Version 1.0

ATN 163

Safety, PK/PD, Acceptability, and Desirability of a Novel HIV Prevention Douche among Adolescent Men (DREAM)

Sponsored by:
The Eunice Kennedy Shriver
National Institute of Child Health and Human Development (NICHD)

with co-funding from:

The National Institute on Drug Abuse (NIDA)

The National Institute of Mental Health (NIMH)

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This protocol will be performed under the following IND#: 126136

After completing the screening evaluation, we will enroll 16 YMSM as participants if they are consented, eligible and able to complete the study visits. At the dosing visit, they will receive a single dose of a tenofovir (TFV) rectal microbicide douche (600mg TFV in 125mL 0.45% NaCl [half-normal saline]) at the Hopkins study site. Prior to using the TFV douche, baseline samples (see Appendix 1) will be collected to establish pre-dose conditions for safety assessments at the Enrollment Visit.

At the Dosing Visit, YMSM will then receive a single dose of TFV douche at the research unit, and, asked to retain the douche without defecation for up to 5 minutes, if possible. Subsequently, participants will be instructed on how to expel the douche contents. The amount of time that the douche was retained and the volume of expelled effluent will be measured. Post-dose observations and data collection will follow at 1, 6, 24, and 72 hours, using a sparse PK sampling design in which plasma and peripheral blood mononuclear cells (PBMC) are collected at each designated time. **Participants are randomized to only one time point for flexible sigmoidoscopy with rectal biopsies at either 1, 6, 24, or 72 hours after dosing (thus, providing 4 subjects at each time point).** Anoscopy, with rectal fluid collection will occur pre-dose and at either 1, 6, 24, or 72 hours after dosing to coincide with the time of each participant's flexible sigmoidoscopy. Microbiome specimens will be collected via anoscopy pre-dose and at 24 hrs post-dose.

Between sampling windows, YMSM will complete a web-survey examining their perceived reactions and comfort using the study douche, factors influencing product use in the future, and comfort with the trial procedures. The survey will be administered after dosing but scheduled not to interfere with other study assessments. Sampling for safety, PK, PD, and acceptability assessments will be collected according to the schedule of events. Phase I Trial participants will complete an IDI as part of their Termination visit.

KEY ROLES

Protocol Identification

Protocol Title: **Safety, PK/PD, Acceptability, and Desirability of a Novel HIV Prevention Douche among Adolescent Men (DREAM)**

Short Title: ATN DREAM

Date: December 10, 2022

Funding Agency, Sponsor, and Monitor Identification

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1. INTRODUCTION

1.1 Rationale

HIV prevention scientists are actively developing and testing the efficacy of next generation PrEP formulations[1-4]. Diverse drug delivery mechanisms could offer choice based on YMSM's sociocultural context, and, facilitate uptake and adherence of products that are behaviorally congruent with their sexual practices. For example, rectal douches[5] may be congruent with routine cleansing practices and behaviors regarded as normative prior to participating in activities where the probability of engaging in RAI is high.[6,7] Thus, it is crucial that next generation biomedical prevention be designed so that they can be delivered via mechanisms that not only deliver enough drug to block HIV transmission, but are also a good behavioral fit with the drug's intended end-users[8]. Achieving consistent and correct use among the product's consumers will require researchers to develop products that are desirable *and* acceptable, both within the context of clinical trials and in the real world.[9-11] However, YMSM's recruitment in these next generation PrEP studies has been limited, particularly among those under the age of 25. Thus, the absence of data focused on product safety, pharmacokinetic (PK)/pharmacodynamic (PD), and acceptability specific to YMSM may delay their uptake once they become available as a new HIV prevention modality.

This ATN protocol seeks to ensure the inclusion of adolescent and young adults' (age 15-24 years) perspectives as next generation biomedical prevention, specifically a rectal douche, is being developed for efficacy. The necessity of extending the testing of new HIV prevention modalities to this proposed adolescent population is motivated by anticipated behavioral differences, not biological ones. The biological differences in existing safety cohort (DREAM-01 with -02 and -03 ongoing) are anticipated to be trivial given the maturation of the GI tract in proposed study participants. We have no expectation of differences in safety or PK readouts in the proposed trial, though capturing these endpoints is essential, nonetheless.

1.2 Background

A safe and effective rectal microbicide used consistently would make an important impact on the HIV pandemic domestically and internationally. Per UNAIDS, there were an estimated 1.7 million new HIV infections globally in 2018 [12]. MSM are a high-risk group for the acquisition of HIV through unprotected receptive anal intercourse (URAI), and serve as an important source of HIV infection for other risk groups. MSM remain the majority of the U.S. epidemic, comprising 63% of incident HIV infections overall and 78% among men [13]. In Baltimore, 46.6% of MSM have HIV, and the seroincidence rate is 11% in young (ages 15-29) African-American MSM [14].

Condom use has had highly variable uptake, with high prevalence of URAI among young MSM, 38-65% of whom report not using condoms [15]. As Unprotected RAI (URAI) is a common mode of transmission of HIV which carries a 20-fold higher risk of transmission than vaginal intercourse, there has consequently been little or no decline in the rates of new HIV infections, particularly in MSM [16]. The relative risk of various sexual practices in transmission of HIV is outlined below in Table 1, with URAI ranking significantly higher than other practices. For these reasons, rectal microbicides are considered an important HIV prevention technology for all practitioners of RAI, not just for MSM.

Table 1: Relative Risk of Unprotected Sexual Practice in HIV Transmission (per sex act)

Behavior	Risk of HIV Infection	Reference
Oral Sex	0.0% (95% CI: 0,1.5)	Page-Shafer K. et al. 2002 [17]
Vaginal Sex	0.001 – 0.02% (95% CI: NA)	Kalichman S. et al. 2002 [18]
Anal Sex	0.25% (95% CI: 0.06,0.49)	Vittinghoff E. et al. 1999[19]

In May 2018, tenofovir disoproxil fumarate (TDF)/emtricitabine (FTC) dosing was approved by the Food and Drug Administration for use as HIV pre-exposure prophylaxis (PrEP) for adolescents.[20] More recently, emtricitabine (FTC)/tenofovir alafenamide (TAF) has been licensed for those at risk for sexual transmission of HIV, excluding those individuals at risk from receptive vaginal sex [21]An essential tool in ending the HIV epidemic, daily oral PrEP is more than 90% effective in preventing HIV infection among men who have sex with men (MSM) with high adherence.[22,23] However, while YMSM are disproportionally infected with HIV, PrEP uptake has been low.[24-27] Moreover, adherence to PrEP among YMSM has been suboptimal as evidenced in ATN 110 and ATN 113 [24,25]. Currently, researchers are exploring whether adherence challenges to daily oral PrEP could be overcome through a rectal douche given its popularity among MSM prior to engaging in receptive anal intercourse (RAI) [28].

The Developing Rectal Enema as Microbicide (DREAM) Program (U19 AI113048) is part of the DAIDS Integrated Pre-Clinical/Clinical HIV Topical Microbicide development program and seeks to advance an on-demand, behaviorally-congruent[8] tenofovir rectal douche for HIV prevention in persons at risk of HIV acquisition due to RAI. Behavioral data with adult MSM[5,8], pre-clinical macaque SHIV challenge studies, and Clinical Phase I PK studies with adults have been completed and encourage further clinical development. We summarize these data below:

Douching Prevalence. DREAM completed a Grindr survey of 5,000 racially/ethnically diverse adult MSM in the US to assess current douche practices and interest in a douche-delivered rectal microbicide, and to inform clinical product development. 80% of MSM frequently/always douched prior to RAI. Interest in a PrEP-delivered douche was high among receptive partners who currently douche (98%) and those who do not douche (94%). 95% of insertive partners were supportive of their receptive partners using such a microbicide douche. At present, however, limited data exist on the rectal douching practices of YMSM. Prior MSM samples reporting their rectal douching (RD) practices had mean ages in the mid-thirties (e.g., 33.9 years [29]; 36.6 years [30]). In a recent study conducted by our team with a sample of high-risk HIV-negative YMSM (N=180; ages 18-24), the mean age of douching onset was 19 years (SD=2.05). Racial/ethnic minority YMSM (50% of our sample) were also more likely to report douching (AOR= 2.24; 95% CI: 1.17, 4.29, p=.015) and to douche more frequently (β=.20, p=.009) than non-Hispanic White counterparts. Given the potential for YMSM to benefit greatly from a rectal PrEP douche in the future, and, recognizing the developmental differences that may be present for this group relative to adults, it is vital that we ensure YMSM's inclusion in these trials. These findings have recently been replicated in a Latin American and African Grindr survey [31].

Developing a behaviorally congruent PrEP douche: Since rectal douching is commonly practiced prior to RAI, this prevention strategy requires only minimal behavior change, as compared to oral or other topical strategies which require adherence, possibly daily, to dose-taking which is not normally part of their sexual practices. Furthermore, in contrast to condoms, enemas are commonly perceived to enhance the sexual experience, rather than detracting from it. Reasons cited for peri-coital enema use included pre-coital hygiene,

partner encouragement, and a belief that a deeply penetrating enema would confer greater protection against HIV than a gel [32]. Patterns of use of enemas underline the importance of the rapid achievement of sustained drug levels in the relevant tissues. Modeling studies by our group indicate oral tenofovir PrEP regimens require one week to achieve protective concentrations of the active drug form, TFV diphosphate (TFV-DP), in tissue. These concentrations can be achieved within 30 minutes after TFV 1% rectal gel dosing [33]. Finally, another marked advantage of topical strategies is the limited systemic absorption of drug, which minimizes adverse long- and short-term systemic side effects.

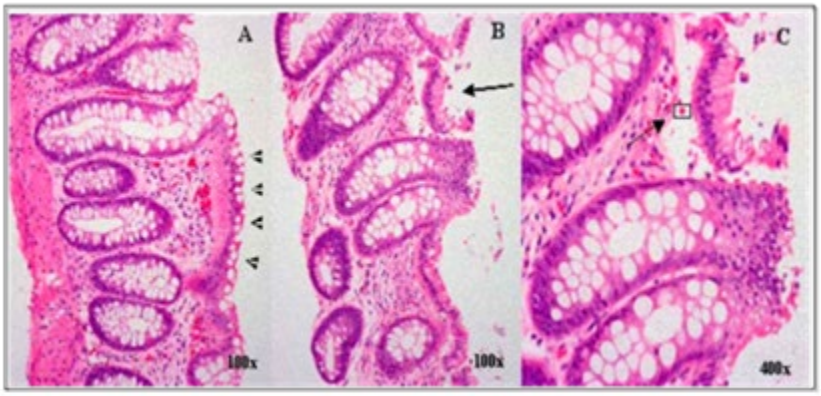


Figure 1: Histological Sections of Healthy Rectosigmoid Biopsies from Routine Colonoscopy

Panel A is uninjured with attached epithelia and prominent goblet cells (arrows). Panel B shows mild injury from colonoscope with epithelial sloughing and mucin depletion. Panel C is a high power view identifying a RBC (box) to emphasize the easy access of an HIV-sized particle.

The rectal columnar epithelium is fragile and extremely vulnerable to HIV-1 infection, in part due to the proximity of sub-epithelial stromal tissues that are densely populated with cells receptive to incident HIV-1 infection, such as dendritic cells (DCs), macrophages and T-cells that express both CD4 and both HIV-1 co-receptors CCR5 and CXCR4 [34,35]. Although the mechanisms of viral uptake and infection across rectal mucosa are not fully established, such physiological and anatomical differences may explain why HIV is more readily transmitted across rectal than across the cervicovaginal genital epithelium (Figure 1). In conjunction with this higher transmission risk of rectal versus vaginal exposure to HIV, there is a higher concentration of TFV achievable in colonic tissue than in vaginal tissue [36,37]. The plasma concentration of TFV associated with 90% protection (EC_{90}) in PK/PD models, 107 ng/mL, was not achieved by the most highly adherent heterosexual subpopulations; achievement of concentrations above 107 ng/mL requires fastidious adherence and daily dosing [38-40]. This is in contrast to the protective effect for MSM which is achieved with only 2 to 4 doses of oral TDF per week [41].

TFV-based HIV PrEP, when taken, provides a high degree of protection against HIV when used as a single daily oral dose in men and women or as a pre- and post-coital vaginal gel dose. Seven randomized controlled trials have tested TFV-based PrEP regimens, and it is now clear that plasma concentration correlates with HIV protection response across these trials [38]. Though heterogeneous estimates of HIV protection with daily TDF/FTC have ranged from 0 to 100%, the variance in results can mostly be explained best by wide-ranging adherence. There is evidence in MSM that protection appears to require less frequent dosing. For example, in iPrEx, the only study focusing on MSM and transgender women, daily oral TDF/FTC provided only 44% protection in the overall study, but 96% protection with evidence of only 4 doses taken per week, on average, as measured by plasma TFV levels [42]. This is in contrast to studies in heterosexuals whose primary route of infection is not rectal, which demonstrated equivalent (>90%) protection requires 6-7 doses per week [38-40]. For heterosexual women, in contrast to MSM, this difference is likely due to the >100-fold higher concentrations of the active drug moiety, TFV-DP, in colon tissue than in vaginal tissue [36,37].

While no efficacy studies to date have evaluated rectal dosing, RMP-02/MTN-006 showed that, compared to a single oral 300mg TDF dose, a single 44 mg TFV rectal gel achieved 90-times higher colon tissue TFV-DP concentrations more rapidly, and with greater protection in *ex vivo* HIV challenge of colon tissue explants [33,43,44].

When compared directly in Partners PrEP, no statistically significant difference emerged with TDF compared to TDF/FTC [39,45]. Given (i) the very high HIV prevention efficacy of TDF-based PrEP taken consistently orally, (ii) more efficient delivery of active drug to the colon with rectal compared to oral dosing, (iii) the large influence of poor adherence on PrEP trial outcomes, (iv) the frequency of unprotected RAI in men and women, (v) pre-existing common behaviors of enema use before and after RAI by many MSM, and (vi) the potential organ toxicity of ongoing systemic oral therapy, we reason that an enhanced TFV enema is a necessary and viable PrEP candidate capable of both high degrees of adherence and efficacy. This protocol is the first step in the DREAM IPCP Program to advance an enema formulation toward full clinical development as a rectal microbicide for adolescent sexual and gender minority youth.

1.3 Description of TFV Rectal Douche Study Product

We propose a phase I clinical study of a dose of a tenofovir (TFV) enema in YMSM. The douche formulation is selected to provide a more behaviorally congruent pre-exposure prophylaxis alternative to both oral daily dosing and rectal gel dosing using pharmacokinetic and behavioral rationale described above. Unique among rectal microbicide studies and in biomedical HIV prevention trials with youth, we propose this initial single dose study of the TFV douche in order to have sufficient evidence to ensure the inclusion of YMSM if subsequent trials demonstrate HIV prevention efficacy and we are successful in demonstrating PK congruence between adults and YMSM in the colon tissue cell concentrations.

The study product active ingredient is TFV (PMPA,9-[(R)-2-(phosphonomethoxy)propyl] adenine monohydrate), an acyclic nucleotide analogue of adenosine monophosphate. Once inside the cell, TFV is phosphorylated by cellular enzymes to form TFV-DP. TFV-DP is a competitive inhibitor of HIV-1 reverse transcriptase (RT) that terminates the growing deoxyribonucleic acid (DNA) chain. TFV-DP is a weak inhibitor of mammalian DNA polymerases α, β, and mitochondrial DNA polymerase. TFV is licensed only as the prodrug form, TFV disoproxil fumarate (TDF) which is available in a 300 mg dosage form as well as in fixed dose combination with emtricitabine (200 mg), also an HIV nucleoside reverse transcriptase inhibitor (NRTI), marketed as Truvada™ and indicated for prevention of HIV infection in persons at risk of HIV infection as well as for treatment in combination with another NRTI and a more potent antiretroviral drug. TFV Powder will be supplied by CONRAD.

The TFV douche product is a clear, colorless fluid with pH 7.0. It contains 660 mg TFV in 125 mL, and has a hypotonic osmolality (~ 145 mOsm/kg). Characteristics of the formulation of the TFV douche which will be tested clinically are specified below in Table 2.

Table 2: Characteristics of Tenofovir Hypotonic, High Dose Douche

	Product C
Dosing	660 mg per 125 mL
Concentration	5.28 mg/mL
Ingredients	
TFV	0.66 g
NaOH 18%w/v (18g NaOH in 100 ml water)	0.75 mL
NaCl, NF	0.412 g
Purified water	124.25 g or mL
Target Osmolality	145sm/kg

1.3.1 Formulation Testing

Table 3 compares the various unit dose formulations of TFV gel that have been studied, compared with the product currently under study.

Table 3: Comparison of 3 TFV 1% Gel Formulations Studied Clinically

Descriptive name	**Vaginal formulation**	**Reduced glycerin vaginal formulation**	**Rectal formulation**	**DREAM-03**
Acronym	**VF**	**RGVF**	**RF**	**-**
TFV dose	1% w/v; 40 mg/3.5 mL	1% w/v; 40 mg/3.5 mL	1% w/v; 40 mg/3.5 mL	5.28 mg/mL (660 mg/125 mL)
Osmolality (mOsmol/kg)	3111	846	479	145
Prior Vaginal Dose Study	CAPRISA 004, VOICE, FACTS 001			
Prior Rectal Dose Study	RMP-02/MTN-006, CHARM-01, CHARM-02	MTN-007, CHARM-01, CHARM-02, MTN-017	CHARM-01, CHARM-02	DREAM-01

1.3.2 Anti-HIV-1 Activity, Resistance, and Cross-resistance

The *in vitro* antiviral activity of unformulated TFV against laboratory and clinical isolates of HIV-1 was assessed in lymphoblastoid cell lines, primary monocyte/macrophage cells and peripheral blood lymphocytes [46]. The 50% effective concentration (EC_{50}) values for TFV were in the range of 0.04 µM - 8.5 µM. TFV displayed antiviral activity *in vitro* against HIV-1 clades A, B, C, D, E, F, G, and O (EC_{50} values 0.5 µM - 2.2 µM) and showed strain specific activity against HIV-2 (EC_{50} values ranged from 1.6 µM to 5.5 µM). HIV-1 isolates with reduced susceptibility to unformulated TFV have been selected *in vitro* [47]. Cross-resistance among certain NRTIs has also been recognized in vitro [48]. Somewhat reassuringly, in clinical trials of TFV-based PrEP and the subsequent rollout of PrEP in at risk populations, less than a handful of cases of acquired TFV-resistance have been reported [42,45,49-53].

1.4 Animal Studies

At least 7 different TFV-containing gel formulations were evaluated in animal toxicology studies prior to conduct of DREAM-01 that also support conduct of DREAM-03. The details of these studies are summarized in three GLP toxicokinetic study reports including the following products:

(1) *Fourteen Day Repeat Dose Toxicology Study of Tenofovir Vaginal Gel in New Zealand White Rabbits following Rectal Administration*, Pacific BioLabs (West Chester, PA), Report Number X8F130G, IND 126,136, Section 6, Attachment 1; Cross-referenced from IND 73,382), which evaluated TFV 1%, 3%, and 10% Vaginal Formulation (High Osmolality)

(2) *14-Day Rectal Irritation Study of Rectal Specific Formulation (RF) of TFV Gel in Rabbits.* MPI Research, (Mattawan, MI), Study Number 1901-001 (IND 126,136, Section 6, Attachment 2; Cross-referenced from IND 113,620), which evaluated TFV 1%, 3%, and 10% Rectal Specific Formulation (Rectal Specific Formulation is referred to elsewhere in this protocol as "RF")

(3) *Rectal Gel (TFV/GRFT) and RGVF of TFV Gel: 28-Day Rectal Dose Toxicity Study in Male and Female Rabbits*, MPI Research (Mattawan, MI), Study Number 1901-002, September 13, 2012 (IND 126,136, Section 6, Attachment 3; Cross-referenced from IND 73,382) which evaluated "RGVF of TFV 1% Gel (referred to elsewhere in this protocol as "RGVF") and "Rectal Gel (1% TFV)" (referred to elsewhere in this protocol as "RF").

The results of these studies are summarized in Section 4.1, 4.2, and 4.3 of the Tenofovir Douche Formulation Investigator Brochure (4th edition, August 5, 2019). Summarizing these three studies, no toxicity signals were identified and the (No Observed Adverse Effect Level) NOAEL was determined to be greater than the highest dose used in each of the studies, namely, 10% of vaginal formulation (VF), and 10% rectal formulation (RF), and 1% reduced glycerin vaginal formulation (RGVF). For the multiple dose level studies of VF and RF, there was an increase in local and systemic TFV concentration, though this was not strictly dose proportional. The FDA found the above TFV gel studies sufficient to allow the DREAM-01 clinical study of 3 TFV douche formulations without specific TFV douche pre-clinical studies.

TFV Enema Administration – Toxicology. The potential toxicity and toxicokinetics of TFV enema formulations are assumed to be comparable to that seen in the studies of TFV gel. Therefore, in accordance with FDA guidance provided in response to our pre-IND submission, Rabbit Rectal Irritation studies were not repeated using the TFV enema formulation. The IND for the first-in-human study of the TFV douche, DREAM-01, referenced these TFV gel studies.

Macaque PK and SHIV Challenge Studies. The TFV douche proposed for this study was evaluated for PK and PD (SHIV rectal challenge) in macaques. [54]

Macaque PK Comparison. The TFV rectal douche was administered as a single dose and compared to human equivalent dosing (22 mg/kg) of oral daily F/TDF PrEP to compare the uptake of TFV into the tissue and systemic circulation. Single TFV HOsm high (5.28 mg/ml) dose douches achieved peak plasma TFV levels similar to daily oral PrEP. Tissues TFV-DP concentrations at the rectal tissue portal of virus entry, however, were markedly higher at 1-24 hours post HOsm douching than continued daily oral PrEP. Importantly, no such oral-rectal dosing difference was noted in distal lymphoid tissues including colonic lymph nodes.[55]

Macaque SHIV Challenge Comparison. Macaques received the same steady-state oral F/TDF and rectal TFV douche dosing in the PK studies as for the SHIV challenge studies. Macaques received a weekly SHIV rectal challenge within one hour of the sham, steady-state oral F/TDF, and rectal TFV douche dosing. The weekly rectal douche one hour before weekly SHIV challenge protected 5 of 6 macaques, while all 6 sham controls were infected and 3 of 6 macaques in the steady-state oral F/TDF daily dosing group were infected by 8 weeks. Our results demonstrate the ability to rapidly deliver protective doses of TFV via the rectal route at portal of virus entry as a potential low cost and safe alternative to oral PrEP.[56]

1.5 Condom Integrity

The RF TFV was shown to be compatible with lubricated and non-lubricated latex condoms using the Magee condom compatibility test based on the Texture Analyzer. A second condom compatibility study with the RF TFV was performed by FHI using the ASTM standard testing protocol (ASTM D7661-10). In this testing, 6 styles of condoms were evaluated: 3 non-lubricated latex (Durex®, Lifestyles®, and Trojan®), 2 polyisoprene (Avanti Bare® and Lifestyles SKYN®), and 1 polyurethane (Trojan Supra®). The condoms were treated with the RF TFV gel and tested for tensile (break force and elongation) and airburst (pressure and volume) properties. Untreated condoms subjected to testing procedures served as a control; condoms treated with a known degradant served as a positive control.

The condom testing data for the RF TFV gel was evaluated for statistical significance using Tukey's multiple range test. Overall, in 20 of the 24 sets of results (4 tests and 6 condoms) there were either no significant

differences between the treated and control groups or the treated group performed significantly better than the control. In the four sets of results in which the treated condoms performed significantly worse than the controls, which were observed for two of the non-lubricated latex condoms and for the polyurethane condom, the differences were much smaller (at least 74% smaller) than those observed after condom exposure to the known degradant. There were no significant differences between the treated and control groups for the polyisoprene condoms.

For DREAM-01 and this exploratory trial in YMSM, the TFV douche will not be used in the context of RAI, condom compatibility studies will be deferred until an appropriate enema formulation candidate has been identified for further product development. For the ½ normal saline (½ NS) solutions to be used as enemas in the context of RAI, the FDA has identified these formulations as consistent with normal medical practice to meet product quality standards (FDA written responses to Pre-IND 126136 meeting request, dated 5/22/15).

1.6 Rectal Microbicide Development in the DREAM Program

DREAM ATN has been developed to dovetail with the on-going DREAM clinical development program of a rectal TFV douche described below. The goal of DREAM ATN is to define the safety, PK, PD, and acceptability of a rectal TFV douche administered to adolescent and young adult MSM. Establishing, as we expect, the PK equivalence of the TFV douche in adolescent, young adult, and adult study participants is designed to accelerate inclusion of YMSM in future TFV douche studies and to provide a PK bridge to YMSM once efficacy of the TFV douche is demonstrated in adults, as is anticipated to be the first population studied in pivotal randomized clinical seroconversion studies leading to market approval by FDA. The necessity of extending the testing of new HIV prevention modalities to this proposed adolescent population is motivated by anticipated behavioral differences, not biological ones. The biological differences in existing safety cohort (DREAM-01 with -02 and -03 ongoing) are anticipated to be trivial given the maturation of the GI tract in proposed study participants. We have no expectation of differences in safety or PK readouts in the proposed trial, though capturing these endpoints is essential, nonetheless. The product to be studied in DREAM ATN is the same as that studied in DREAM-01 (completed), DREAM-02 (recruiting), and DREAM-03 (recruiting). For context, these studies are summarized here.

DREAM-01 (completed) is the initial clinical study in the DREAM Program and designed as a single ascending dose study to demonstrate safety, acceptability, and feasibility of exceeding pre-established colon tissue cell TFV-DP concentration targets (associated with full protection in iPrEx) for one week. Preliminary results of DREAM-01 indicate that objectives were met or exceeded (details below).

DREAM-01 compared three different douche formulations including iso-osmolar Product A (220 mg TFV in 125 mL normal saline [0.9% NaCl]), iso-osmolar Product B (660 mg TFV in 125 mL normal saline), and Product C (660 mg TFV in 125 mL half-normal saline [0.45% NaCl], a hypo-osmolar formulation. Each participant received each of the 3 formulations to allow paired comparisons for safety and PK. We enrolled 18 healthy HIV-uninfected adult MSM, to identify the dose and osmolarity that achieves target colonic tissue cell TFV-DP concentrations to exceed iPrEX tissue EC_{90}, based on levels from 4 PO TDF doses/ week. Semi-intensive sampling of plasma and PBMCs and sparse sampling of rectal fluid and colon tissue was performed. Ex vivo explant susceptibility to HIV infection was assessed via colon biopsies, and acceptability was assessed via questionnaires and in-depth interviews. A within-subject comparison of safety, PK, PD, distribution, & acceptability of 3 TFV douche formulations was conducted. [57]

The douche was demonstrated to be safe and well tolerated, with high acceptability to participants. All three products were generally well-tolerated. There were three Grade 3 Adverse Events (AE), one in each study phase, none of them deemed to be product related. There were a total of two AE's that were deemed product-related (rectal dryness and blood-tinged enema effluent, in a participant with history of internal hemorrhoids), and both were Grade 1 (See Table 4). Adverse events were also not dose related.

Table 4. Aggregate adverse events and histology results from DREAM-01 (n=18)

	While on Study	During Baseline	During Phase 1	During Phase 2	During Phase 3
		No Study Product	Study Product A	Study Product B	Study Product C
		(Visit 2)	(Visits 3-7)	(Visit 8-11)	(Visit 12-16)
Participants who experienced an AE [3]					
Grade 1	29	0	13	8	8
Grade 2	14	1	8	2	3
Grade 3	3	0	1	1	1
Grade 4	0	0	0	0	0
Grade 5	0	0	0	0	0
Total	46	1	22	11	12
Total # of AEs reported [8]					
Grade 1	60	0	29	10	21
Grade 2	20	1	10	2	7
Grade 3	3	0	1	1	1
Grade 4	0	0	0	0	0
Grade 5	0	0	0	0	0
Total	83	1	40	13	29

Histology. Rectal biopsies from baseline (pre-dose) and post-dose at times associated with PK and PD sampling (1, 3, 6, 24, 72, and 162 hours) and displayed in Table 5. AE refers to a scale established for evaluation of inflammatory bowel disease.

Table 5. Rectal biopsies from baseline (pre-dose) and post-dose at times associated with PK and PD sampling (1, 3, 6, 24, 72, and 162 hours)

Product	Post-Dose Hours	N	Grade		Epithelial Denudation		Lamina Propria Hemorrhage	
			Median (IQR)	p value*	Median (IQR)	p value	Median (IQR)	p value
Baseline	pre-dose	21	0.0 (0.0, 1.0)	-	0.0 (0.0, 1.0)	-	1.0 (0.0, 1.0)	-
A	1-6	21	1.0 (0.0, 1.0)	0.79	0.0 (0.0, 1.0)	1.00	1.0 (0.0, 1.0)	1.00
	24	6	0.5 (0.0, 1.3)	1.00	0.0 (0.0, 2.0)	0.31	1.0 (1.0, 1.0)	0.50
	72	8	0.5 (0.0, 1.0)	1.00	0.0 (0.0, 0.0)	0.63	0.0 (0.5, 1.0)	1.00
	168	6	0.0 (0.0, 0.3)	1.00	0.0 (0.0, 0.3)	1.00	1.0 (0.0, 1.0)	1.00
B	1-6	19	1.0 (0.0, 1.0)	0.31	0.0 (0.0, 0.0)	0.79	1.0 (0.0, 1.0)	0.51
	24	6	1.0 (0.8, 1.0)	0.75	1.0 (0.0, 1.0)	0.50	1.0 (0.0, 1.0)	0.63
	72	8	0.5 (0.0, 1.0)	1.00	0.0 (0.0, 0.0)	1.00	1.0 (0.0, 1.0)	1.00
	168	5	1.0 (0.0, 1.0)	1.00	0.0 (0.0, 0.5)	0.63	1.0 (1.0, 1.0)	1.00
C	1-6	16	0.0 (0.0, 1.0)	0.69	0.0 (0.0, 0.8)	0.59	1.0 (1.0, 1.0)	0.22

	24	6	1.0 (1.0, 1.3)	0.63	0.5 (0.0, 1.3)	1.00	1.0 (0.8, 1.0)	0.25
	72	6	0.5 (0.0, 1.0)	1.00	0.0 (0.0, 1.0)	1.00	0.5 (0.0, 1.0)	1.00
	168	5	0.0 (0.0, 0.5)	1.00	0.0 (0.0, 0.0)	1.00	1.0 (1.0, 1.0)	1.00

Acceptability. All three DREAM Products were found acceptable to participants, including Product C, the hypo-osmolar, high dose formulation to be used in this trial. Participants indicated high baseline anticipated likelihood of using the products before receptive anal intercourse for HIV prevention, with 15/17 participants indicating at baseline that they would be likely or very likely to use such products.

PK-PD. Plasma concentration vs. time course indicates median TFV concentration at all times after the TFV douche fall below the median steady-state plasma TFV trough concentrations associated with 4 doses per week (HPTN 066 as reference population) (Figure 2). TFV-DP in colon tissue cells extracted from colon biopsies vs. time. TFV 660 mg dose, half-normal saline (HNS) achieves similar peak TFV-DP concentrations as TFV 660 mg in normal saline (NS) formulation, but the rate of rise is faster based on data one hour after dosing. All products provide concentrations within 1 hour after dosing that exceeds the target concentration, sustained across the first 24 hours before falling to levels at (TFV 220 mg NS) or several fold above (TFV 660 mg, NS or HNS) target concentrations by 3 days post-dose. This decline is faster than the TFV-DP decay previously observed in PBMCs. Viral p24 antigen (accumulated over 14 days in the tissue supernatant) from explants collected after *in vivo* TFV douche dosing and *ex vivo* biopsy HIV challenge in tissue culture. The median p24 antigen declines to very near lower limit of assay quantitation by 1 hour after dosing where it remains until increasing back nearly to baseline values by 72 hours, generally mirroring the TFV-DP colon tissue cell TFV-DP pattern. Compared to baseline, the group of all biopsies collected 1-24 hours after dosing reduced HIV viral replication in *ex vivo* colon tissue explants by median (IQR) 0.77 $\log_{10}$ (0.20, 0.81) ($p<0.01$).

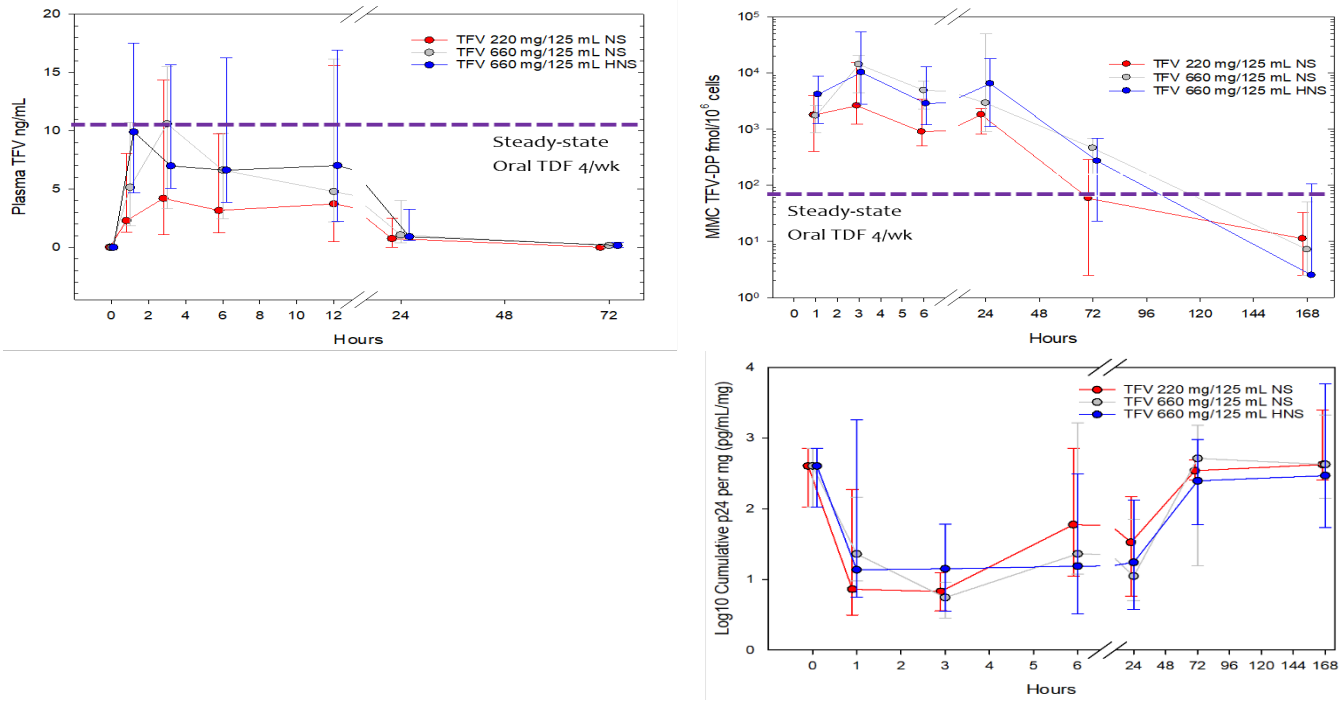


Figure 2. PK and PD readouts from the DREAM-01 clinical study. All values are median and interquartile range. *Upper Left Panel*. Plasma concentration vs. time course with 4 oral F/TDF doses per week as reference (HPTN 066 as reference population). *Upper Right Panel*. TFV-DP in colon tissue cells extracted from colon biopsies vs. time. Same TFV-DP 4 dose per week

reference for oral F/TDF dosing reference as for plasma. *Lower Right Panel*. Viral p24 antigen (accumulated over 14 days in the tissue supernatant) from explants collected after *in vivo* TFV douche dosing and *ex vivo* biopsy HIV challenge in tissue culture.

Summarizing, DREAM-01 provided supportive evidence that a rectal TFV douche provides potential for a novel, behaviorally-congruent delivery method for condomless RAI PrEP which is safe, acceptable, and well-tolerated. In addition, a single dose results in minimal systemic concentrations rapidly (1-3 hrs) reduced HIV replication *ex vivo* in explants. Colon cell TFV-DP concentrations exceeded the steady-state colon tissue cell concentrations associated with >90% efficacy (based on modeling iPrEx and smaller pharmacokinetic studies) by >10-fold and were higher with each escalation step in a dose-dependent manner (A<B<C). Findings support further clinical development of Product C (the formulation planned for DREAM ATN) as on demand, behaviorally congruent TFV douche for PrEP.

DREAM-02 (recruiting, open to enrollment) assesses (objective 1) the effect of a TFV douche before and after simulated sex on drug and ejaculate distribution in the GI tract as well as (objective 2) the effect of a second non-medicated douche on an initial TFV-medicated douche (given high frequency of multiple sequential douches in practice[58]). Regarding douche-sex sequencing (objective 1), we hypothesize that douching after sex will increase the distribution of HIV surrogates within the lower GI tract and may lead to a mismatch of drug and HIV surrogate distribution, possibly, reducing rectal douche effectiveness. This information will be essential to the design of phase 2 extended safety studies of our TFV douche, especially with regard to providing guidance for research participants and study counselors. Regarding the effect of a second non-medicated douche (objective 2), we hypothesize that a non-medicated douche will modestly reduce the tissue TFV-DP concentrations following a single medicated douche due to luminal displacement and colorectal tissue dilution of the prior TFV-medicated dose, similar to observations of the impact of sex on TFV-DP in cervicovaginal tissues in MTN-011 [59]. As such, DREAM-02 will provide data complementary to the 3 dose sequences planned for DREAM-03. DREAM-02 is open for enrollment at Johns Hopkins.

DREAM-03 (recruiting, open to enrollment) is the logical multiple dose safety and PK study which follows the DREAM-01 single ascending dose study and will be done simultaneously with the DREAM-02 study on coital timing. DREAM-03 is currently partially enrolled at Johns Hopkins and will soon be open for enrollment at University of Pittsburgh. Behavioral data from the DREAM Project indicates that MSM commonly use several sequential douches precoitally, prior to RAI. The effects of several sequential TFV douches on tissue integrity, tissue TFV-DP concentrations, plasma concentrations, and other rectal safety outcomes are unknown. Furthermore, it is unknown whether douching first with non-medicated douches followed by TFV-containing douches (or vice versa) has any impact on safety or tissue concentrations achieved. Therefore, DREAM-03 is designed to understand the effects of multiple sequential douche doses on safety and PK/PD endpoints. Together DREAM-01, DREAM-02, DREAM-03, and data from other DREAM Projects will inform the design and labeling of an optimal TFV douche for further clinical testing.

1.7 DREAM ATN Design Rationale

According to our working target product profile and our clinical niche as an on demand PrEP product, the TFV douche is being developed to provide protective concentrations in colon tissue cells for a similar duration as that achieved using the on demand oral F/TDF 2+1+1 regimen demonstrated equally effective with daily F/TDF in Ipergay.[60] Therefore, sampling for PK and safety will occur over 72 hours based on our model-based PK estimates. In addition, we intend to establish the congruence of PK between adult in the DREAM studies and the YMSM population to be recruited in this DREAM ATN protocol. Accordingly, we propose a slightly shorter total PK sampling duration (3 days) when compared to DREAM-01 (7 days). This

will enable documentation of concentrations throughout the first day when DREAM-01 indicated sustained concentrations well above target concentrations in colon tissue cells as well as the initial decay in concentration that occurs between 24 and 72 hours when colon tissue TFV-DP median concentration falls nearer to the 4 dose per day target concentration.

To achieve these goals, blood (plasma and cells), colon tissue (tissue homogenate and cells), and rectal fluid will be collected using a relatively sparse sampling scheme that balances a desire for rich PK data collection and feasibility. Altogether, each participant will have 5 pharmacokinetic blood samples, 2 rectal fluid samples, and 2 biopsy sessions (up to eight pinch biopsies at baseline and 20 pinch biopsies at one time point following dosing) for the enema product studied. The total number of pinch biopsies collected following enema formulation dosing (n=20) in adolescents is well below the number collected in adult participants (n=120) in DREAM-01.

Pre-dose baseline visit tissue biopsies, plasma and blood will be collected to provide comparisons for pre and post dosing in challenge studies and to establish that no oral PrEP is being taken, respectively. After a single dose of the TFV douche (previously referred to as Product C in DREAM-01) we will collect samples at pre-dose, 1, 6, 24, and 72 hours to establish the early rate of increase, peak concentrations, and initial decay of TFV and TFV-DP in all matrices. Plasma and PBMC will be collected from each participant at all of these timepoints. Rectal fluid sampling is partially destructive of local matrix, so, we are planning only two rectal fluid samples per participant in the first 72 hours, with each participant contributing two samples among the four blood sampling times (1, 6, 24, and 72 hours). Colon tissue biopsies will be collected at each of these times, but each participant will be assigned to only one of these post-dose biopsy times, using a sparse sampling approach and population PK analysis. Aggregate estimates for PK parameters will be used. Non-linear mixed effect modeling will be attempted, but the total sample size may be insufficient to generate robust PK parameter estimates.

Note: Gastrointestinal (GI) sampling procedures for PK/PD assessment (e.g. anoscopy, flexible sigmoidoscopy with biopsy) will not be conducted in participants less than age 18. Participants older than age 18 will be prioritized to undergo GI assessment, but if this adversely impacts accrual feasibility, sample collection may be limited to plasma/PBMC only.

2. STUDY OBJECTIVES

The global objective of this study is to identify the safety, PK, PD, and acceptability of a single dose of the Product C douche. Study objectives and endpoints are summarized in Table 6.

Table 6: Study Objectives and Endpoints

Objectives	Endpoints
Primary	
• To describe YMSM's acceptability of a novel HIV prevention douche, prior to and after using a one-time dose of the TFV douche.	• Questionnaires/CASI • In-depth interviews (IDIs)
• To quantify the colonic tissue cell TFV-DP concentration following TFV douche administration among YMSM	• Colonic tissue cell TFV-DP concentrations at 10-20 cm from the anal verge
• To assess safety of the TFV douche and quantify any association with clinical toxicity or adverse events	• Adverse Events (clinical or laboratory) Grade 2 or higher
Secondary	

• To compare systemic, tissue, and luminal PK of TFV and TFV-DP.	• Descriptive statistics of analyte-matrix concentration: o Plasma TFV o PBMC TFV-DP o Colon tissue homogenate TFV and TFV-DP o Colon tissue total cell (MMC) TFV-DP o Rectal fluid TFV concentration
Exploratory	
• To examine the association of YMSM's behavioral practices on DREAM TFV douche acceptability.	• Questionnaires/CASI • In-depth interviews (IDIs)
• To explore the impact of the TFV douche on colonic tissue infectability with HIV	• *Ex vivo* explant challenge with evaluation of p24 antigen production in infected biopsies.
• To explore the impact of the TFV douche on various tissue-level outcomes including histology and the regional microbial community of the colon	• Colon biopsy histology • 16 S ribosomal RNA gene sequencing • Changes in relative abundance of microbiota before and after dosing

2.1 Primary Objectives

Acceptability
To describe YMSM's acceptability of a novel HIV prevention douche, prior to and after using a one-time dose of the Product C douche.

Pharmacokinetics
To quantify the colonic tissue cell TFV-DP concentration following TFV douche administration among YMSM

Safety
To assess safety of the TFV douche product and quantify any association with clinical toxicity or adverse events

2.2 Primary Endpoints

Acceptability
Participant self-report of product acceptability, liking the product, and likelihood of use in the context of receptive anal intercourse

Pharmacokinetics
Colon tissue MMC TFV-DP concentrations from 1 hr to 72 hours post-dose

Safety
Adverse Events Grade 2 or higher clinical and laboratory adverse events as defined by the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 2.1 dated Jul 2017 and Addendum 3, Rectal Grading Table for Use in Microbicide Studies.

2.3 Secondary Objectives

Systemic, tissue, and luminal PK of TFV and TFV-DP

To compare systemic, tissue, and luminal PK of TFV and TFV-DP in multiple matrices, including plasma, PBMC's, colon tissue, and rectal fluid.

2.4 Secondary Endpoints

Descriptive statistics of analyte-matrix concentration:

- Plasma TFV
- PBMC TFV-DP
- Colon tissue homogenate TFV and TFV-DP
- Colon tissue total cell (MMC) TFV-DP
- Rectal fluid TFV concentration

2.5 Exploratory Objectives

Behavioral
To examine the association of YMSM's behavioral practices on TFV douche acceptability.

Ex Vivo
To explore the impact of the TFV douche on colonic tissue infectability with HIV

Histology
To explore the impact of the TFV douche on various tissue-level outcomes including histology and the regional microbial community of the colon

2.6 Exploratory Endpoints

Behavioral
Participants' self-reported prior experiences and comfort using rectal products, as well as douching or other rectal hygiene practices.

Participants' self-reported reactions to the study product and administration method, including perceptions of their partners' reactions and future intentions to use product with partners before sex.

ExVivo
Ex vivo colon tissue explant HIV challenge

Histology
Colon tissue histology semi-quantitative scoring system reflecting extent of columnar epithelium loss

Microbiome
Colon microbiota will be characterized using 16 S ribosomal RNA gene sequencing. Changes in relative abundance of microbiota before and after product dosing.

3. STUDY DESIGN

The purpose of the overall study is to ensure the inclusion of adolescent and young adults' perspectives as next generation biomedical prevention, specifically a rectal douche (TFV microbicide; 660mg TFV in 125mL half-normal saline), during its clinical testing and development for safety and efficacy.

We will recruit YMSM who have expressed interest in being contacted to screen for eligibility into a Phase I HIV prevention biomedical trial. We expect to initially **consent and** screen 48 YMSM living in the greater Baltimore Metropolitan Area to enroll 16 evaluable participants. YMSM who are willing and cleared to participate in the Trial visit will be asked to use a single dose of the TFV douche at the Hopkins study site. Prior to using the douche, baseline samples (see Appendix 1) will be collected to establish pre-dose conditions for safety assessments as well as sample collection for TFV analyte concentration in multiple matrices for participants taking oral TDF/FTC PrEP. These concentrations will be used to adjust post-dose PK and PD assessments. Participants will also complete a post-dose survey and an in-depth interview (IDI) regarding product acceptability and experiences participating in the study at their termination visit.

As a second aim of this protocol, we will also conduct in-depth interviews with individuals who choose not to participate in the Phase I Safety Trial or who were not eligible after medical screening (N=32) in order to examine perceived barriers and facilitators to their willingness to participate in biomedical trials, and examine their suggestions to maximize YMSM's engagement in future biomedical trials.

Note: Gastrointestinal (GI) sampling procedures for PK/PD assessment (e.g. anoscopy, flexible sigmoidoscopy with biopsy) will not be conducted in participants less than age 18. Participants older than age 18 will be prioritized to undergo GI assessment, but if this adversely impacts accrual feasibility, sample collection may be limited to plasma/PBMC only.

3.1 Study Population & Recruitment

Participants will be between 15-24 years old HIV-negative YMSM living in the Baltimore area. We expect that, at a minimum, a third of the sample screened will identify as a racial/ethnic minority. We will use both face-to-face recruitment through ongoing events and clinic referrals, as well as online advertisements through social media recruitment. The combined recruitment strategy has yielded great success in prior behavioral and biomedical trials, and is considered the standard for recruiting diverse samples of YMSM.

3.2 Sample Size

We will recruit 48 YMSM who gave permission to be contacted and expressed initial interest in an in-person Screening Visit. These 48 YMSM come from a larger online survey (n=500) focused on YMSM's willingness to participate in biomedical trials. We will then aim to screen and consent approximately 48 YMSM in-person to enroll 16 YMSM for the Phase I Safety Trial.

3.3 Time to Complete Accrual

Accrual is expected to be complete within approximately 9 months.

3.4 Expected Duration of Participation

Each participant will be on study for approximately 2-3 months. The total duration of the study will be approximately 1 year. (Note: This is contingent upon meeting stated Product target concentrations.)

3.5 Study Randomization, Stratification, or Description of Non-Random Assignment Procedures

This is an open-label, unblinded study. In order to assure that sparse sampling for tissue drug concentrations is evenly distributed among the study population, each research participant will be randomized and assigned a specific sampling schedule.

4. STUDY POPULATION

The inclusion and exclusion criteria in Sections 4.1 and 4.2 will be used to ensure the appropriate selection of study participants.

4.1 Inclusion Criteria

- Between the ages of 15-24 at Screening
- Cisgender male who has sex with other men
- Willing and able to communicate in English
- Willing and able to provide informed consent to take part in the study
- Participant demonstrates capacity to comprehend, evaluate, reason, and express a choice about their participation in study
- For youth ages 15-17, have parent or caregiver consent to take part in the study
- Willing and able to provide adequate locator information
- Express initial interest in participating in a douching study
- Understand and agree to local HIV/STI reporting requirements
- HIV-1 uninfected at screening as documented by HIV-1 antigen/antibody 4th generation testing
- Willingness and availability to attend all study visits, barring unforeseen circumstances
- Per participant report at screening, consensual RAI in prior 6 months
- Live in or around the Baltimore area.
- Willing to abstain from insertion of anything (drug/medication, digits, penis, object, sex toy, or douche) into the anorectum for 72 hours before and after each study visit and 7 days after the biopsy collection.
- Willing to refrain from aspirin, vitamins and herbal supplements, and NSAID use for one week before and after each study biopsy visit
- Agrees not to participate in other research studies involving drugs and/ or medical devices for the study's duration

4.2 Exclusion Criteria

- Participation in research studies involving drugs, medical devices, genital products, or vaccines within 30 days of the Enrollment Visit.
- History of Hepatitis B infection, as documented by positive HBsAg at screening
- ≥ Grade 2 laboratory abnormality at baseline as defined by *Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1 - July 2017, and Addendum 3 (Rectal Grading Tables for Use in Microbicide Studies)*
- Self-report of having used TDF 300 mg/FTC 200 mg (Truvada®) or TAF 25 mg/FTC 200 mg (Descovy®) as HIV PrEP or Truvada as PEP within three weeks of dosing visit. Note: Plasma TFV/FTC concentrations will be measured at the Enrollment Visit (Visit 2). Participants with quantifiable plasma tenofovir/emtricitabine concentrations will not proceed to the tenofovir douche administration and pharmacokinetic sampling portion of the protocol. These participants may continue to participate in the In-Depth Interview portion of the protocol.
-

- Significant colorectal symptom(s) as determined by medical history or by participant self-report (including but not limited to presence of any unresolved injury, infectious or inflammatory condition of the local mucosa, history of inflammatory bowel disease, presence of symptomatic hemorrhoids, and presence of any painful anorectal conditions that would be tender to manipulation)
- At screening: participant- reported symptoms and/or clinical or laboratory diagnosis of active rectal infection requiring treatment per current CDC guidelines. Infections requiring treatment include Chlamydia (CT), gonorrhea (GC), syphilis, active HSV lesions, chancroid, genital sores or ulcers, and, if clinically indicated, genital warts. HSV seropositivity with no active genital lesions is not an exclusion criterion. (Note: Allow one re-screening after documented treatment (30 days) in cases where urethral and/or rectal GC/CT identified at screening. Individuals with newly diagnosed syphilis may re-screen with documented evidence of adequate treatment).
- History of an underlying clinically significant cardiac arrhythmia or renal disease (including creatinine clearance <60 mL/min using Cockcroft-Gault equation)
- Serum phosphate < 2.3 mg/dL
- History of significant gastrointestinal bleeding
- Current use of warfarin or heparin or other anticoagulant medications associated with increased risk for bleeding following mucosal biopsy (Daily high dose aspirin [>81 mg], or NSAIDs may be allowed if in the judgement of the investigator the medications may be safely withheld prior to flexible sigmoidoscopy)
- Use of systemic or anorectal immunomodulatory medications within 4 weeks of enrollment or planned use at any time during study participation
- Per participant report, use of any rectally administered products containing N-9 (including condoms) or investigational products within 4 weeks of enrollment, or planned use of either at any time during study participation
- Known allergic reaction to TFV or other components of the test articles
- Current known HIV-positive partner(s)
- History of recurrent urticaria
- Symptoms suggestive of acute HIV seroconversion at screening and enrollment
- Any other condition or prior therapy that, in the opinion of the investigator, would preclude informed consent, make study participation unsafe, make the individual unsuitable for the study or unable to comply with the study requirements.

4.3 Recruitment

Potential study participants will be recruited from an ATN online survey focused on YMSM's willingness to participate in biomedical trials. Briefly, this online survey is recruiting 500 cisgender men ages 15 to 24 who report same-sex attractions and behaviors and who live in the greater Baltimore Metropolitan Area. Once consented online, participants complete a series of domains including demographics (such as race/ethnicity, income, and education level), general mood, overall health, and sexual health behaviors. After completing the survey, participants are asked whether they would like to be contacted to learn more about future HIV prevention trials. Only those who agree to be recontacted will be screened for eligibility into a Phase I HIV prevention biomedical trial. The Hopkins site staff will contact potential participants and invite them to an in-person screening visit. We foresee needing to screen about 48 YMSM to yield an enrollment of 16 YMSM who have consented, are eligible and able to complete the study visits of our Phase I clinical trial.

We will make every effort to ensure that we carryover the diversity of our sample for the biomedical trial. For example, site staff will prioritize the outreach to racial/ethnic minority YMSM when they call to schedule the initial in-person visit. We expect that, at a minimum, a third of the sample screening in-person at the initial

site visit will identify as a racial/ethnic minority. By prioritizing the scheduling for racial/ethnic minority YMSM, we will be able to medical screen a larger proportion of racial/ethnic minority YMSM. Addressing structural challenges keeping YMSM from participating in a biomedical trial. We also recognize that racial/ethnic minority YMSM in Baltimore may also be more likely to experience greater socioeconomical barriers to study participation. To facilitate YMSM's ability to circumvent challenges to participating in the biomedical trial, we also have created financial support systems to offset structural barriers (e.g., paying for transportation, meals and an overnight stay). This is crucial as we recognize that racial/ethnic YMSM may require more time to successfully enroll in the DREAM Phase I trial (e.g., require time to re-screen if treated for STIs given the greater HIV/STI incidence in the community).

4.4 Retention

Once participants enroll in this study, the study site will make every effort to retain them for the duration of follow-up in order to minimize possible bias associated with loss-to-follow-up. A retention rate of 90% will be targeted. The study staff is responsible for developing and implementing local standard operating procedures to target this goal. Components of such procedures include:

- Thorough explanation of the study visit schedule and procedural requirements during the informed consent process, and re-emphasis at each study visit
- Thorough explanation of the importance and inter-relatedness of all the product dose and sampling phases to the overall success of the study
- Use of appropriate and timely visit reminder mechanisms (via email and/or telephone/text)
- Immediate and multifaceted follow-up (i.e. phone/text, email, and/or any other communication methods available at the disposal of site's study staff) on missed visits
- The protocol principal investigator in consultation with the site investigator will determine if research participants who fail to complete all biopsy collection sessions should be replaced. Replacement subjects would be assigned the same sampling schedule as the subject they are replacing.
- Any participant with quantifiable plasma TFV/FTC or peripheral blood mononuclear (PBMC) TFV-DP/FTC-TP concentrations at the Enrollment Visit (Visit 2) will be replaced. Participants with quantifiable plasma tenofovir/emtricitabine concentrations at Visit 2 will not proceed to the tenofovir douche administration and pharmacokinetic sampling portion of the protocol. PBMC TFV-DP/FTC-TP concentrations will be measured periodically. Participants with quantifiable TFV/DP/FTC-TP concentrations may continue to the TFV douche administration and pharmacokinectic sampling portion of the protocol, but may be replaced once the PBMC TFV-DP/FTC-TP results are available.

4.5 Co-enrollment guidelines

As indicated in Section 4.2, participants must not take part in other research studies involving drugs, medical devices, genital or rectal products, or vaccines after the Screening Visit and while taking part in ATN DREAM unless approved by the Safety and Monitoring Committee (SMC). Should any participant report concurrent participation in contraindicated studies after enrolling in ATN DREAM, the IoR/designee will consult the SMC regarding potential safety considerations associated with co-enrollment.

5. STUDY PRODUCT

Tenofovir Enemas in JHU Research Unit

The tenofovir douche will contain TFV 660 mg in 125 mL hypo-osmolar solution at half the osmolarity of iso-osmolar solution. The compounded formulations will be placed in commercially available 125 mL enema bottles. The enema bottles to be used are intended for single-use only and are disposable.

5.1 Administration

Each research unit dose of the study product will be administered by a study site investigator or a designated sub-investigator. After douche dosing, research participants will be instructed to retain the fluid without defecation for five minutes, if possible. Five minutes following douche administration by study staff, research participants will be instructed to expel the douche contents. The time of the douche expulsion will be recorded. The douche effluent will be collected and volume measured so that a single 1 mL sample of this effluent may be assessed for TFV concentration. The results will be volume and dose-corrected (based on administered dose) to establish the % retention of the enema volume post-expulsion. Any deviations or occurrences of note during product administration will be noted in the participant study record.

5.2 Study Product

Tenofovir (PMPA,9-[(R)-2-(phosphonomethoxy)propyl] adenine monohydrate) is a nucleotide analogue belonging to the class of acyclic phosphonomethylether nucleotides with potent activity against retroviruses. Study product should be stored at room temperature 25ºC (77°F). After the pharmacist has prepared 150 mL of the enema solution, 20-25 mL of the solution will be placed in a container and sent to the University of Pittsburgh/Magee Women's Research Institute for tenofovir identity/assay, osmolality and pH testing.

The ATN Dream Product contains TFV 660 mg in 125 mL hypo-osmolar solution at half the osmolarity of iso-osmolar solution. This study product will be administered in the research unit at Visit 3.

5.3 Study Product Supply and Accountability

5.3.1 Product supply

The tenofovir douches will be compounded at the study site pharmacy (JHU). The Pharmacy Procedures Manual will specify the detailed compounding instructions. A designated Pharmacist of Record (PoR) will be available at the study site. At JHU, the PoR will also be the compounding pharmacist in the designated compounding areas. The enemas may be prepared in advance within the established guidelines based on available stability data. The API will be provided by CONRAD and sent directly to the site PoR or the compounding pharmacist.

Where possible, all raw materials will be provided from the same manufactured lot. The raw materials will be provided in bulk and will require measurement at each enema preparation. The raw materials (Table 7) that will be provided to each site for preparation of research unit enemas include:

Table 7. Study drug supply raw materials

Material	Raw Material Appearance
Tenofovir	White powder
Sodium chloride, NF	Pellets

Sodium hydroxide, 18% (w/v)	White granular powder
0.1N HCl	Colorless clear solution
0.1N NaOH	Colorless clear solution

5.3.2 Product accountability

All study products will be available to the study staff through the designated site pharmacy or compounding pharmacy, once they have been labeled appropriately by the PoR. The PoR is required to maintain complete records of all study products received and dispensed.

5.4 Study Product Dispensing

Study products will be dispensed from the pharmacy to the designated study staff for an enrolled participant only upon receipt of a written prescription from an authorized prescriber as noted on the FDA 1572 form. Pre-procedural cleansing enemas will be administered by study participants. Study product enemas will be administered to subjects by study staff, in order to ensure maximal retention of study product in the desired anatomic compartment, and maximum comparability.

5.5 Adherence to Administration Guidelines

Research unit doses of study product will be administered either by the study site investigator or a designated co-investigator, with time of administration recorded in the study chart and on the CRF. Anything of note during this process will be recorded in the source documents.

5.6 Concomitant Medications and Procedures

With the exception of medications listed as prohibited, enrolled study participants may use concomitant medications during study participation. All concomitant medications, over-the-counter preparations, vitamins and nutritional supplements, and herbal preparations reported throughout the course of the study will be recorded in the source documents and on case report forms designated for that purpose.

5.7 Prohibited Medications and Procedures

Participants will be advised to refrain from RAI or any practices which include rectal insertion of any product, including those used during sexual intercourse (drug/medication, penis, other objects, sex toy, or enema) for 72 hours before and after the research unit study product dose and 7 days after each flexible sigmoidoscopy with biopsy collection.

Study participants will be prohibited from using the following medications throughout the study period: heparin (including Lovenox®), warfarin, Plavix® (clopidogrel bisulfate), immunomodulary medications, rectally administered products containing N-9 (including over-the-counter products), or investigational products. Other drugs, such as aspirin or NSAIDS that are associated with increased likelihood of bleeding following mucosal biopsy, should be restricted for use prior to flexible sigmoidoscopy as required by clinical guidelines.

Based on the tenofovir U.S. prescribing information, concomitant use of tenofovir with medications that decrease renal function or compete for active tubular secretion may increase concentrations of tenofovir or other renally eliminated medications. Therefore, oral PrEP in the prior 21 days (based on self-report) will not be allowed.

If participants report using any of these medications or products while on study or within the specified time frame noted, their study participation will be put on hold, pending discussions with study site investigator, DREAM Program Chair who is the IND Sponsor, and/or the study medical officer. Study staff will actively review medication use with each research participant during screening and throughout study participation. Participants will also be queried as to their adherence regarding insertion of rectal products 72 hours before and after study product exposure and 7 days after each biopsy collection visit.

5.8 Recommended Procedures

Condoms are recommended for use by all participants enrolled in this study. The study site will provide male condoms to participants in quantities expected to be sufficient for the study period. These condoms will not be coated with any type of spermicide. Using condoms provided by the study will be recommended for all sexual encounters during the study period. In the event that a participant needs additional supply of condoms between visits, he may request these from study sites at any time. If, after completing the study, the study participant wishes to receive prescribed oral HIV PrEP, the study team will assist with making a referral to an appropriate clinical provider.

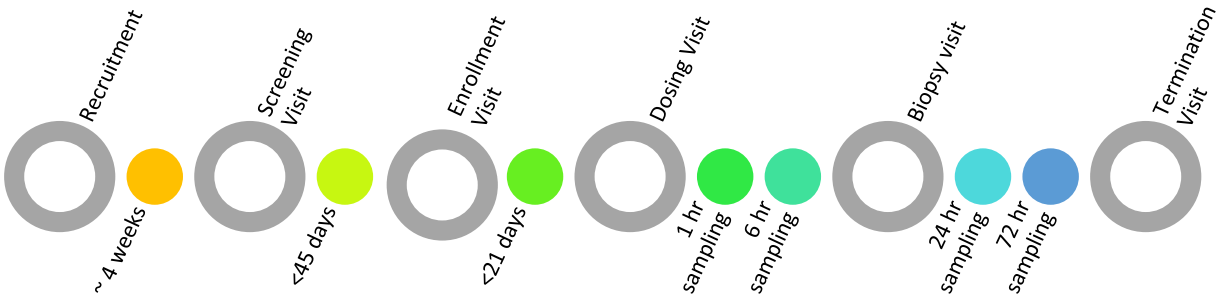
6. STUDY PROCEDURES

6.1 Study overview

This section includes information on visit-specific study procedures. An overview of the study visits and evaluations is available in protocol Appendix I: Schedule of Events. Unless otherwise specified, the laboratory procedures listed in this section are performed at the local study site laboratories.

As needed per institutional policy, participants may be required to undergo COVID-19 symptom screening prior to all visits. If a participant screening is positive, the participant will be tested for COVID-19. Additionally, participants may be required to undergo pre-procedure COVID-19 testing within 72hrs of any biopsy visit. If the screening or pre-procedure results indicate COVID infection, the study visit (including the procedure visit) will be canceled. If negative, the participant will be allowed to move forward. Positives will be able to move forward once cleared.

Figure 3. ATN Dream Study Visit Schedule



6.2 Recruitment: Pre-Screening

We will recruit YMSM who have expressed interest in being contacted to screen for eligibility into a Phase I HIV prevention biomedical trial. As part of participant outreach and recruitment strategies, study staff can pre-screen potential study participants at either on-site or off-site locations. During these interactions, study staff may explain the study to potential participants and ascertain elements of presumptive eligibility (e.g., willingness to use the study products, willingness to adhere to the study requirements, etc.), to be confirmed at an on-site screening visit. Process information (e.g., number of potential participants contacted, number presumptively eligible) may be recorded and stored at the study site. Procedures and documentation will comply with local IRB requirements. Participants will be asked to share their contact information and preferred mode of communication so that study team members can schedule an in-person Screening visit at Johns Hopkins University. Eligible YMSM will be contacted by site staff to schedule an in-person visit at the ATN site. To be accommodating to the needs of young participants, multiple visits may be scheduled to complete all required screening procedures, if necessary.

6.3 Visit 1: Screening Visit

A Screening Visit will take place up to 45 days prior to the Enrollment Visit. The study team will explain the procedures for the Phase I Safety and Acceptability Trial, and answer questions from prospective participants.

At this visit, YMSM (n≈48) will be consented to participate in Screening Procedures, which includes HIV/STI testing, and a brief, online survey regarding their sexual behaviors, prior experiences and comfort using rectal products, and douching or other rectal hygiene practices. Only participants who consent to participating in the Phase I trial will then undergo a medical exam. It is important to underscore that participants receive risk reduction counseling as part of both screening and enrollment procedures, including condoms and lube and discussion with site staff about daily oral PrEP.

YMSM who are <18 years old must be accompanied by a parent/guardian at this visit. Written informed consent will be obtained at the screening before any procedures are initiated. For participants who do not meet the eligibility criteria, screening will be discontinued once ineligibility is determined. Participants not meeting the eligibility criteria may be rescreened later, if appropriate, as in the case of a concomitant medication washout period. In such cases, the length of the washout period will be determined by the PI based on safety considerations and the elimination half-life of the concomitant medication. Screening labs may be repeated to determine eligibility.

- YMSM consenting and found to be evaluable for participation in the Phase I Trial (n≈16) will be scheduled for their Dosing visit once all HIV/STI labs have been confirmed, leaving no more than 45 days between the Screening and Baseline visits.
- YMSM not interested in enrolling or not eligible for enrollment in the Clinical Trial (n≈32) will be invited to participate in an online-facilitated in-depth interview (IDI) as part of the Screening visit (though not required to be completed on the same day as screening procedures). This interview will examine concerns, perceived barriers and challenges when invited to participate in a biomedical clinical trial to inform efforts focused on their engagement in future trials. The IDI will be conducted by a trained interviewer located at UPenn using video-conferencing.
- YMSM whose HIV tests are reactive will not be interviewed or be able to participate in the Phase I trial. The site staff will focus on linking these individuals to HIV-related care.

The following activities (Table 8) will take place at the in-person screening visit:

Table 8: Visit 1 – Screening Visit

Component	Procedure/Analysis
Administrative	• Obtain written informed consent • Assess eligibility • Obtain demographic and locator information • Provide counseling and condoms: o HIV/STI risk reduction counseling o HIV pre- and post- test counseling o Provide a copy of pre- and post- visit reminders o Participant education: flexible sigmoidoscopy procedure with biopsy & rectal fluid collections
Clinical	• Obtain complete medical history, documenting pre-existing conditions • Review and record concomitant medications • Perform general physical exam • Obtain vital signs – blood pressure (BP), heart rate (HR), temperature (temp), height, and weight
Safety Labs and STI Screening*: blood	• Collect blood specimens for: o Complete Blood Count (CBC) with differential o Comprehensive Metabolic Panel (CMP), PO4 o HBsAg o HIV-1antigen/antibody 4th generation testing (confirmatory tests as needed) o Syphilis testing (confirmatory test as needed)

Safety Labs and STI Screening*: urine and rectal	• Collect urine for toxicology screen • Collect urine sample for GC/CT (NAAT) • Collect rectal swab for GC/CT (NAAT) • Collect swab for HSV, if ulcerations are present on exam
Behavioral	• YMSM not interested in or not eligible for enrollment, In-depth interview regarding future participation in HIV biomedical trials

*Screening laboratory values are valid up to 45 days.

6.4 Visit 2 - Enrollment Visit (Day 0)

Visit 2 must take place ≤45 days after Visit 1. If more than 45 days pass before Visit 2, participants will need to be re-screened and re-consented. If deemed eligible based on the results from the screening procedures, participants will be contacted to schedule an Enrollment visit and asked to refrain from RAI and the insertion of anything in their anorectum for 72 hours prior to the visit. YMSM will complete a baseline web-survey examining questions on participants' prior experiences and comfort using rectal products, as well as douching or other rectal hygiene practices.

Baseline samples (see Appendix 1) will be collected to establish pre-dose conditions for safety assessments as well as sample collection for TFV analyte concentration in multiple matrices for participants taking oral TDF/FTC PrEP. Prior to proceeding to Visit 3 (Dosing Visit), the participant's plasma will be analyzed for tenofovir/emtricitabine. Individuals with quantifiable tenofovir/emtricitabine concentrations will be excluded from participation in the tenofovir douche administration and pharmacokinetic sampling.
These concentrations will be used to adjust post-dose PK and PD assessments. The following activities (Table 9) will take place at the Enrollment visit:

Table 9: Visit 2 – Enrollment Visit

Component	Procedure/Analysis

Administrative	• Confirm participant understood and signed informed consent
	• Review/update locator information
	• Confirm eligibility
	• Ensure participant complied with pre-visit instructions
	• Provide counseling and condoms:
	  ○ HIV/STI risk reduction counseling
	  ○ Provide a copy of pre- and post- visit reminders
	  ○ Participant education: flexible sigmoidoscopy procedure with biopsy & rectal fluid collections

Clinical	• Review/update medical history • Review/update concomitant medications • Complete AE assessment • Review any available test results • Perform symptom-directed physical exam, including rectal exam, as needed • Collect vital signs (HR, BP, temp)
Safety Labs: blood	• HIV-1 antigen/antibody 4th generation testing (confirmatory tests as needed)
Cleansing Enema	• Cleansing enema will be administered, in preparation for flexible sigmoidoscopy
Blood for PK, Plasma and PBMC	• Plasma and PBMC's collected to measure baseline TFV/FTC concentrations
Rectal specimens for PD and Histology (Not conducted in participants < age 18; Prioritized for participants age 18 and above)	• COVID-19 sigmoidoscopy pre-screening per institutional policy • Perform flexible sigmoidoscopy with biopsy collection for explant evaluation/histology/banking
Behavioral	• Participant will complete Baseline Behavioral Survey Note: The Baseline Behavioral Survey may be completed at any time from the enrollment visit (Visit 2) until just prior to TFV douche dosing om Day 1 (Visit 3).

6.5 Visit 3 - Dosing Visit (Day 1)

Visit 3 must take place > 7 days and ≤21 days after Visit 2 (the Enrollment Visit) and ≤45 days from the Screening Visit 1. Prior to using the TFV douche, baseline samples (see Appendix 1) will be collected to establish pre-dose conditions for safety assessments as well as sample collection for TFV analyte concentration in multiple matrices for participants taking oral TDF/FTC PrEP. These concentrations will be used to adjust post-dose PK and PD assessments.

YMSM will then receive a dose of the TFV douche at the research unit, and, asked to retain the douche without defecation for 5 minutes, if possible. Subsequently, participants will be instructed on how to expel the douche contents. The amount of time that the douche was retained and the volume of expelled effluent will be measured. Post-dose observations and data collection will follow at 1, 6, 24 and 72 hours, using a sparse sampling design in which paired plasma and peripheral blood mononuclear cells (PBMC) are collected at all PK sampling times.

Participants are assigned to a single time point for flexible sigmoidoscopy with rectal biopsies at either 1,6, 24, or 72 hours after dosing (thus, providing 4 subjects at each time point). Anoscopy, with rectal fluid collection will occur pre-dose and at either 1, 6, 24, or 72 hours after dosing, coinciding with each participant's flexible sigmoidoscopy. Microbiome specimens will be collected via anoscopy pre-dose and at 24 hrs post-dose.

Between sampling windows, YMSM will complete a web-survey examining their perceived reactions and comfort using the study douche, factors influencing product use in the future, and comfort with the trial procedures. The survey will be administered after dosing, but scheduled not to interfere with other study assessments. Sampling for safety, PK, PD, and acceptability assessments will be collected according to a schedule of events (see Appendix 1). A summary of the proposed activities to take place at this visit is noted below:

Table 10: Visit 3 –Dosing Visit (Day 1)

Component	Procedure/Analysis
Administrative	• Confirm participant understood and signed informed consent • Review/update locator information • Confirm eligibility • Ensure participant complied with pre-visit instructions • Provide counseling and condoms: o HIV/STI risk reduction counseling o Provide a copy of pre- and post- visit reminders o Participant education: flexible sigmoidoscopy procedure with biopsy (for participants assigned to 24 hr sampling) & rectal fluid collections
Clinical	• Review/update medical history • Review/update concomitant medications • Review any available test results • Perform symptom-directed physical exam, including rectal exam, as needed • Complete AE assessment • Collect vital signs (HR, BP, temp)
Safety Labs: blood	• Collect blood specimens for o CBC with differential o CMP o HIV-1 antigen/antibody 4th generation testing (confirmatory tests as needed) • If indicated, syphilis (confirmatory tests as needed)
Safety Labs[1]: urine and rectal	• Collect urine sample for GC/CT (NAAT), if indicated • Collect rectal swab for GC/CT (NAAT), if indicated • Collect swab for HSV, if indicated
Study Product Administration	• Investigator or designated sub-investigator will administer study product to participants
Blood for PK, plasma, and PBMC	• **Pre-Dose** (within 30 min prior to dosing) • **Post-Dose:**** o 1 hr ± 15 min o 6 hr ± 60 min
Rectal Specimens: Douche effluent	• Collected to measure volume and TFV/FTC concentration
Rectal Specimens: Biopsies for PK/PD, histology (age 18 and above only)	• COVID-19 sigmoidoscopy pre-screening per institutional policy • Perform flexible sigmoidoscopy with biopsy collection at 1 hr ± 40 min, or 6 hr ± 40 min post-dose per each participant's designated specimen collection schedule **
Rectal Specimens: Rectal Fluid for PK (Not conducted in participants < age 18; Prioritized for participants age 18 and above) and Microbiome (collected in all participants)***	• **Collect rectal swab for microbiome assessment** • Collect rectal fluid **pre-dose for all participants and at** 1 hr (± 15 min) or 6 hr (± 60 min) post-dose per each participant's designated specimen collection schedule**
Behavioral	• Participant will complete Research Unit Dose Acceptability Questionnaire

[1] Safety labs will be drawn prior to dosing and initiation of any specimen collections.

* Post-dose PK collection time points are approximate. Every effort will be made to adhere to the timeline, however, due to logistics, timing will not be exact and specimen collections may be missed (i.e. during biopsy collection). **Biopsies taken depend on participant's individual sampling schedule

** Biopsies and post-dose rectal fluid collection taken depend on participant's individual sampling schedule

*** Microbiome and rectal fluid collection should be done prior to dosing or enema administration and microbiome collection should precede rectal fluid collection.

6.6 Visit 4 – 24 hour Sampling Visit (Day 2) and Visit 5 - 72 hour Sampling Visit (Day 4)

Visit 4 takes place the day after dosing study product (Day 2) and Visit 5 takes place on third day after the dosing of study product (Day 4). Visit 4 and 5 takes place in the research unit. The following activities will be completed at these visits:

Table 11: Visit 4 (Day 2) and Visit 5 (Day 4)– Sampling Visit

Component	Procedure/Analysis (Day 2)	Procedure/Analysis (Day 4)

Administrative	• Review/update locator information
	• Ensure participants complied with pre-visit instructions
	• Provide counseling and condoms:
	  ○ HIV/STI risk reduction counseling
	  ○ Provide a copy of pre- and post- visit reminders
	  ○ Participant education: flexible sigmoidoscopy procedure with biopsy & rectal fluid collections
		• Review/update locator information
		• Ensure participants complied with pre-visit instructions
		• Provide counseling and condoms:
		  ○ HIV/STI risk reduction counseling
		  ○ Provide a copy of pre- and post- visit reminders
		• Participant education: flexible sigmoidoscopy procedure with biopsy & rectal fluid collections

Clinical	• Review/update medical history • Review/update concomitant medications • Review any available test results • Complete AE assessment • Perform symptom-directed physical exam, including rectal exam, as needed •	• Review/update medical history • Review/update concomitant medications • Review any available test results • Complete AE assessment • Perform symptom-directed physical exam, including rectal exam, as needed •
Blood for PK, plasma, and PBMC	• 24 ± 2 hr post-dose blood draw	• 72 ± 4 hr post-dose blood draw
Cleansing Enema (Not conducted in participants < age 18; Prioritized to participants age 18 and above)	N/A	Cleansing enema will be administered, in preparation for flexible sigmoidoscopy
Rectal Specimens: Biopsies for PK/PD (Not conducted in participants < age 18; Prioritized for participants age 18 and above)	• Perform flexible sigmoidoscopy with biopsy collection at 24 ± 2 hrs post-dose per each participant's designated specimen collection schedule (moderate sedation may be used for pediatric participants less than 18 years)	• Perform flexible sigmoidoscopy with biopsy collection at 72 ± 4 hrs post-dose per each participant's designated specimen collection schedule (moderate sedation may be used for pediatric participants less than 18 years)
Rectal Specimens: Rectal Fluid for PK measurement (Not conducted in participants < age 18; Prioritized for participants age 18 and above) and microbiomeassessment* (collected in all participants)	• Collect rectal fluid at 24 ± 2 hr • Collect rectal swab for microbiome assessment*	• Collect rectal fluid at 72 ± 4 hr post-dose per each participant's designated specimen collection schedule

* Microbiome and rectal fluid collection will be collected prior to dosing or enema administration and microbiome collection should precede rectal fluid collection.

6.7 Visit 6- Termination Visit- 96 hour (Day 5 + 3 days)

The Termination Visit ideally should occur at 96 hours (+3 day window) for all participants. The term visit should be at least 24 hours after the last scheduled biopsy. This visit will serve as the participant's study termination.

During their Termination Visit, participants will complete an exit in-depth interview (IDI) that will explore their experiences participating in the study, user-centered suggestions for product design and delivery, factors influencing product use in the future, and suggestions to maximize YMSM's engagement in future biomedical trials, including how to overcome perceived barriers and challenges based on their experiences. The IDI will be conducted using video-conferencing by a trained interviewer located at the University of Pennsylvania.

If a participant withdraws from study participation early or is withdrawn early from study participation by the study team, they will be asked to come in for an Early Termination Visit and the all the activities conducted at the Termination Visit will also be conducted for the Early Termination Visit.

Activities to take place at the Termination Visit include:

Table 12: Termination Visit

Component	Procedure/Analysis
Administrative	• Review/update demographic and locator information • Review STI risk reduction counseling and provide condoms
Clinical	• Review/update medical history • Review/update concomitant medications • Review any available test results • Complete AE assessment • Symptom-directed physical exam as needed • Obtain vital signs (HR, BP, Temp)
Safety Labs and STI Screening: blood	• Collect blood specimens for: ○ CBC with differential ○ CMP ○ HIV-1 antigen/antibody 4th generation testing (confirmatory tests as needed) * ○ Syphilis Testing (confirmatory test as needed) * ○
Safety Labs and STI Screening: urine and rectal	• Collect urine sample for GC/CT (NAAT) * • Collect rectal swab for GC/CT (NAAT) * • Collect swab for HSV, if indicated *
Other Specimens (rectal fluid, etc.)	• Collect specimens as appropriate/indicated (if the participant is willing) *
Behavioral	• Completion of exit in-depth interview

*If indicated and/or per local standard of care.

6.8 Exit Follow-up Phone Call

After the final study visit, follow-up call or early termination visit, a final contact may be required to provide laboratory test results (e.g. GC/CT) and post-test counseling. Anyone testing positive for HIV or an STI will be linked to appropriate care. All contacts will be documented in participant study records.

6.9 Interim Visits

Interim visits may be performed at any time during the study. All interim contacts and visits will be documented in the source documentation and on applicable case report forms. Some Interim Visits may occur for administrative reasons. For example, the participant may have questions for study staff. Other interim contacts and visits may occur in response to AEs experienced by study participants. When interim contacts or visits are completed in response to participant reports of AEs, study staff will assess the reported event clinically and provide or refer the participant to appropriate medical care (see Table 13).

Table 13: Interim Visit

Component	Procedure/Analysis
Administrative	• Review/update locator information • Review STI risk reduction counseling and provide condoms
Clinical	• Review/update medical history • Review/update concomitant medications • Review any available test results • Complete AE assessment • Symptom-directed physical exam as needed • Obtain vital signs (HR, BP, Temp)
Specimens	• Collect specimens as appropriate/indicated

6.10 Participants Who Withdraw or Are Withdrawn from the Study

If the participant withdraws or is withdrawn from the study after receiving study product, an Early Termination Visit will be conducted, if possible. The protocol principal investigator in consultation with the site investigator will determine if research participants who fail to complete all biopsy collection sessions should be replaced. Replacement subjects would be assigned the same sampling schedule as the subject they are replacing.

Any participant with quantifiable plasma TFV/FTC or PBMC TFV-DP/FTC-TP concentrations at the Enrollment Visit (Visit 2) will be replaced.

Participants with quantifiable plasma tenofovir/emtricitabine concentrations at Visit 2 will not proceed to the tenofovir douche administration and pharmacokinetic sampling portion of the protocol. These participants may continue to participate in the In-Depth Interview portion of the protocol.

PBMC TFV-DP/FTC-TP concentrations will be measured periodically. Participants with quantifiable TFV/DP/FTC-TP concentrations may continue to the TFV douche administration and pharmacokinetic sampling portion of the protocol, but may be replaced once the PBMC TFV-DP/FTC-TP results are available.

6.11 Participants Who HIV Seroconvert

Participants will be tested for HIV exposure and queried for high-risk behaviors. If there is a concern of possible HIV exposure, HIV and STI screening may be offered at any study visit. If a participant self-reports

seroconversion while on study, or if one of the HIV tests performed during the study protocol is confirmed positive, study staff will provide counseling and direct the participant to the appropriate HIV care.

Study staff will also request documentation of lab results or, failing availability of test results, will perform HIV Testing, with confirmatory testing as needed. These results will be kept in the subject's study chart and recorded on study case report forms (CRFs).

Participants will be withdrawn from the study if they test positive for HIV antibody or RNA. Any participant who tests positive for HIV while enrolled in the study will be immediately removed from the study and referred to the appropriate care provider for additional counseling and treatment as necessary. HIV 4th generation EIA and RNA test results will be available prior to any subsequent study intervention. No additional doses of study product or take-home enemas will be dispensed. Participants will be compensated for their study participation up until the time of removal.

6.12 Behavioral and Acceptability Evaluations and Procedures

The following behavioral assessments will be conducted at select visits.

6.12.1 Phase I Behavioral Questionnaire (Enrollment Visit)

Note: The Baseline Behavioral survey may be completed at any time from the enrollment visit (Visit 2) until just prior to TFV douche dosing on Day 1 (Visit 3).

The baseline behavioral data will be primarily descriptive of demographic variables (age, gender-identity, sexual orientation, racial background, education, and income), HIV test history and status, sexual behaviors and PrEP awareness, technology use, substance use, psychological distress and well-being, and rectal douche practices. It will assess participants' risk perception for HIV or STI transmission, and willingness to participate in biomedical trials. Baseline behavioral data will be collected via a web-based self-interview, also known as a web-based computer self-interview (WCSI), to be completed by the participant at Visit 1.

Baseline evaluations/measures

- Demographic Information
- Socioeconomic
- Housing
- General Health and Well-Being
- Relationships
- HIV and STI Testing
- Sexual Behaviors & Condom Use
- PrEP Use, Awareness, and Willingness
- Technology Use and Social Media
- Substance Use
- Psychological Distress
- Willingness to participate in biomedical trials
- Rectal Douching Practices
- Motivation for Clinical Trial Participation

6.12.2 Research Unit Dose Acceptability Questionnaire (Dosing Visit)

The Research Unit Dose Acceptability Questionnaire will be administered following study product use in-clinic. It will assess participants' psychosocial well-being, attitudes about the enema, how acceptable it is to them and their anticipated likelihood of using it in the future if it provided protection against HIV transmission, and product recommendations. It will be collected via WCSI using a computer connected to the Internet.

Research Unit Dose Acceptability evaluations/measures

- Psychosocial
- Product Acceptability
- Attitudes towards Enema Bottle
- Volume Issues
- Likelihood of Future Product Use
- Recommendations

6.12.3 In-Depth Interview (Screening Visit or Termination Visit)

Interviews with individuals who do participate in the Safety Trial will explore their experiences participating in the study, experiences using a rectal douche, user-centered suggestions for product design and delivery, factors influencing product use in the future, and suggestions to maximize YMSM's engagement in future biomedical trials, including how to overcome perceived barriers and challenges based on their experiences.

- Benefits and barriers to participating in a clinical trial
- Challenges and opportunities to ensure inclusion of YMSM in biomedical trials
- Strategies to optimize participation in future biomedical trials
- Experiences participating in a rectal microbicide clinical trial
- Acceptability of study product
- Discussion of clinical trial participation with social/sexual networks
- Product Recommendations

Interviews with individuals who do not participate in the Safety Trial will explore their experiences participating in the study, experiences using a rectal douche, factors influencing product use in the future, and suggestions to maximize YMSM's engagement in future biomedical trials, including how to overcome perceived barriers and challenges based on their experiences.

- Benefits and barriers to participating in a clinical trial
- Challenges and opportunities to ensure inclusion of YMSM in biomedical trials
- Strategies to optimize participation in future biomedical trials
- Discussion of clinical trial participation with social/sexual networks

We will use semi-structured guides in the videoconferencing interviews to examine YMSM's perceived benefits and barriers to participating in a clinical trial, identify challenges and opportunities to ensure

inclusion of YMSM in biomedical trials, and explore youth-driven perspectives on what strategies could optimize participation in future biomedical trials. Research assistants at the University of Pennsylvania will transcribe all interviews and import them into Dedoose, a platform commonly used to organize and manage qualitative data.

We will approach the data from a phenomenological framework given our interest in understanding the lived experiences of YMSM in their communities. Initial reading and open coding of the transcripts will be done by the primary analysis team at the University of Pennsylvania, and brought to meetings for review, comparison, and refinement. As thematic codes are identified in the data, they will be included in the codebook. This systematic process will lead to the creation of a coding structure that will involve a hierarchical set of constructs that account for the patterns seen in the data.

After creating a codebook, we will code several transcripts jointly as a team to ensure that the meaning, interpretation, and analytic approach are uniform across coders. We will discuss themes, resolve difficulties or concerns that may arise while coding, and make changes accordingly. Comparative analyses will be conducted to clarify differences among subgroups of YMSM (e.g., YMSM under age 21 vs those who are 21 or over). The use of negative case analysis and testing of rival hypotheses are methodological strengths that ensure the credibility and trustworthiness of the data.

6.13 Clinical Evaluations and Procedures

The following history and physical exam components will be conducted at select visits.

Medical History:

- Each participant will have a complete review of their medical history at screening and will be asked about any changes to their medical history at each visit.
- Concomitant medications will be reviewed and recorded at screening and updated as needed at every study visit.

Physical Exam:

- A complete review of systems will be completed at screening and a targeted physical exam will be done during study participation as needed.
- Height and Weight (at screening only)
- Vital signs
    - Heart Rate/Pulse
    - Blood Pressure
    - Temperature

Rectal Exam, Cleansing Enema, and Rectal Specimen Collection:

The participant will be positioned in the left lateral decubitus position for the following procedures:

- Rectal exam: The examiner will conduct a visual examination of the anus and surrounding area and note any abnormality. The examiner will then insert a lubricated (Good Clean Love, Good Clean Love, Inc. Eugene, OR) gloved finger into the anal canal and sweep around the internal anal circumference.
- Rectal specimen collection: Swabs for GC/CT will be inserted into the rectum and placed in contact with the rectal wall, turned 360 degrees and then removed.
- Rectal fluid will be collected by using a swab/sponge inserted through the anoscope to come into contact with the rectal wall for 2 minutes to absorb rectal fluid.

- A pre-procedural cleansing enema will be self-administered prior to flexible sigmoidoscopy for baseline and 72 hour post-dosing assessment.
- Rectal biopsies collection via flexible sigmoidoscopy: Up to 20 biopsies will be taken via a flexible sigmoidoscope at approximately 10-20 cm from the anal verge using large-cup biopsy forceps. The number of biopsies to be collected will vary depending on individual subject schedule and study site. Biopsies will be performed at different times in order to limit the number of total biopsies for each participant, but still achieve a rich post-dose sampling picture overall given the 6 different biopsy sampling times across all participants.

6.14 Laboratory Evaluations

6.14.1 Clinical Laboratory Testing

The local clinical laboratory or designee will run the following, as indicated:

- Blood specimens:
  - CBC with differential
  - CMP, which includes blood urea nitrogen (BUN), creatinine (Cr), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase test (ALP), sodium (Na), potassium (K), calcium (Ca), carbon dioxide (CO2), chloride (Cl), glucose, albumin, total bilirubin, and total protein
  - PO4
  - HBsAg
  - HIV-1 antigen/antibody 4th generation testing
  - Syphilis Testing
- Urine specimens:
  - Urine GC/CT (NAAT)
  - Urine immunoassay screen for: Amphetamine/Ecstasy, Barbiturates, Benzodiazepines, Cocaine Metabolite, Codeine/Morphine, THC, Oxycodone/Oxymorphone, Fentanyl
- Rectal swabs:
  - Rectal GC/CT (NAAT)
- Suspicious lesions or ulceration swab
  - HSV PCR/NAAT

6.14.2 Research Laboratory Testing

The JHU research laboratories will run the following, as indicated:

- Blood specimens:
  - Plasma PK
  - PBMC PK
- Douche effluent
  - TFV/FTC concentration
- Rectal fluid
  - PK
- Colonic tissue PK
  - PK (total tissue and isolated MMC)
  - *Ex vivo* HIV challenge of colon tissue explants

- Histology

University of Maryland research laboratory will run the following as indicated

- Rectal fluid
  - Microbiome

6.15 Specimen Collection, Handling, and Processing

Due of the congruency of ATN's mission with that of HIV/AIDS Networks supported by the NIAID Division of AIDS (DAIDS), NICHD has adapted and implemented several DAIDS policies and requirements for the ATN. Study sites will adhere to the standards of DAIDS Laboratory Requirements and site standard operating procedures for proper collection, processing, labeling, transport, and storage of specimens at the local laboratories.
(http://www.niaid.nih.gov/LabsAndResources/resources/DAIDSClinRsrch/Documents/gclp.pdf)

Specimen collection, testing, and storage at the site laboratories will be documented per standard site practice. In cases where laboratory results are not available due to administrative or laboratory error, sites are permitted to re-draw specimens that are intended for use in the screening as well as ongoing safety assessments process. Specimens will be handled in accordance with Requirements for DAIDS Sponsored and/or Funded Laboratories in Clinical Trials.

6.16 Storage of Specimens for Future Use

Study staff will store all specimens collected according to site SOPs. Specimens will not be labeled with any personal identifiers. Storage of all study samples will follow local standard operating procedure to ensure the anonymity and confidentiality of the trial research participants. Specimens remaining at the end of the study will be transferred to a designated central bio-repository after all protocol-required and quality assurance testing has been completed. Study participants' permission to do so will have been documented by signing informed consent which will include appropriate IRB-approved language. All screening samples for individuals who do not eventually enroll in the study will be destroyed.

6.17 Biohazard Containment

As the transmission of blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate blood and secretion precautions will be employed by all personnel in the drawing of blood and shipping and handling of all specimens for this study, as currently recommended by the United States Centers for Disease Control and Prevention. All infectious specimens will be transported in accordance with US regulations 42 Code of Federal Regulations (CFR) 72.

6.18 Laboratory Sample Management

During sample processing, all laboratory samples are handled according in accordance with good clinical laboratory practices.

7. CLINICAL MANAGEMENT & ASSESSMENT OF SAFETY

Guidelines for clinical management and product hold/discontinuation are outlined in this section. In general, the study site investigator has the discretion to hold study product at any time if he or she feels that continued product use would be harmful to the participant, or interfere with participant's acute medical condition (such as e.g. viral gastroenteritis) or with treatment deemed clinically necessary according to the judgment of the investigator. This section serves as a protocol overview; please see Data Safety and Management Plan for additional details.

7.1 Safety Monitoring

The study site investigator is responsible for continuous close safety monitoring of all study participants and for promptly alerting the DREAM Protocol Chairs and the protocol team if unexpected concerns arise. A panel of experts, the ATN Medical Officer (MO), amongst others may serve as the Safety Monitoring Committee (SMC).

Study status reports will be submitted to the study leadership every month for review. These reports will include all adverse events reported for the study, regardless of their relationship to the study products. The study team will meet, as needed, throughout the period of study implementation to review the safety data, discuss product use management, and address any potential safety concerns.

The SMC will receive standard data reports on a quarterly basis. The chair of the SMC will lead biannual calls to discuss study progress, and convene additional SMC meetings as needed based on interim reporting. The SMC will monitor several other factors, including HIV seroconversion, changes in kidney function, behavioral disinhibition, medication adherence, persistence in PrEP care, and differential loss to follow-up.

7.2 Clinical Data Safety Review

A multi-tiered safety review process will be followed for the duration of this study. The study site investigators are the first layer and are responsible for the initial evaluation and reporting of safety information at the participant level, and for promptly alerting the MO and ATN DREAM PSRT if unexpected concerns arise. In the event of any study participant experiencing an AE ≥ Grade 3, the study site investigator will review and discuss the appropriate study management with the PSRT, the IND Sponsor, and ATN MO.

During the trial, the PSRT will review status reports described above and conduct calls to clarify the data as appropriate. The content, format and frequency of the safety reports will be agreed upon in advance of study implementation. If necessary, external experts representing expertise in the fields of microbicides, biostatistics, HIV transmission, and medical ethics may be invited to review the events.

If the protocol team has serious safety concerns, they can request additional (ad hoc) reviews of data by the independent ATN Study Monitoring Committee (SMC), comprised of specialists in gastroenterology, infectious diseases, and adolescent medicine. Otherwise, the SMC will review participant safety data as part of its regular reviews. The SMC may recommend that the study proceed as designed, proceed with design modifications, or be discontinued. Members of the SMC will be independent investigators with no interest (financial or otherwise) in the outcomes of this study. If at any time a decision is made to discontinue enrollment and/or study product use in all participants, the Site IoR will notify the responsible IRB(s)/IEC(s) expeditiously. The IND holder, Dr. Anton, will assume responsibility for the

reporting of Serious Adverse Events to the FDA and regulatory agencies outside the U.S., as appropriate per 21 CFR 312.32 and 21 CFR 312.33.

7.3 Criteria for Early Termination of Study Participation

Participants may voluntarily withdraw from the study for any reason at any time. The site investigator also may withdraw participants from the study to protect their safety and/or if they are unwilling or unable to comply with required study procedures. Participants also may be withdrawn if the study sponsor, government or regulatory authorities (including the Office of Human Research Protections), NICHD or site IRBs terminate the study prior to its planned end date. Every reasonable effort is made to complete a final evaluation of participants who withdraw or are withdrawn from the study prior to completing follow-up. Study staff members will record the reason(s) for all withdrawals in participants' study records.

7.4 Grading System

Due of the congruency of ATN's mission with that of HIV/AIDS Networks supported by the NIAID Division of AIDS (DAIDS), NICHD has adapted and implemented several DAIDS policies and requirements for the ATN. The Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1 dated July 2017 and Addendum 3 (Rectal Grading Tables for Use in Microbicide Studies) will be the primary tools for grading adverse events for this protocol. Adverse events not included in those tables will be graded by the DAIDS AE Grading Table, Version 2.1 dated July 2017. In cases where an AE is covered in multiple tables, Addendum 3 (Rectal Grading Table for Use in Microbicide Studies) will be the grading scale utilized.

The DAIDS AE Grading Tables are available on the RSC website at https://rsc.niaid.nih.gov/clinical-research-sites/daids-adverse-event-grading-tables

EAE Reporting Period

- The expedited AE reporting period for this study is defined as the entire study duration for an individual participant (from enrollment until the participant's final study contact).
- After the protocol-defined AE reporting period, unless otherwise noted, only Serious Adverse Events (SAE) will be reported to NICHD if the study staff becomes aware of the events on a passive basis (from publicly available information).

7.5 Adverse Events Definitions and Reporting Requirements

7.5.1 Adverse Events

An adverse event is defined as any untoward medical occurrence in a clinical research participant administered an investigational product and which does not necessarily have a causal relationship with the investigational product. As such, an AE can be an unfavorable or unintended sign (including an abnormal laboratory finding, for example), symptom or disease temporally associated with the use of an investigational product, whether considered related to the product. This definition is applied to all the study groups, and is applied to all groups beginning from the time of enrollment. The term "investigational product" for this study refers to all study products listed in Section 5.

Study participants will be instructed to contact the study site staff to report any AEs they may experience at any time between enrollment and completion of their participation. In the case of a life-threatening event, they will be instructed to seek immediate emergency care. Where feasible and medically appropriate, participants will be encouraged to seek evaluation where the study clinician is based, and to request that the clinician be contacted upon their arrival. Study site will obtain written permission from the participant to obtain and use records from non-study medical providers to complete any missing data element on a CRF related to an adverse event. All participants reporting an untoward medical occurrence will be followed clinically, until the occurrence resolves (returns to baseline) or stabilizes.

The study site investigators will determine AE resolution or stabilization in their best clinical judgment, but may seek medical consultation regarding follow-up or additional evaluations of an AE from the ATN MO and/or PSRT. Study site staff will document in source documents and case report forms all AEs reported by or observed in enrolled study participants regardless of severity and presumed relationship to study product. The Addendum 3 (Rectal Grading Tables for Use in Microbicide Studies) to DAIDS AE Grading Table Version 2.0 will be the primary tool for grading adverse events for this protocol. Adverse events not included in that table will be graded by the standard DAIDS AE Grading Table, Version 2.0 dated November 2014. In cases where an AE is covered in multiple tables, Addendum 3 (Rectal Grading Table for Use in Microbicide Studies) will be the grading scale utilized. Please see Data Safety and Management Plan for additional details.

7.5.2 Serious Adverse Events

The OHRP guidance, "Guidance on Reviewing and Reporting Unanticipated Problems Involving Risks to Subjects or Others and Adverse Events," (OHRP, Appendix 10-09) defines a serious adverse event (SAE) as any adverse event that:

- Results in death,
- Is life-threatening,
- Requires impatient hospitalization or prolongation of existing hospitalization,
- Results in persistent or significant disability/incapacity,
- Results in a congenital anomaly/birth defect, or
- Is an important medical event that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above.

All serious adverse events will be reported to NICHD, as per the ATN Manual of Policies and Procedures, as well as the IRB of record. Please see Data Safety and Management Plan for additional details.

7.5.3 Adverse Event Relationship to Study Product

The ATN Manual of Procedures and Policies (MOPP) notes in Section 10 that ATN investigators must determine how to assess AEs. Consistent with other HIV biomedical prevention trials, the relationship of all AEs to study product will be assessed per the *Manual for Expedited Reporting of Adverse Events to DAIDS (Version 2.0, dated January 2010)*, the Investigator's Brochures, and clinical judgment. Per the *Manual for Expedited Reporting of Adverse Events to DAIDS (Version 2.0, dated January 2010*), the relationship categories that will be used for this study are:

- ***Related***: There is a reasonable possibility that the AE may be related to the study agent(s)
- ***Not related****:* There is not a reasonable possibility that the AE is related to the study agent(s)

When an AE is assessed as "not related" to study participation, an alternative etiology, diagnosis, or explanation for the AE should be provided. If new information becomes available, the relationship assessment of any AE will be reviewed again and updated, as required.

7.6 Expedited Adverse Event (EAE) Definitions and Reporting Requirements

7.6.1 Expedited Adverse Event Reporting

Per ATN policies, SAEs of any severity grade, AEs of severity grade 3 or higher that are determined to be related to study participation or intervention, and UEs that are severity grade 3 or higher will be reported immediately.

7.6.2 EAE reporting procedures specific to this protocol

Events that meet SAE criteria and are unexpected (not previously reported to the agency or included in the IB) must be reported in an expedited manner. The IND Sponsor will assume responsibility for the reporting of Serious Adverse Events to the FDA and regulatory agencies outside the U.S., as appropriate per 21 CFR 312.32 and 21 CFR 312.33. Copies of this final report will be filed with the DREAM Regulatory Core.

If the event is assessed as reportable to the FDA in an expedited manner, the DREAM Regulatory Core will draft the report in narrative format and will distribute to the ATN MO and the IND Sponsor for review and approval. Once the review is complete, the DREAM Regulatory Core will generate a final version of the expedited safety report and will provide it to the IND Sponsor with a cc to the ATN MO via secure e-mail submission at the following address:

Peter A. Anton, MD.
E-cubed Consulting, LLC
7777 Hollywood Blvd, #309
Los Angeles, CA 90046

In case of a fatal or life-threatening suspected adverse reaction, the IND Sponsor will notify the responsible review division of the FDA by telephone or facsimile transmission as soon as possible but in no event later than 7 calendar days after the receipt of the respective adverse event information and determination that it meets the respective criteria for reporting. Any additional information received must be sent to the agency as soon as possible, but no more than 15 days after receipt of the information.

7.6.3 Expedited Adverse Event (EAE) Reporting Requirements for this Study

The study agent, for which expedited reporting is required, is:

- Rectal TFV douche – 5.28 mg/mL (*660 mg in 125 mL) in hypo-osmolar solution

7.7 Dose Modification

This is a single dose administration protocol. Adverse event assessments will be made in collaboration with the Protocol Safety Review Team (PSRT), who will review all study AEs, with special attention to the events

and toxicities assessed to be greater than Grade 2. The determination as to whether dose administration in additional participants is justifiable and compatible with the mandate for participant safety will be based on this assessment. Please see detailed Data Safety and Management Plan for additional information.

7.8 Discontinuation of Study Product(s) in the Presence of Toxicity

Each participant will receive one dose of the study product, administered in clinic. If study site investigator has an acute concern about the safety of any product administration, may withdraw the participant from the study. Please see Data Safety and Management Plan for additional details.

7.9 Reporting of Adverse Reactions to the Responsible IRBs

The study site investigator will report adverse reactions to the responsible IRBs in accordance with respective IRB policies and procedures.

Follow-up information to a reported adverse event will be submitted to the IRB as soon as the relevant information is available. If the results of the study site investigator's follow-up investigation show that an adverse event that was initially determined to not require reporting to the IRB does, in fact, meet the requirements for reporting, the study site investigator will report it as soon as possible in accordance with respective IRB policies and procedures. Please see Data Safety and Management Plan for additional details.

7.11 Withdrawal of Subjects Due to Adverse Events

If a participant has a Grade 2 or higher AE associated with the study, he has met a study end point and will be referred to local care and treatment services. He may return to the research clinic for additional counseling and other support services, as needed. Continued study participation would be of no added benefit; thus follow-up visits will be discontinued and the participant will be considered terminated from the study. An Early Termination Visit will be conducted, if the participant is willing. Please see Data Safety and Management Plan for additional details.

7.12 Social Harms Reporting

Although study sites make every effort to protect participant privacy and confidentiality, it is possible that participants' involvement in the study could become known to others, and that social harms may result (i.e., because participants could become known as HIV-infected or at "high risk" for HIV infection or participants could potentially disclose their sexual orientation). For example, participants could be treated unfairly or discriminated against, or could have problems being accepted by their families and/or communities and/or employer.

Social harms that are judged by the study site investigator to be serious or unexpected will be reported to responsible site IRB at least annually or according to their individual requirements. In the event that a participant reports social harm, every effort will be made by study staff to provide appropriate care and counseling to the participant, and/or referral to appropriate resources for the safety of the participant as needed.

7.13 Monitoring untoward effects associated with or resulting from study

Site staff must first follow their own IRB's procedure for reporting and managing untoward effects.

There are three types of untoward effects to be identified: (1) those related to the participant, (2) those related to the study staff and (3) those related to the neighborhood or community where the study is being implemented.

First, the study will catalogue any untoward effect related to the participant. Reporting is required for occurrences including social harms, psychological distress and serious life threatening events such as suicide attempts. These may be immediately apparent to the study staff, such as the participant's emotional upset requiring referral for counseling; or they may be delayed and reported later to study staff, such as physical harm to an individual for having participated in the study. Study staff will notify the team of these untoward effects using the iTech QNS accessible through the iTech website (https://itechnetwork.org). Study staff will be briefed during the training on the scope of possible untoward effects and instructed to report events.

Second, study staff may encounter untoward events during sessions that personally affect them. Training and guidance will seek to minimize this risk. Nonetheless, an assessment of the cost of conducting this study must include cataloguing these events as well. The protocol chairs should be notified of these events so that they may be immediately addressed, evaluated, and guidance modified or expanded to minimize similar risk to other staff.

All untoward events related to the participant or study staff will be graded for severity by site study staff according to the definitions outlined in Table 10-1 (Appendix VI), as per ATN procedures. Reporting of all UEs graded 3 or higher will be expedited. Please see Data Safety and Management Plan for additional details.

8. DATA COLLECTION AND SITE MONITORING

8.1 Development of Protocol and Case Report Forms

The Protocol Team, in collaboration with the ATN iTech Management Core and the iTech Analytic Core, is responsible for the development of this protocol as well as the Case Report Forms (CRFs) needed to collect the information required to implement this protocol.

8.2 Data Records

Participant-related study information will be identified through a study ID number (SID) and participant code on all participant CRFs, audio files, transcripts, and Computer Assisted Self Interviewing (CASI) files. Participant code consists of the participant's first initial and two-digit date of birth. Participant names or other personal identifying information will not be used on any study documents and will be redacted from any qualitative transcripts. Participants enrolled in the Phase I will also receive a Trial ID number (TID) to identify their assigned PK sampling schedule. Participant names and their SID/TID will be stored separately from other study information, accessible only to designated study staff, iTech site monitors, and representatives from the NICHD. Original source documents for individual participants will be accessible only to the study staff. Data from original source documents will be transcribed on CRFs as applicable.

8.3 Research Staff Training

All proposed study staff have participated in the required trainings in participation and conduct of studies that involve human subjects, and any future study staff will do so upon hiring. Training for all research staff includes (but is not limited to) an overview of the study, study procedures and human subjects issues (informed consent process, confidentiality), a demonstration of all technology components, methods for establishing comfort with the sensitive issues, including discussion of sexual behaviors, that will likely arise in the course of the assessments, review of the study instruments, their required elements and the inherent flexibility built into them, Human Subjects Protection, Good Clinical Practice, Child Abuse History Clearance, FBI Criminal History Background Check, informed consent, quality management, confidentiality, and reporting of adverse events.

8.4 Data Collection

8.4.1 CRFs

Study monitoring data, including information about eligibility, demographic data and monitoring untoward effects, will be collected on CRFs. All CRFs for this study will be available for download from the iTech Box account, a secure content management platform.

8.4.2 Web-Survey Data

Data collected using a web-based computer-assisted survey method will be on a portable computer, tablet, or mobile phone via an internet-based application. All survey data will be collected using SurveyGizmo. Data

will remain confidential; no personal identifying information will be collected during the computer session. The participant's unique study ID will be used in order to link the interview responses to the participant's CRF data. We use SSL encryption for transfers of information online and data will be stored in the secure, HIPAA-compliant servers of Qualtrics. The Emory team maintains a business partner HIPAA agreement with SurveyGizmo.

8.4.2.1 Data Security

To ensure data privacy, as soon as data is entered (in real-time), it will be encrypted during transmission using Secure Socket Layer (SSL) technology. The data will then be immediately stored in a secure database on an server within the Emory data center.

We clean, manage, and download the survey data directly from the SurveyGizmo application interface. The survey data collected through SurveyGizmo is encrypted while in transfer using SSL certificates, encrypted at the disk level in the database servers, and encrypted at the row level. Access to the SurveyGizmo account where the data can be downloaded is password-protected with a dual authentication password. The data collected through our web application is encrypted while in transfer using SSL certificates and access to the administrative web application where the data can be downloaded is password-protected using sufficiently complex passwords for each approved user.

8.4.3 BlueJeans Platform Description

For the in-depth interviews that occur over video chat, the BlueJeans platform – or a comparably secure videoconferencing platform - will be used. BlueJeans is compatible with PCs, Macs, and any device with a web browser, including tablets and smartphones. Unlike other video-chat platforms (e.g. Skype), BlueJeans is HIPAA-compliant. BlueJeans includes the following functions to protect users:

Media handling and encryption. In WebEx, Vidyo, Tandberg, and Polycom architectures, media is sent to a server (also called a video relay or MCU). Although encryption is applied from the user's computer to these servers, the servers still have full access to the user's media. In contrast, Blue Jeans does not record or store your information; they simply encrypt and transmit it. Blue Jeans Network does not store any video conference content, in any format. This security infrastructure means Blue Jeans has no control over the content shared via video conference. BlueJeans supports standards-based encryption (AES-128) for video conferencing.

Infrastructure and network security. BlueJeans employs a wide range of security management. This includes network firewalls throughout the infrastructure to create security zones for different applications and services. BlueJeans also deploys proxy servers that terminate all third party/customer traffic at a proxy layer. All web traffic passes through industry-leading load balancers to protect against a suite of application attack vectors. Beyond the firewall, proxy servers, and load balancers, BlueJeans also periodically scans for network, port, and application-level vulnerabilities. Vulnerability scans are conducted by a leading third-party SaaS provider, in addition to some special-purpose, in-house scanning tools. Furthermore, all of the third-party applications and operating system software is checked for security advisories and is patched periodically. Routers, firewalls, load balancers, and proxy application servers are all configured to mitigate numerous types of DOS attacks. BlueJeans also engages with third party consultants to perform penetration testing of the service.

No-install client. Video conferencing software clients tend to be large and to leave a big footprint on the user's system. Almost all of them require administrator permissions to install.
Once the client software gains administrator permissions, they can severely compromise computer security. BlueJeans offers a browser-based conferencing system that does not require administrator permissions or installation.

BlueJeans offers the HIPAA-required Business Associate Agreement (BAA) where BlueJeans agrees to be responsible for keeping all patient information secure and to immediately report any breach of personal health information. In this study, University of Pennsylvania will maintain a BAA with BlueJeans, and this will be extended to cover the proposed activities. The BlueJeans sessions will include identifying information (e.g., images of the participant, voice recordings). All identifying information will be stripped from the data transcripts before they are sent to the analysis team for content analysis.

8.4 Data Submission

8.4.1 CRFs

During study conduct, the SRVs will maintain the CRFs in secured locations, and transmit CRF data to the AC either electronically using DFexplore or by submitting scanned paper forms using DFSend. DFexplore and its DFdiscover platform is a leading multi-site database environment for HIV RCT that can receive and transcribe CRF data via scanned PDFs, or allow for direct electronic data entry. It provides for monitoring form completion and data quality, and a system for data querying and resolution with SRVs, while maintaining an audit trail. The AC uses DFexplore for MSM studies and RCTs and data is maintained by the parent company DF/net on a cloud-based server with Microsoft Azure.

8.4.2 Audio Data

In-depth interviews will be audio-recorded to allow for verbatim transcription of the interview and checked for accuracy and completion. We will format all transcripts and import them into qualitative data analysis software, Dedoose. Data analysis will be primarily conducted by Dr. Bauermeister and other DREAM study team members at the University of Pennsylvania when appropriate. One year after study completion, audio recordings will be destroyed.

8.4.3 Survey Data Transmission

Only authorized users will be able to access and open the survey through SurveyGizmo. To ensure data privacy, as soon as data is entered (in real-time), it will be encrypted during transmission using Secure Socket Layer (SSL) technology. The data will routinely be downloaded and stored in a secure database on an Emory server within the AC data center.

8.4.4 Retention data

N/A

8.5 Data Quality Assurance

Investigators receiving federal funding must adhere to the Code of Federal Regulations (CFR) to protect research participants and produce reliable study information. Sites participating in research sponsored by the

NICHD need to have an internal quality assurance (QA) plan that will identify problems and correct errors in research study records.

Behavioral data will be collected via Qualtrics. Qualtrics is a HIPAA-compliant, web-based electronic data collection tool that ensures data quality through the standardization of data collection procedures. The PIs and study staff will be responsible for data monitoring and quality assurance. There will be ongoing communication within study team to monitor data collection, participant safety, and compliance with the study protocol.

8.6 Role of Data Management

The iTech AC will provide instructions concerning the recording of study data on the CRFs and entry of the data into the AC data management systems. Behavioral components of the application (e.g., recruitment, video interviews, transcription and analysis) will be overseen by the UPENN team. Clinical components of the application will be overseen by the JHU team. The iTECH AC will also offer data monitoring support for the online screeners, data management, and statistical support for trial analyses.

8.7 Study Site Monitoring and Record Availability

Site monitors from the MC and AC may visit participating study sites to review a selected portion of the individual participant records, including assent/consent forms, CRFs and supporting source documentation to ensure the protection of study subjects, compliance with the protocol, and accuracy and completeness of records. Regulatory files, as required, will also be inspected to ensure that regulatory requirements are being followed.

The site investigator will make study documents (e.g., consent forms, case report forms) *and pertinent hospital or clinic records* readily available for inspection by the local IRB, the site monitors, the NICHD, the Office of Human Research Protection (OHRP), or the sponsor's designee for confirmation of the study data.

9. STATISTICAL CONSIDERATIONS

9.1 Overview and Summary of Design

This is an exploratory, open label study to compare the safety, PK, PD, and acceptability of the formulation of a TFV microbicide enema. The goal of the study is to describe the pharmacokinetic parameters in a population of a TFV douche currently under development in a population of young men who have sex with men (YMSM).

Eligible YMSM will be contacted by site staff to schedule an in-person Screening Visit at the ATN site. A Screening Visit will take place up to 45 days prior to the Baseline enrollment Visit. At this visit, YMSM (n≈48) will learn about the Phase I trial and be invited to participate in the study. All participants will be consented and those who agree to participate in the Phase 1 portion the study will complete Screening Procedures, which includes a medical exam, HIV/STI testing, and a brief, online survey regarding their sexual behaviors, prior experiences and comfort using rectal products, experiences and willingness to participate in biomedical trials, and douching or other rectal hygiene practices. YMSM will be considered enrolled in the trial and scheduled for their PK/PD visit once all HIV/STI labs have been confirmed. YMSM who choose not to participate in the Phase I trial or who are ineligible to participate due to labs results (n≈32) will be invited to participate in an online-facilitated in-depth interview (IDI). The screening, enrollment, dosing, and termination visits will take place at Johns Hopkins University.

After completing the screening evaluation, we will enroll 16 evaluable participants. YMSM who are willing and cleared to participate in the Trial visit will be asked to use a single dose of a tenofovir (TFV) rectal microbicide douche (600mg TFV in 125mL 0.45% NaCl [half-normal saline]) at the Hopkins study site. Prior to using the TFV douche, baseline samples (see Appendix 1) will be collected to establish pre-dose conditions for safety assessments.

YMSM will then receive a single dose of TFV douche at the research unit, and, asked to retain the douche without defecation for 5 minutes, if possible. Subsequently, participants will be instructed on how to expel the douche contents. The amount of time that the douche was retained and the volume of expelled effluent will be measured. Post-dose observations and data collection will follow at 1, 6, 24, and 72 hours, using a sparse PK sampling design in which plasma and peripheral blood mononuclear cells (PBMC) are collected at each designated time. Participants are randomized to only one time point for flexible sigmoidoscopy with rectal biopsies at either 1, 6, 24, or 72 hours after dosing (thus, providing 4 subjects at each time point). Anoscopy, with rectal fluid collection will occur pre-dose, 1, 6, 24 or 72 hours after dosing to coincide with the time of each participant's flexible sigmoidoscopy. Microbiome specimens will be collected via anoscopy pre-dose and at 24 hrs post-dose.

Between sampling windows, YMSM will complete a web-survey examining their perceived reactions and comfort using the study douche, factors influencing product use in the future, and comfort with the trial procedures. The survey will be administered after dosing but scheduled not to interfere with other study assessments. Sampling for safety, PK, PD, and acceptability assessments will be collected according to the schedule of events. Phase I Trial participants will complete in an IDI as part of their termination visit.

9.2 Study Endpoints

Study endpoints to be assessed are each paired with specific objectives as listed in Section 3 Objectives & Endpoints.

9.3 Study Hypotheses

This protocol and derived data will provide information that will address, even if not completely answer, the following study hypotheses:

- YMSM will find the product to be acceptable and behaviorally congruent with their rectal hygiene practices
- The TFV douche will be safe and acceptable when applied rectally
- The TFV douche will exceed target colon MMC concentrations for a period of 1 hour to 72 hours post-dose
- Concentrations in various compartments after enema will provide an important parallel to concentrations seen after TFV gel (historic control from previous study) and will provide correlating data to a prior study carried out in adult MSM.

9.4 Accrual and Sample Size

We expect to screen 48 YMSM willing to participate in the in-person Screening Visit. We derive this estimate based on prior experiences in rectal microbicide trials through the Microbicide Trials Network (MTN). We will need to screen and consent approximately 48 YMSM in-person. Assuming a 1:3 ratio of willingness to enroll in a biomedical clinical trial, we will enroll 16 YMSM (n=8 between the ages of 15-19; n=8 between the ages of 20-24) for the Phase I Safety Trial.

A sample of 16 YMSM enrolled in the DREAM ATN trial will allow for maximal comparability with DREAM-01, which included the same product in 18 adults. Comparing 8 participants of DREAM ATN to counterparts in each arm of DREAM-01 provides 80% power, with 2-sided type I error of 5% to detect a difference of 1.0 standard deviations.

9.5 Randomization and Blinding

Enrolled participants will not be randomized, as this is by necessity an open-label study. This will be an unblinded study.

9.6 Data Analysis

For primary endpoints, sensitivity analyses will be performed by age group (15 to 19-year-old YMSM; 20 to 24-year-old YMSM). PK estimates will be reported overall and per group, given need to examine whether there are significantly different TFV-DP rectal tissue levels across these two age groups. Safety data will be reported in the aggregate for the overall population, as safety is not expected to differ significantly by age group. As noted above, sample size is conditioned to a great degree on having a comparable, complete, and relevant cohort for comparison to DREAM-01. Descriptive statistics and graphics will be used to summarize the characteristics of endpoints across each step of the study. Continuous measures will be summarized by

means and standard deviations or median and interquartile range depending on data distribution. For binomial endpoints, 95% exact confidence intervals will be calculated. Given the small sample size and possible non-normality of the data, non-parametric tests will be used to test for differences among (Kruskal-Wallis) and between (Wilcoxon rank sum test) test formulations.

Willingness to engage in biomedical clinical trials: We will examine YMSM's willingness to engage in biomedical clinical trials through several data sources, including: IDIs from YMSM who screen in-person yet refuse to enroll in the Phase I Trial (n=32), as well as trial participants' experiences in the trial and future willingness to participate in future biomedical trials (n=16). We will use semi-structured guides in the videoconferencing interviews to examine YMSM's perceived benefits and barriers to participating in a clinical trial, identify challenges and opportunities to ensure inclusion of YMSM in biomedical trials, and explore youth-driven perspectives on what strategies could optimize participation in future biomedical trials. Research assistants at the University of Pennsylvania will format all transcripts and import them into Dedoose, a platform commonly used to organize and manage qualitative data. We will approach the data from a phenomenological framework given our interest in understanding the lived experiences of YMSM in their communities. Initial reading and open coding of the transcripts will be done by the primary analysis team at the University of Pennsylvania, and brought to meetings for review, comparison, and refinement. As thematic codes are identified in the data, they will be included in the codebook. This systematic process will lead to the creation of a coding structure that will involve a hierarchical set of constructs that account for the patterns seen in the data. After creating a codebook, we will code several transcripts jointly as a team to ensure that the meaning, interpretation, and analytic approach are uniform across coders. We will discuss themes, resolve difficulties or concerns that may arise while coding, and make changes accordingly. Comparative analyses will be conducted to clarify differences among subgroups of YMSM (e.g., YMSM under age 20 vs those who are 20 or over). The use of negative case analysis and testing of rival hypotheses are methodological strengths that ensure the credibility and trustworthiness of the data.

Acceptability: To examine the YMSM's overall product acceptability of the TFV douche formulation after rectal administration, as determined by participants' self-report of likelihood of product use if shown to be effective, we will examine the outcome in both continuous and dichotomous forms. We define the acceptability as a mean score of 3 or higher on 4-point continuous acceptability measures (1= completely unacceptable; 2= somewhat unacceptable; 3= somewhat acceptable; 4= highly acceptable) that we argue is the minimal clinically meaningful threshold for acceptability. Under such design, we will test the null hypothesis that the testing product has a true mean acceptability score greater than or equal to 3. If the null hypothesis is rejected, we will conclude that the product under consideration does not have good acceptability in the current format among YMSM. Conversely, if the null hypothesis is not rejected, we cannot rule out the possibility of a truly acceptable product and further confirmatory testing would be of interest. In such case, the Type I error is the probability that we mistakenly declare the testing product is not acceptable when in reality it is acceptable. We will conduct a one-sample, one-sided t-test to test above null hypothesis. We will also conduct a paired-sample t-test to determine whether YMSM's acceptability of a TFV douche changed between before and after using it. As is conventional in early phase trials, we set the type I error at 0.10 and will not adjust for multiple comparisons. Trial participants will complete an IDI (~45 minutes) at the Termination Visit. This IDI will include questions exploring user-centered suggestions for product design and delivery, acceptability and influence to use the study product in the future, douching history and education, and experiences participating in the study, among other topics. Suggestions for product improvement will also be collected. Major components of both survey and IDI assessments have been used successfully and validated in prior ATN and rectal microbicide trials (e.g., MTN-006, MTN-007, MTN-017, MTN-026, MTN-033) by Dr. Bauermeister, and are currently being used in MTN-035.

Safety: To evaluate the safety of a single dose of a TFV douche when applied rectally, as measured by the number of ≥Grade 2 adverse events (AEs) as defined by the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events. For the safety analysis, the number and the frequency of all adverse events (AEs) will be tabulated. A single summary outcome of this type (yes/no) can be reasonably assumed to follow a Bernoulli distribution. Assuming a true underlying rate of one or more participants experiencing AEs is between 0.01 and 0.20 in a sample of 16 YMSM, there would be a 81% chance that one or more participants might experience an AE when the true rate is at least 10% and a probability less than 15% when the true rate is no more than 1%. Unless the true rate is at least 3%, the probability of two or more subjects experiencing AEs will be quite small – less than 5%. Additional safety analyses will also tabulate the number and type of AEs observed overall, and by severity, site, and study product. AEs that lead to discontinuation of study participation will be tabulated separately.

Pharmacokinetics: To characterize the systemic and compartmental pharmacokinetics of a TFV douche following rectal application, as measured by TFV concentrations in plasma, rectal fluid and rectal mucosal tissue homogenates and cells. Pharmacokinetic parameters will be compared to data from studies using rectal dosing of TFV in DREAM-01 and other oral TFV dosing studies in adults, which also included colorectal biopsies. Pharmacokinetics in blood plasma and PBMCs, colon tissue homogenate and cells, and rectal fluid for the TFV-based douche will be evaluated after rectal administration. The primary analysis will use non-compartmental methods to estimate pharmacokinetic parameters, including peak concentration (C_{max}, time to peak concentration (T_{max}), area under the matrix concentration-time curve from 0 to infinity ($0 \rightarrow \infty$), and terminal elimination half-life. For sparsely sampled matrices (colon tissue homogenate, colon tissue cells, and rectal fluid), we will use population PK methods to build PK models describing individual PK parameter estimates and typical values for the population along with significant covariates (possibly weight, age, pharmacogenetics, creatinine clearance). Tenfovir and TFV-DP assays will be performed by the Clinical Pharmacology Analytical Laboratory (CPAL) at Hopkins and are validated following FDA bioanalytical guidance and approved by an NIH-cross network Clinical Pharmacology Quality Assurance (CPQA) program before used in clinical studies. Primary PK analyses will be testing for (1) differences in PK measures between the two age groups (Kruskal-Wallis) and (2) comparison with DREAM-01 PK results.

Secondary/Exploratory Endpoints:

Acceptability correlates. We will also examine previously identified correlates of product acceptability, including participants' prior experiences and comfort using rectal products, as well as douching or other rectal hygiene practices. We will also explore reactions to the study product and administration method, including participants' perceptions of their partners' reactions and future intention to use product if found to be effective. These assessments will allow investigators to identify product attributes likely to challenge and/or facilitate future sustained use when applied rectally by participants.

Explant studies. Cumulative p24 produced per biopsy from tissue culture supernatants collected 4, 7, 10, and 14 days after ex vivo HIV challenge will be the dependent variable in pharmacodynamic modeling. Each post-dose result will be compared to the pre-dose baseline. Concentration-response PK-PD analyses will be explored to estimate baseline cumulative p24 concentration (E_0), maximum inhibition of cumulative p24 suppression (I_{max}) and concentration associated with half-maximal inhibitory effect (IC_{50}).

Histology and Microbiome. The association of mucosal parameters with study products will be examined. Changes between time points and the baseline visit will be calculated and used as the outcome for the analysis. Additional exploratory analysis will be largely dependent on tissue models of pharmacokinetic and

pharmacodynamic parameters. These are currently in development as an overall objective of the DREAM Program and will be largely dependent upon findings related to the primary objectives; they will be fully developed only subsequent to achieving the primary objectives.

9.7 Missing Data

All reasonable efforts will be made to obtain complete data for all participants; however, missing observations will occur due to missed visits, participants lost to follow-up, or noncompliance. Research participants who fail to complete dosing and evaluations relevant to all study product doses for any reason may be replaced at discretion of the study site investigator after consultation with the Sponsor. Data for PK and distribution will be required of all periods of the study or the research participant will be replaced.

Several procedures will be used to conduct data analysis when data for either outcomes or covariates are missing. The first step will be to assess the extent and pattern of missing data. If data are missing for only a few cases, then data analysis will be conducted only on study participants with complete data. However, when such a strategy would result in loss of data from a substantial proportion of participants, or if this approach would lead to biased or inaccurate results, then some form of imputation will be performed. The form of imputation used will depend on the nature of the data that are missing. For example, data that are collected repeatedly might be imputed using the “last value carried forward” method; and in some instances, interpolation between neighboring points might also be used. When the primary endpoint is missing, one data analysis will be conducted using only cases with the endpoint. Subsequent analysis will be done where missing endpoints are imputed. Hot-deck imputation or regression imputation may also be used in this context.

As previously noted, all available data will be used for safety purposes. All available data will also be used for descriptive purposes. Formal statistical comparisons between study products, however, must necessarily be limited to subjects who complete use of study products. The between-product comparisons are critical to allow the use of the data in this study to guide future rectal microbicide development. Accordingly, the protocol principal investigators in consultation with the site investigators will determine if research participants who fail to complete all PK collection sessions should be replaced. Replacement subjects would be assigned the same sampling schedule as the subject they are replacing.

The familywise error rate corrections will be used to adjust for multiple comparisons.

Missing data adjustment methods will be investigated using multiple imputation and sensitivity analyses in the event of possible informative missingness.

10. DATA HANDLING AND RECORDKEEPING

10.1 Data Management Responsibilities

Due to the congruency of ATN's mission with that of HIV/AIDS Networks supported by the NIAID Division of AIDS (DAIDS), NICHD has adapted and implemented several DAIDS policies and requirements for the ATN. Study case report forms will be developed by the study team and the Emory Analytic Core. Quality control reports and queries routinely will be generated and distributed to the study sites for verification and resolution prior to reporting data to NICHD.

10.2 Source Documents and Access to Source Data/Documents

All study sites will maintain source documents and data in accordance with *Requirements for Source Documentation in DAIDS Funded and/or Sponsored Clinical Trials:* (http://www.niaid.nih.gov/LabsAndResources/resources/DAIDSClinRsrch/Documents/sourcedocpolicy.pdf).

Each investigator will maintain and securely store complete, accurate, and current study records throughout the study. Per US regulations, for all investigational products tested, the investigators will retain all study records on site for at least two years following the date of marketing approval for the study product for the indication in which it was studied. If no marketing application is to be filed or if the application is not approved, the records must be retained until two years after the investigation is discontinued and the US FDA is notified.

Study records must be maintained on site for the entire period of study implementation. Thereafter, instructions for record storage will be provided by Sponsor. No study records may be moved to an off-site location or destroyed prior to receiving approval from both NICHD and Sponsor.

10.3 Quality Control and Quality Assurance

Quality control and quality assurance procedures will be performed by all study sites as specified in *Requirements for Clinical Quality Management Plans at DAIDS Funded and/or Supported Clinical Research Sites*: https://www.niaid.nih.gov/sites/default/files/qmppolicy.pdf

10.4 Study Coordination

Study implementation will follow this protocol, which may not be amended without prior written approval from the DREAM Program Chair, who is also the IND Sponsor, and Dr. Bill Kapogiannis - ATN Medical Officer. Study implementation will also be guided by a common study-specific procedures manual that provides further instructions and operational guidance on conducting study visits; data and forms processing; specimen collection, processing, and shipping; AE assessment, management and reporting; dispensing study products and documenting product accountability; and other study operations.

Close coordination between protocol team members is necessary to track study progress, respond to queries about proper study implementation, and address other issues in a timely manner. Rates of accrual, retention, follow-up, and AE incidence will be monitored closely by the team.

11. CLINICAL SITE MONITORING

Due of the congruency of ATN's mission with that of HIV/AIDS Networks supported by the NIAID Division of AIDS (DAIDS), NICHD has adapted and implemented several DAIDS policies and requirements for the ATN. Study monitoring will be carried out by representatives from the ATN iTech Management and Analytic Core. Site monitoring visits will be conducted to assess overall study compliance, as required per *Requirements for On-Site Monitoring of DAIDS Funded and/or Sponsored Clinical Trials*, GCP, and FDA regulations 21 CFR Part 312:

https://www.niaid.nih.gov/sites/default/files/onsitemonitor_reqs.pdf

Study monitors will visit the site to complete the following:

- Assess compliance with the study protocol, Good Clinical Practices (GCP) guidelines, and applicable regulatory requirements, including US CFR Title 45 Part 46 and Title 21 Parts 50, 56, and 312
- Review informed consent forms, procedures, and documentation
- Perform source document verification to ensure the accuracy and completeness of study data
- Verify proper collection and storage of biological specimens
- Verify proper storage, dispensing, and accountability of investigational study products
- Assess implementation and documentation of internal site quality management procedures
- Assess site staff training needs

Site investigators will allow study monitors to inspect study facilities and documentation (e.g., informed consent forms, clinic and laboratory records, other source documents, case report forms), as well as observe the performance of study procedures. Investigators also will allow inspection of all study-related documentation by authorized representatives of NICHD, the IND Sponsor, and US regulatory authorities. A site visit log will be maintained at the study site to document all visits. The outcomes of the monitoring visits and the subsequent reports of resolutions of any identified problems will be provided to the Sponsor of the IND application.

12. HUMAN SUBJECTS

This study will be conducted in compliance with the protocol, ICH Good Clinical Practice guidelines, and 45 CFR Part 46.

The investigators will make efforts to minimize risks to participants. Volunteers and study staff members will take part in a thorough informed consent process. Before beginning the study, the investigators will have obtained IRB approval, and the protocol will have been submitted to the appropriate regulatory agencies involved in this trial. The investigators will permit audits by the NIH, Sponsor, Office for Human Research Protections (OHRP), the FDA or any of their appointed agents and other local, US, and international regulatory entities.

12.1 Institutional Review Boards

The Johns Hopkins University Institutional Review Board (JHM IRB) will serve as the single IRB of record, and will be responsible for assuring that this protocol, the associated site-specific informed consent documents, and study-related materials meet regulatory standards. Any amendments to the protocol, informed consent documents, and other study-related materials must be approved by the single IRB of record.

Subsequent to the initial review and approval, the JHM IRB must review the study at least annually. The study team will submit safety and progress reports to the IRB at least annually and within three months after study termination or completion. These reports will include the total number of participants enrolled in the study, the number of participants who completed the study, all changes in the research activity, and all unanticipated problems involving risks to human subjects or others.

12.2 Protocol Registration

Prior to implementation of this protocol, and any subsequent full version amendments, the study site must have the protocol and the protocol consent form(s) approved, as appropriate, by their local institutional review board (IRB)/ethics committee (EC) and any other applicable regulatory entity (RE). Upon receiving final approval, the site will submit all required protocol registration documents to the ATN Analytic Core (iTech) . The ATN Analytic Core (iTech) will review the submitted protocol registration packet to ensure that all of the required documents have been received.

Site-specific informed consent forms (ICFs) *WILL NOT* be reviewed or approved by the ATN Analytic Core (iTech), and sites will receive an Initial Registration Notification when the ATN Analytic Core (iTech) receives a complete registration packet. Receipt of an Initial Registration Notification indicates successful completion of the protocol registration process. The site will not receive any additional notifications from the ATN Analytic Core (iTech) for the initial protocol registration. A copy of the Initial Registration Notification should be retained in the site's regulatory files.

Upon receiving final IRB/EC and any other applicable RE approval(s) for an amendment, the study site should implement the amendment immediately. The site is required to submit an amendment registration packet to the ATN Analytic Core (iTech). The ATN Analytic Core (iTech) will review the submitted protocol registration packet to ensure that all the required documents have been received. Site-specific ICF(s) *WILL NOT* be reviewed and approved by the ATN Analytic Core (iTech) and sites will receive an Amendment Registration Notification when the ATN Analytic Core (iTech) receives a complete registration packet. A copy of the Amendment Registration Notification should be retained in the site's regulatory files.

12.3 Study Coordination

Study implementation will be directed by this protocol, which may not be amended without prior written approval from the Protocol Chairs and ATN Medical Officer. Study implementation will also be guided by a common Study-Specific Procedures (SSP) Manual that provides further instructions and operational guidance on conducting study visits; data and forms processing; specimen collection, processing, and shipping; AE assessment, management and reporting; dispensing study products and documenting product accountability; and other study operations. Standardized study-specific training will be provided by JHU investigators to the site by the SDMC, LC and other designated members of the Protocol Team.

Close coordination between protocol team members is necessary to track study progress, respond to queries about proper study implementation, and address other issues in a timely manner. The PSRT and SMC will address issues related to study eligibility and AE management and reporting as needed to assure consistent case management and documentation. Rates of accrual, adherence, follow-up, and AE incidence will be monitored closely by the protocol team, PSRT, and the SMC.

12.3 Risk-Benefit Statement

12.3.1 Risks

It is not expected that this trial will expose human subjects to unreasonable risk.

Breach of Confidentiality

A potential risk to participants is violation of confidentiality. We will take the utmost caution to protect the confidentiality of all responses. We will minimize this risk by maintaining confidentiality and discretion throughout all iTech research procedures and data management and analysis. We will develop procedures to minimize indirect disclosure that a participant is participating in an HIV- related research study, or a study that enrolls MSM. For each mode of contact information, we will ask specifically whether anyone else potentially has access to that mode of communication, and if it is acceptable to leave a non-specific message about participation in a health study. No study-related messages will ever mention HIV prevention or the nature of the research study. Additionally, all scripts for email, text message, and telephone contact with participants will be reviewed and approved by the Johns Hopkins IRB before being used for contact with participants.

Data Security

Participants may be concerned about the security of their data, particularly since it is collected and stored electronically. The iTECH Analytic Core has significant experience developing security protocols for Internet-based studies, and we will take a variety of steps to ensure participant security, including using a dedicated server behind a firewall, encryption of data, separation of identifiers from responses, and password-protected access to data. Therefore, we believe that this risk will be minimal.

We will secure study data with all appropriate physical, electronic and operational protections. Data will be stored in a physically secure environment. All data files will have encryption and strong password protection. Any identifiable data will either be stored on secure servers or will be on fully encrypted laptops. Access to data will be on a role-based standard; only those study staff who require access to each type of data to complete their study-related roles will be allowed access. All study staff will be trained in security and confidentiality procedures, and will sign a confidentiality agreement before receiving access to any participant data.

General
Phlebotomy and insertion of peripheral intravenous catheters (PIVs) may lead to discomfort, feelings of dizziness or faintness, and/or bruising, swelling and/or infection, and vein irritation. Less common risks from PIVs include local site infiltration or clotting. Very rarely, PIV's can be associated with a venous air embolism. Disclosure of STI status may cause sadness or depression in volunteers. Participation in clinical research includes the risks of loss of confidentiality and discomfort with the personal nature of questions.

Although all efforts will be made to protect participant privacy and confidentiality, it is possible that participant's involvement in the study could become known to others, and that social harms may result (i.e. become known as HIV-infected or at "high risk" for HIV infection). For example, participants could be treated unfairly or discriminated against, or could have problems being accepted by their families, employer, and/or communities.

Participants in sites requiring partner notification in response to diagnosed STIs or HIV infection could have problems in their relationships with their sexual partners. Participants also could have problems in their partner relationships associated with use or attempted use of study products. In addition, participants could misunderstand the current experimental status of the study gels (i.e., their unknown safety and unproven efficacy) and as a result increase their HIV risk behaviors while in the study.

Rectal Swabs
There is the risk of mild discomfort from this procedure, in addition to a slight risk of bleeding.

General Risks of Douches
The main risk from having an enema is temporary discomfort. A hollow plastic tube about the thickness of a pencil will be used to administer approximately 120-125 mL of enema fluid into the rectum. This may cause a "bloated" or "cramping" feeling. Some air may be pumped into the rectum as well, causing flatulence. The tube is small, but it might cause some anal or rectal discomfort if the subject has any hemorrhoids or other painful conditions. There is a remote possibility that the enema tip could cause perforation.

Tenofovir Formulation Enemas
TFV has been administered as a rectal topical agent in several prior studies as summarized in the introduction. The very high osmolality of the VF gel was associated with some GI side effects in RMP-02/MTN-006. In subsequent studies (CHARM-01, CHARM-02, MTN-017) lower osmolality formulations (RGVF and RF) have not been associated with these earlier VF symptoms. These lower osmolality formulations have been associated with excellent safety profiles with no clear association of AEs with study product.

To date, the TFV douche has been well tolerated. In the DREAM-01 study, two of 85 reported adverse events, blood spotting in effluent immediately after douching, and rectal dryness were deemed related to the study product. Both were graded as mild.

Flexible Sigmoidoscopy with Biopsies
Flexible sigmoidoscopy is a commonly practiced medical procedure and the endoscopic procedures done in this trial will not involve any unusual risks or discomforts. The risks associated with these procedures include mild discomfort and the feeling of having a "bloated stomach". Endoscopic biopsies are painless and heal quickly within 3 days. On extremely rare occasions, the endoscopic procedure or biopsies may lead to pain, infection (sepsis), bleeding or perforation of the gastrointestinal tract. Perforation occurs approximately once out of every

1 in 1,000 to 10,000 procedures. If this extremely rare complication occurs, antibiotics and surgery to repair the tear may be necessary.

Risks of Moderate Sedation (if required)
Risks include amnesia during the period of sedation, and occasionally shivering, mild nausea or over-sedation can occur. It is extremely rare for any other complications to occur, but over-sedation may also cause respiratory depression and changes in blood pressure that, if not monitored correctly, could become life-threatening. Participants are continuously monitored during sedation and an anesthesiologist is available to optimize participant safety. A period of sleepiness may persist for several hours after the procedure.

Anoscopy and Luminal Fluid Collection
It is possible that the participant may experience mild discomfort, embarrassment or, rarely, pain (should they have another condition that is already causing pain in the area) during this procedure. If there is any pain experienced during the procedures, the study clinician will adapt or stop the procedure.

Risks of Computer-Assisted Questionnaire
There may be discomfort or embarrassment related to questions dealing with sexual behaviors and personal habits. If some of the questions upset or make the research participant uncomfortable, they may choose not to answer them. Participants will also be able to refuse to answer any question that makes them uncomfortable.

12.3.2 Benefits

Participants in this study will experience no direct benefit. Participants and others may benefit in the future from information learned from this study. Specifically, information learned in this study may lead to the development of safe and effective interventions to prevent HIV acquisition and transmission. Participants also may appreciate the opportunity to contribute to the field of HIV prevention research.

Participants will receive HIV/STI risk reduction counseling, HIV and STI testing, and physical and rectal examinations. Participants may be provided or referred for STI treatment free of charge. In addition, STI testing and treatment may be offered and/or referrals may be provided for their partners. For other medical conditions identified as part of the study screening and/or follow-up procedures, participants will be referred to other sources of care available in their community. Some participants may have the opportunity to access expedited treatment and benefit from decreased morbidity due to early diagnosis and treatment of abnormalities identified during tests, examinations, and referrals

12.4 Informed Consent Process

It is the responsibility of the study site investigator to ensure that the Elements of Informed Consent (21 CFR 50.25 and ICH GCP 4.8.10) and Health Insurance Portability Accountability Act (HIPAA) guidelines are followed and documented in the source document file. The process for obtaining informed consent from potential research participants should be clearly documented and appropriately filed within the site's standard operating procedures.

Written informed consent will be obtained from each study participant prior to initiation of any study procedures. Consent forms will explain the purpose of the study, the procedures to be followed, and the risks and benefits of participation, in accordance with all applicable regulations. The informed consent process will

cover all elements of informed consent required by research regulations, inclusive of the following topics of importance to this study:

- The unknown safety and unproven efficacy of the study products
- The need to practice safer sex behaviors regardless of study treatment group
- The importance of adherence to the study visit and procedures schedule
- The potential medical risks of study participation (and what to do if such risks are experienced)
- The potential social harms associated with study participation (and what to do if such harms are experienced)
- The limited benefits of study participation
- The distinction between research and clinical care
- The right to withdraw from the study at any time

The consent/assent form describes all study procedures, including confidentiality and privacy, information about potential risks, discomforts, benefits of participation, and information regarding who they can contact with further questions. It also states that participation is voluntary, that participants may decide not to take part or to withdraw from the study at any time without penalty or loss of any benefit to which they might otherwise be entitled, and that study participation is in no way related to being able to access or continue getting care or services at any participating study site. Participants can refuse to answer any question, and can withdraw from the study at any time. The PIs, Co-PIs, or designee at each site will review all informed consents and assents.

If during the trial, a consent revision is being presented, including new information that might affect the research participant's willingness to participate, participants will be informed of the revisions. If a research participant terminates the study and consent form revision occurs after their participation has ended, they do not need to sign the revised consent form.

Written informed consent also will be obtained for long-term specimen storage and possible future testing. In obtaining and documenting informed consent, the investigators and their sub-investigators will comply with applicable local and US regulatory requirements and will adhere to GCP and to the ethical principles that have their origin in the Declaration of Helsinki. Study staff must document the informed consent process in accordance with the Requirements for Source Documentation in ATN Funded and/or Sponsored Clinical Trials available in Chapter 8 of ATN MOPP (https://sites.cscc.unc.edu/atn/system/files/private-docs/MOPP08_Data%20Management.pdf), NIAID policy (https://www.niaid.nih.gov/sites/default/files/daids-sourcedocpolicy.pdf), and NIAID requirements: (https://www.niaid.nih.gov/sites/default/files/sourcedocappndx.pdf).

Participants will be provided with copies of the informed consent forms if they are willing to receive them. The study site is responsible for developing study informed consent forms for local use, based on the templates in the Appendices that describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation, in accordance with all applicable regulations.

12.4.1 Parental consent and inclusion of minors

This research will involve adolescent MSM between 15-17 years old. The age range is critical for this research because rates of HIV-related risk behaviors being in adolescence and peak as youth transition into adulthood, and interventions delivered during this time can have the potential to prevent future escalation and

consequences associated with HIV. Given the limited inclusion of youth in HIV biomedical prevention trials, this study has the potential for significant gains for this age group. For participants between the ages of 15 and 17 years, we will request that they are accompanied by a parent and/or legal guardian. These participants will also be asked to assent to participate in study activities.

Similar to prior PrEP trials with youth at the Site, a licensed social worker will serve as the Participant Advocate (JHSPH IRB00007369) and will be present for all study-related procedures if the parent/legal guardian is unable to attend. We will ascertain the extent to which parental permission influences YMSM's willingness to participate in biomedical trials among the subsample who is eligible yet decides not to enroll in the study using in-depth interviews (n=32), as well as those who enroll in the trial (n=16).

12.4.2 Assessing for decisional capacity

The informed consent process will include an assessment of each potential participant's understanding of concepts identified by the protocol team as essential to the informed consent decision. For all participants, the research assistant (RA) reviews the informed consent/assent to assess the youth's decisional capacity and ability to provide consent/assent prior to signing, using a 2-step process. First, the RA determines if the person understands the study goals by asking "Can you tell me what this study is about?" In step 2, potential participants will be asked questions designed to assess their capacity to understand, appreciate, reason with, and express a choice about participation in our specific protocol. Participants will be asked to: name things they will be expected to do during the study; explain what they would do if they no longer wished to participate in the study**;** explain what they would do if they experienced distress during the study; and identify potential risks for participating in the study. For youth who cannot answer these questions, the RA will go back and review the relevant elements of consent with the participant again and repeat the process. Youth who appear not to understand after repeated review will not be enrolled in the study.

12.5 Participant Confidentiality

All study procedures will be conducted in private and every effort will be made to protect participant privacy and confidentiality to the extent possible. The study site will implement confidentiality protections that reflect the local study implementation plan and the input of study staff and community representatives to identify potential confidentiality issues and strategies to address them. In addition to local considerations, the protections described below will be implemented at all sites.

All laboratory specimens, questionnaires, evaluation forms, reports, and other records will be identified by a coded number and initials only, to maintain participant confidentiality. All records with personally-identifying information will be kept in a locked, limited access area (such as a locked file cabinet). All computer entry and networking programs will be done with coded numbers and initials only. Clinical information will not be released without written permission of the participant (and parent or legal guardian, when applicable), except as necessary for monitoring by the iTECH MC or NICHD.

All study-related information will be stored securely at the study site. All participant information will be stored in locked file cabinets in areas with access limited to study staff. Data collection, process, and administrative forms, laboratory specimens and other reports will be identified by a coded number only, to maintain participant confidentiality. Forms, lists, logbooks, appointment books and any other listings that link participant ID numbers to other identifying information will be stored in a separate, locked file in an area with limited access. All local databases will be secured with password-protected access systems. Participants'

study information will not be released without their written permission, except as necessary for review, monitoring, and/or auditing by:

- Representatives of the US Federal Government, including the US Food and Drug Administration (FDA), the US Office for Human Research Protections (OHRP), NIH, and/or contractors of the NIH
- Representatives of the Sponsor
- DREAM IND Regulatory Program
- Site IRB
- JHU legal representatives and other JHU staff

12.5.1 Certificate of Confidentiality

This research specifically targets a vulnerable population, children (YMSM ages 15-17). We will take every available step to minimize the risk of identifying/linking data being subpoenaed, stolen, or inadvertently released. First, the iTech has obtained a Certificate of Confidentiality from the NIH. Second, all research staff members are required to complete ethical clearance certification regarding protection of human's subjects through their relevant IRBs. Third, all studies will have documented procedures to safeguard against the risk of the linking information being stolen by keeping such information in a locked spaces to which only essential study personnel who have completed CITI certification for human subjects research ethics training (http://citiprogram.org) will have access.

Per Section 2012 of the 21st Century Cures Act as implemented in the 2017 NIH Certificates of Confidentiality Policy, all ongoing or new research funded by NIH as of December 13, 2016 that is collecting or using identifiable, sensitive information is automatically issued a CoC. As noted on the NIH website (http://grants.nih.gov/grants/policy/COC/faqs.htm#187), a Certificate of Confidentiality will help the research team "...avoid compelled 'involuntary disclosure' (e.g., subpoenas) of names and other identifying information about any individual who participates as a research subject (i.e., about whom the investigator maintains identifying information) during any time the Certificate is in effect."

12.6 Special Populations

This section outlines considerations made for the inclusion or exclusion of special populations in this study.

12.6.1 Women

Only healthy male volunteers with a history of recent RAI will be included in this study, as the rationale for use of the enema as rectal microbicide is based on established behaviors in MSM related to pre-RAI enema use. The enema use behaviors in women are far less well studied and, where data do exist, it suggests women do not use enemas in the same manner as MSM. This selected MSM population also comprises a large portion of the target population for rectal microbicide use in the United States.

12.6.2 Children

The NIH has mandated that children be included in research trials when appropriate. This study meets *Justifications for Exclusion* criteria for younger children as set forth by the NIH. Because of the need to understand douching as a potential PrEP option for adolescents, this study plans to enroll adolescents defined as 15 -24 years old.

12.7 Compensation

Pending IRB approval, participants will be compensated for their time and effort in this study, and/or be reimbursed for travel to study visits, child care, and time away from work according to site's practice. Reimbursement amounts will be specified in the site's informed consent form.

Participants will also receive a per diem for food and round trip fare to the study site ($110 total), as well as having their hotel covered by study.
Participants who do not complete visit 4 will receive a pro-rated amount for visits completed (at $25 per visit completed). All participants who participate in the study will be offered the opportunity to participate in an in-depth interview. This is regardless of which visit is completed. Participants will be provided food and transportation at each study. If participants need to stay overnight for a visit (for example, between visit 3 and 4), the study will also cover a one night's hotel stay.

12.8 Communicable Disease Reporting

Study staff will comply with all applicable local requirements to report communicable diseases including HIV identified among study participants to local health authorities. Participants will be made aware of all reporting requirements during the study informed consent process.

12.9 Access to HIV-related Care

12.9.1 HIV Counseling

HIV test-related counseling will be provided to all potential study participants who consent to undergo HIV screening to determine their eligibility for this study. Testing will be performed in accordance with the algorithm in Appendix II. Counseling will be provided in accordance with standard HIV counseling policies and methods at the participating study site and additionally will emphasize the unknown efficacy of the study products in preventing HIV infection. In accordance with NIH policies, participants must receive their HIV test results to take part in this study. Referral for additional counseling related to testing or diagnosis will occur if needed or requested by the participant.

12.9.2 Care for Participants Identified as HIV-infected

Participants will be provided with their HIV test results in the context of post-test counseling. Per site SOPs, participants found to be HIV-infected will be referred to available sources of medical and psychosocial care and support, and local research studies for HIV-infected adults.

12.10 Study Discontinuation

This study may be discontinued at any time by NICHD, Sponsor, US FDA, the OHRP, or site IRB.

13. PARTICIPANT MANAGEMENT

13.1 Tracking Participants / Follow-up

Participants will be contacted to schedule their visits and IDIs. Multiple contact methods will be used for participants to remind them about their scheduled visits, (e.g., phone calls, alternative email addresses, text messages, Facebook/other social media accounts). Participants will be asked whether or not messages can be left for any phone numbers they provide. They will be informed that messages will not contain any information regarding the nature of the project.

13.2 Intervening on “Social Harms"

All sites have specific policies governing the treatment of human subjects. These policies specify that medical and psychological assistance will be available in the immediate environment in the event a participant should experience any adverse reactions resulting from study procedures.

While participants will be informed that they may refuse to answer any question at any time, responses or reactions to certain questions may indicate distress on the part of the participants. If at any time during the study, a participant divulges that he or she is at risk for harm, including but not limited to being abused or experiencing violence, if harm is suspected or likely, or if the participant states he or she is suicidal/homicidal, measures will be taken to ensure his or her safety. Reporting will be done as appropriate to the situation and the legal statutes, including reporting to child protection agencies or other appropriate agencies and referrals will be provided to appropriate support, counseling or treatment resources.

13.3 Criteria for Premature Study Discontinuation

The principal investigator has the authority to withdraw any participant at any time if it their opinion it would be in the best interest of the participant. The participant will be informed of this withdrawal and explained the rationale. Withdrawal will be documented in the study tracking system.

Subjects will be prematurely discontinued from the study if any of the following occurs:

a. The subject withdraws consent/assent;
b. The study is cancelled by the *NIH (or iTech, or other administrative entity)*;
c. The study is cancelled for other administrative reasons; or
d. Death of the subject.

Participants may end their participation in the study at any time. No further data collection will occur from the date the decision is made to permanently discontinue the subject from the study. Participants who experience distress during the survey can access our list of community referrals or contact the research staff using the information provided to the participant during the consent/assent process. Any unexpected adverse events that meet the New Safety Information reporting criteria will be immediately reported to the UNC-CH IRB and the respective sites’ IRBs if applicable. All study activities will halt pending UNC-CH IRB review and recommendations if necessary. If a participant withdraws from the research, all data collected will be immediately destroyed and will not be used in subsequent analysis.

If a participant withdraws or is removed from the study, the DREAM Study Stop Form will be completed.

14. PUBLICATION OF RESEARCH FINDINGS

NICHD and ATN policies will govern publication of the results of this study. Any presentation, abstract or manuscript will be made available for review by the study sponsors prior to submission.

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16. APPENDICES

APPENDIX I: SCHEDULE OF STUDY VISITS AND EVALUATIONS

		Screening Visit	Enrollment Visit	Dosing & Sampling Visits			Final/Early Termination Visit	Follow-up
Visit #		1	2	3	4	5	6	Phone Call
Visit Day			≤ 45 days from Screening	Day 1 >7d and ≤21 Days from Enrollment	Day 2 (24hrs)	Day 4 (72hrs)	Day 5 (+3 days) (96hrs)	if needed
ADMINISTRATIVE								
Informed consent		X						
Assessment of Eligibility		X	X	X	X	X	X	
Review Demographics & Locator Information		X	X	X	X	X	X	
Review and update Medical History		X	X	X	X	X	X	
Review Concomitant Medications		X	X	X	X	X	X	
AE Assessment			X	X	X	X	X	
Query for Compliance with Pre-Visit Instructions			X	X	X	X	X	
Provide Pre/Post Visit Reminders		X	X	X	X	X	X	
Review STI Risk Reduction Counseling & Provide Condoms		X	X	X	X	X	X	
Participant education: flexible sigmoidoscopy procedure with biopsy & rectal fluid collections		X	X	X	X	X		
Review lab results			X	X	X	X	X	X
CLINICAL								
Physical Exam	Complete	X						
	Symptom-Directed		X	X	X	X	X	
	VS (BP, HR, Temp, HTC‡, Wt‡)	X	X	X	X	X	X	
Cleansing Enema			X			X		
SAFETY LABS								
Blood	CBC w/ Diff	X		X			X	
	CMP	X		X			X	
	PO4	X						

	HBsAg	X						
Urine	Toxicology Screen††	X						
		Screening Visit	Enrollment Visit	Dosing & Sampling Visits			Final/Early Termination Visit	Follow-up
Visit #		1	2	3	4	5	5	Phone Call
Visit Day			≤ 45 days from Screening	Day 1 >7d and ≤21 Days from Enrollment	Day 2 (24hrs)	Day 4 (72hrs)	Day 5 (96hrs)	If needed
STI TESTING								
Blood	HIV Testing†	X	X	X			X	
	Syphilis Testing†	X	X†††	X†††			X†††	
Urine	GC/CT NAAT	X	X†††	X†††			X†††	
Rectal Swab	GC/CT NAAT	X	X†††	X†††			X†††	
Swab	HSV PCR NAAT	X†††	X†††	X†††			X†††	
DOSING/STUDY PRODUCT								
Study Product Administration				X				
STUDY SPECIMEN COLLECTION††††								
Blood PK/PBMC[1]	Pre-dose		X	X				
	1hr			X				
	6hr			X				
	24hr				X			
	72hr					X		
Rectal Biopsies*[2] (Not conducted in participants < age 18; Prioritized for participants ≥18 years)	Pre-dose		X					
	1hr			X				
	6hr			X				
	24hr				X			
	72hr					X		
Douche Effluent				X				
Microbiome[3]	Pre-Dose			X				
	24hr				X			
Rectal Fluid*[4] (Not conducted in participants	Pre-Dose			X				
	1hr			X				

< age 18; Prioritized for participants ≥18 years)	6hr			X				
	24hr				X			
	72hr					X		
BEHAVIORAL								
In-Depth Interview (Ineligible)		X						
Baseline Behavioral Survey[5]			X					
Dose Acceptability Questionnaire				X				
Acceptability In-Depth Interview							X	

- Height and Weight only at screening

† Confirmation testing as needed – refer to appendices for HIV and syphilis testing algorithms

†† Urine immunoassay screen for: Amphetamine/Ecstasy, Barbiturates, Benzodiazopines, Cocaine Metabolite, Codeine/Morphine, THC, Oxycodone/Oxymorphone, Fentanyl

††† If clinically indicated

†††† Collection time points are approximate. Every effort will be made to adhere to the timeline, however, due to logistics, timing will not be exact and specimen collections may be missed (i.e. during biopsy collection) – these departures from the timeline will not be considered deviations and will not be recorded/reported as such

* According to individual participant sampling schedule

1 Sampling includes blood plasma and PBMC

2 Biopsies in complete sets (10-20 cm from the anal verge) will be performed, to evaluate the concentration range based on prior enema distribution studies. Moieties of sampled drug will include TFV, TFV-DP, and dATP. Biopsies will be processed for PK (tissue homogenate, total MMC (cells), histology, and *ex vivo* HIV explant challenge

A total of up to 5 biopsies will be collected at the baseline visit, post-dosing, up to 20 biopsies will be collected according to each participant's individual sampling schedule.

3 Swab for microbiome should be collected prior to dosing, enema administration or rectal fluid collection

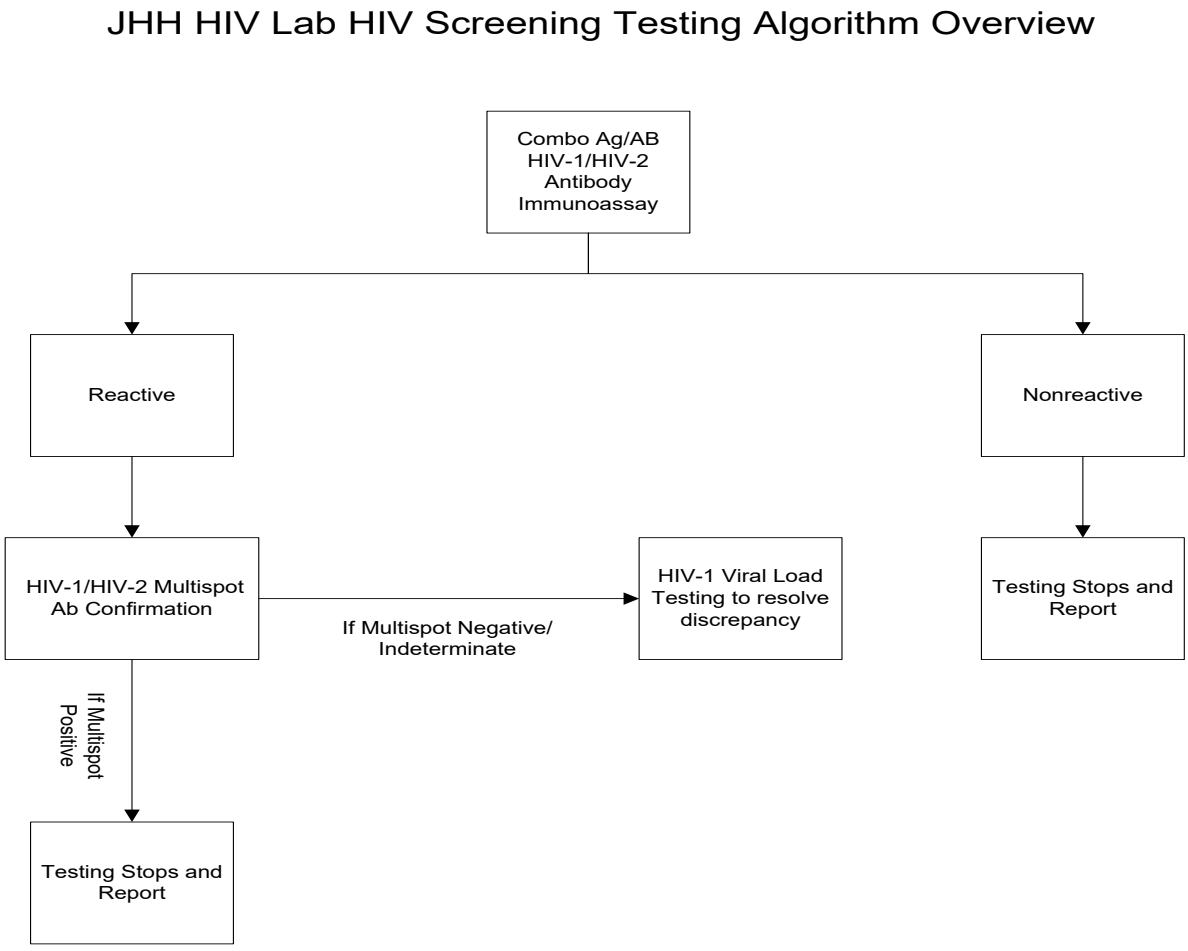
4 Rectal fluid will be collected on all participants pre-dose

5 The Baseline Behavioral survey may be completed at any time from the enrollment visit (Visit 2) until just prior to TFV douche dosing om Day 1 (Visit 3).

*

APPENDIX II: HIV ALGORITHM

In accordance with CDC guidelines for HIV serology testing, The Johns Hopkins Hospital HIV Laboratory has moved to a 4th generation assay which is a more sensitive and specific assay. The 4th generation assay is a combined antigen and antibody assay for HIV1 and HIV2 serological detection. All reactive samples will be confirmed with a rapid multi-spot assay for HIV-1 and HIV-2. The Western blot confirmatory assay is no longer performed. Any discrepant results will go on to HIV RNA viral load testing. As with the previous HIV serology, no reactive results will be released prior to confirmatory testing.



APPENDIX III: TOXICITY TABLES

Due of the congruency of ATN's mission with that of HIV/AIDS Networks supported by the NIAID Division of AIDS (DAIDS), NICHD has adapted and implemented several DAIDS policies and requirements for the ATN. The *Division of AIDS Rectal Grading Table for Use in Microbicide Studies (DAIDS AE Grading Table Addendum 3, Clarification dated May 2012)* will be the primary tool for grading adverse events for this protocol.

Adverse events not included in the *Rectal Grading Table* will be graded by the most current *Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1. (July 2017).* In cases where an AE is covered in both tables, *DAIDS AE Grading Table Addendum 3* will be the grading scale utilized.

All Division of AIDS grading tables are available online at:
https://rsc.niaid.nih.gov/clinical-research-sites/daids-adverse-event-grading-tables

APPENDIX IV: SEVERITY GRADING DEFINITIONS FOR UNTOWARD EVENTS (UES)

SOURCE: ATN MANUAL OF POLICIES AND PROCEDURES ON SAFETY AND ADVERSE EVENT REPORTING (MOPP 10)

Table 10-1: Severity grading definitions for untoward events (UEs)

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING	GRADE 5 DEATH
Event	Mild symptoms causing minimal or no interference with usual social & functional activities with intervention not indicated	Moderate symptoms causing greater than minimal interference with usual social & functional activities with intervention indicated	Severe symptoms causing inability to perform usual social & functional activities with intervention or hospitalization indicated	Potentially life-threatening symptoms causing inability to perform basic self-care functions with intervention indicated to prevent permanent impairment or persistent disability	Death
Participant UE Example: Inadvertent disclosure of study participation to participant's partner	Participant experienced discomfort that resolved without intervention	Participant experienced moderate stress in relationship with partner. Resolution: Resolved after provision of educational resources for the participant to share with partner	Participant experienced verbal harassment by partner and unable to work. Resolution: Resolved after referral to couples support services or other mental health resources	Participant experienced physical assault by partner. Resolution: Required immediate medical treatment, mental health referral, and legal advice	Death
Staff UE Example: Verbal or physical threat or harm to study staff by study participant or guardian	Staff member experienced discomfort that resolved without intervention	Staff member experienced moderate distress. Resolution: Resolved after site leadership intervened	Staff member experienced major distress. Resolution: Required security/police to be notified or mental health referral	Staff member experienced life-threatening physical harm. Resolution: Required immediate medical treatment, mental health referral, and legal advice	Death

Adapted from Appendix 10-3.