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The Pharmacokinetics, Pharmacodynamics, and Mucosal Responses to Maraviroc-Containing PrEP Regimens in Men who have Sex with Men

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Abstract

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Objective: HPTN 069/ACTG A5305 was a study of 48-week oral PrEP regimens in MSM and transgender women A rectal substudy was included to evaluate drug concentrations in rectal compartment *vs.* blood, gut-associated lymphoid tissue (GALT) responses to four antiretroviral (ARV) PrEP regimens (maraviroc (MVC), MVC + emtricitabine (FTC), MVC + tenofovir disoproxil fumarate (TDF), and TDF + FTC), and to determine whether ARV exposure was associated with *ex vivo* suppression of HIV infection in colorectal explants.

Methods: CCR5 genotype was characterized using PCR. At baseline and at weeks 24, 48, and 49, GALT phenotype was characterized by flow cytometry, rectal biopsies were challenged with HIV-1_{BaL}, and tissue and plasma pharmacokinetics were measured via mass spectrometry.

Results: Exposure to MVC was not associated with increased expression of CD4+/CCR5+ HIV target T-cells. Significant *ex vivo* viral suppression compared to baseline was seen at Weeks 24 and 48, ranging from 1.4 to 1.8 $\log_{10}$ for all study regimens except the MVC-alone arm which did not show statistically significant viral suppression at Week 48. Tissue concentrations of tenofovir (TFV), TFV-diphosphate (DP), and FTC were correlated with viral suppression.

Conclusions: MVC-containing HIV PrEP regimens did not increase GALT CD4+ T-cell activation or the CD4+/CCR5+ phenotype. No virologic suppression was seen with MVC-alone at Week 48 compared to combination regimens, suggesting MVC monotherapy might be less effective than combination ARV PrEP regimens.

Keywords

HIV; PrEP; men who have sex with men (MSM); MVC; tenofovir disoproxil fumarate; FTC; phase 2 clinical trial; pharmacokinetic; pharmacodynamic; explant challenge; gut-associated lymphoid tissue

Introduction

Several studies have demonstrated the safety and effectiveness of tenofovir disoproxil fumarate (TDF) / emtricitabine (FTC) in the prevention of HIV infection [1–5]. However, use of TDF is associated with modest reductions in renal function and bone density [6–8] and may be contraindicated in some individuals. Maraviroc (MVC) is approved for the treatment of chronic HIV infection, but is only active against CCR5-tropic virus and its use requires prior characterization of the patientś viral tropism [9]. MVC has an excellent safety profile [10]. Given its limited use in the treatment of HIV infection MVC may be a valuable drug for the prevention of HIV infection [11].

MVC has been evaluated in the non-human primate (NHP) model of HIV infection. Interestingly, MVC was not associated with prevention of infection when given orally despite high concentrations of MVC in the rectal tissue [12] but was effective when given topically [13]. It is not clear whether this was due to differences between NHP and human CCR5 receptor biology (because the receptor off-rate of MVC in monkeys is 10 times faster) or species differences in pharmacokinetics (PK) [14]. A number of treatment studies where MVC has been used to intensify ARV regimens have failed to demonstrate significant changes in T-cell activation phenotype or the proportion of CCR5 positive T-cells in blood [15–17]. One notable exception is a paper by Hunt el al. which reported that MVC

intensification was associated with increases in T-cell activation in blood and gut derived T-cells [18]. A PrEP drug that induces these immunological changes in genital or rectal mucosal tissue might paradoxically increase rather than decrease the risk of HIV infection by upregulating cell surface markers that facilitate infection.

Tissue based PK / pharmacodynamic (PD) assays are increasingly being used to characterize the efficacy of ARV PrEP agents. The candidate ARV agent is added to tissue samples *in vitro* which are then challenged with virus to determine whether the PrEP agent has ARV activity [19, 20]. A modification of this assay is to deliver the drug *in vivo* and to then acquire colorectal tissue that is subsequently exposed to virus *ex vivo* [21]. Using an *in vitro* colorectal explant model, Fletcher, et al., demonstrated only modest and poorly sustained viral suppression for MVC compared to studies of other drugs [22]. Two single-dose studies were also unable to demonstrate MVC efficacy using an *ex vivo* colorectal explant challenge assay, although it has been postulated that this effect might be due to diffusive loss of drug from the explant tissue during the viral incubation period, and to a lesser extent faster receptor dissociation rate, or other technical factors [23, 24].

A tissue substudy was included in the HPTN 069/ACTG 5305 study to further evaluate the immunological and virological effects of MVC within the more vulnerable and activated colorectal tissue compartment in HIV negative individuals.

Methods

Study Design and Participants:

The study was conducted in the United States, sponsored by the Division of AIDS (DAIDS) of the U.S. National Institutes of Health (NIH) through the HIV Prevention Trials Network (HPTN) and co-sponsored by the AIDS Clinical Trials Group (ACTG).

The HPTN 069/ACTG 5305 study was a prospective, randomized, double-blinded multicenter clinical trial that evaluated the safety and tolerability of four ARV regimens used as PrEP in at-risk, HIV-uninfected men who have sex with men (MSM), cis, and transgender women [11, 25]. Participants were randomized to receive MVC-alone, MVC+FTC, MVC +TDF, or TDF+FTC (control) for a period of 48 weeks. The following doses were administered; MVC (300 mg), FTC (200mg), and TDF (300 mg). The study was blinded and so all participants received three tablets once daily.

A tissue substudy was nested within the parent study to evaluate compartmental PK/PD and changes in GALT T-cell phenotype associated with the four ARV regimens. Participants enrolled in the tissue substudy had samples collected at baseline, at two time points while on study (Weeks 24 and 48), and after a one-week washout period (Week 49), to characterize the compartmental pharmacological, immunological, and virological consequences of exposure to MVC-alone compared to the other three combination study arms.

Eligible participants were born male, at least 18 years old, and self-reported condomless anal intercourse with at least one man known to be HIV-infected, or of unknown HIV serostatus within 90 days prior to study entry. Eligible participants met baseline laboratory safety

criteria including a calculated creatinine clearance ≥70 mL/min (Cockcroft-Gault) within 45 days of study entry, a non-reactive HIV-1 antibody test, and a plasma HIV-1 RNA below detection within 14 days of study entry. Participants were excluded if they used any ARV drug within 90 days prior to screening (e.g., for PrEP or post-exposure prophylaxis [PEP]), reported recent injection drug use, or had a reactive hepatitis B surface antigen. The study was reviewed and approved by the institutional review boards at each of the participating sites; all participants provided written informed consent.

Procedures:

In the parent study, drugs were discontinued at Week 48 and a final visit was conducted at Week 49. At each study visit, interval history, targeted physical examination, safety laboratories, adherence assessments, risk-reduction counseling, condom distribution, and HIV testing were conducted, and study drugs were dispensed. In addition, rectal fluid and blood samples were collected and stored for drug concentration measurements. Rectal fluid was collected using cellulose sponges (ULTRACELL®, Aspen Surgical Caledonia, Michigan). Flexible sigmoidoscopy was performed at Baseline and at Weeks 24, 48, and 49 with collection of 20 rectal biopsies acquired at approximately 15 cm from the anal verge. Biopsies (8 mm x 2 mm x 1 mm from large-cup, endoscopic biopsy forceps; Microvasive Radial Jaw #1589, outside diameter 3.3 mm, Boston Scientific, Marlborough, MA) were collected and immediately placed into 15 mL of tissue culture medium (RPMI 1640, Irvine Scientific, Santa Ana, CA). Tissue samples were transported to the local laboratories in Pittsburgh, Los Angeles, and Baltimore. The PK tissue samples were weighed and frozen and sent in batches to Baltimore for PK analysis. The explant challenge experiments were set up immediately locally as described below. The tissue samples for flow cytometry were sent overnight to Pittsburgh for cell isolation and flow analysis as previously described [26].

CCR5 genotyping

CCR5 genotyping was performed on peripheral blood mononuclear cell (PBMC) derived DNA samples using a PCR-based technique as previously described [27].

Flow cytometry

Mucosal mononuclear cells (MMC) were isolated from rectal biopsies using a combination of mechanical and enzyme digestion [26]. Flow cytometric analysis was performed on a BD™ LSRFortessa cytometer (BD Biosciences, San Jose, CA). All antibodies were purchased from BD Biosciences, San Jose, CA (PerCP-CD45, Clone 2D1; Pacific Blue-CD3, Clone UCHT1; Alexa Fluor 700-CD4, Clone RPA-T4; APC-H7-CD8, Clone SK1; PE-Cy7-CD69, Clone FN50; FITC-HLA-DR, Clone L243; PE-CF594-CD38, Clone HIT2; FITC-Ki-67; APC-CD184 (CXCR4), Clone 12G5; and PE-CD195 (CCR5), Clone 2D7). Cells were stained with LIVE/DEAD® Fixable Aqua stain fluorescence (Life Technologies, Eugene, OR) to define viable cells.

Pharmacodynamic assays

Rectal biopsies were collected and transported to the laboratory for *ex vivo* infection within 30 minutes using a common viral stock of HIV-1_{BaL} (10^5 $TCID_{50}$) as previously described

[21]. HIV-1_{BaL} is a CCR5 tropic virus that has been used extensively in the explant challenge model [28]. Results were adjusted for initial biopsy weight and reported as 14-day cumulative HIV-1 p24 antigen (p24 HIV antigen ELISA; Alliance; Perkin-Elmer Life Sciences Inc., Boston, MA) where the assay's lower limit of quantification (LLOQ) was 10 pg/mL. Non-detectable cumulative p24 measures were converted to LLOQ/2 prior to log transformation. Concentration-response plots used median values of 4 replicate biopsies for each time point. Plotting of Baseline (no drug) concentrations were imputed by back extrapolation of linear regression slope based only on the on-drug values so that the slope was not influenced by the imputed values, but enabled visualizing the relative value in the same plots.

Pharmacokinetic assays

MVC, FTC, TFV, and TFV-DP were quantified via validated liquid-chromatographic-tandem mass spectrometric (LC-MS/MS) methods in plasma, PBMCs, rectal fluid, and rectal tissue [29–31]. Assay LLOQs were as follows: plasma MVC: 0.5 ng/mL, FTC and TFV: 0.31 ng/mL; PBMC FTC-TP: 0.1 fmol/sample; TFV-DP: 2.5 fmol/sample; rectal fluid (collected on the Merocel sponge) MVC: 0.5 ng/sponge, FTC: 5 ng/sponge, TFV: 1.25 ng/sponge; tissue MVC: 0.20 ng/sample, FTC: 0.25 ng/sample, TFV: 0.05 ng/sample, TFV-DP: 50 fmol/sample. Results for PBMCs were normalized to number of PBMCs analyzed and reported as fmol/10^6 cells (study LLOQs normalized to cell counts ranged from ~ 1.25 to 5 fmol/10^6 cells); results for rectal fluid and tissue samples were normalized to net sponge or tissue weights and reported as drug concentration per mg of sponge or tissue, respectively.

Statistical analysis

Drug concentration data for each drug and biologic matrix were summarized using non-parametric descriptive statistics. Values below the LLOQ were imputed as LLOQ/2. Comparisons among regimens were compared first with the Kruskall-Wallis test followed by pairwise comparison of statistically significant results with Wilcoxon rank sum testing which was also used to test differences between study visits (Week 24, 48, and 49). Adherence categories were based on Week 24 and 48 drug concentrations of plasma TFV and PBMC TFV-DP indexed adherence benchmarks from a directly observed dosing PK study (HPTN 066) [31].

Correlations between drug concentration results and pharmacodynamic results (p24 antigen) were evaluated using Spearman correlation test. Concentration-response relationships between drug concentrations (PK) and median cumulative 14-day p24 antigen (PD) were evaluated first with linear regression by pooling all on-study drug observations (all participants and visits with both PK and PD data) in the analysis. The effect of each drug or regimen on viral replication was assessed using generalized estimating equations (GEE; SAS version 9.4, Cary NC) using cumulative p24 antigen for each biopsy as the dependent variable with independent variables including drug concentration or assigned regimen, and study visit. In addition, several E_{max} models of drug concentration and p24 antigen (modeling E_{max} and EC_{50} with and without E_0 and/or Hill coefficient) were explored to describe concentration-response relationship.

Results

Study population

406 HIV-uninfected men and transgender women were enrolled in the parent study [11]. A subset of 59 MSM were enrolled into the tissue sub study (Figure 1) which was conducted at three study sites (the University of Pittsburgh, Pittsburgh, PA, USA (n=20), the University of California Los Angeles, Los Angeles, CA, USA (n=20), and Johns Hopkins University, Baltimore, MD, USA (n=19). Participant retention was good and 213/236 (88.8%) of scheduled endoscopies were performed.

Of the 59 men enrolled in the tissue substudy, 55 (93.2%) participants had the CCR5 wild type genotype and four (6.8%) were heterozygous (2 subjects randomized to the MVC + FTC arm; two to the TDF + FTC arm). There was no evidence of reduced explant viral replication from any of the heterozygous participants at Baseline (p=NS). Cumulative explant supernatant HIV-1 p24 following 14 days of incubation was 889 ± 1179 pg/mL) for heterozygotes compared to wild type participants (695 ± 607 pg/mL).

Mucosal cell phenotype

When the MVC-alone arm was compared to the TDF+FTC control arm, there were significant reductions in the prevalence of the following phenotypes; CD8+/CCR5+ (Week 24: −11.1%, p=0.01) and Ki67+ (Week 24: −4.0%, p=0.04). When the pooled MVC-containing arms (MVC-alone, MVC+FTC, and MVC+TDF) were compared to the TDF +FTC arm, there were significant reductions in the following phenotypes; CD8+/CCR5+ (Week 24: −12.1%, p=0.002 & Week 48: −20.4%, p=0.03), CD8+/CXCR4+/CCR5+ (Week 24: −10.0%, p=0.02 & Week 48: −13%, p=0.04) and CD8+/Ki67+ (Week 49: −3.42%, p=0.01). Other significant changes in cell phenotype within and across study arms are summarized in Table 1. There were no significant changes in the CD4+/CCR5+ T cell subset.

Pharmacodynamics

The MVC-alone arm showed median 0.6 $\log_{10}$ pg/mL cumulative p24 antigen reduction at Week 24 (p=0.03) but no change from Baseline at Week 48 (p=0.48). In contrast, there was significant viral suppression (reduced median cumulative 14-day p24 antigen) in all 3 combination ARV arms' colorectal explants at both Weeks 24 and 48 compared to each patient's baseline results (all p<0.02) with median decrease of 1.6 (MVC+FTC), 1.8 (MVC +TDF), and 1.4 (TDF+FTC) $\log_{10}$ pg/mL (Figure 2). All three combination arms remained significantly suppressed at Week 49 (after 1 week of washout) compared to Baseline although the level of suppression was significantly less than at Week 48.

Pharmacokinetics

For all drug analytes and matrices, 13% or fewer samples were below the limit of quantification (BLQ) at Week 24 and 48. One week after all dosing stopped (Week 49) BLQ percentages varied widely ranging from 6–32% for plasma FTC and TFV and PBMC TFV-DP and FTC-TP, but greater than 50% for all others (meaning BLQ median values): MVC plasma and rectal fluid (RF) from 59–69%, and all tissue analytes 77–97%. There were high

levels of adherence to the daily dosing estimated as greater than 95% of participants taking 4 or more doses in the week prior to the Week 24 and 48 study visits using plasma TFV (4.2 ng/mL), plasma FTC (4.6 ng/mL), or PBMC TFV-DP (9.9 fmol/10^6 cells) benchmarks; using FTC-TP this estimate was 67–71% [31]. Adherence in the MVC only arm cannot be quantified as benchmarks have not been established.

Pharmacokinetic data, expressed as median (Interquartile range), across all matrices are summarized in Table 2. In general, there were no consistent differences in any given drug concentration across arms containing the drug. The few exceptions to this, noted in Table 2 footnotes, were inconsistent between parent and phosphorylated metabolite or inconsistent between Week 24 and 48 visits. Similarly, there were no changes over time from Weeks 24 to 48 in the combination arms; by contrast, in the MVC-alone arm, median plasma MVC dropped 2-fold from Weeks 24 to 48 ($p=0.016$). Median MVC tissue and rectal fluid concentrations fell by larger proportions (5-fold and 8-fold, respectively), but the changes in these matrices were not statistically significant and had numerous BLQ values. All Week 49 values (drugs and matrices) were below those of Week 48; notably, median tissue concentrations for all ARVs are BLQ (data not shown). For each of the three drugs, correlations were high between parent and phosphorylated metabolite in blood plasma, PBMC, and tissue (all $R>0.84$, all $p<0.001$).

Pharmacokinetics and pharmacodynamic relationship

The relationship between tissue drug concentration and explant infection is displayed graphically in Figure 3, characterized by 14-day cumulative HIV-1 p24 from each subject at each biopsy time point. When each drug analyte concentration (MVC, FTC, TFV, and TFV-DP) in each matrix (plasma, PBMC, and tissue) are pooled from each of the 2 or 3 study arms in which it was used, each analyte was modestly and negatively correlated with cumulative 14-day p24 antigen concentrations (all $R<-0.4$, all $p<0.001$); the highest correlations were between tissue drug concentrations and p24 antigen (all $R<-0.69$, all $p<0.001$). Using tissue concentrations for each analyte tested (MVC (Figure 3A), FTC (Figure 3B) TFV (Figure 3C), and TFV-DP (Figure 3D)), regardless of the presence of another drug in the assigned regimen, linear regression indicated that each analyte is associated with a significant decrease in p24 antigen (with R^2 ranging from 0.39 (MVC) to 0.64 (FTC) (Figure 3A–3D). However, if MVC tissue concentrations from only the MVC-alone arm are examined, the linear regression is not statistically significant (Figure 3A.).

In the GEE analysis where the relative impact of all three drug's tissue concentrations were assessed simultaneously, each of the parent drugs demonstrated a statistically significant suppression of cumulative p24 (negative values) compared to baseline (Table 3; all 3 $p\leq0.05$). For the multivariate comparison of drug regimens (Table 3), compared to the TDF + FTC reference regimen, the MVC-alone arm demonstrated a greater cumulative p24 value or a much smaller p24 reduction ($p=0.0001$); the MVC combination regimens also trended toward statistical significance with cumulative p24 values intermediate between the TDF + FTC reference and the MVC-only arms. (both $p=0.08$). There were no differences between on drug visits (Weeks 24 and 48). We were not able to fit any drug concentration data to any E_{max} model.

Discussion

The HPTN 069 / ACTG 5305 tissue substudy evaluated the effects of four candidate PrEP regimens on GALT immunology, determined whether PrEP drug exposure was associated with inhibition of viral replication in the *ex vivo* explant challenge model, and characterized the compartmental PK of the four PrEP regimens. The key findings from the study were that there was no evidence of increased activation or CCR5 expression on CD4+ T-cells at Weeks 24 or 48 in any of the PrEP regimens, and that exposure to MVC-alone was associated with little or no explant viral suppression unlike the combination regimens.

A total of 59 participants were enrolled in the HPTN 069 / ACTG 5305 tissue sub study. Participation in the tissue substudy required completion of four flexible sigmoidoscopies and 213/236 (88.8%) of scheduled endoscopies were performed. This high level of procedural completion is in keeping with a recent review by Chiu et al. that demonstrated that repeated flexible sigmoidoscopic collection of intestinal tissue in Phase 1 PK/PD studies is safe and well tolerated by study participants [32].

The majority of the tissue sub study participants had the wild type CCR5 genotype although four participants were heterozygous for the CCR5 Δ32 mutation, which has been associated with slower disease progression and lower viral load [33]. However, in the explant infection model used here, CCR5 Δ32 heterozygosity was not associated with greater suppression of CCR5-tropic HIV-1 viral replication.

MVC has been investigated to intensify ART regimens in HIV-1-infected patients with suboptimal CD4+ recovery. In the majority of these studies, exposure to MVC decreased peripheral T-cell activation [16, 34–36]. In contrast, a placebo-controlled study by Hunt et al. demonstrated an increase in rectal CD4+/CD38+/HLA-DR+ and CD4+/CD38+/HLA-DR+ T-cells [18]. A variety of explanations have been used to explain the divergent results from these studies, the majority of which were not placebo controlled. It has been postulated that the decreased T-cell activation might have resulted from improved antiretroviral adherence, as a consequence of study participation, and concomitant decreases in viral load [18]. Other explanations include population characteristics, and sample processing [16, 18]. It is also unclear whether similar changes in GALT T-cell activation would occur in HIV-uninfected study participants. It is reassuring that we found no evidence that MVC increased GALT CD4+ T-cell activation which might increase, rather than decrease the risk of HIV acquisition if MVC is used as a PrEP agent.

Adherence in this sub-study was very high for TDF and FTC containing regimens, estimated as greater than 95% of participants taking four or more doses per week based on established PK benchmarks; adherence was consistent between the Weeks 24 and 48 visits. The MVC plasma concentration dropped 2-fold between the Weeks 24 and 48 visits. This could be due to reduced adherence in the days prior to the week 48 visit, but we have no other objective measure to confirm this. Consistent with the falling concentration in plasma and tissue, there was an increase in explant infection in the MVC arm between Weeks 24 and 48. After a one-week washout period the proportion of tissue samples with analytes that were BLQ rose substantially with only 3–33% of samples quantifiable. This affected our ability to assess the

PK-PD relationship, because so many values had to be imputed, thus, reducing analytical precision. However, because most plasma and PBMC concentrations were still above the LLOQ at Week 49, it is reasonable to assume the p24 suppression seen at Week 49 (intermediate between baseline and Weeks 24/48) is due to residual TFV-DP and FTC-TP in tissue based on correlations between matrices and other studies indicating persistence of these analytes for one week after the final dose.

A key finding in this study was the observation that explant infection with HIV-1 was poorly suppressed, if at all, in the MVC-alone arm at Weeks 24 and 48 compared to the TDF+FTC (standard PrEP regimen) control arm; there were also similar trends (not statistically significant) in the other two regimens that contained MVC (MVC+TDF and MVC+FTC). The *ex vivo* explant challenge model has been used to characterize the effect of oral, topical, and injectable ART on HIV replication in rectal, cervical, and vaginal tissue [21, 37–39]. In the majority of studies, exposure to ARV agents was associated with inhibition of explant infection. However, in the MWRI-01 evaluating long acting (LA) rilpivirine (RPV) [21] viral inhibition was seen in colorectal tissue, but not in cervical or vaginal tissue. Subsequent experiments suggested that the cervicovaginal explant model requires higher drug concentrations than the colorectal model to inhibit infection and that PK exposure associated with LA-RPV was lower in cervicovaginal tissue compared to colorectal tissue [40]. A second study evaluating the PK/PD profile of a dapivirine (DPV, an investigational non-nucleoside reverse transcriptase inhibitor)/MVC combination intravaginal ring demonstrated efficacy for the DPV alone and the DPV/MVC combination ring but failed to demonstrate any efficacy for the MVC-alone ring in cervical tissue [38]. The authors postulated that the absence of any PD effect in cervical tissue was due to negligible drug exposure caused by MVC efflux through the P-glycoprotein MDR1 transporter system [41].

The lack of PD effect with MVC has been recapitulated in other studies. Using an *in vitro* system, optimal viral inhibition was only seen when colorectal tissue explants were continuously exposed to MVC [22]. Two clinical studies evaluating single-dose oral MVC also showed incomplete or absent colorectal explant protection from HIV infection [23, 24]. In the study by Coll, *et al.,* the authors demonstrated that explant tissue lost approximately 60% of MVC within one hour of culture [24]. It is, therefore, possible that the negative explant findings in the HPTN 069 / ACTG 5305 substudy reflect a technical challenge (such as loss of drug from explant tissue prior to infection), rather than an intrinsic failure of MVC ARV potency. This issue will be further investigated in the CHARM-03 study (NCT02346084), a Phase 1 PK/PD study where explant PK was characterized in snap-frozen explant tissue as well as in tissue that had been cultured for 2 hours.

During the course of the parent study, there were 5 HIV seroconversions, 4 of which occurred in participants randomized to the MVC-alone arm [11]. While this was not statistically significantly different among the 4 PrEP regimens studied, this substudy was not powered to detect any, but very large differences among study arms. Given the uncertainty about the utility of the explant model in assessing the ex vivo efficacy of MVC it would be inappropriate to correlate MVC explant efficacy with the HIV seroconversions that occurred in this study.

In summary, this study found that MVC exposure in HIV-negative individuals is not associated with increased T-cell activation that might limit its acceptability as a PrEP agent. Our explant data suggest that MVC-alone might be less effective than combination ARV PrEP regimens, but other studies indicate the explant challenge assay may not be suitable for characterizing the pharmacodynamic profile of MVC in rectal tissue.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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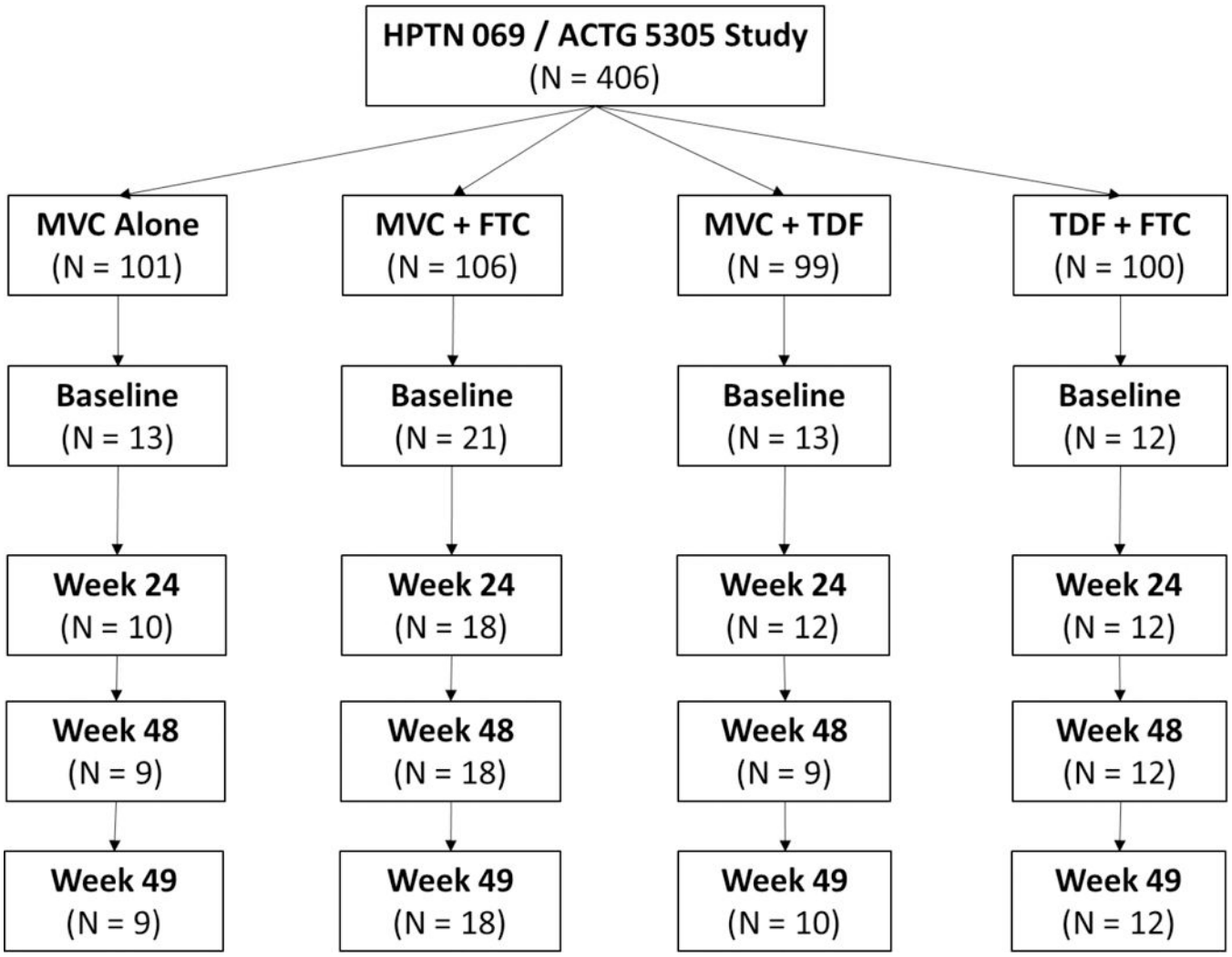

Figure 1.
Trial profile

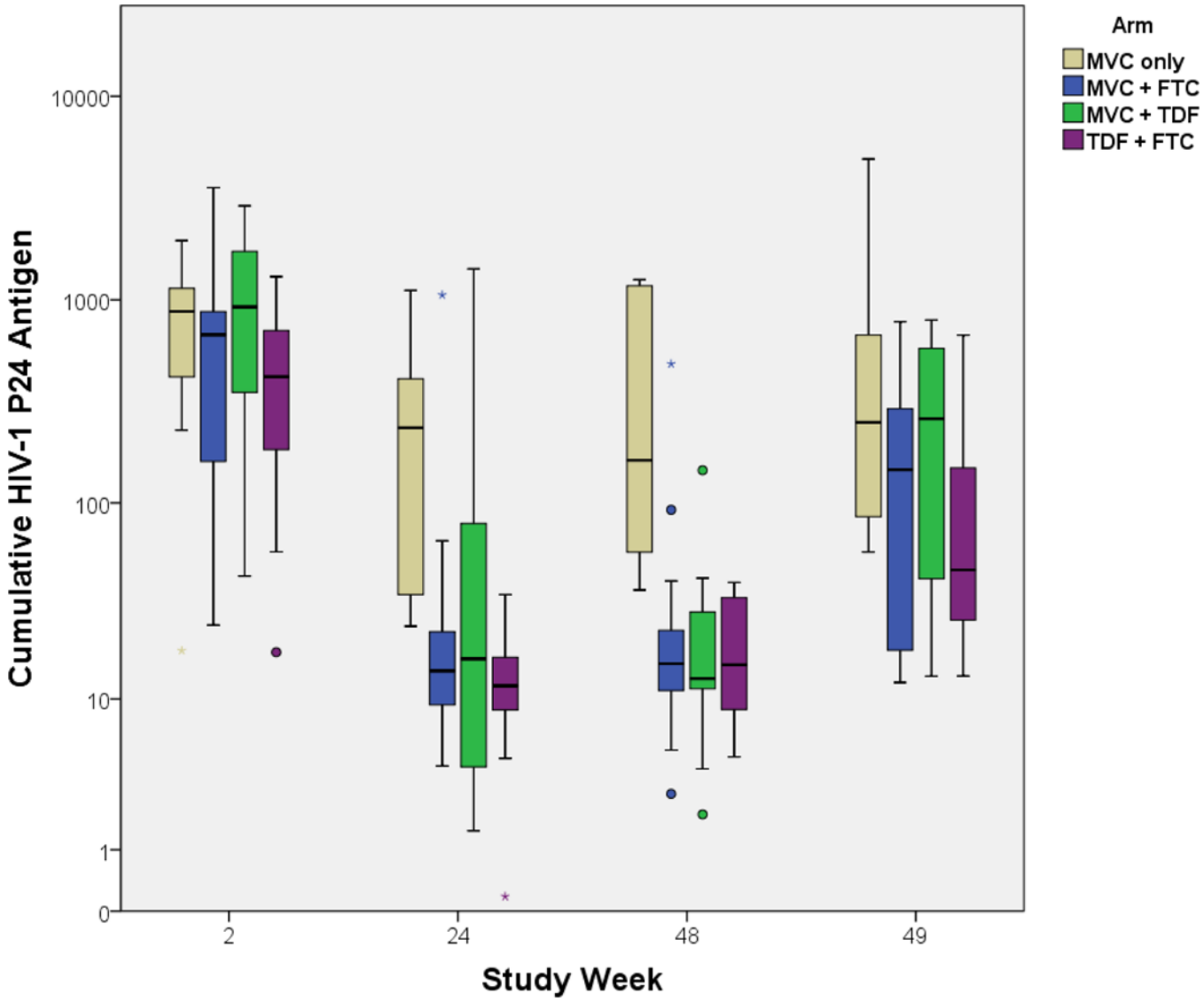

Figure 2.
Explant Infection

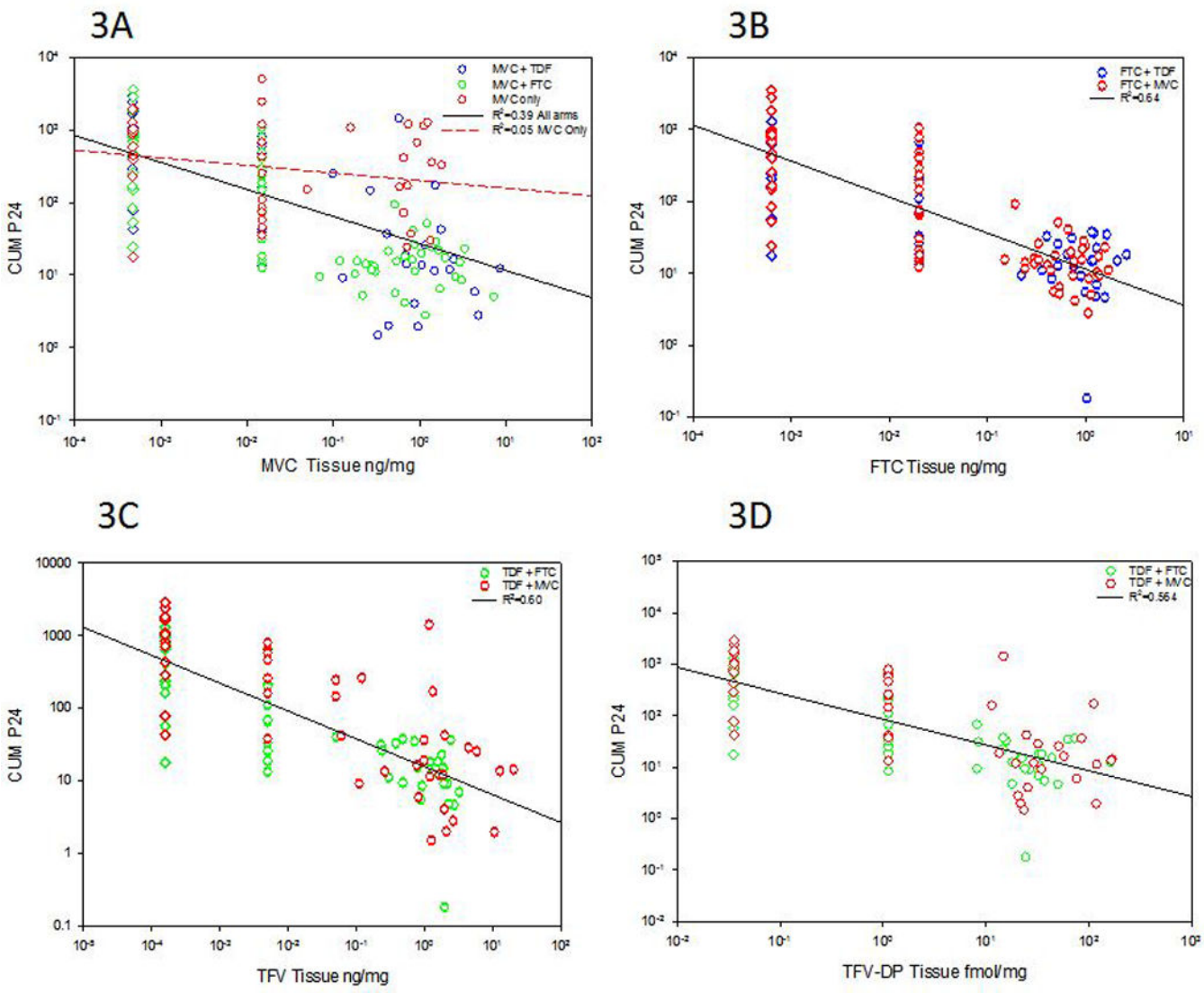

Figure 3.
Explant tissue PK/PD profile.

Table 1

Summary of significant changes in GALT T-cell phenotype

	MVC			MVC + FTC			MVC + TDF			TDF + FTC		
Comparison	**Marker**	**Change**	**P-Value**	**Marker**	**Change**	**P-Value**	**Marker**	**Change**	**P-Value**	**Marker**	**Change**	**P-Value**
W24 vs. BL	CD8/CXCR4/ CCR5	+13.5%	0.04	CD3/CD4	+6.9%	0.02	CD3/CD4	+10.0%	0.02			
				CD3/CD8	−13.6%	0.0001	CD3/CD8	−13.5%	0.04			
				CD8/CXCR4	+9.9%	0.01						
				CD8/CXCR4/CCR5	+12.6%	0.003						
W48 vs. BL	CD8/CXCR4/CCR5	+15.5%	0.01									
W48 vs. W24				CD8/CXCR4	−6.8%	0.01	CD4/Ki67	−3.4%	0.03			
				CD8/CXCR4/CCR5	−6.8%	0.01						
W49 vs. BL	CD3	+15.1%	0.03	CD8/CD69	−6.7%	0.03	CD8/CD69	−15.5%	0.02	CD8/Ki67	−3.3%	0.03
	CD8/CD38	+5.6%	0.002	CD4/CD69	−7.5%	0.03						
W49 vs. W24				CD4/CCR5	−7.3%	0.01	CD8/CD38	−3.0%	0.03	CD4/CD38	+5.4%	0.03
				CD4/CD69	−7.9%	0.001				CD4/CD38/HLA-DR	+11.8%	0.04
				CD8/CD69	−6.1%	0.04				CD8/Ki67	−2.5%	0.02
				CD8/CXCR4/CCR5	−10.7%	0.01						
				CD4/CXCR4/CCR5	−9.2%	0.01						
W49 vs. W48				CD4/CD69	−5.5%	0.01				CD4/CD38/HLA-DR	+10.2%	0.04
				CD8/CD69	−4.9%	0.01						

Table 2

Weeks 24 and 48 pharmacokinetic data for all analytes and matrices tested (Median and interquartile range)

		#Pooled		MVC		MVC + FTC		MVC + TDF		TDF + FTC	
Analyte	**Units**	**Week 24**	**Week 48**	**Week 24**	**Week 48**	**Week 24**	**Week 48**	**Week 24**	**Week 48**	**Week 24**	**Week 48**
Plasma MVC	ng/mL	15.1 (7.0, 19.3)	13.3 (4.6, 31.0)	*17.5 (9.3, 24.8)	12.9 (1.0, 19.0)	15.0 (6.8, 18.5)	13.0 (4.9, 31.9)	7.6 (4.1, 17.8)	25.3 (11.9, 73.7)		
Rectal Fluid MVC	ng/mL	4.7 (1.4, 14.7)	1.0 (0.4, 12.8)	3.5 (0.4, 20.7)	1.2 (BLQ, 1.8)	4.2 (0.7, 14.1)	1.2 (0.4, 11.4)	6.9 (3.0, 14.8)	5.2 (1.1, 22.5)		
Tissue MVC	ng/mg	0.7 (0.3, 1.4)	0.8 (0.2, 1.5)	0.8 (0.7, 1.4)	0.2 (BLQ, 0.8)	0.7 (0.3, 1.4)	0.7 (0.2, 1.6)	0.7 (0.4, 1.5)	1.5 (0.8, 2.3)		
Plasma TFV	ng/mL	75 (30, 126)	68 (39, 109)					49 (19, 82)	68 (49, 131)	113 (40, 135)	65 (37, 101)
PBMC TFV-DP	fmol/10^6 cells	60 (39, 82)	50 (34, 83)					47 (20, 67)	47 (30, 83)	**77 (56, 127)	50 (35, 95)
Tissue TFV	ng/mg	1.2 (0.8, 2.1)	1.7 (0.7, 2.1)					1.2 (0.9, 5.0)	1.9 1.1, 2.7)	1.5 (0.7, 1.9)	1.4 0.3, 2.1)
Tissue TFV-DP	fmol/mg	26 (17, 60)	27 (17, 42)					43 (16, 105)	27 (21, 72)	24 (17, 41)	25 (15, 37)
Plasma FTC	ng/mL	161 (52, 316)	190 (50, 368)			88 (43, 207)	134 (50, 346)			***307 (121, 699)	208 (66, 460)
PBMC FTC-DP	pmol/10^6 cells	5.6 (3.0, 8.4)	5.8 (3.3, 8.7)			5.2 (2.7, 7.8)	5.2 (3.5, 9.6)			6.9 (5.3, 12.2)	6.5 (2.4, 9.3)
Tissue FTC	ng/mg	0.5 (0.3, 1.1)	0.8 (0.4, 1.2)			0.5 (0.3, 1.0)	0.7 (0.3, 1.0)			***1.1 (0.6, 1.6)	0.9 (0.6, 1.3)

BLQ, below lower limit of assay quantitation

#Concentration data pooled across all arms containing the specified drug.

*p=0.016 v. Week 48 (MVC only)

**p=0.03 v. MVC + TDF, Week 24

***p=0.014 v. MVC+ FTC, Week 24

Table 3

Pharmacokinetic-Pharmacodynamic relationship among study drugs and among study regimens using GEE analysis.

Parent Drug Concentrations	Estimate (95% Confidence Interval)	P value
Tissue MVC	−32 (−62, −1)	0.04
Tissue TFV	−25 (−50, 0)	0.05
Tissue FTC	−137 (−235, −38)	0.01
Drug Regimens		
MVC only	557 (269, 845)	0.0001
MVC + FTC	53 (−6, 111)	0.08
MVC + TDF	100 (−14, 213)	0.08
TDF + FTC	–	REF