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Higher Colorectal Tissue HIV Infectivity in Cisgender Women Compared to MSM Before and During Oral Pre-Exposure Prophylaxis

Short Title: Mucosal HIV Infectivity Gender Differences

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Conflicts of Interest:

IMG is an employee of Orion Biotechnology, Ontario, Canada

RJL has served on advisory boards for Gilead Sciences and Merck, Inc, and received honoraria from Roche and Janssen.

KHM has served on scientific advisory boards for Gilead Sciences and Merck, Inc. and has received unrestricted research grants from Gilead Sciences, Merck, Inc, and Janssen.

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Abstract

Objective: Compare HIV-negative cisgender women (CGW) to men who have sex with men (MSM) for mucosal tissue differences in pharmacokinetics, HIV infectivity, and cell phenotype.

Design: Substudy of HPTN 069/ACTG A5305, 48-week study of three oral candidate pre-exposure prophylaxis regimens: maraviroc, maraviroc/emtricitabine, and maraviroc/tenofovir disoproxil fumarate (TDF) compared to a TDF/emtricitabine control group.

Methods: Plasma, peripheral blood mononuclear cells, and cervical and colorectal tissue biopsies were collected at Baseline (no drug), Week 24 and 48 (on drug), and Week 49 (one-week post-drug). Drug concentrations were assessed in all matrices. HIV infectivity was assessed using tissue biopsy "explants" challenged with HIV ex vivo followed by HIV p24 measurement. Flow cytometry evaluated colorectal cell phenotype.

Results: Thirty-seven CGW and 54 MSM participated. CGW's colorectal explant p24 was higher than MSM before (0.31 $\log_{10}$, p=0.046), during (1.01-1.19 $\log_{10}$, p=0.016), and one week after (0.61 $\log_{10}$, p=0.011) study drug dosing. Pooling regimens, cervical explant p24 did not differ among visits. CGW had higher plasma maraviroc and colorectal tissue tenofovir diphosphate and lower colorectal tissue emtricitabine (all $p<0.005$) compared to MSM. Each study drug's cervical tissue concentrations were >10-fold below paired colorectal concentrations ($p<0.001$). Cell phenotype sex differences included 4% higher CD38+/CD8+ cells at baseline and 3-7% higher CD69+/CD8+ cells throughout Weeks 24-49 in CGW compared to MSM ($p<0.05$).

Conclusion: Colorectal explants in CGW demonstrated greater HIV infectivity than MSM with and without study drugs. Small differences in adherence, drug concentration, and colorectal tissue flow cytometry cannot fully explain this difference.

Key Words:maraviroc; emtricitabine; tenofovir; pharmacokinetics; pharmacodynamics ; flow cytometry; mucosal tissue

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Introduction

HIV Pre-Exposure Prophylaxis (PrEP) has proven effective in specific populations with oral tenofovir disoproxil fumarate (TDF)/emitricitabine (FTC), tenofovir (TFV) alafenamide (TAF)/FTC, vaginal tenofovir (TFV) gel, vaginal dapivirine ring (DPV), and injectable long acting cabotegravir [1-9]. As with contraception, having multiple PrEP choices likely increases uptake, persistence, and adherence of PrEP regimens with product choices influenced by many variables beyond efficacy [10-13]. Biological and pharmacological variables, which vary between men and women, also influence HIV acquisition and prevention, but are infrequently compared directly.

HPTN 069/ACTG 5305 was a prospective safety and tolerability trial evaluating maraviroc (MVC) alone or in combination with FTC or TDF in comparison to TDF/FTC, the only PrEP regimen proven effective at that time. Non-overlapping side effect profiles and coverage of community-acquired TFV or FTC resistance were considered potential benefits of MVC-containing regimens. Perhaps, the most significant challenge to PrEP product development is the inability to evaluate concentration-response in Phase 2 prior to Phase 3 efficacy trials. Substitutes have included site of HIV acquisition assessments of mucosal tissue antiretroviral pharmacokinetics (PK), pharmacodynamic (PD) concentration-response evaluations using ex vivo HIV challenge of mucosal tissue as a potential indicator of clinical HIV protection, and immunological changes that might affect HIV infectivity. Sex-based differences in colorectal tissue, an important route of HIV acquisition in men who have sex with men (MSM), transgender women (TGW), and cisgender women (CGW), are not well studied.

We conducted a mucosal tissue substudy within HPTN 069/ACTG 5305 that compared CGW to MSM with respect to PK, PD, and mucosal immunology. This substudy represents one of the largest studies comparing colorectal and cervical tissue and a unique opportunity to compare these variables in CGW and MSM. We report important differences in colorectal tissue susceptibility to HIV infection between MSM and CGW both before and during antiretroviral dosing.

Methods

Study design and Population

We report the mucosal tissue PK, PD, and flow cytometry results of CGW (first reported here) in comparison to previously reported MSM results [14]. This tissue substudy was nested within HPTN 069/ACTG 5305 study (NCT01505114), a Phase 2 prospective, randomized, controlled, double-blind trial of the safety and tolerability of three candidate PrEP regimens - MVC 300mg, MVC 300mg/FTC 200mg (MVC/FTC), and MVC 300mg/TDF 300mg - compared to a control group who received TDF 300mg/FTC 200mg [15, 16]. Substudy participants followed the same study protocol plus collection of (1) plasma and peripheral blood mononuclear cells (PBMC), cervicovaginal fluid (CVF), and rectal fluid (RF) at Weeks 24, 48, and 49 for PK, and (2) cervical and colorectal biopsies for PK, ex vivo

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HIV infectivity (PD), and colorectal tissue flow cytometry at Baseline, Week 24, 48, and 49. All study sites were in the US.

Sample Processing

Plasma, PBMC, RF, and colorectal biopsy collection and processing was previously reported [14]. Participants self-collected CVF with a Dacron swab; two exocervical biopsies were collected using Tischler forceps (reported previously) [17]. Blood plasma, PBMC lysates, CVF, RF, and biopsy homogenates were immediately frozen, stored, and later shipped to JHU for drug concentration analysis. Frozen explant supernatants (batch shipped) and flow cytometry samples (overnight shipped) went to University of the Pittsburgh for analysis.

Pharmacology

Drug concentration analysis used ultra-performance liquid chromatograph tandem mass spectrometry (UPLC-MS/MS)[18-20]. CVF, RF, and tissue results were reported as mg drug per mg of sample; lower limit of quantitation (LLOQ) listed for these matrices is based on median sample weights (**Table 1**).

Ex vivo HIV Challenge.

Cervical and colorectal biopsies were placed in tissue culture media, challenged with HIV for 2 hours, and supernatant harvested over 10-14 days [17, 21]. Differences in cervical and colorectal methods, respectively, include biopsy number (one vs. four), HIV challenge dose ($5x10^4$ vs. $1x10^4$ $TCID_{50}$), supernatant sampling (4, 7, 10 days vs. 4, 7, 10, 14 days), analysis volume (0.7 vs. 0.5 mL). Unit of analysis was median biopsy cumulative log_{10} p24 antigen concentration. LLOQ for p24 was 12.5 pg/mL.

Cell Phenotype

The isolation of colorectal biopsy mononuclear cells and flow cytometry analysis for CD3+, CD4+, CD8+, and CD45+ (surface [FCS] and intracellular markers [FCI]) and CD38+, CD69+, HLA-DR+, CCR5+, CXCR4+, Ki67+ (FCS only) was described previously [14, 22].

Data Analysis

Non-parametric descriptive statistics, comparisons tests for differences among regimens or study visits (Kruskall-Wallis or paired Friedman tests) or between sex at birth, regimens, or visits (Wilcoxon ranked sum tests), and correlation (Spearman test) were used.

We defined daily "adherence" using HPTN 066 thresholds for TFV and FTC; "percent adherence" is the percent of participants meeting this definition [20]. The MVC adherence threshold, 4.6 ng/mL, was determined by the lowest concomitant MVC concentration in TFV and FTC adherent participants on combination regimens. The MVC threshold demonstrated

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93% sensitivity and 78% specificity identifying daily adherence defined by TFV and FTC thresholds.

Drug concentration vs. p24 response modeling used the sum of molar tissue drug concentrations to account for combination drug regimens. Modeling explored (1) 2, 3, and 4-parameter I_{max} models (E_0 baseline p24 without drug, I_{max} maximum p24 change on drug, IC_{50} molar drug concentration at half-maximal effect, and slope term [Hill coefficient]), (2) weighting schemes for heteroscedasticity, (3) + biopsy weight adjustment, (4) + MVC concentration correction (x0.18) to account for PK (no incubation) vs. PD (2-hr incubation) processing differences [23], and (5) + imputation of baseline and/or BLQ values. Goodness-of-fit was assessed using the correlation matrix, coefficient of variation, and Schwartz and Akaike information criterion (Phoenix WinNonlin v.8, Certara, Cary, NC).

Results

Enrollment and Participant Characteristics

The substudy included 37 CGW, whose PK, PD, and cell phenotype results are first reported here, and the previously reported cohort of 54 MSM (**Figure1**) [14]. The analysis set of 91 participants, excluded six enrolled substudy participants without post-dose PK, PD, or flow cytometry sampling. Paired comparisons of baseline and active drug visits included 84 participants and excluded seven CGW without baseline biopsies.

CGW height was lower than in the MSM group ($p<0.001$), but age, weight, and creatinine clearance were similar (Supplemental Table 1, http://links.lww.com/QAD/C102). Pooling sex groups, there were no differences in these variables across study arms. Nineteen CGW (51%) reported use of hormonal contraceptives, including depot medroxyprogesterone acetate (N=3) and other progestins (N=16), of which six were also on estrogens.

Adherence

Across regimens, 79% of CGW met the protocol definition of adherence, 12% lower than the 90% adherent in MSM ($p=0.045$). Overall adherence changed little from Week 24 (87%) to Week 48 (85%).

Pharmacokinetics

For on drug periods (Week 24 and Week 48), plasma, PBMC, rectal fluid, cervicovaginal fluid, and colorectal tissue concentrations fell below the LLOQ in 0 to 20% of samples depending on sex and matrix-analyte pair, without CGW-MSM differences (**Table 1**). Sex differences were observed in plasma MVC (1.7-times higher in CGW), colorectal tissue FTC (2.3-times higher in MSM), and colorectal tissue TFV-DP (12.2 times higher in CGW, all $p<0.005$). When excluding non-adherent participants, the only additional PK difference was a 59% higher PBMC TFV-DP in MSM ($p=0.044$). The differences in plasma MVC (1.6-times higher in CGW, $p<0.001$), colorectal tissue FTC (2.0-times higher in MSM, $p=0.016$),

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and colorectal tissue TFV-DP (13.8-times higher in CGW, p<0.001) remained statistically significant and of similar magnitude. We observed no PK differences among the four regimens when pooling study week across all participants (**Table 1**).

In contrast to all other matrices, cervical tissue drug concentrations fell below the LLOQ in 43-91% of samples. Paired median colorectal to cervical tissue concentration ratios, using the analyte-matrix LLOQ where necessary for BLQ values, indicate differences of 12-times (MVC only), 276-times (TFV), 3-times (FTC), and 93-times (TFV-DP), though with only 8-11 observations per drug analyte.

Tissue Cell HIV Infectivity

Biopsy weight differences were observed among regimens for cervical tissue at Week 24 (p=0.05), colorectal tissue at Weeks 48 (p=0.048) and 49 (p=0.028), and between MSM and CGW (each week p<0.002). Therefore, explant p24 results are biopsy weight-adjusted. However, the weight-adjusted and non-weight adjusted explant p24 antigen values were highly correlated for both cervical (r=0.963) and colorectal tissue (r=0.983). In addition, there were trivial differences in goodness-of-fit assessments in PK-PD modeling whether or not using biopsy weight-adjusted p24.

For the 29 CGW with pre- and post-dose cervical biopsies, cervical explant p24 expression was not different across study visits when pooling drug regimens (**Figure 2**). When comparing on drug visits to baseline, the only difference was a one $\log_{10}$ p24 reduction in the MVC/FTC arm at Week 24 (p=0.03). We found greater p24 suppression in FTC containing arms compared to others ($p < 0.05$). The frequency of greater than one $\log_{10}$ reductions from baseline was (Week 24 and 48 range): MVC only 0%, MVC/FTC 0-33%, MVC/TDF 0-25%, and TDF/FTC 38-44% (**Figure 3**). Concomitant use of progestin-containing birth control was associated with lower cervical explant p24 at baseline, median $\log_{10}$ (IQR) 1.76 (0.96, 2.29) pg/mL/mg, when compared to CGW without progestin use, 2.65 (1.97, 3.31) pg/mL/mg (p=0.009). There was no consistent relationship (seen at both Week 24 and 48) with hormonal contraceptive use and p24 results for either cervical or colorectal tissue.

In cervical tissue concentration-response modeling, the data best fit a 2-parameter sigmoid I_{max} model with mean (95% confidence interval) E_0 2.1 (1.8, 2.3) $\log_{10}$ p24 antigen (pg/mL/mg) and IC_{50} 1.20 (0.09, 2.31) pmol/mg. While the coefficient of variation for these estimates is acceptable (6% and 46%, respectively) and the parameter estimate statistically significant, the IC_{50} estimate should be viewed tentatively since only 7% to 40% of cervical tissue PK results were above the LLOQ and only 20% of those values were observed above the estimated IC_{50}.

For the 11 CGW with colorectal explant p24 results (**Figure 2**), biopsy weight-adjusted colorectal tissue explant p24 expression was not different at any visit (Week 24, 48, or 49) compared to Baseline. The MSM reductions at Week 24, 48, and 49, were 1.8, 1.5, and 0.7 $\log_{10}$, respectively (all p<0.001) (**Figure 2**). When compared to MSM, CGW p24 was higher

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at every visit, including pre-drug baseline: Baseline 0.31 $\log_{10}$ difference (p=0.046), Week 24 1.01 $\log_{10}$ difference (p=0.015), Week 48 1.19 $\log_{10}$ difference (p=0.016), and Week 49 0.61 $\log_{10}$ difference (p=0.011).

Combining all participant data, statistically significant p24 reductions relative to baseline were observed at all visits (p<0.001). For on drug periods (Weeks 24 and 48), there were differences among study drug regimens (both p<0.001), with reductions compared to baseline seen in FTC- and TDF-containing regimens at Week 24 and 48 (all p<0.001) and FTC-containing regimens at Week 49 (p<0.033). The frequency of reductions from baseline greater than one $\log_{10}$ on drug for each regimen was (range of Week 24 and 48 visits): MVC only 18-23%, MVC/FTC 73-80%, MVC/TDF 64-82%, and TDF/FTC 79-80% (**Figure 3**).

In colorectal concentration-response modeling, the data best fit a 3-parameter I_{max} model with mean (95% confidence interval) E_0 2.7 (2.5, 2.9) pg/mL/mg, IC_{50} 0.55 (0.22, 0.87) pmol/mg tissue, and I_{max} 2.2 (1.9, 2.4) pg/mL/mg. The coefficients of variation for the three parameters were 6%, 30%, and 4%, respectively. In contrast to cervical tissue explant modeling, 64% of colorectal tissue drug concentrations (including Week 49) were above the LLOQ and 81% of these were greater than the estimated IC_{50} (**Figure 3)**. Direct comparisons between cervical tissue and colorectal tissue p24 results should not be made due to methodological differences. (Note: the best colorectal and cervical model fits included uniform weighting, MVC dilution correction, and LLOQ/10 imputation for baseline drug concentrations. IC_{50} estimates were sensitive to imputation of drug values below LLOQ, so, these were excluded from modeling.)

Colorectal Tissue Cell Phenotype

When comparing flow cytometry results in CGW (11 participants with 39 observations) to MSM (54 participants with 214 observations) including all regimens at each study visit, the only difference at Baseline was 4% higher CD38+/CD8+ FCS (activated suppressor cells) in CGW compared to MSM (p=0.029). With only 2 or 3 CGW per regimen, we did not test regimen-specific differences between sexes. On study drug, the only consistent sex-based difference (same direction of statistically significant change) was CD69+/CD8+ FCS (tissue resident memory suppressor cells) at Week 24, 48, and 49 (all CGW to MSM ratios < 7%, all p<0.046). CD69+/CD8+ cells were also increased 3% at Baseline in women using progestins for contraception (p=0.03).

Pooling colorectal tissue results (both sexes), differences among study weeks were observed only for CD3+ FCI (all regimens and MVC-containing regimens, p<0.01), CCR5+/CD8+ FCS (MVC-containing regimens, p=0.001), CCR5+/CXCR4+/CD8+ FCS (all regimens and MVC-containing regimens, p<0.01), and CD69+/CD4+ FCS (all regimens and MVC-containing regimens p<0.001). In general, CD3+, CCR5+/CD8+, and CCR5+/CXCR4+/CD8+ (all FCS) rose at one or more of weeks 24, 48, and 49 compared to baseline in at least one drug regimen. Compared to earlier visits, CD69+/CD4+ FCS fell at Week 49, though only in MVC containing regimens. Nearly all the individual participant

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changes in these few surface markers were between a 2-fold increase or 50% decrease (**Figure 4)**.

Discussion

This tissue substudy substantially extends PK, ex vivo HIV infectivity, and tissue flow cytometry observations in CGW on oral antiretroviral candidate PrEP regimens, enabling direct comparison to HIV seronegative MSM in the same study. Our key finding is increased HIV infectivity of colorectal tissue biopsies of CGW compared to MSM following ex vivo HIV challenge. CGW values were 2-fold higher at baseline (0.31 $\log_{10}$) and increased on antiretrovirals. Four earlier PrEP studies including both sexes were too small to compare HIV infectivity differences; we were only able to make comparisons by including more women and pooling all regimens [23-26]. The impact and mechanism of this sex difference remains to be understood but underscores the need for critical inclusion of populations at risk of HIV very early in PrEP development.

The baseline HIV infectivity difference cannot be explained by any immunologic measure we assessed as the only consistent CGW vs. MSM difference was a very modest seven percent higher CD69/CD8 FCS, a difference also seen in MTN-007 [27]. Our immunological findings are limited, however, without cytokines or immunohistochemistry to provide absolute cell subset numbers and anatomic co-localization of cell subsets [28].

HIV infectivity differences cannot be fully explained by pharmacologic differences because (1) p24 differences were seen before study drug dosing, (2) higher FTC concentrations in MSM colorectal tissue are too small to explain the difference given the PK/PD modeling, and (3) higher colorectal tissue TFV-DP would be expected to confer reduced HIV infectivity in CGW, making this finding incongruous with the HIV infectivity observation. The CGW colorectal TFV-DP concentrations, however, fall within the previously reported range, 206 and 1329 fmol/mg, for daily oral TDF dosing; this leaves MSM colorectal tissue TFV-DP results as the anomaly, more consistent with single dose results[20, 24, 29, 30].

We suggest hormonal differences as the source of the HIV infectivity differences, but only as an explanation by exclusion. Exogenous progestin in our study, however, was associated with reduced, not increased HIV infectivity at baseline. Two other clinical studies designed to compare the impact of a specific progestin, depot medroxyprogesterone acetate (DMPA), reported either no difference or an increase in active drug concentrations in cervical tissue in the presence of DMPA and no impact on cervical tissue HIV infectivity. However, neither study assessed the DMPA impact on colorectal tissue pharmacology or HIV infectivity [31, 32]. We had too few CGW using DMPA to assess this.

CGW have a four-fold greater risk of HIV acquisition through unprotected receptive anal intercourse (URAI) compared to MSM in one meta-analysis, though this should be viewed tentatively as it is derived from one retrospective observational study in heterosexual couples compared to three prospective studies in MSM [33-37]. No prospective clinical studies

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directly compare URAI risk in CGW and MSM, which would provide the strongest evidence comparing URAI HIV transmission risk in CGW and MSM to corroborate the meta-analysis and our ex vivo HIV infectivity findings. Obtaining such definitive evidence is doubtful given few seroconversion endpoint studies including both CGW and MSM/TGW, challenges capturing reliable CGW anal sex data, and confounding of much higher frequency of receptive vaginal sex compared to anal sex in CGW.

Some have argued the need for higher antiretroviral drug concentrations for oral TDF/FTC PrEP in CGW compared to MSM to achieve the same level of protection as in MSM [38, 39]. These studies attributed HIV acquisition differences to TDF/FTC PK differences in cervicovaginal compared to colorectal tissue - abundantly evident in our present study - since systemic active drug concentrations (PBMC TFV-DP and FTC-TP) do not differ between CGW and MSM [29, 40]. Our findings suggest there may also be physiologic, possibly hormonal differences in colorectal HIV infectivity that may be relevant in TGW on gender-affirming hormonal therapy (GAHT). Reductions in TFV and FTC analytes have been reported inconsistently in TGW on GAHT [29, 40-42].

Our finding of no HIV infectivity suppression in cervical or colorectal tissue explants for the MVC only arm concurs with several studies of MVC by both oral, rectal, and vaginal dosing routes, using a variety of methods [19, 23, 43, 44]. This finding may also further explain findings from our main study results where 5 of 406 MSM acquired HIV; 4 of these 5 seroconverters were randomized to the maraviroc only arm [16]. In several reports, including ours, combination of MVC with either TDF or FTC (oral) or with dapivirine (vaginal ring) reported significant HIV suppression indicating the assay performed as expected to indicate HIV suppression in comparison to MVC alone, suggesting consistency in findings [19, 45]. However, several groups have reported that MVC is not fairly tested by explant HIV challenge due to substantial loss of MVC during incubation in culture media. This loss is diminished with TFV-DP and FTC-TP since they are trapped within cells [23, 44, 46]. We are not aware of similar testing of DPV in vitro, but it is highly lipophilic and probably more resistant to loss during the HIV incubation step. Taking account of this loss of MVC from tissue, we improved our concentration-response model fitting. These MVC findings highlight the critical importance of understanding the impact of analytical conditions on both physiology and pharmacology before application in clinical studies.

Even excluding the MVC only arm, the three combination drug regimens also failed to consistently suppress HIV replication in our cervical explants. Again, interesting to consider this in light of the main study finding where none of 188 women study participants acquired HIV [15]. Others also reported no HIV suppression with a single dose MVC/TDF oral dose using similar HIV challenge methods [43]. Significant HIV suppression has been reported with a single MVC/TDF oral dose, though using double the MVC/TDF dose, a higher HIV challenge titer, and RNA, not p24, as primary readout [45]. Extending the comparison to vaginal PrEP products, single agent formulations indicate substantial reductions of 1.1-1.5 $\log_{10}$ (TFV film and gel) to 1.0-1.7 $\log_{10}$ reduction (DPV film and gel)[19, 47-50].

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The magnitude of p24 suppression in the combination arms, ranging from 0.56 to 0.86 $\log_{10}$ reduction, was consistent with a 0.68 $\log_{10}$ reduction after 8 weeks of daily oral TDF/FTC dosing in a study entirely of MSM, MTN-017, which used the same PK and PD methods. The only other oral study of TDF or FTC with colorectal explant testing in women, RMP-02/MTN-006, did not show any p24 reduction 30 minutes after a single TDF/FTC dose using the same PK and PD methods [24]. As with the cervicovaginal results, rectal TFV dosing exceeds the p24 suppression in colorectal tissue compared to oral TDF/FTC dosing; examples include a 1.06 $\log_{10}$ p24 reduction after a week's daily dosing of a near iso-osmolar TFV gel optimized for rectal use and a single dose of a TFV douche, 1.9 $\log_{10}$ [26, 52].

Our greatest limitation is the small number of CGW on each regimen with colorectal biopsies, preventing regimen-specific CGW-MSM comparisons, especially for PK endpoints. Still, we report the largest HIV prevention study of CGW colorectal explant p24 analysis. The inability to assess MVC's antiviral effect in the explant challenge model further limited assessment of HIV infectivity to only three arms. The practical limitation of very few cervical biopsies captured when compared to colorectal biopsies reduces pharmacologic assay sensitivity in cervical tissue, thus, making it difficult to quantitatively understand how much lower drug concentrations were in cervical tissue compared to colorectal tissue. Assays that are more sensitive are now available, which could add precision to the colorectal-cervical PK comparisons.

In summary, we identified significantly higher HIV infectivity of colorectal tissue in CGW when compared to MSM, seen before, during, and after study drug dosing, consistent with at least one meta-analysis of clinical studies. At best, these CGW vs. MSM differences are only partly attributable to immunologic and pharmacologic measures we assessed. More work is needed to understand the mechanism of this difference and to understand any impact on PrEP dosing recommendations for CGW who have URAI. Our results also reinforce the need for earlier comparative studies of HIV risk and PrEP interventions in all people at risk of HIV acquisition.

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Figure Legends

Figure 1. Study enrollment and outcome availability summary for the parent HPTN 069/ACTG A5305 study and the mucosal tissue substudy that enrolled 91 participants from within the larger parent study. Pharmacokinetic samples were contributed by all substudy participants. Cervical biopsies were contributed for PK and PD (explant) analysis by all of the CGW, but only some provided rectal tissue biopsies. Flow cytometry was performed only on rectal tissue. Abbreviations: MSM, men who have sex with men; TGW, transgender women; CGW, cisgender women; MVC, maraviroc; FTC, emtricitabine; TDF, tenofovir disoproxil fumarate. MSM may be truncated to MSM for space.

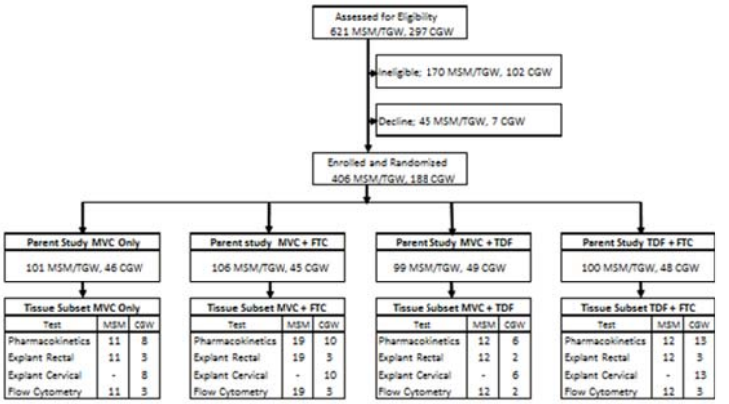

Figure 2. Explant p24 antigen concentration (cumulative biopsy weight-adjusted) change from baseline by tissue type, gender group, and antiviral drug regimen (median and interquartile range). Values are difference between active (on drug or post-drug) phase (week 24, 48, or 49) $\log_{10}$ p24 minus baseline (pre-drug) $\log_{10}$ p24 (equivalent to $\log_{10}$ of active phase divided by baseline p24). Zero value reference line indicates no change compared to baseline. Key: black diamond, all genders and all regimens; brown diamond, CGW all regimens; blue (open) diamond, MSM all regimens. Regimen specific groups (CGW for cervical tissue and all genders for rectal tissue) are blue circle MVC only, green up triangle MVC + FTC (M/F), cyan down triangle MVC + TDF (M/T), and red diamond TDF + FTC (T/F). Triplets in each coded grouping are (left to right) week 24, week 48, and week 49 relative to baseline. Asterisks indicate p value < 0.05 for Wilcoxon signed rank test comparing baseline to each of three active periods (week 24, 48, and 49).

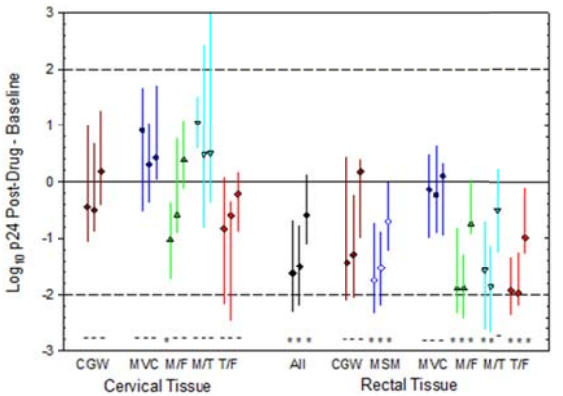

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Figure 3. Antiretroviral concentration vs. p24 antigen response plots of 120 cervical tissue (left) and 243 rectal tissue (right) homogenates. Y-axis is $\log_{10}$ cumulative p24 antigen with biopsy weight-adjustment. X-axis is sum of molar tissue concentration (pmol/mg) for the one or two antiretroviral drugs in the assigned regimen. Closed symbols represent cisgender women participants and open symbols are men who have sex with men and transgender women participants. Colors and symbol indicate the four drug regimens (blue circle, MVC only; green up triangle, MVC/FTC; green down triangle, MVC/TDF; red diamond, TDF/FTC). Drug concentrations below the limit of assay quantitation (BLQ) and at baseline (BL) are imputed as LLOQ/2 and LLOQ/10, respectively, of the median drug LLOQ; these are offset to avoid overlap. Gender group median $\log_{10}$ p24 concentrations associated with BLQ and BL are indicated with horizontal bars (dark yellow CGW, blue MSM). Pharmacodynamic I_{max} model fit predicted values are shown (black line).

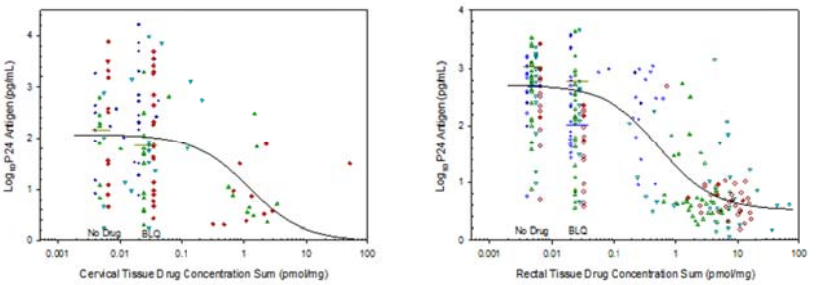
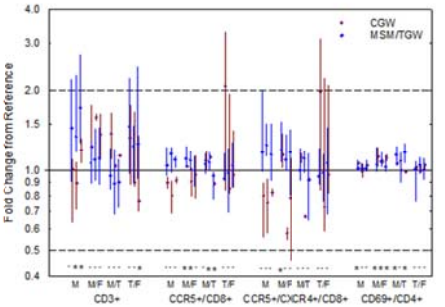


Figure 4. Colorectal tissue flow cytometry changes over time relative to a reference visit (baseline or post-drug) expressed as a ratio on the y-axis are displayed by several layers of groupings along the x-axis, starting with surface marker subset, study arm, study week (numerator of ratio), and gender (MSM beside CGW). The four flow cytometry markers displayed all had visit-to-visit temporal changes among regimens (Friedman test $p<0.05$). Within each marker subsets, each study arm is indicated as M (MVC only), M/F (MVC and FTC), M/T (MVC and TDF), and T/F (TDF and FTC). Within each regimen grouping, temporal changes are indicated by the ratio of marker expression percent at a given visit divided by a temporal reference value, either Baseline or Week 49 depending on which ratio was statistically significant. For CD3+ FCI, CCR5+/CD8+ FCS, and CCR5+/CXCR4+/CD8+ FCS, the ratios (left to right) are Week 24, Week 48, and Week 49 divided by pre-drug baseline. For CD69/CD4 FCS, the ratios (left to right) are Baseline, Week 24, and Week 48 divided by Week 49. Symbols are medians and error bars are either interquartile range for the larger MSM cohort (blue diamond and error bars) or range for the smaller 2-3 person CGW within a regimen (dark red diamond). Asterisks along the x-axis indicate paired Wilcoxon rank sum test $p<0.05$ for each of 3 observation times compared to the reference visit for pooled CGW, MSM, and TGW genders; dashes indicate $p>0.05$.



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