical trial simulation (26).

Colorectal vs. cervicovaginal variation.

Multiple groups have reported substantial cervicovaginal versus colorectal differences in tissue PK of active form of TFV (TFV diphosphate [TFV-DP]) and FTC (FTC triphosphate [FTC-TP]) and deoxynucleotide triphosphates (dNTP), dATP and dCTP, respectively, the natural competitors of NRTIs for HIV reverse transcriptase (27, 28). A complex PK picture

emerges indicating an important role for both drugs in both anatomic locations, more rapid phosphorylation and clearance of FTC than TFV, 10 to 100-fold higher concentrations of the active TFV-DP in colorectal tissue and 100-fold higher active FTC-TP in cervicovaginal tissue, and 10-fold lower dNTPs concentrations in colorectal tissue. While there are competing methods for incorporation of the dNTP data into complex PK-viral dynamics models of these interactions (18, 27, 28) the data help explain the need for at least 6–7 weekly doses of TDF/FTC in women (whose primary risk is receptive vaginal intercourse [RVI]), less frequent 4 per week dosing in MSM and TGW (whose primary risk is receptive anal intercourse [RAI]), and how a only a few doses appears to provide rapid protection in MSM and TGW as in Ipergay (15). Another source of PK variability is that the specific intracellular kinases that phosphorylate TFV and FTC vary anatomically among the cells in blood, cervicovaginal tissue, and colorectal tissue; the kinases are also genetically polymorphic (29–31).

Vaginal microbiome and genital inflammation.

An abnormal non-lactobacillus dominant vaginal microbiome was associated with reduced cervicovaginal lavage fluid (CVL) and plasma TFV concentrations in different studies, as well as a 3-fold reduction in HIV protection in the CAPRISA 004 study of TFV vaginal gel (32, 33). Some post hoc analysis concerns were allayed by finding non-lactobacillus dominant microbiome in similar proportions of high and low adherence groups and both TFV and placebo arms. These changes were not seen with oral TDF/FTC dosing or vaginal dosing of DPV, though there may be other microbiome impacts on DPV (34–36). Bacteria possess a variety of enzymatic mechanisms for drug metabolism, including hydrolases, oxidoreductases, lyases, and transferases with established impact on dozens of licensed drugs (37). Genital inflammation, defined by presence of pro-inflammatory cytokines in cervicovaginal lavage (CVL) fluid, was associated with no protective benefit of TFV gel in CAPRISA 004 compared to a 75% relative risk reduction in women without genital inflammation (38). These findings indicate a need for prospective assessment of microbial metabolism of PrEP drug candidates, reassessment of drug concentration-based adherence measures in trials of topical drugs, and consideration of inflammation as an explanatory variable in future clinical trials.

Unique PrEP development challenges

Despite the RCT proven efficacy and population level impact of oral TDF/FTC PrEP, there remains a substantial demand for alternative PrEP methods. Some acceptability assessments indicate one-quarter of persons at risk prefer on demand, coitally-related dosing to daily dosing (25, 39–41). Long-acting formulations, e.g., the DPV IVR, free users from the challenges of sustaining daily pill-taking behavior. On demand formulations (e.g., Ipergay peri-coital dosing) link PrEP to the time of risk. Behaviorally-congruent formulations add ARVs to products that are commonly used before sex, e.g., sexual lubricants and douches. Choice among PrEP options should increase overall HIV prevention just as contraceptive choice increased contraceptive effectiveness (42, 43).

Continued PrEP product development to expand product choice is complicated by the convergence of numerous challenging obstacles (Table 2). Most of all, the inability to assess concentration-response for HIV prevention in a traditional phase II proof-of-concept study greatly hinders product development requiring reliance upon imperfect pre-clinical and ex vivo surrogates. Development of chemoprevention for some infectious diseases can use clinical challenge studies to provide proof-of-concept, for example, as can be done with prevention of malaria using treatable parasite challenge study designs (44). Pharmacology and virology knowledge have grown substantially during the PrEP development decades, but fundamental PrEP product development questions about site of entry, timing and location of early infectious events, and persistence of infectious virions remain unanswered. The inability to quantitatively monitor sexual exposures in order to link risk exposure to dosing events hinders our understanding of relationships between HIV risk, drug exposure, and protective response.

Given the unique development challenges, the unproven status of PrEP as an HIV prevention strategy, and the mismatch between the unmet medical need and financial ability to afford PrEP, investing in PrEP drug development was a very high risk endeavor until the last few years. Likely for these reasons, large pharmaceutical companies watched PrEP trials encouragingly, but largely, from the sidelines. Now that governments and philanthropy have greatly de-risked PrEP by funding numerous studies proving PrEP ARV efficacy in diverse populations and funding high impact implementation projects, large pharmaceutical companies are entering the playing field with greater energy and funding. Gilead's ongoing DISCOVER trial compares two Gilead products, TDF/FTC vs. TAF/FTC, and is the first RCT funded fully by a large pharmaceutical company. ViiV/GSK is also an important funding partner in HPTN studies. Hopefully, the substantial progress in surmounting the complex PrEP development hurdles (discussed below) encourages and informs both greater industry engagement and continued government-philanthropic-industry collaboration to improve PrEP choices.

Proof-of-Concept Alternatives

In the antiretroviral (ARV) treatment setting, proof-of-concept requires only a few dozen HIV-infected persons in brief one to two week multiple ascending dose studies providing concentration-response data to greatly reduce the risk and uncertainty of performing pivotal RCTs in hundreds of HIV patients later. By contrast, PrEP development lacks clinical concentration-response options in phase II that would ordinarily encourage and guide regimen selection for phase III. Further complicating trial designs, because HIV incidence rates in the RCT study populations were below 10%, over 90% of phase III research participants are not contributing to event comparisons, thus, requiring sample sizes in the thousands (Table 1). Now in the age of head-to-head RCTs (DISCOVER, HPTN 083, HPTN 084, AMP) with active control arms having much smaller incidence, RCT sample sizes are ballooning to many thousands and quarter billion dollar price tags. Of necessity, new methods were developed to advance pharmacometric modelling and viral challenge models with animals and ex vivo human cervicovaginal and colorectal mucosal tissue. In the early years of PrEP development, these surrogate methods were often explored only in parallel

with RCTs, but have been used more recently to inform product design earlier in development (Table 3).

Pharmacokinetic Method Development

Enhancing Pharmacokinetic Methods.—From the earlier HIV treatment development programs, repurposed ARVs for PrEP began development armed with historical data on PK in blood elements. The analytical efforts supporting the PK/PD assessments of the primary PrEP clinical RCTs (Table 1) were performed at five academic labs: Kashuba at UNC-Chapel Hill (CAPRISA 004, FEM-PrEP, FACTS 001), Anderson at University of Colorado (iPrEx, Ipergay), Bumpus at Johns Hopkins (Partners PrEP, TDF2, Bangkok TFV Study), Marzinke at Johns Hopkins (VOICE, ASPIRE, Ring Study), and Khoo at University of Liverpool (PROUD). Lab quality is high as all of these labs participate in one of two ongoing assay quality review programs with proficiency testing and cross-validation work between the two quality review programs (45, 46). Methods for assessing the female genital tract (Kashuba) and intracellular pharmacology (Anderson) pre-dated PrEP development, but colorectal pharmacology methods were still needed to address PrEP-relevant mucosal pharmacology. Methods were developed to simultaneously assess intracellular dNTPs with active NRTIs and to perform tissue CD4+ T cell subset isolation (28, 47–52). Mucosal PK studies, limited to only a few timed biopsies per research participant, sometimes with near simultaneous cervicovaginal and colorectal biopsies, required sparse sampling designs and posed challenges recruiting healthy volunteers (49, 51, 53–62). These studies helped tremendously to explain the differences between protecting individuals during vaginal sex when compared to anal sex and were essential for rational alternative formulation development.

Pharmacokinetic-Viral Dynamic Response Modelling.—Using clinical data, Cottrell (UNC) and Chen (Colorado), separately, developed pharmacometric models that aligned well with RCT outcomes (27, 28). The one narrow exception to this alignment is under-predicting the high degree of success of oral TDF alone in women in Partners PrEP – 71% in the intent-to-treat analysis and 100% in their nested adherence enhancement substudy (9, 63). In parallel with the above clinical PK sampling efforts, Duwal, von Kleist, and colleagues at Freie Universität Berlin, have continued to evolve top-down and bottom-up molecular mechanism of action models, which correctly predicted later empiric intracellular PK work and their evolving models increasing align with RCT outcomes as additional empiric data became available (18, 64, 65). They also note the complementary roles of TDF and FTC in PrEP, though propose alternative molecular models for incorporation of dNTP data to account for non-linear and saturable relationships that differ between dATP/TFV-DP and dCTP/FTC-TP pairs. Their models address important virologic variables affecting PrEP efficacy (e.g., seminal viral load) and provide a rich modular framework readily employable for much needed clinical trial simulations to optimize future trial design of emerging PrEP candidates. Most of the above modelling has been focused on oral dosing with some assumptions about PBMC versus tissue cell differences. Given the wealth of topical PrEP development innovations (see below), models to inform their development are badly needed.

Luminal Drug and Viral Kinetics.—For topical PrEP drug development, quantitative methods were developed to assess distribution of drugs and viral surrogates within the female genital tract and distal colon to insure ARV distribution in space and time matched or exceeded that of HIV surrogates. Barnhart and Pretorius (University of Pennsylvania) used MRI in the first microbicide distribution studies (66). Our group built on his work and prior nuclear medicine methods in using SPECT/CT to describe the distribution of substances applied to the vagina or rectum, including cell-free and cell-associated HIV surrogates after simulated vaginal and anal sex to identify the luminal distribution of interest for microbicides (67–70). We also dosed radiolabeled candidate microbicides followed by a radiolabeled HIV surrogate to assure the coincident distribution of microbicide and HIV target within the distal colon, demonstrated the suitability of enema formulations, and raised concerns about the feasibility of delivering adequate intraluminal microbicide gel when applied as an anal lubricant (59, 71, 72). Three-dimensional tube-fitting of SPECT data enabled quantitative descriptions of drug and HIV surrogate distribution which proved useful when comparing among competing rectal microbicide candidates (58, 69, 71, 73).

Non-Clinical Viral Challenge Models

In the absence of clinical surrogates of protection, macaque, mouse, and ex vivo human challenge models evolved during this period of PrEP development to enable more robust testing of candidates to guide clinical development (Table 3; described in Supplemental Materials). All of these methods have put forward EC₅₀ or EC₉₀ estimates to compare with clinical trial results as a type of validation. One feature of all these methods, both a necessity and a limitation, is that the challenge (ranging from one to twenty weekly occurrences) are designed to achieve 100% infection to minimize both time to infection and sample size. In reality, the riskiest sexual behavior, unprotected RAI, has a much lower estimated HIV infection risk per exposure of only 1 in 72 (CDC).

Vaginal Product Underperformance

Adherence incompletely explains PrEP outcomes.

Correcting for adherence in the VOICE gel arm, protection increased to between 60% and 88% relative risk reduction in analyses by Dai (Poisson model, adherence defined as detectable plasma TFV ever or at 3 months) and Ruberman (targeted maximum likelihood estimation method, adherence defined as detectable plasma TFV at all observations) (74, 75). On demand dosing of the same gel in CAPRISA 004 showed 73% protection when adherence was high (76). ASPIRE DPV IVR adherence adjustments increased the relative risk reduction estimate as high as 65% (using residual ring DPV concentration) (77). Though far better than the planned intent-to-treat findings of low or no protection in these studies, these adherence-adjusted estimates fall short of the 100% relative risk reduction in the enhanced adherence cohort of Partners PrEP (Table 1) (63). Additional causes need to be identified to enable development of more effective vaginal products or study designs.

Poor rectal ARV penetration.

Daily vaginal dosing of the TFV 1% gel achieved very low TFV-DP concentrations in rectal tissue, detectable in only 15% of women (27 fmol/mg LLOQ), indicating concentrations

well below simultaneous vaginal tissue concentrations (166 fmol/mg) (78). These rectal tissue concentrations after vaginal dosing predict very low, if any, protection from RAI for women using these vaginal products. Because RAI carries a much greater HIV transmission risk per exposure, e.g., 20-fold as a midpoint of numerous estimates, only one anal exposure among 20 total sexual exposures (5%) reduces the efficacy ceiling to only 50% protection in a vaginal microbicide trial. One anal exposure among fifty sexual exposures (only 2%) still drops the efficacy ceiling to 87%. While data in RCTs doesn't have sufficient granularity to estimate RAI frequency among all sexual exposures, the percent of women practicing RAI during these RCTs can range up into double digits. Perhaps worse, if a woman anticipated RAI and thought to apply the TFV gel rectally, loss of colorectal epithelial integrity due to the hyperosmolar gel could result in an increased risk of HIV infection (79).

In contrast to TFV and DPV, a multidrug pod-IVR demonstrated emtricitabine and maraviroc colorectal tissue concentrations greater than the in vitro protein-adjusted IC_{90} in women (80). Other ARVs in development may also diffuse more efficiently into vaginal and rectal tissue, potentially providing protection for vagina and rectum with application of a vaginal product. Another alternative would be to develop a product suitable for dosing in both the vagina and the rectum to provide women the flexibility of protecting themselves wherever needed. For this purpose, as noted, the TFV vaginal gel used in RCTs is not suitable for rectal dosing due to hyperosmolarity and GI side effects (51). Rectal DPV gel studies, used as a sexual lubricant and with an applicator, are ongoing (MTN-026, MTN-033).

Microbiome.

The previously discussed non-lactobacillus dominant microbiome effect on TFV gel efficacy, but not oral TDF efficacy, indicates an opportunity to improve TFV gel efficacy through treatment of bacterial vaginosis (32, 34, 36). In addition, the impact of bacterial vaginosis on TFV indicates that plasma TFV-based adherence estimates in VOICE, as in CAPRISA 004, are underestimates depending on the frequency of women with non-lactobacillus dominant microbiome, often near 50%. Microbiome surveillance, both vaginal and rectal, are warranted in future microbicide product development.

Toxicity.

Vaginal products trade off benefits of avoiding systemic toxicity and on demand use with potential mucosal toxicity given high cervicovaginal concentrations. With a narrow therapeutic window, high levels of adherence may, paradoxically, see reduced protection. Many clinical toxicity measures have been employed including visual examination, colposcopy, and mucosal permeability assessment, as well as assays of cytokines, MTT tissue viability, and various 'omics measures among others. The challenge is correlating post-dose changes in these tests with actionable toxicity requiring dropping a product or reducing a dose.

One might define actionable toxicity simply by two criteria, (1) clinically impactful changes, e.g., symptoms sufficient to reduce adherence or (2) changes insufficiently high to counterbalance high level HIV protection. Recently, 8 of 12 women randomized to the

active, but not placebo, arm of a TDF IVR developed vaginal erosions, symptomatic in half the women (81). This presents a clear case for halting development of the product (and increased scrutiny of related products) given our simple rejection criteria of symptoms or offsetting HIV transmission risk. Nonoxynol-9, cellulose sulfate, and Pro2000 all had in vitro safety signals during development or coincident with their respective clinical trials, though of uncertain clinical significance at the time (82–84). Nonoxynol-9 and cellulose sulfate increased HIV infection and Pro 2000 provided no protection when advanced to clinical trials, providing helpful, if retrospective and indirect, validation of in vitro toxicity tests (85–88). The current challenge is deciding when to halt development as unproven tests return abnormal results, e.g., proteomic or metabolomic changes, without the benefit of clinical evidence to validate the predictive value of candidate safety markers.

For rectal microbicides (discussed later) there are still no efficacy trial results to inform the validity of theoretical toxicity markers. Theoretically, colorectal tissue is highly vulnerable to toxicity because the single columnar epithelium is both more fragile and less thick a physical barrier when compared to the stratified squamous epithelium lining the vast majority of the exposed mucosal surfaces in the lower female genital tract. Before HIV-specific antiretrovirals were studied, rectal dosing of hyperosmolar, N9-containing spermicides (e.g., K-Y®Plus, ForPlay®, Advantage 24®) as well as hyperosmolar, non-medicated sexual lubricants (e.g., Replens® and ID Glide®) resulted in epithelial cell exfoliation within 15 to 90 minutes after dosing and still notable 12 hours after dosing (79, 89, 90); some products were also associated with GI symptoms. Vaginal use of these same products and rectal use of non-medicated, iso-osmolar gels (appropriate negative controls) were not associated with these histological findings or symptoms, consistent with the theoretical concern of greater epithelial vulnerability of colorectal mucosa compared to cervicovaginal mucosa. It is not hard to imagine that loss of the epithelial lining would increase risk of HIV infection, though this has not been proven. When more subtle alterations in colorectal physiology and function were later noted in clinical rectal microbicide studies – including cytokine expression, permeability, proteomics, and HIV infectivity of tissue explants – it remains challenging to interpret these theoretical toxicity signals in the absence of knowing their impact on HIV seroconversion.

Systemic or mucosal protection

Essential, yet unresolved, scientific questions for PrEP development include: what is the relevant site of PrEP drug action – systemically circulating cells, mucosal tissue cells, somewhere in between like lymph nodes – and the target ARV concentration that confers protection. Several lines of empirical data support a relatively more important role for mucosal than systemic drug concentration as most variation in outcomes is best explained by mucosal differences rather than systemic:

- (1) NHP challenge studies (albeit with single site of infection and perfect adherence) indicate both vaginal and rectal microbicides with a single drug provide higher levels of protection from SHIV challenge compared to oral dosing of the same single drug despite lower systemic drug concentrations after

topical dosing compared to oral dosing and higher tissue concentrations with topical dosing.

- (2) Women in VOICE, correcting for adherence, demonstrated worse protection with oral TDF and TDF/FTC dosing than vaginal TFV gel dosing. Oral TDF dosing achieves cervicovaginal cell TFV-DP concentrations 1–2-fold higher than PBMC TFV-DP concentration; Topical TFV dosing achieves 100-fold higher cervicovaginal tissue cell TFV-DP concentrations with undetectable PBMC TFV-DP concentrations (far below oral dosing PBMC TFV-DP median 32 fmol/10⁶ cells) indicating a relatively more important role of mucosal concentrations (74, 75).
- (3) Assuming similar adherence, oral TDF/FTC dosing prevents colorectal infection with less frequent dosing (4 per week) compared to preventing vaginal infection (6–7 doses per week), despite higher risk of infection with RAI. Mucosal TDF and FTC PK and dNTP differences between colorectal and cervicovaginal tissue provide a plausible explanation these outcome difference (22, 28–31, 49, 51, 52, 91). However, because systemic NRTI and dNTP PK is no different in MSM (primary RAI risk) compared to women (primary RVI risk), systemic concentration cannot contribute to differences in PrEP effectiveness in MSM and women.
- (4) When modelling plasma TFV concentration-seroconversion response relationships across all of the primary RCTs using daily dosing, most of the variation in the relationship is explained by adjusting for anatomic differences of TFV-DP concentrations at mucosal sites of HIV acquisition and the higher RAI risk in MSM; both are mucosal differences, not systemic (17).

Ongoing RCTs of long-acting injectable cabotegravir (HPTN 083/084) and oral TAF/FTC (DISCOVER) may contribute greatly to understanding the relative importance of systemic and mucosal drug concentrations; both cabotegravir and TAF have much lower mucosal tissue concentrations compared to their systemic concentrations whereas their RCT comparator, TDF, achieves higher mucosal (cervicovaginal and, especially, colorectal) than systemic concentrations (92, 93). If mucosal tissue concentration is dominant as the evidence above indirectly suggests, then topical products, with far higher mucosal tissue concentrations, remain viable formulation options for expanding PrEP choices.

Based on our efficacy eroding variables list, the focus for new product development should shift to: designing products that maximize adherence, developing products that protect both vaginal and rectal infection or can be applied to both locations, selecting only research participants whose route of infection aligns with the product studied, and rigorously diagnosing and treating bacterial vaginosis (when relevant for a specific drug, like vaginally dosed TFV). For example, on demand, behaviorally-congruent rectal microbicide trials in MSM and TGW will avoid most of these issues if adherence is high (as it increasingly has been since iPrEx and should continue to be with behaviorally-congruent formulations). Trials of same rectal microbicides in women could enroll only women who do not practice RAI (a challenge, especially in high risk commercial sex settings). Alternatively, trials of promising vaginal microbicide candidates (if not demonstrating good vaginal to rectal

penetration) could include effective rectal microbicides for all women to have RAI to protect those who need them and to remove anal sex as a variable contributing to reduced vaginal PREP efficacy.

Last and next generation TFV microbicide development

Early vaginal TFV gel development.

The development path for the TFV 1% vaginal formulation (termed VF to distinguish it from later formulations) moved through clinical development to RCTs with very few preliminary studies (Figure 2, top right). The phase I PK evaluation (HPTN 050) was completed without the benefit of single or multiple ascending dose evaluations. The phase II extended safety study (HPTN 059) was completed without proof-of-concept (explained above) and development moved into three pivotal phase III RCTs (on demand dosing in CAPRISA 004, FACTS 001 intended to be the confirmatory test, and daily dosing in VOICE) (7, 11, 12, 94, 95). VF development didn't have the advantage of prior TFV gel treatment trials demonstrating efficacy, well-described multiple anatomic compartment PK, knowledge of HIV distribution or tissue PK impact of semen within the female genital tract after sex, viral challenge studies (macaque, BLT mouse, or ex vivo explant with in vivo dosing) prior to phase III vaginal gel efficacy studies (though all were available after or in parallel with vaginal gel RCTs) (53, 61, 67, 96–99). There are no published examples of iterative product optimization or comparisons to select from among competing TFV gel candidates. In the phase II safety trial, HPTN 059, soft signals of VF product limitations appeared (e.g., a minority of participants liked the product or found it easy to use) that might have led to development of a more agreeable formulation (95). Assuming those symptoms might have impacted product adherence, investigators had only self-reported adherence to help interpret the findings absent objective adherence measures either not used (EMS) or not yet developed (drug concentrations). These VF trials were much of the impetus to develop quantitative PrEP-specific adherence measures to detect and improve adherence. Only in the aftermath of the TFV gel trials that didn't show protection were these early signals better understood as possibly clinically significant. This is not unlike unvalidated toxicity signals seen in early non-ARV vaginal microbicide development whose impact became clearer after subsequent large trials demonstrated enhanced risk of HIV infection.

Rectal microbicide demand.

Initiation of rectal microbicide development lagged years behind vaginal microbicide development. Rectal microbicides are in demand as alternatives to oral PrEP due to the very high risk of unprotected RAI in MSM and TGW as well as significant minorities of MSM and TGW (27% in MTN-017) indicating oral daily TDF/FTC as their least preferred regimen (compared to on demand and daily gel regimens); these participants also indicated displeasure with the rectal gel applicator in the gel arms of the study (100). Important numbers of MSM and TGW struggle to maintain adherence on PrEP as, for example, most of PrEP demonstration project failures are in persons without detectable ARVs in their blood. MSM and TGW advocates and community level discussions have long indicated a high level of interest for on demand, behaviorally-congruent PrEP with lubricants or anal douches. Proof of concept for on demand strategies is supported by Ipergay's highly

protective on demand oral TDF/FTC regimen (86% relative risk reduction) and CAPRISA 004's on demand VF gel achieving high levels of protection as well (73% relative risk reduction) when adherence was very high (15, 76). Survey research indicates a large majority of MSM (85–95%) almost always use lubricants with anal sex and 67–80% always or almost always use rectal douches (enemas) prior to anal sex (101). A recent online survey of 4,751 MSM conducted on Grindr, a popular gay dating app, indicated that among the 78% of respondents who had RAI in the past 3 months, 80% used a rectal douche before sex. Nearly all respondents who currently douche (98%) or do not douche (96%) indicated they would likely use an effective rectal microbicide; 95% of respondents practicing insertive anal sex indicated support of their receptive partners using a rectal microbicide douche (102). Numerous studies of female sex workers, another high risk group, also indicate high frequencies of sexual lubricant and vaginal douching use. Accordingly, lubricants and douches emerge as desirable candidates for addition of ARV to piggy-back HIV protection onto existing sexual product use.

Rectal method development.

Because microbicide products for rectal use followed years after the start of the TFV vaginal gel program, rectal development benefitted not only from the lessons of vaginal microbicide development, but also from many clinical, pre-clinical, and laboratory methods that greatly enabled quantitative, comparative, and iterative assessments of rectal microbicide candidates against a desired target product profile. These methods (Figure 2, Left panel) included mucosal tissue CD4+ T cell intracellular pharmacology, imaging studies of gel and semen distribution in the lower GI tract, and non-invasive toxicity assessment using gut permeability (48, 49, 68–70, 79). Pre-clinical BLT and NHP models greatly matured during this time as well as the ex vivo explant challenge model and all of them indicated rectal TFV gels conferred rectal protection (Supplemental Materials). Acceptability assessments tailored to implementation in short, limited drug exposure studies, using “take home” doses for use with sex partners and *in mano* evaluations to explore tactile experiences with the products were implemented, refined, and applied in first in human vehicle and medicated product studies (51, 58, 59). While some of these methods were specific to rectal microbicide development, many were also highly informative for parallel development of vaginal products.

Rectal TFV Gel Development.

The first clinical study of rectal TFV, RMP-02/MTN-006 (Anton Microbicide Development Program [MDP] within the IP/CP-HTM Program collaborating with MTN), used the hyperosmolar VF formulation (3,111 mOsm/kg) under parallel study in CAPRISA 004 and VOICE. Previously, rabbit rectal irritation studies indicated no toxicities with the gel and the gel protected 6 of 9 macaques from rectal SHIV challenge (103). As was true for all rectal product studies that followed, RMP-02/MTN-006 included a wider array of safety and PK assessments than vaginal TFV gel studies at similar stages of development, largely because more methods were now available (51). In only 30 minutes, a single rectal dose of VF achieved impressive rectal tissue cell TFV-DP concentrations 6-fold greater than steady-state concentrations associated with oral dosing which achieved high levels of protection (51).

However, lower GI symptoms after VF rectal dosing, attributed to the hyperosmolar gel, ended development of VF for rectal use.

The combination of adverse events associated with hyperosmolar VF (RMP-02/MTN-006) and earlier studies of hyperosmolar sexual lubricants and enemas causing of colorectal epithelial cells led to development of reduced glycerin vaginal formulation (RGVF) with lower, but still hyperosmolar, characteristics (836 mOsm/kg) (58, 79, 104). Phase I MTN-007 demonstrated a clean side effect profile for RGVF of TFV (105). In an extended safety and acceptability study of the RGVF gel applied rectally, MTN-017, side effects remained very low, adherence very high, and on demand dosing of the gel was preferred to daily gel dosing (25). Participants were not so enamored of the rectal gel applicator, borrowed from the vaginal development program, and consensus among investigators was that applicator dosing would not be acceptable in extended RCTs (41, 100). MTN-014 demonstrated poor penetration of TFV into vaginal tissue after rectal RGVF dosing, indicating a future TFV rectal product applied rectally would not likely perform well protecting vaginal HIV infection (78).

In parallel with the RGVF studies and out of concern for hyperosmolar toxicity, interest grew in an iso-osmolar, rectal optimized gel. A head-to-head clinical study of four different iso-osmolar non-medicated rectal semi-solid and liquid vehicles, developed by Rohan, et al., indicated a clearly superior formulation based on high levels of acceptability, including use during anal sex at home, GI distribution, safety, and ex vivo explant protection even without adding an ARV (59, 106). This vehicle was modified to accommodate addition of 1% TFV in a more nearly iso-osmolar (479 mOsm/kg) rectal formulation, named RF. CHARM 01 and CHARM 02 directly compared the 3 TFV 1% formulations (VF, RGVF, and RF) applied rectally and found RF was superior to or equivalent to the other two formulations in all categories – PK, PD, acceptability, and safety (62, 71). In these studies, RGVF achieved higher tissue TFV-DP concentrations than RF, but performed slightly less well than RF in the ex vivo explant HIV challenge, raising concern that local toxicity with the hyperosmolar RGVF may have eroded its protective effect compared to the more nearly iso-osmolar RF – possibly a bell shaped efficacy curve. At this point, neither RGVF nor the vaginal applicator have plans for advancement to efficacy studies. While the more promising RF remains viable, it needs a method to deliver a dose rectally given the displeasure with the applicator developed for vaginal use uncovered in MTN-017.

Lubricant formulation development.

RF gel would be a strong candidate for a medicated anal lubricant microbicide. Lubricant doubling as microbicide provides an on demand, behaviorally-congruent option, has long been sought by the gay community, and solves the vaginal applicator acceptability problem. However, study of an over-the-counter anal lubricant (Wet Original™), radiolabeled and applied as a lubricant with only fingers and phallus, indicate highly inefficient (<10%) intrarectal dose delivery (as seen on SPECT/CT) compared to use of a gel applicator (72). Ongoing MTN-033 evaluates the colorectal tissue distribution of dapivirine gel applied as an anal lubricant with results expected in 2019. Future development of anal lubricants as microbicide depends greatly on this study.

Enema formulation development.

An ARV-medicated enema as rectal douche before anal sex also provides an on demand, behaviorally-congruent rectal microbicide option. In parallel with rectal gel development, another Anton MDP study demonstrated the safety and desired colonic distribution of an iso-osmolar douche that avoided the significant toxicity of a hyper-osmolar Fleet™ enema and performed well as a douche in sexual settings (58). A TFV hypo-osmolar enema formulation (EF), has emerged from an optimization program in mice and macaques into clinical development (DREAM IP/CP-HTM Program) (107, 108). Villinger, et al., demonstrated an EF dose one hour prior to weekly SHIV rectal challenge in macaques confers high levels of protection (5 of 6 macaques) when compared to daily oral TDF dosing (3 of 6 protected) (109). In a clinical single ascending dose study (DREAM-01), EF achieved colorectal cell TFV-DP concentrations 1 to 3 hours after a single douche that exceed by 100 to 1,000-fold the steady-state protective concentrations achieved with oral TDF, suppresses HIV infection in ex vivo explants, and maintains a high degree of acceptability and safety (110). Imaging studies to evaluate coincident EF and semen colorectal distribution following simulated RAI (DREAM-02) and multiple douches in rapid sequence, more consistent with typical pre-RAI douching practice (DREAM-03) are planned for 2019. Clinical trial simulation to optimize phase III clinical trials are currently underway building upon existing adherence, PK, and viral dynamics models.

Next Generation ARV PrEP Strategies

Improving adherence drives next generation PrEP innovation and can be categorized by two major contrasts: (1) long-acting versus short-acting and (2) systemic versus local application (further differentiated into vaginal or rectal). Strategic categories of pipeline products are summarized in Table 4 with regard to how they stack up to the eight PrEP development challenges discussed above. A select list of numerous ARV PrEP products in clinical development within these categories (Table 5) encourages the hope that new product choices, suited to a broader range of individual needs will prevent infections more broadly. At the intersection of PrEP and contraception, at least eight multipurpose prevention technologies (MPT), designed to prevent HIV and pregnancy in a single product, are currently in varied stages of development. One long-acting MPT development challenge worth noting is the complexity of differing release rates and subsequent pharmacokinetics of ARV and contraceptive from the same product vehicle. MPT trial design is further complicated by multiple primary prevention outcomes, each with different incidence rates. Product and trial optimization usually requires de-optimization of two individual drug formulations or trial designs to accommodate the multiple MPT goals.

Short-acting Local

The most extensively tested short-acting local PrEP products are the vaginal and rectal TFV microbicides contrasted above where the rationale and proof-of-concept evidence for on demand strategies was discussed. Struggles with oral dosing adherence, desire to avoid systemic side effects, or preference for more behaviorally-congruent formulations that fit seamlessly within sexual behaviors drive demand for vaginal and rectal formulations. The

behavioral congruence is highly attractive because it doesn't require the adoption of a new medicating behaviors. Rather, it only requires typical use of products already commonly used, but which have been enhanced by addition of effective ARVs.

Vaginal pipeline.

The goal of most of these development programs is improved adherence through simpler, optimally on demand use, and possibly behaviorally-congruent use as vagina lubricant or douche. On demand formulations in clinical development include TFV and DPV fast-dissolving films similar to OTC vaginal contraceptive films (Hillier's FAME IP/CP-HTM Program). Both the DPV film and TFV film performed similarly to the comparator gels. Single dose films achieved concentrations near or above steady-state concentrations with the DPV IVR and TDF oral dosing, respectively, indicating potential for on demand use (56, 57, 60, 61). CONRAD and MTN are co-developing a TFV/elvitegravir combinations as a vaginal (and rectal) fast-dissolve insert for on demand use.

Rectal pipeline.

At present, 6 antiretrovirals (3 approved for treatment [TFV, elvitegravir, and maraviroc] and 3 others [dapivirine, MIV-150, IQP-0528]) in 4 different dosing formulations (gel with applicator, gel as lubricant, douche, fast-dissolving insert) have recently or will soon be studied in clinical PK and safety studies. Griffithsin and 5P12-RANTES bind HIV in the colorectal lumen and are nearing clinical development. Among these products, there may be candidates that could meet the needs of an on demand and behaviorally-congruent formulation, overcome the two sites of risk protection need, or serve as MPT with activity against HIV and other viruses (PC-1005) or could be suitable for co-formulation as MPT with other agents. The challenge with so many options will be deciding from among competing candidates and developing more user-friendly formulations for on demand application that avoid or improve upon past applicator designs.

Long-Acting Local

The monthly DPV IVR is currently under EMA review. Rings containing DPV, TFV, TDF, MVC, or FTC alone or in combination using matrix, reservoir, and pod-IVR configurations are also in clinical development. As mentioned, the pod-IVR design achieved potentially protective concentrations of FTC and MVC in rectal fluid, possibly solving the two of infection-one site of application problem (80). An MPT IVR combining DPV and levonorgestrel designed to last 90 days achieved safety and drug concentration targets in a 14-day clinical study (MTN-030/IPM041) (111). A 90-day ring study is ongoing (MTN-044).

Short-Acting Systemic

Oral.

Gilead is competing their oral daily TAF/FTC fixed dose combination head-to-head against their highly effective oral daily TDF/FTC PrEP in a double-blind, randomized trial, DISCOVER. In combination with other ARVs, TAF proved effective in the HIV treatment

setting and has greater potency due to more efficient penetration of susceptible CD4+ T cells in blood with intracellular transformation of TAF into TFV by cathepsin A. TAF/FTC, but not TAF alone, proved highly effective in NHP challenge studies, but hasn't been tested in BLT mouse or ex vivo HIV challenge studies. One potentially important difference between TAF and TDF in DISCOVER is that TDF may achieve higher TFV-DP concentrations than TAF in cervicovaginal and colorectal cells (Table 3) (93, 112). Cathepsin A is differentially expressed anatomically and may be the cause of this difference. The clinical importance depends on both the contribution of FTC and the relative importance of systemic vs. mucosal ARV concentrations as discussed above. The superior PK characteristics consistent with weekly oral dosing of Merck's EFdA make it a strong future competitor of the TFV prodrugs (113).

Long-Acting Systemic

Injectables.

In the 1950s, adding benzathine turned penicillin's 30 minute half-life into a 336 hour effective half-life long-acting (LA) injectable. Long-acting (LA) injectable contraceptive depot medroxyprogesterone acetate (DMPA) is dosed IM quarterly due to a 30 day effective half-life. DMPA also provides useful lessons for long-acting PrEP development. Using different nanoformulation methods, LA rilpivirine (RPV-LA), a non-nucleoside reverse transcriptase inhibitor (NNRTI), and LA cabotegravir (CAB-LA), an integrase strand transfer inhibitor (ISTI), are paired in phase 3 development for HIV treatment. Rilpivirine-LA is not being pursued further for PrEP, likely due to several challenges: low barrier to resistance, cold chain requirement, and lower mucosal tissue RPV concentrations compared to blood. CAB-LA is a low solubility, nanomilled crystalline drug suspended in an aqueous vehicle and avoids several RPV-LA limitations (higher resistance barrier and no cold chain). Using bi-monthly IM injections, CAB-LA is being compared to daily oral TDF/FTC in two phase 3 RCTs. In an abundance of caution, a one month oral CAB lead-in is being used to rule out side effects before the first long-acting injection. Because of concern for antiviral resistance due to persistence of CAB in the blood after dosing stops (over a year in a significant number of research participants), cessation of CAB-LA dosing is followed by 48 weeks of daily oral TDF/FTC. These leading and trailing oral requirements are challenges in light of CAB-LA's principle role to avoid daily oral PrEP dosing. In the phase 2 safety studies, injection site reactions were common, but mostly minor with few related discontinuations (114, 115).

While outside our small molecule ARVs focus, LA injectable formulations of broadly neutralizing monoclonal antibodies (bnMAbs) are in advanced clinical development. Rare HIV-infected "elite" neutralizers evolve neutralizing antibodies naturally and serve as the basis for engineering more broadly neutralizing, more potent, and longer half-life antibodies. These antibodies both neutralize envelope and recruit effector cells to kill HIV infected cells. bnMAbs protect macaques from rectal SHIV challenge (116). The phase 2b Antibody Mediated Prevention (AMP) Study (HVTN 074/HPTN 085) is now enrolling 2,700 MSM and TGW to receive VRC01 or placebo in bi-monthly IV infusions for 72 weeks. AMP's placebo-controlled design is quite unlike HPTN 083/084 and DISCOVER which compare

CAB-LA and TAF/FTC, respectively, head-to-head with standard of care oral TDF/FTC. In contrast, AMP assures oral TDF/FTC availability to all study participants at no charge, though outside the study. Numerous other bnMAbs in less advanced clinical development boast broader, more potent, and longer half-life profiles with promise for less frequent subcutaneous dosing and greater efficacy.

Implantable.

Subcutaneously implanted devices, designed to very slowly release ARVs over months or years, would dramatically extend PrEP duration, avoid adherence challenges, and greatly reduce healthcare system interactions. Several groups have reservoir (TAF, CAB) and matrix (EFdA) implants in pre-clinical development, some with promise for yearly implants in humans (117–119). In particular, EFdA, a nucleoside reverse transcriptase translocation inhibitor (NRTTI), is very well suited for implantation with sub-nM antiviral potency and longer intracellular triphosphate half-life than even TFV-DP. EFdA protects macaques from SHIV challenge with daily or weekly dosing (113). These implant programs typically use existing contraceptive implant trocars obviating the need for additional device development. Some groups are exploring biodegradable devices which trade off a removal procedure with the design challenge of optimizing drug release and device degradation rates to minimize multiple simultaneously indwelling devices as they degrade.

Conclusion.

The development of highly effective oral TDF/FTC as PrEP has led to impressive and rapid population level reductions in HIV incidence among MSM. PrEP efficacy in women has been less impactful due to poor adherence and other issues specific to women. These limitations reinforce the need for improved PrEP formulations that provide a choice of effective options suited to heterogeneous individual needs in the very complex intersection of sexual intimacy and HIV risk. Clinical pharmacology has been fruitfully engaged as an essential discipline to develop novel quantitative PK and PD assessments unique to PrEP development, guide early clinical development, and to optimize future trial design through modelling and simulation. The current PrEP pipeline is rich with early stage products in a wide variety of ARV and formulation options which align with the needs and desires of people who are most in need of PrEP. The combination of unmet PrEP product need, waning governmental support, successful PrEP development examples, and clear evidence of markets and state level support for PrEP programs should further encourage public-private industry partnerships to accelerate further PrEP development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

HIV prevention methods include strategies to reduce the infectious burden in the infected partner and reduce susceptibility in the uninfected “at risk” partner. PrEP is one of many highly effective methods to reduce HIV infection working in a complementary manner with these other prevention methods.

Figure 2.

Comparison of the vaginal TFV and rectal TFV microbicide product development pathways. Left column indicates numerous methods developed primarily to enhance rectal product development which later influenced vaginal product development, but not in a timely way for TFV 1% vaginal gel development. Four different TFV microbicide products (middle text indicating VF, RGVF, RF, and EF) were developed in temporal sequence (top to bottom). The traditional development stages for both vaginal (top, tan boxes) and rectal (lower, blue boxes) include a brief list of design elements for PK, safety, and acceptability becoming increasingly complex. Red boxes indicate development “no go” decisions. Light blue boxes indicate needed future studies to advance rectal microbicide products.

Table 1. Randomized controlled clinical trials of antiretroviral drugs powered to detect differences in HIV seroconversion

Study	Population	Drug	Per ^a	Freq ^b	PY ^c	N	Incid ^d CTRL	Incid ARV	RRR All (95% CI)	RRR Drug ^e Detected (95% CI)	NNT ^f	Resistance #(FTC:#TDF)	AE ^g
iPrEX	MSM, TGW	TDF/FTC	Oral	Daily	3324	2499	3.9	2.2	44 (15, 63)	92 (40, 99)	59	0 (0,0) [2 (1,0)] ^h	N
PROUD	MSM, TGW	TDF/FTC	Oral	Daily	465	544	9.0	1.2	86 (64, 96)	ND ⁱ	13	2 (2,0)	-
Ipergay	MSM, TGW	TDF/FTC	Oral	Sex ^j	431	400	6.6	0.9	86 (40, 98)	ND	18	0	GI, Renal
Partners PrEP	Serodiscord M&W	TDF TDF/FTC	Oral Oral	Daily Daily	7830	4758	2.0	0.7 0.5	67 (44,81) 75 (55, 87)	86 (57, 95) 90 (56, 98)	75	2 (1,1) [5 (4,1)]	PMN, GI, F
CDC TDF2	Hetero. M&W	TDF/FTC ^k	Oral	Daily	1563	1219	3.1	1.2	62 (22, 83)	50% SC ^l , 80% NSC	67	1 (1,1)	N, V, CNS
FEM-PrEP	Hetero. Women	TDF/FTC	Oral	Daily	1407	2120	5.0	4.7	6 (-41, 52)	No diff.	-	4 (4,0)	N, V, ALT
VOICE	Hetero. Women	TDF TDF/FTC	Oral Oral	Daily Daily	1661 1411	2977	4.2 4.6	6.3 4.7	-49 (-130, 4) -4 (-49, 30)	No diff. No diff.	- -	3 (3,0)	Cr
CAPRISA 004	Hetero. Women	TFV	V ^m gel	Sex ⁿ	1341	889	9.1	5.6	39 (4, 60)	>1,000 CVF †RRR	29	0 [0]	-
FACTS 001	Hetero. Women	TFV	V gel	Sex ⁿ	3036	2059	4.0	4.0	0 (-40, 30)	48 (1, 73) †CVL	-	-	-
VOICE	Hetero. Women	TFV ^o	V gel	Daily	2054	1992	6.8	6.0	15 (-20, 40)	34 (13, 87)	-	0	-
ASPIRE	Hetero. Women	DPV	IVR ^p	30d	4280	2629	4.5	3.5	27 (1, 46)	37 (12, 56) ^q 67 (23, 84) ^r	50	DPV NS ^s	-
Ring Study	Hetero. Women	DPV	IVR	30d	2805	1959	6.1	4.1	31 (1, 51)	NS	100	DPV NS	-
Bangkok	PWID M&W	TDF	Oral	Daily	9665	2413	0.68	0.35	49 (10, 72)	70 (2, 91)	303	0	-

^aRoute^bFrequency^cPerson-years^dIncidence, annual^eLower limit of assay quantitation (LLOQ) iPrEX, 10 ng/mL, all other studies plasma 0.31 ng/mL, unless otherwise noted.^fNNT, number needed to treat to prevent one infection

^gAdverse events (AEs): N, nausea; V, vomiting; F, fatigue; CNS dizziness; PMN, neutropenia; GI, gastrointestinal; Cr, creatinine elevation

^hNumbers in brackets based on more recent availability of “454 sequencing.”

ⁱND, not done, insufficient data published to compare

^jOn demand: TDF/FTC 2 doses prior to sex, 1 dose daily x2 after sex

^kSmall number with TDF alone initially, switched to TDF/FTC

^lSC seroconverter, NSC non-seroconverter

^mV vaginal gel

ⁿOn demand: first dose within 12 hours before sex, second dose within 12 hours after sex

^oVOICE has 3 active arms, oral TDF alone, oral TDF/FTC, TFV vaginal gel

^pTVR intravaginal ring

^qplasma TFV >95 pg/mL

^rresidual ring DPV definition of adherence

^sNNRTI resistance, not significant v. placebo; frequency greater than NRTIs in TDF/FTC studies. ASPIRE 10% (10/96) PBO; 12% (8/68) DPV; Ring Study DPV 18% (14/77); PBO 16% (9/56)

Table 2.**PrEP Product Development Challenges**

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- Sex behaviors, power, & HIV stigma complexity influence product adherence & development
 - Phase 2 proof-of-concept HIV challenge requires reliance on HIV challenge substitutes
 - Viral challenge validation incomplete - humanized mice, non-human primate, ex vivo tissue
 - Pharmacology and virology knowledge of HIV infection location and timing incomplete
 - Exposure event monitoring not objective, limits connection with medication exposures
 - Pharma leadership and funding limited
-

Table 3.

Supporting evidence of effect during development

Drug	Form ^a	Established Indication ^b	Clinical Mucosal PK ^c	Explant ^d (in vitro dosing)	Explant ^e (in vivo dosing)	Mouse	Macaque	Phase III Outcomes
N9	VR gel	Contracep	CVF, MRI	Protective VR <i>some toxic VR*</i>	ND	ND	Protect V	Enhances HIV, toxic
Cellulose Sulfate	V gel	Contracep	CVF, MRI	Protect V <i>some toxic V</i>	ND	ND	Protect V	Possibly enhance HIV, toxic, AEs
Pro2000	V gel	NT	CVL	Protective VRP <i>some toxic VR*</i>	ND	ND	Protect V	No protection
TDF/FTC ^f	oral	HIV Rx	R/B = 4.1 V/B = 2.1	-	SD TDF No R V ND	ND	Protect R V ND	Protect VR (Licensed)
TFV	V gel	NT	V/B = 183	Protect V	Protect V	Protect V	Protect V	Protect V
TFV	R gel	NT	R/B = 159 SPECT/CT	Protective R	Protect R	Protect R	Protect R	Phase II only
TFV	R enema	NT	R/B = 8,560 SPECT/CT	Protective R	Protect R	ND	Protect R	Phase I only
TAF/FTC ^f	PO	HIV Rx	R/B = 0.22 V/B = 0.12	-	ND	ND	Protect R	DISCOVERY Enrolling
DPV	IVR	NT	V/B = 8,963	Protect VR	Protect V	Protect V	ND	Protect V (EMA Review)
CAB-LA	IM	HIV Rx POC w/RPV	R/B = 0.08 V/B = 0.22	ND	ND	ND	Protect VR	HPTN 083/084 Enrolling
RPV-LA	IM	HIV Rx POC w/CAB	R/B = 1.2 V/B = 0.7	Protect VR	Protect R No protect V	Protect V	ND	Phase II only
VRC01	IV	NT	ND	Protective VRP	Protect VR	Protect VR	Protect VR	AMP Phase 2B

Bold, “positive result”; italic, “poor results”; bold italic, “results pending.”

Green “positive result”; red “poor results”; yellow “results pending”

^aFormulation: V vaginal, R rectal^bEstablished indication: contracep, contraception; NT, not tested; Rx, treatment; POC proof-of-concept^cClinical Mucosal PK: B, blood (PBMC for NRTI [TDF, TFV, FTC]; plasma for others); R, colorectal tissue (cells for TDF, TFV, TAF, FTC, if available); V = cervicovaginal tissue (cells for TDF, TFV, TAF, FTC, if available); P, penile tissue; CVF, cervicovaginal fluid. R/B, colorectal tissue cell to PBMC ratio; V/B, cervicovaginal to blood ratio. Red, green color coding assumes tissue dominant site of PREP action. MRI, magnetic resonance imaging of gadolinium label in vehicle; CT, SPECT/CT luminal distribution of radiolabel dissolved in vehicle.^dExplant tissue (in vitro dosing, ex vivo challenge) used for both efficacy and toxicity assessments: findings vary across studies and by toxicity test. *shedding of epithelium, MTT assay reduced viability, N9 necrosis of epithelial cells and lamina propria

Explant tissue (in vivo dosing, ex vivo challenge): SD TDF No R pro, single dose oral TDF only does not provide protection. ND, not done.

FTC part of more effective oral regimen. Mouse and macaque results based on both TFV product and FTC. PK based on TFV prodrug only (TFV-DP).

Table 4.

PrEP Formulation Pros & Cons. Eight categories of comparison are briefly summarized for each of five types of PrEP products, licensed and in development. (There are no long-acting rectal products in development.) The categories are described in the text and appear in the same order in each section to facilitate comparison across formulation types. Text color is used to differentiate pros (bold), cons (italic), and intermediate or conditional impact (bold and italic).

	Systemic	Topical – Vaginal	Topical – Rectal
Short-Acting	<i>Superior efficacy (86–100%)</i> <ul style="list-style-type: none">• <i>High daily/low on demand burden</i>• <i>New dosing behaviors</i>• <i>RVI & RAI coverage</i>• <i>Days to protection</i>• <i>Short PK tail</i>• <i>Systemic toxicity</i>• <i>No local (mucosal) toxicity</i>• <i>Modest healthcare system burden</i>	<i>Modest efficacy (39–88%)</i> <ul style="list-style-type: none">• <i>Reduced adherence burden</i>• <i>New dosing behaviors</i>• <i>Site specific coverage (unless RAI)</i>• <i>Few hours to 1 dose protection</i>• <i>Short PK tail</i>• <i>Reduced systemic toxicity</i>• <i>Local toxicity with some</i>• <i>Low healthcare system burden</i>	<i>Untested RM efficacy</i> <ul style="list-style-type: none">• <i>Reduced adherence burden</i>• <i>Behaviorally-congruent for some (lube, douche)</i>• <i>Site specific (MSM/TGW) (unless RVI)</i>• <i>1 hour to 1 dose protection (models)</i>• <i>Short PK tail</i>• <i>Reduced systemic toxicity</i>• <i>Local toxicity inapparent (so far)</i>• <i>Low healthcare system burden</i>
Long-Acting	<i>Untested efficacy</i> <ul style="list-style-type: none">• <i>Reduced adherence burden</i>• <i>No self-dosing behavior issues</i>• <i>RVI & RAI coverage</i>• <i>Days to protection (ex. bnMAbs)</i>• <i>Long PK tail</i>• <i>Systemic toxicity</i>• <i>Incision/injection site reaction</i>• <i>Increased HCS burden (IM/IV, AEs)</i>	<i>Modest efficacy (27–75%)</i> <ul style="list-style-type: none">• <i>Reduced adherence burden</i>• <i>New dosing behaviors</i>• <i>Site specific coverage (unless RAI)</i>• <i>Days to protection</i>• <i>Short PK tail</i>• <i>Reduced systemic toxicity</i>• <i>Local toxicity inapparent</i>• <i>Low HCS burden</i>	

AE, adverse event; bnMAbs, broadly neutralizing monoclonal antibodies; HCS, healthcare system; PK, pharmacokinetic; PrEP, pre-exposure prophylaxis; RAI, receptive anal intercourse; RM, rectal microbicide; RVI, receptive vaginal intercourse.

Table 5.

Select HIV PrEP Pipeline by Formulation

	Systemic	Topical – Vaginal	Topical – Rectal
Short-Acting	Oral <ul style="list-style-type: none"> • Daily TDF/FTC • On demand TDF/FTC (MSM/TGW only) • EFdA 	Gel <ul style="list-style-type: none"> • TFV BAT24 (<i>mITT & post hoc</i>) • TFV daily (<i>post hoc</i>) • Giffithsin/carageenan • PC-1005^a Fast-dissolving film <ul style="list-style-type: none"> • TFV film Fast-dissolving insert (tablet) <ul style="list-style-type: none"> • TFV/EVG 	Gel <ul style="list-style-type: none"> • TFV • Maraviroc • Dapivirine • IQP-0528 • PC-1005 (<i>MIV-150, Zn, CG</i>) Giffithsin/carageenan <ul style="list-style-type: none"> • Dapivirine Lubricant <ul style="list-style-type: none"> • Dapivirine Douche <ul style="list-style-type: none"> • TFV Fast-dissolving insert (tablet) <ul style="list-style-type: none"> • TFV/EVG
Long-Acting	Injectable IM <ul style="list-style-type: none"> • CAB-LA Implantable SC <ul style="list-style-type: none"> • TAF • CAB • EFdA Infusion IV/Injection SC <ul style="list-style-type: none"> • bnMAbs (<i>multiple</i>) 	Intravaginal ring <ul style="list-style-type: none"> • Dapivirine (DPV) • TFV • TDF • Maraviroc (MVC) • DPV/MVC • Pod-IVR TFV/FTC/MVC • IVR TFV/LNG MPT 	Key <ul style="list-style-type: none"> • Clinical efficacy established • Clinical trial ongoing/complete • Clinical trial pending • <i>Pre-clinical testing</i>

Abbreviations, mechanism of action

TFV, tenofovir, nucleotide reverse transcriptase inhibitor (NtRTI)

TDF, tenofovir disoproxil fumarate, NtRTI

TAF, tenofovir alafenamide fumarate, NtRTI

FTC, emtricitabine, nucleoside reverse transcriptase inhibitor (NRTI)

EFdA, 4'-ethynyl-2-fluoro-2'-deoxyadenosine, Nucleoside reverse transcriptase translocation inhibitor (NRTTI)

CAB, cabotegravir, integrase strand transfer inhibitor (INSTI), LA long-acting IM formulation

EVG, elvitegravir, INSTI

MVC, maraviroc, CCR5 receptor inhibitor

DPV, dapivirine, non-nucleoside reverse transcriptase inhibitor (NNRTI)

IQP-0528, NNRTI

MIV-150, NNRTI

Zn, zinc acetate

CG, carageenan, luminally active, blocks HIV binding to cells

Griffithsin, luminally active HIV binding to prevent HIV interaction with cells

bnMAb, broadly neutralizing monoclonal antibodies, neutralize envelope and recruit effector cells to kill HIV infected cells

LNG, levonorgestrel, hormonal contraceptive