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Repeated Administration of High Dose Depot Medroxy Progesterone Acetate does not Alter $SHIV_{SF162p3}$ Viral Kinetics and Tenofovir Pharmacokinetics when Delivered via Intravaginal Rings

Priya Srinivasan[1], **Jining Zhang**[2], **Chuong T. Dinh**[1], **Ryan S. Teller**[3], **Janet M. McNicholl**[1], **Patrick F. Kiser**[3], **Betsy C. Herold**[4], **James M. Smith**[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA

[2]Total Solutions Inc., Atlanta, GA

[3]Department of Biomedical Engineering, Northwestern University, Evanston, IL

[4]Department of Pediatrics and Microbiology-Immunology, Albert Einstein College of Medicine, Bronx, NY

Abstract

Background—Intravaginal rings (IVR) for HIV prevention will likely be used by women on depot medroxy progesterone acetate (DMPA) hormonal contraception. We used pigtailed macaques to evaluate the effects of DMPA on tenofovir disoproxil fumarate (TDF) IVR pharmacokinetics and viral shedding.

Methods—Mucosal tenofovir (TFV) levels were compared in $SHIV_{SF162p3}$-negative DMPA-treated (n=4) and normally cycling (n=4) macaques receiving TDF IVRs. Plasma viremia and vaginal shedding were determined in groups of $SHIV_{SF162p3}$-positive DMPA-treated (n=6) and normally cycling (n=5) macaques.

Results—Similar median vaginal fluid TFV concentrations were observed in the DMPA-treated and cycling macaques over 4 weeks (1.2×10^5 and $1.1. \times 10^5$ ng/mL respectively). Median plasma viremia and vaginal shedding AUC of the DMPA-treated (2.73×10^7 and 8.15×10^4 copies/mL respectively) and cycling macaques (3.98×10^7 and 1.47×10^3 copies/mL respectively) were statistically similar.

Conclusions—DMPA does not affect TDF IVR pharmacokinetics or SHIV shedding.

Keywords

DMPA and HIV; Tenofovir pharmacokinetics; Mucosal HIV shedding; Nonhuman primates; SHIV

Corresponding author: James M. Smith, 1600 Clifton Road, M.S. A25, Atlanta, GA, 30333; 404-639-1312 (phone); 404-639-4676 (fax); ajo9@cdc.gov.
MS. PRIYA SRINIVASAN (Orcid ID : 0000-0001-8426-6532)

Introduction

Globally, 52% of those living with HIV are women. In areas of high HIV incidence, such as sub-Saharan Africa, women account for 57% of persons infected with HIV [1]. With no effective vaccine foreseeable in the near future, antiretroviral (ARV) therapy and preexposure prophylaxis (PrEP) play a pivotal role in controlling the epidemic [2]. The lack of efficacy due to poor adherence in the recent clinical trials with oral ARVs (VOICE, FEM-PrEP) and moderate to suboptimal efficacy with topical gels (CAPRISA, FACTS-001 and VOICE) in women demonstrate a need for providing PrEP delivery options for women to improve adherence [3–6]. As shown with contraceptive preferences, women may choose HIV-prevention strategies based on their individual choices and their socioeconomic conditions [6–8]. Availability of multiple dosage forms may help improve adherence and thereby increase protection [9, 10]. Different topical PrEP delivery modalities such as intravaginal rings (IVRs), vaginal films, inserts and soft-gel capsules are being developed and tested [11–16]. The recent successes obtained in two clinical trials with IVRs delivering the potent non-nucleoside reverse transcriptase inhibitor, dapivirine, of greater than 65% reduction in HIV acquisition among women with high adherence (ASPIRE) and a 37% risk reduction among women over 21 (The Ring Study) highlight the potential of IVRs to increase adherence [9, 17, 18]. These successes have helped accelerate the development of other IVR/drug combinations.

Along with PrEP for HIV, a safe and effective long-acting contraceptive is vital to all women, and in particular to at-risk and HIV infected women to reduce the accompanying risk of unwanted pregnancy, maternal deaths and vertical transmission. A study comparing the cost-effectiveness of contraception use to nevirapine administration to HIV-positive mothers estimates that 28.6% more HIV positive births could be averted with contraception [19]. Depot medroxy progesterone acetate (DMPA), or Depo-Provera, administered as a three- month 150 mg intramuscular injection is a highly effective contraceptive with a 0.2% failure rate when used as recommended and represents 43% of the modern contraceptive method used in sub-Saharan Africa, the epicenter of HIV-1 infections [20–22].

We evaluated the efficacy of PrEP IVRs delivering tenofovir disoproxil fumarate (TDF, prodrug of tenofovir, TFV) in a non-human primate pigtailed macaque model in the presence or absence of DMPA [11, 12, 23]. In DMPA-naïve macaques, the IVR maintained levels of 1.8 x 10^5 ng/ml of TFV in vaginal fluid and tissues (approximately 80 times greater than the *in vitro* IC_{50}), which are fully protective against repeat low-dose challenge with $SHIV_{SF162p3}$ [23]. More recently, we showed that the TDF IVR can also significantly protect pigtailed macaques that received high-dose DMPA treatment every 6 weeks against 12 weekly vaginal $SHIV_{SF162p3}$ exposures [11]. The dose of DMPA was 30 mg per macaque, a slightly higher dose in mg/kg than the human dose of 150 mg. All control animals receiving a placebo ring became SHIV infected, whereas 5 of 6 animals receiving the TDF IVR were protected [11]. Since women at risk for HIV frequently use hormonal contraception (HC), it is important to study the biological effects of HC on mucosal drug pharmacokinetics (PK) and determine if such contraceptives modify PrEP PK [24, 25]. Moreover, as some women using DMPA and using an IVR delivering drugs for PrEP may

become HIV-infected, if IVR use is suboptimal, it is important to know if DMPA use would increase HIV shedding in such women.

In the current study we characterize, in depth, the impact of DMPA on the PK of the TDF IVR in SHIV naïve macaques. Data were compared to that obtained among cycling macaques that received the same TDF IVR [26]. In addition we evaluate safety of the IVR in such animals, using a panel of cytokine assays. To model the possibility of breakthrough infections in women using DMPA who may have suboptimal IVR use, we evaluated if macaques that received DMPA and became SHIV infected had higher plasma viral loads and increased viral shedding than normally cycling SHIV positive pigtailed macaques from a previous study [23].

Methods

Humane Care Guidelines

All macaques were housed at the Centers for Disease Control and Prevention, an AAALAC accredited facility, according to the Guide for the Care and Use of Laboratory Animals (National Research Council of the National Academies, 2010) under biosafety level-2 containment conditions and the study was approved by the CDC Institutional Animal Care and Use Committee (IACUC).

DMPA and TDF IVR safety and PK

Four SHIV-negative DMPA-treated (30 mg intramuscularly, every 6 weeks) pigtailed macaques were enrolled in a two-arm crossover PK (TDF IVR and placebo IVR) study. Plasma progesterone levels were monitored (weeks 0 to 4.5) to determine if repeated DMPA administration suppressed endogenous progesterone production (data not shown) [11]. These macaques served as their own controls and completed both arms of the study with a five-week washout period in between the crossovers. The rings were inserted on day zero (one week after DMPA administration) and left in place for 28 days. Vaginal secretions were collected with eight Ultracell surgical sponges (3.5 x 4 mm, Beaver-Visitec, Waltham, MA) on weeks −1, 0, 1, 2, 3, 4, 4.5 for drug content and cytokine analysis. TDF and TFV were quantitated by liquid chromatography-mass spectrometry in vaginal secretions [27]. The data were compared to that obtained among cycling macaques that received the same TDF IVR and was published previously [26]. We monitored for mucosal inflammation by the measurement of cytokines using a Milliplex™MAP (Millipore, Billerica, MA, USA) fluorescent multiplexed bead-based assay as previously described [28, 29].

DMPA and SHIV viral kinetics

We previously evaluated the effectiveness of the TDF IVR in a rigorous challenge model combining repeated DMPA administration of 30 mg every six weeks and twelve weekly vaginal exposures to $SHIV_{SF162p3}$ [11]. In the six DMPA placebo IVR control animals, all became SHIV-infected and cervicovaginal lavages (CVL) for measurement of mucosal viral shedding were collected beginning after two consecutive positive plasma viral load measurements. The plasma viremia and mucosal shedding among the DMPA-treated pigtailed macaques was compared to that obtained from five SHIV-infected, HC-naïve

cycling control macaques from a previous study [23]. The plasma viral load and the viral shedding values for all macaques were determined by RT-PCR with a lower limit of 50 copies/mL [30].

Data Analysis

PK—Wilcoxon matched-pairs signed-rank test was used to compare the proximal and distal vaginal fluid TFV levels obtained at weeks 1–4 among the DMPA-treated macaques. One-way analysis of variance with Bonferroni adjustment for multiple comparisons was employed to compare TFV levels in vaginal secretions between the DMPA and cycling macaques [26]. The changes in cytokines and chemokines of the TDF and the placebo IVR macaques were monitored by Friedman test of the log-transformed values. These were followed by Wilcoxon signed-rank test with false discovery rate (FDR) adjusted p-values for post-hoc pairwise comparisons between each of 5 time points (weeks 1, 2, 3, 4, 4.5) and −1 and 0.

Viral kinetics—Samples tested using the viral load assay that were below the limit of detection were given the value of 50 copies/mL. The cumulative plasma viral load and the cumulative viral shedding in CVL of the DMPA controls were compared to that of $SHIV_{SF162p3}$ positive normally cycling macaques [11, 23]. The cumulative viremia and viral shedding was estimated as the area under the curve (AUC) [31]. The viremia and viral shedding AUC were included as a continuous variable and the two groups compared with Wilcoxon rank sum test [32].

Results

DMPA and TDF IVR safety and PK

Vaginal fluid was obtained from the DMPA-treated macaques at weeks 0, 1, 2, 3 and 4 to quantitate the mucosal TFV levels and these were compared to the median values for a similar time frame reported among cycling macaques (n=6) that received the same TDF IVR from a previous study [26]. Median vaginal fluid TFV concentration over 4 weeks among the DMPA-treated macaques (40 samples) was 1.2×10^5 (range, 7.5×10^0 – 7.8×10^5) ng/mL, similar to that seen in the cycling macaques (96 samples), 1.1×10^5 (2.9×10^3 – 1.2×10^6) ng/mL from a previous study (Fig 1) [26]. As shown in figure 1 the distribution of TFV proximal to the IVR (median, 1.67×10^5 ng/mL, range 4.51×10^4 to 4.51×10^5 ng/mL) among the DMPA-treated macaques were higher than the distal vaginal fluid samples (median, 3.65×10^4 ng/mL, range 7.5×10^0 to 7.8×10^5 ng/mL) though the weekly proximal vs. distal difference was not statistically significant (Wilcoxon matched-pairs signed-rank test, p=0.1250).

We monitored mucosal cytokines to determine what effect, if any, the TDF and placebo IVRs had on proinflammatory cytokine production. Vaginal fluid was obtained at weeks −1, 0, 1, 2, 3, 4, and 4.5 for the analysis. Friedman tests found differences over time in IL-8 and GCSF (TDF IVR) and IL-1β, IL-8, IL-15, MIP-1β, and GM-CSF (placebo IVR). FDR adjusted p-values for post-hoc pairwise comparisons between each of the 5 time points (weeks 1–4.5) and weeks −1 and 0, suggest changes in these cytokines were due to

differences among time points (weeks 1–4.5) signifying that the effects are not due to ring placement (Tables 1 and 2) [26].

DMPA and SHIV viral kinetics

The median plasma viremia AUC during the first 17 weeks of infection of the six DMPA-treated macaques [2.73 x 10^7 (2.79 x 10^5– 4.47 x 10^8 copies/mL)] was similar (Wilcoxon rank sum test, p= 0.9307) to the median for the five macaques that were infected during the regular menstrual cycle [3.98 x 10^7(1.42 x 10^6 – 4.75 x 10^7copies/mL)], though a trend for a higher set point is seen among the DMPA-treated macaques (Fig 2a). Mucosal viral shedding AUC of the DMPA-treated macaques [8.15 x 10^4 (4.45 x 10^2– 1.13 x 10^6copies/mL)] were higher than those of the regularly cycling pigtailed macaques [1.47 x 10^3(2.6 x 10^2– 1 x 10^4 copies/mL)] though it did not reach statistical significance (Wilcoxon rank sum test, p= 0.1255) (Fig 2b).

Discussion

We demonstrate using the well-established pigtailed macaque model that repeated DMPA administration to macaques at a mg/kg dose that is greater than the dose used for humans does not affect TDF IVR PK in SHIV-negative macaques. Furthermore, we observed that the IVR does not induce inflammatory cytokines in the presence of DMPA. We also show that repeated DMPA does not affect SHIV plasma viremia and vaginal shedding.

With greater acceptance to PrEP delivered via IVR in areas where DMPA and other hormonal contraceptives are widely used, it is imperative to study potential interactions between drug PK and HC and determine if such contraceptives modify IVR PrEP PK and vice versa [24, 25]. While effects of DMPA on some other PrEP modalities have been conducted, no studies published thus far have evaluated the effects of DMPA on IVR PK. A sub-study analysis among female participants on DMPA and males (whose female partners were HIV-positive and on DMPA) on oral PrEP, either receiving daily TDF or TDF/emtricitabine, found that the risk reduction rates were similar as compared to those receiving the ARV but not on DMPA [33]. This is consistent with a previous finding in pigtailed macaques where DMPA did not diminish oral TDF/emtricitabine efficacy [34]. However, these studies cannot address the impact of DMPA on long-acting topical PrEP. In our study, the repeated administration of DMPA at 30 mg every 6 weeks to pigtailed macaques did not alter mucosal TFV levels delivered via IVRs. TFV vaginal fluid levels were found to be equivalent in cycling macaques receiving the TDF IVR, with a similar distribution of proximal being greater than distal, regardless of whether DMPA-treated or not. The levels were comparable to those shown to be effective in protecting macaques against low-dose challenges with $SHIV_{SF162p3}$ in the presence and absence of DMPA [11, 23]. These data taken together suggest that TDF IVR will be highly effective in women using DMPA.

We did not address in this study whether the TDF IVR affected HC PK. This is an important issue, as contraceptives are metabolized by cytochrome P450 enzymes which are known to be modulated by many ARVs [35, 36]. In women, the efficacy of contraceptives such as DMPA were found to be relatively stable with oral ARV usage [35]. Similarly, PrEP with dapivirine IVR was found not to inhibit HC effectiveness among women who received

injectable or implant HC [37]. Since plasma TFV is not detected in animals receiving TDF IVR in the presence or absence of DMPA, we would similarly not expect any negative impact of the TDF IVR on HC PK.

A number of studies have demonstrated a strong association between DMPA use and altered genital inflammatory status and increased recruitment of HIV target cells at mucosal entry sites [38, 39]. Mucosal cytokines were monitored throughout the study to determine what effect, if any, the TDF and placebo IVRs had on proinflammatory cytokine production. Mucosal cytokine changes were noted at some time points. However, FDR adjusted p-values for post-hoc pairwise comparisons suggested changes in these cytokines were due to differences among time points (weeks 1–4.5), signifying that the effects were not due to ring placement.

All prevention modalities from oral to topical PrEP will potentially have breakthrough infections owing to suboptimal use and lack of adherence [2, 3, 9, 17]. The macaque model provides an unique opportunity to help address concerns regarding suboptimal or intermittent PrEP dosing and thus inform human studies of the needs to improve delivery to achieve safe and efficacious ARV concentrations to prevent HIV infections [27, 40]. To mimic the possibility of breakthrough infections among women who use DMPA and who may use the IVR suboptimally, we evaluated if DMPA-treated macaques that became $SHIV_{SF162p3}$ infected had higher plasma viral loads and vaginal shedding than normally cycling $SHIV_{SF162p3}$ positive pigtailed macaques, using data from a previous study [23]. These studies are hard to conduct in clinical trials, as women who may become HIV positive would be referred to begin treatment with ARV. Studies of HIV shedding are important as an increase of HIV-1 RNA in genital secretions was shown to increase sexual transmission of HIV, and use of injectable contraceptives was shown to increase HIV-1 RNA levels in the genital tract [41]. A recent study analyzing plasma viral suppression and genital HIV shedding among 1,079 HIV positive women commencing HIV treatment from three clinical trials in South Africa demonstrated that HC does not delay the time to viral suppression in plasma and does not increase genital shedding [42].

Repeated high-dose DMPA administration to pigtailed macaques did not cause a significant increase in plasma viremia in the limited sample size studied. The median plasma viremia AUC was similar to that reported among $SHIV_{SF162p3}$ positive normally cycling macaques, though a trend for a higher set point is seen among the DMPA-treated macaques. Similarly, a study of a lower dose of DMPA in pigtailed macaques did not cause an increase in plasma viremia in $SHIV_{SF162p3}$ positive macaques [43]. In the present study, an insignificant increase in vaginal shedding was observed among the DMPA-treated macaques, when compared the normally cycling SHIV-positive macaques. Similarly, a low DMPA dose did not cause an increase in mucosal shedding in $SHIV_{SF162p3}$ positive macaques [43].

Unlike human cross-sectional data where the effects of DMPA on plasma viremia and shedding may not be accurately estimated owing to the long gap between sample collections, the repeat low-dose pigtailed macaque challenge model allows for longitudinal and repeated sampling. The absence of a statistically significant increase in plasma viremia and shedding in this rigorous model (combining a high dose of DMPA with repeated $SHIV_{SF162p3}$

administration) during the first 17 weeks of infection suggests that DMPA may not enhance the risk of HIV transmission to others during acute infection.

There are some key limitations to our study such as small sample size, and lack of true DMPA controls without an IVR. The effects of the placebo ring, if any, on the plasma viremia and vaginal shedding in DMPA-treated macaques cannot be separated. However, the absence of IVR related increases in mucosal cytokines would suggest that the IVR may have minimal effects on shedding. Additionally, we do not report on concentrations of vaginal tissue TFV or the intracellular active form of TFV, tenofovir diphosphate levels.

In summary our results demonstrate that repeated DMPA administration does not have undesirable effects on HIV kinetics and TDF IVR PK in our model. These findings further support the clinical evaluation of the TDF IVR even among DMPA-treated women. The model presented here could be useful for defining interactions between the PK of other potential drugs for topical PrEP and other types of HC in a rigorous setting, and assess the effects if any of the contraceptive on ARV efficacy to prevent HIV infection.

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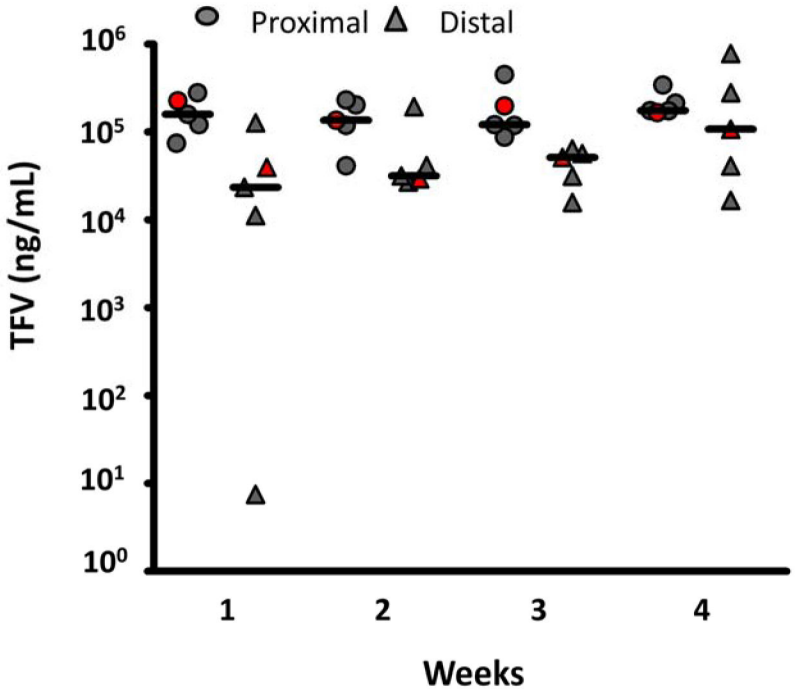

Fig 1.

Tenofovir levels are undistinguishable between DMPA-treated and cycling macaques: TFV levels were measured from vaginal secretions obtained proximal (gray circle) and distal (gray triangle) to the IVR throughout the study (weeks 1, 2, 3, 4). Each symbol (gray circle and gray triangle) represents a single animal receiving DMPA and the median values are indicated by bars. Red symbols (red circle-proximal and red triangle- distal) indicate median TFV values obtained in vaginal fluids from six cycling macaques from a previous study [26]. The individual TFV vaginal fluid levels in cycling macaques are not shown here and have been published previously [26]. One-way ANOVA with Bonferroni adjustment for multiple comparisons was employed to compare TFV levels in vaginal secretions between DMPA and cycling macaques. The mucosal TFV concentration among the DMPA-treated macaques were similar to that seen in the cycling macaques.

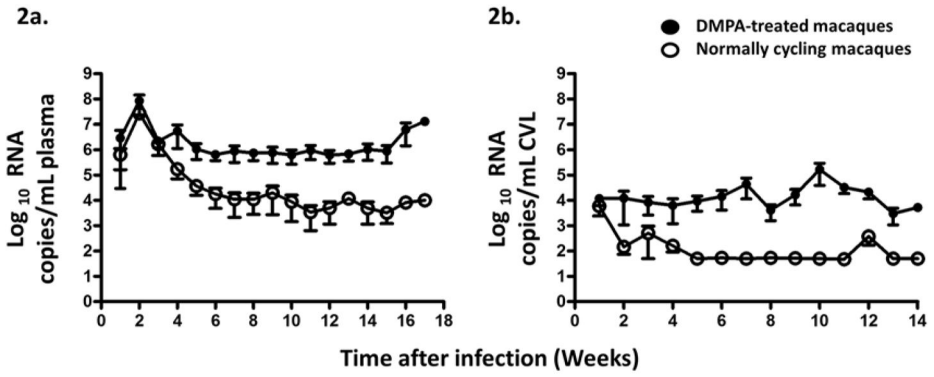

Fig 2.

Viral RNA kinetics (mean with SEM) in SHIV positive DMPA-treated and regular cycling pigtailed macaques: Plasma viremia (a), and mucosal viral shedding (b) were determined by RT-PCR. Samples that were below the limit of detection were given the value of 50 copies/mL. The cumulative viremia and viral shedding were estimated as the area under the curve (AUC). The viremia and viral shedding AUC were included as a continuous variable and the two groups compared with Wilcoxon rank sum test. Although there is a trend of higher plasma viral load and vaginal shedding with the DMPA group, the differences were not found to be statistically significant.

Table 1

Cytokine/chemokine concentrations (pg/mL) in the cross-over PK studies DMPA-treated TDF IVR phase (n=4)

Cytokine/ chemokine[1]	Baseline		TDF IVR treatment (week)- median *(range)*				Post IVR
	−1	0	1	2	3	4	4.5
IL-1β	570.5 *(1.22–19,987)*	392.5 *(1.22–4,104)*	1,145 *(1.22–22,977)*	2,998 *(121.4–7,224)*	139 *(1.22–6,272)*	180.6 *(64.13–960.4)*	103.7 *(1.22–435.5)*
IL-1Ra	21,238 *(8,205–93,052)*	25,169 *(14,268–37,633)*	22,793 *(9,779–38,325)*	24,148 *(18,868–30,903)*	17,804 *(14,954–23,920)*	15,520 *(8,793–24,518)*	15,503 *(11,870–23,892)*
G-CSF	337.3 *(120.9–6,054)*	736.8 *(500.9–3,983)*	409.7 *(278.1–629.1)*	333.8 *(246.7–407.4)*	316.3 *(147.9–342.6)*	370 *(215.7–811.8)*	37.86 *(1.22–94.99)*
IFNγ	37.27 *(5.51–894)*	58.48 *(10.11–253.5)*	171.9 *(1.22–333.2)*	182.1 *(40.96–240.2)*	36.18 *(1.22–240.8)*	56.56 *(1.22–160.7)*	41.18 *(0.75–83.61)*
IL-8	4468 *(374.6–57,999)*	16,721 *(637.9–31,843)*	50,440 *(9,977–93,966)*	51,791 *(7,778–159,840)*	24,375 *(9,004–87,990)*	26,292 *(9,959–74,025)*	3,448 *(738.9–10,184)*
IL-4	201.3 *(2.44–234.3)*	217.4 *(15.72–396.2)*	297.3 *(276.9–1,133)*	266.1 *(159.2–1,309)*	338.7 *(114.8–382.5)*	232.3 *(215.5–245.9)*	114.6 *(2.44–202)*
IL-6	53.47 *(1.22–3,258)*	342.8 *(14.52–3,211)*	404.9 *(36.54–824.5)*	703.1 *(147.2–1,829)*	497.7 *(127.4–2,172)*	1,527 *(60.34–4,098)*	58.43 *(1.22–2,101)*
IL-12/23p40	60.4 *(21.32–1,083)*	94.04 *(69.47–413.7)*	233.4 *(65.66–593.8)*	343.9 *(21.7–390.1)*	112.8 *(9.17–407.6)*	221.7 *(70.7–381.5)*	40.34 *(1.22–71.03)*
IL-13	41.08 *(1.22–301.9)*	109.7 *(1.22–282.4)*	179.4 *(1.22–250.1)*	208.9 *(1.22–235.2)*	129.7 *(65.09–178.8)*	251.2 *(140.2–378.3)*	39.66 *(1.22–96.47)*
IL-15	53.2 *(6.66–389.9)*	113.6 *(23.19–218.3)*	115.1 *(64.32–256.3)*	162.6 *(41.34–229)*	85.95 *(54.13–187.1)*	136.3 *(29.14–199.6)*	44.25 *(11.04–95.57)*
IL-17	16.71 *(1.65–94.48)*	18.41 *(8.82–71.64)*	29.95 *(7.99–131.1)*	57.99 *(4.12–89.12)*	17.42 *(7.86–48.52)*	21.88 *(4.83–50.34)*	3.52 *(1.22–10.34)*
MCP-1	366.3 *(86.74–3,187)*	1,593 *(947.7–2,538)*	1,458 *(1,038–1,964)*	2,598 *(991.2–11,729)*	1,767 *(330.9–4,946)*	1,455 *(523.9–6,700)*	610.3 *(139.1–3,168)*
MIP-1β	42.68 *(1.22–458.7)*	164 *(22.24–371.8)*	195.8 *(53.48–331.5)*	250.1 *(38.91–1,081)*	218.5 *(1.22–394.1)*	151.3 *(32.93–747.2)*	63.96 *(43–144.1)*
sCD40L	217.2 *(1.22–2,329)*	215.7 *(1.22–980)*	515.5 *(1.22–1,187)*	453.4 *(134.7–690.6)*	205.1 *(17.4–566.8)*	265.2 *(202.3–529.8)*	166.9 *(17.09–404.6)*
TNF-α	373.3 *(1.22–15,959)*	2,103 *(345.3–11,363)*	4,536 *(1.22–11,109)*	1,857 *(265.8–10,009)*	1,282 *(473.6–15,859)*	2,739 *(1,233–4,900)*	727.8 *(74.06–3,409)*
VEGF	749.4 *(182–7,359)*	943.5 *(510.5–3,846)*	3,499 *(306.2–7,171)*	3,615 *(922.5–28,031)*	1,779 *(421.6–8,776)*	3,352 *(773.5–7,171)*	314 *(1.22–822)*
IL-18	6,540 *(885.4–19,683)*	7,937 *(1,211–12,572)*	6,300 *(985.1–33,403)*	6,200 *(2,826–23,395)*	3,834 *(3,501–22,528)*	3,681 *(3,027–5,104)*	5,014 *(834.6–21,093)*

Cytokine/ chemokine[1]	Baseline		TDF IVR treatment (week)- median *(range)*				Post IVR
	−1	0	1	2	3	4	4.5
GM-CSF	10.95 *(1.71–29.03)*	7.94 *(3.31–15.36)*	12.85 *(2.66–20.54)*	31.8 *(29.95–38.83)*	16.52 *(7.48–49.01)*	18.36 *(2.99–42.62)*	7.85 *(1.12–10.34)*

[1] Friedman tests found differences over time in IL-8 and GCSF. FDR adjusted p-values for post-hoc pairwise comparisons between each of 5 time points (wk 1 –4.5) and wk −1 and wk 0, suggest changes in these cytokines were due to differences among time points (wk 1–4.5) signifying that the effects are not due to ring placement.

Table 2

Cytokine/chemokine concentrations (pg/mL) in the cross-over PK studies DMPA-treated placebo IVR phase (n=4)

Cytokine/ chemokine[2]	Baseline		Placebo IVR treatment (week)- median *(range)*				Post IVR
	−1	0	1	2	3	4	4.5
IL-1β	431.4 *(30.51–750.6)*	23.92 *(6.21–472.8)*	204.1 *(48.79–935.4)*	8,797 *(907.2–16,869)*	5,279 *(193–14,354)*	1,533 *(73.19–25,762)*	44.3 *(0.69–1,171)*
IL-1Ra	25,072 *(15,508–35,990)*	19,992 *(11,038–25,617)*	28,723 *(26,576–43,642)*	18,764 *(13,283–25,963)*	30,142 *(23,734–33,269)*	25,583 *(14,398–32,146)*	21,732 *(19,762–23,302)*
G-CSF	1,528 *(381.2–15,190)*	274.3 *(155.1–1,228)*	516.4 *(284.3–607.5)*	4,703 *(1.22–9,884)*	1,158 *(254.1–2,688)*	1,024 *(618.3–2,907)*	231.7 *(44.33–1,012)*
IFNγ	61.54 *(42.4–273.4)*	30.25 *(3.89–48.79)*	42.82 *(1.22 –301)*	336.7 *(1.22–769.1)*	260.1 *(14.78–530.6)*	116.4 *(1.22–265.8)*	27.16 *(1.22–128.3)*
IL-8	11,139 *(2,685–76,909)*	1,873 *(1,383–9,939)*	19,661 *(4,273–34,038)*	38,897 *(21,985–63,484)*	45,562 *(5,868–58,710)*	18,720 *(3,236–39,890)*	3,113 *(649.8–20,641)*
IL-4	183.7 *(101–628.6)*	42.64 *(2.44–198.5)*	155.6 *(80.56–230.3)*	309.1 *(159.4–581.6)*	190.9 *(159.4–251.3)*	301.9 *(123.4–425.1)*	187.4 *(2.44–228.9)*
IL-6	315.9 *(67.34–87,137)*	54.43 *(1.22–235.4)*	245.3 *(73.32–293.7)*	901.1 *(67.34–2,665)*	369 *(104.8–598.3)*	264.7 *(128.4–318.5)*	1.22 *(1.22–108.3)*
IL-12/23p40	107.5 *(46.39–521.9)*	52.58 *(4.23–96.37)*	76.21 *(1.22–573.4)*	590.2 *(58.55–1,151)*	390.8 *(34.04–820.4)*	166.1 *(22.46–1,186)*	57.31 *(1.22–127.8)*
IL-13	81.65 *(1.22–185.3)*	30.15 *(1.22–106.4)*	161.4 *(1.22–268)*	180.1 *(1.22–367.9)*	252.3 *(1.22–309.2)*	191.5 *(1.22–346.8)*	132.9 *(1.22–253.6)*
IL-15	103.4 *(17.77–164.6)*	102.1 *(40.61–185.8)*	123.7 *(68.83–436.7)*	256.2 *(94.87–520.1)*	232.8 *(94.83–359)*	165.4 *(78.11–204.5)*	35.19 *(18.79–139.5)*
IL-17	13.31 *(2.32–40.85)*	10.75 *(5.59–13.6)*	10.18 *(1.22–37.74)*	74.12 *(9.29–179.7)*	57.75 *(5.42–141.8)*	59.74 *(20.14–78.6)*	13.16 *(1.22–34.83)*
MCP-1	578.5 *(399.9–15,190)*	387.5 *(168.7–3,641)*	590.1 *(393.9–36,812)*	3,166 *(464.1–10,125)*	928.5 *(342.8–1,559)*	597.9 *(153.2–890)*	348.8 *(104.2–1,745)*
MIP-1β	158.6 *(43.77–1,823)*	20.41 *(1.22–87.06)*	110.2 *(68.27–403.2)*	410 *(177.9–660.7)*	297 *(72.01–912.1)*	156.9 *(81.9–400)*	71.43 *(6.92–221.9)*
sCD40L	417.6 *(66,21–3,331)*	65.45 *(1.22–335.7)*	596.4 *(125,1–1,102)*	1,013 *(63.97–2,481)*	989.5 *(79.14–2,325)*	587.4 *(129.5–931.1)*	88.66 *(63.1–874.6)*
TNF-α	472.8 *(418.6–1,382)*	105 *(1.22–974.3)*	677 *(175.3–755.8)*	4,115 *(513.1–9,300)*	1,944 *(552.2–9,025)*	1,541 *(680–2,759)*	202.1 *(1.22–1,416)*
VEGF	1,440 *(849.4–17,947)*	703 *(429–994.2)*	1,363 *(1.22–2,828)*	2,673 *(718.1–5,572)*	3,233 *(609.6–4,904)*	2,232 *(537.8–3,290)*	783.8 *(327.6–2,052)*
IL-18	4,686 *(1,613–1,341,000)*	4,954 *(863.3–64,661)*	10,371 *(8,209–20,574)*	5,924 *(718.5–34,228)*	32,500 *(2,303–36,430)*	5,162 *(3,056–10,860)*	6,032 *(762.8–46,852)*

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Cytokine/ chemokine[2]	Baseline		Placebo IVR treatment (week)- median *(range)*				Post IVR
	−1	**0**	**1**	**2**	**3**	**4**	**4.5**
GM-CSF	14.81 *(6.66–91.56)*	5.2 *(4.38–5.34)*	10.18 *(5.24–22.82)*	22.08 *(8.23–32.53)*	11.79 *(10.78–14.05)*	8.15 *(5.81–19.36)*	5.62 *(4.83–6.68)*