

Single dose topical inserts containing tenofovir alafenamide fumarate and elvitegravir provide pre- and post-exposure protection against vaginal SHIV infection in macaques



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Summary

Background Vaginal products for HIV prevention that can be used on-demand before or after sex may be a preferable option for women with low frequency or unplanned sexual activity or who prefer not to use daily or long-acting pre-exposure prophylaxis (PrEP). We performed dose ranging pharmacokinetics (PK) and efficacy studies of a vaginally applied insert containing tenofovir alafenamide fumarate (TAF) and elvitegravir (EVG) in macaques under PrEP or post-exposure prophylaxis (PEP) modalities.

Methods PK studies were performed in 3 groups of pigtailed macaques receiving inserts with different fixed-dose combinations of TAF and EVG (10/8, 20/16 and 40/24 mg). PrEP and PEP efficacy of a selected insert was investigated in a repeat exposure vaginal SHIV transmission model. Inserts were administered 4 h before (n = 6) or after (n = 6) repeated weekly SHIV exposures. Infection outcome was compared with macaques receiving placebo inserts (n = 12).

Findings Dose ranging studies showed rapid and sustained high drug concentrations in vaginal fluids and tissues across insert formulations with minimal dose proportionality. TAF/EVG (20/16 mg) inserts were selected for efficacy evaluation. Five of the 6 animals receiving these inserts 4 h before and 6/6 animals receiving inserts 4 h after SHIV exposure were protected after 13 challenges (p = 0.0088 and 0.0077 compared to placebo, respectively). The calculated PrEP and PEP efficacy was 91.0% (95% CI = 32.2%–98.8%) and 100% (95% CI = undefined), respectively.

Interpretation Inserts containing TAF/EVG provided high protection against vaginal SHIV infection when administered within a 4 h window before or after SHIV exposure. Our results support the clinical development of TAF/EVG inserts for on-demand PrEP and PEP in women.

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Research in context

Evidence before this study

A monthly vaginal ring releasing Dapivirine (DPV-VR) is the first topical HIV prevention method to be submitted for regulatory approval and is currently recommended by the World Health Organization (WHO) as an additional prevention option for women at substantial risk of acquiring HIV. Two Phase III studies found DPV-VR reduced women's risk of HIV-1 infection by about 30% with no safety concerns. Open-label extension studies estimated effectiveness increased by over 50% among women who consistently used the DPV-VR, suggesting adherence can impact its public health benefit. On-demand topical products provide an alternative option for women who do not need daily PrEP or prefer not to use long-acting products. Here we assessed in a validated macaque model of vaginal simian HIV (SHIV) transmission the pharmacokinetics and efficacy of a vaginal insert that is discreet, portable, affordable, and can be easily self-administered as an on-demand PrEP or PEP option for women.

Added value of this study

Topical inserts provide a convenient dosage form that is well suited for on-demand vaginal or rectal use for individuals who may on occasion have unprotected sex and thus prefer not to take daily or long-acting PrEP regimens. We show the biological efficacy of an insert co-formulated with a fixed-dose combination of tenofovir alafenamide fumarate (TAF) and elvitegravir (EVG) for on-demand PrEP or PEP using a stringent macaque model of repeated vaginal exposures that mimic women at high risk of HIV infection.

Implications of all the available evidence

Our findings provide preclinical proof-of-concept for TAF/EVG inserts as a promising topical on-demand HIV prevention product that can be self-administered before or after sex, which may better align with different needs among HIV prevention users. Additional clinical trials are needed to determine if these products will be effective in preventing HIV infections in women.

Introduction

Ending the HIV epidemic domestically and globally continues to be a high public health priority. While groundbreaking biomedical strategies have led to remarkable progress towards preventing and treating HIV on a global scale, UNAIDS reported that an estimated 1.5 million individuals worldwide acquired HIV in 2020.¹ In sub-Saharan Africa where the majority of the global HIV infections occur, adolescent girls and young women (AGYW) are particularly vulnerable and account for nearly 25% of new infections.² UNAIDS' goal to end the AIDS epidemic by 2030 will require significant progress to reduce HIV incidence among AGYW. Daily pre-exposure prophylaxis (PrEP) with oral emtricitabine and tenofovir disoproxil fumarate (FTC/TDF) is part of the HIV combination prevention package and can significantly reduce HIV acquisition.³⁻⁵ However, low levels of uptake and adherence particularly in younger women has limited the effectiveness of oral PrEP.^{3,6} A bi-monthly injectable long-acting (LA) formulation of cabotegravir (CAB LA) and a monthly vaginal ring releasing dapivirine have clinically advanced as alternative PrEP options for women seeking constant long-term protection and were subsequently found to be effective as PrEP agents in Phase III trials.⁷⁻⁹

On-demand products provide a different option for women who do not need daily PrEP or prefer not to use long-acting products continuously. An oral on-demand FTC/TDF regimen is available for men who have sex with men, but not for women, and requires a more complex, multi-dose regimen.¹⁰ Topical products such as vaginal inserts that are discreet, portable, and can be easily self-administered may represent a desirable

on-demand PrEP or post-exposure prophylaxis (PEP) option for women. These products have several advantages including rapid local absorption and bioavailability, increased drug concentrations in cervicovaginal fluids and tissues, and low systemic drug exposures.^{11,12} Vaginal inserts are approved dosage forms commonly used by women for treatments of reproductive tract conditions such as vaginal yeast infection, infertility, and vaginal atrophy.^{11,13} CONRAD has developed an advanced insert containing the tenofovir (TFV) prodrug tenofovir alafenamide fumarate (TAF) and the integrase inhibitor elvitegravir (EVG), that is designed to be a user friendly single dose regimen that can be self-administered prior to or after coitus for the prevention of HIV and Herpes Simplex Virus Type 2 (HSV-2).¹¹ TAF adds a multipurpose prevention component to the inserts since TFV diphosphate (TFV-DP) has activity against both HIV and HSV-2.^{14,15}

The addition of an integrase strand transfer inhibitor (INSTI) to TAF, a nucleotide reverse transcriptase inhibitor (NtRTI), provides not only an additional mechanism of action but the option of post-coital administration for protection. INSTIs target a late stage of the virus replicative cycle occurring at least 6–8 h after virus entry, thus extending the window of prophylactic activity and enabling the use as PEP (14). A post-coital dosing window beyond what is provided by NRTIs provides a more flexible dosing option that may better align with the needs of different users. Proof-of-concept studies have demonstrated high PEP protection by vaginal raltegravir gel applied 3 h after vaginal SHIV challenge.¹⁶ The *in vivo* protection was associated with high local drug concentrations that were 4–5 orders of

magnitude higher than those needed for *in vitro* inhibition, highlighting the advantage of topical delivery.¹⁷

When administered pre-exposure, previous macaque studies have shown that 1% TFV gel completely protected macaques against vaginal SHIV infection and identified TFV-DP levels in vaginal lymphocytes (~1800 fmol/10⁶ cells) that were associated with full *in vivo* protection.¹⁸ TFV-DP levels dropped 3 days after gel application to a median of 252 fmol/10⁶ cells (range, 196–295), and were associated with 74% efficacy against vaginal SHIV acquisition.¹⁸ A 1% TFV gel also protected macaques from rectal SIV infection, but protection was lost when administration of the gel was delayed 2 h after the SIV challenge, highlighting the limitations of NtRTIs alone for PEP use.¹⁹ By combining in a vaginal insert the highly potent NtRTI, TAF, with a later-stage INSTI, EVG, we aim to provide women a simplified option for protection that can be administered pre or post coitus with a flexible dosing window. Here we investigated the pharmacokinetics (PK) and efficacy of our TAF/EVG insert in macaques modelling on-demand topical HIV prevention in women. We conducted dose-ranging studies with inserts formulated with different fixed-dose concentrations of TAF/EVG for dose selection and subsequent investigation of PrEP and PEP efficacy using a macaque model of vaginal SHIV transmission.

Methods

Virus stock

SHIV_{162p3} (SIVmac239 backbone with an HIV-1 subtype B, CCR5-tropic envelope) was obtained from the National Institutes of Health AIDS Research and Reference Reagent Program and was propagated in peripheral blood mononuclear cells (PBMCs) from pigtailed macaques as previously described.²⁰ Individual 1 mL aliquots containing 50 TCID₅₀ were stored in liquid nitrogen and thawed on ice prior to each challenge.

Antiretroviral drugs and insert formulations

Vaginal inserts contained 3 different fixed-dose combinations of TAF/EVG: low-dose [10/8 mg (smaller sized insert)], mid-dose [20/16 mg (dose and size insert tested clinically)] and high-dose [40/24 mg (same size insert as mid-dose)]. The inserts contained 11.2, 22.4 and 44.8 mg of TAF fumarate salt form, which was equivalent to 10, 20 and 40 mg of TAF free base in low-, mid-, and high-dose inserts, respectively. The mid-dose and high-dose inserts weighed approximately 500 mg each compared to the smaller low-dose inserts (~250 mg). Inserts were manufactured by University of the Sciences (Philadelphia, PA) for CONRAD/EVMS (Norfolk, VA). TAF and EVG were obtained from Gilead Sciences Inc. (Foster City, CA) through material transfer agreements with CONRAD/EVMS.

Dose ranging pharmacokinetic studies

Twelve normal cycling female pigtailed macaques (median age (yr) and weight (kg) [12.5 and 8.6, respectively]) were split into three study groups of four animals per group. The number of animals per group were selected to account for potential variability in drug metabolism which may be due, but not limited, to differences age, weight, or menstrual cycles. Animals in group 1 (avg age/weight; 12.7 yr and 9.2 kg), 2 (avg age/weight; 11.6 yr and 9.7 kg), and 3 (avg age/weight; 14.6 yr and 8.4 kg) received the low-dose (10/8 mg), mid-dose (20/16 mg), and high-dose (40/24 mg) TAF/EVG inserts, respectively. A pediatric speculum and tweezers were used to gently place inserts in the posterior vagina at the fornix near the cervix. Vaginal biopsies were collected at 2 h, 4 h, or 24 h after insert application. Biopsy collections were staggered over several weeks (one time point per week) to minimize animal discomfort. Overall, macaques were dosed on five separate occasions over an 8-week period. The time points and number of specimens collected for each matrix (blood plasma, vaginal fluids, and vaginal pinch biopsies) are detailed in Fig. 1.

Efficacy of TAF/EVG inserts against vaginal SHIV infection in pigtail macaques

Based on the findings from the dose ranging studies, we selected TAF/EVG (20/16 mg) inserts to investigate protective efficacy against vaginal SHIV infection as PrEP or PEP. Protection was assessed in groups of cycling pigtail macaques repeatedly exposed to low doses of SHIV_{162p3} (50 TCID). Both weight and age were not significantly different among the placebo, PrEP and PEP groups ($P = 0.494$ for age, $P = 0.609$ for weight, by Kruskal–Wallis test). In PrEP studies, macaques received inserts 4 h before each vaginal challenge; 6 animals received TAF/EVG (avg age/weight; 11 yr and 8.9 kg) and 7 received a placebo insert (avg age/weight; 14 yr and 9.2 kg). Insert dosing and vaginal challenges were repeated once per week for up to 13 weeks. Blood was collected at the time of each SHIV challenge (4 h post dosing) to monitor for SHIV infection and drug levels.

After an appropriate drug washout period of 6 months, the 5 uninfected animals and 6 additional naive macaques were enrolled in a subsequent study to evaluate the efficacy of the inserts as PEP. Of the 5 uninfected animals in the PrEP study, 4 were used in the treatment and 1 in the placebo group. In this study design, inserts were administered 4 h after each weekly vaginal SHIV challenge; 6 macaques received TAF/EVG and 5 received placebo. Vaginal challenges and insert dosing were repeated once per week for up to 13 weeks. Both studies were done by the same personnel using the same virus stock, inoculum dose, and procedures. For PEP studies, blood was collected at 7 days post dose,

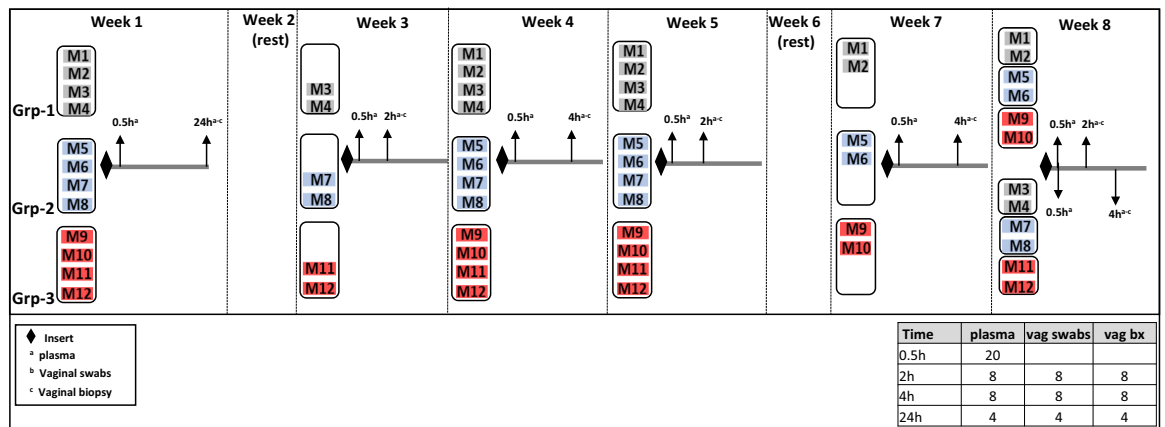


Fig. 1: Study design. Three groups of four macaques (n = 12 total; M1–12) received vaginal inserts containing a fixed-dose combination of TAF/ EVG at 10/8 mg (low-dose; M1–4), 20/16 mg (mid-dose; M5–8) and 40/24 mg (high-dose; M9–12). Dosing intervals, samples collected (plasma, vaginal swabs, and vaginal biopsies), collection time points, and frequency of collection are indicated in the table.

which represents a time point immediately prior to each SHIV challenge to monitor for SHIV infection and drug levels.

SHIV RNA in plasma was quantified using a real time-PCR method with a sensitivity of 50 RNA copies per milliliter.²¹ Serologic testing was done using a synthetic-peptide EIA (Genetic Systems HIV-1/HIV-2 plus O; Bio-Rad). The estimated time of infection was defined as 1 week prior to the first detection of SHIV RNA in plasma assuming a 1-week eclipse period between infection and detection of SHIV RNA.^{18,21} Animals were considered protected if they remained seronegative and negative for SHIV RNA during the 13 weekly virus challenges and a follow up period of 16 weeks.

Specimen collection

Whole blood (2 cc) was collected into K2EDTA-coated tubes for the isolation of plasma and PBMCs. Duplicate vaginal fluid samples were collected by inserting a pre-weighed swab (Fisherbrand™ Polyester-Tipped) vaginally (3–5 cm) and maintaining in place for 1–2 min to allow for absorption. All swabs were transferred to a collection tube, weighed, and immediately placed on ice until stored frozen at –70 °C. Vaginal tissue samples were collected using biopsy forceps with a 3.3 mm jaw (Radial Jaw™ 3 Biopsy Forceps, Boston Scientific or equivalent) at three sites, 0.5 cm (proximal), 1.5 cm (medial), and 3 cm (distal) from the cervix, with two pinches collected per site. Tissues were placed in a screwcap tube and maintained on ice. Vaginal biopsies were then blotted with sterile gauze to remove blood and mucus, weighed, and snap frozen. Biopsies from each site (proximal, medial, distal) were pooled, transferred to tared screwcap tubes, and the total weight was recorded.

The tubes were then snap-frozen on dry ice and stored at –80 °C.

Analysis of drug concentrations in plasma, vaginal fluids, and vaginal biopsies

The concentrations of EVG, TAF, and TFV were measured by HPLC—MS/MS as previously described [33]. Briefly, analytes were extracted from 0.1 mL of plasma, a single swab, or 0.25 mL of tissue homogenate by protein precipitation using 500 µL of methanol containing an internal standard [13C-labeled TFV, deuterium-labelled TAF (Moravex, Inc., Brea, CA), and deuterium-labelled EVG (EVG-d6) (Toronto Research Chemicals, Toronto, Canada)]. Drug concentrations measured in vaginal swabs were converted to ng/mL based on the net weight (assuming 1 µL = 1 mg). The lower limit of quantification (LLOQ) of the assays in plasma was 10 ng/mL for TFV and TAF and 5 ng/mL for EVG. The LLOQ of all 3 analytes in vaginal fluids was 10 ng/mL and 1 ng/mg in tissue homogenates. All calibration curves had r² values greater than 0.99.

Analysis of TFV-DP concentrations in vaginal biopsies and PBMCs

TFV-DP was measured in vaginal tissues and PBMCs as described previously.²² TFV-DP concentrations were measured with an automated online weak anion-exchange solid-phase extraction method coupled with ion-pair chromatography-MS/MS. Briefly, TFV-DP was extracted from PBMCs (~3 million; viability >80%) or tissue homogenates (~15 mg) by protein precipitation with 500 µL of 80% methanol containing 1000 ng of 13C-labeled TFV-DP as internal standards. PBMC samples with low yield (<1 million) or poor viability (<50%) were not tested for TFV-DP levels. Samples were extracted

overnight at -80°C , centrifuged, and dried to completion. Samples were then reconstituted in $100\ \mu\text{l}$ of $50\ \text{mM}$ ammonium acetate buffer (pH 7.0) and centrifuged at $17,000\ \text{g}$ to remove insoluble particulates. Calibration curves were generated from standards of TFV-DP spiked into 80% methanol over the range from 0.25 to $10\ \text{nM}$. The LLOQ for TFV-DP was $100\ \text{fmol/sample}$. All calibration curves had r^2 values greater than 0.99.

Statistics

Pharmacokinetic parameters were estimated using GraphPad Prism 9 and presented as median values. Parameters include maximum concentration (C_{max}), time of C_{max} (T_{max}), and area under the concentration–time curve from 2 to 24 h ($\text{AUC}_{2-24\ \text{h}}$). Analysis of infection rate between placebo controls were compared using Wilcoxon rank-sum exact test and the log-rank test was used to compare time to infection. For efficacy measurements, a group size of 6 animals per treatment group was used to compare differences between treated animals and controls. We performed a simulation study to determine the power of various study designs to detect a shift in the time (number of weekly challenges) to infection between the treatment and control groups of macaques given weekly challenges using the log-rank test. A key question we considered is whether increasing the number of macaques in each group meaningfully improves statistical power. Because we do not have a specific *a priori* value for the true shift, we considered a variety of scenarios. In each scenario, we assumed the number of macaques in control (n_1) and the treatment groups (n_2) to be equal. We set the mean (number of weekly challenges) to infection as $\mu_1 = 4, 5, 9, \text{ or } 10$ in the control group and $\mu_2 = 12 \text{ or } 13$ in the treatment group, giving a shift (difference) of $\delta = 2, 3, 4, 8, \text{ and } 9$ challenges to represent potential real scenarios. We set the common standard deviation in the number of weekly challenges to infection to $\sigma_1 = \sigma_2 = 1, 2, 4, \text{ and } 5$. In each simulated trial, we generated random normal data from the above means and standard deviations and rounded each number up to the nearest positive integer, corresponding to the way data are observed in whole weeks. We simulated 10,000 data sets for each scenario, computed log-rank test p-value for each, then compared survival times of the treatment and control groups. Next, we computed the average rejection rate at the 0.05 significance level for each scenario to obtain the simulated power. [Supplemental Table S1](#) displays the sample size and power calculations for the different combinations of the mean number of weekly challenges for each group (μ_i), the difference between the two group means (δ), the standard deviation of challenges within each group (σ_i), and the sample size of each group (n_i). In most of the recent studies carried out by our group, we observed that the shift between the treatment and control groups were much

larger than 4 but standard deviations for both groups were small. This indicates that we had $>80\%$ power for most scenarios even when we had 6 macaques in both treatment and control groups. For some extreme scenarios, we might have less than 80% power with small sample size ([supplemental Table S1](#)).

Infection probabilities in treated and placebo controls were compared with Fisher's exact test using SAS Version 9.4. A Kaplan–Meier graph of survival and log-rank test were used to compare the survival distribution between animals receiving TAF/EVG and placebo inserts. SHIV RNA detection was used as the censoring variable for the survival distributions. Hazard ratios were calculated to investigate differences over time between the probability of infection in the TAF/EVG treatment groups compared to the probability of infection in the placebo group over time. Efficacy was calculated as 1-relative risk of infection. A 95% confidence interval was also calculated around efficacy calculations using an exact method due to small sample sizes. Kruskal–Wallis tests were used to determine if there were any differences in weight (kg) and age (months) between the three groups (placebo, PrEP, and PEP).

Ethics

All animal procedures were conducted at the Centers for Disease Control and Prevention (CDC) and performed under approved Institutional Animal Care and Use Committee (IACUC) (protocols 3000- and 3002-DOBMONC). Sexually mature female pigtailed macaques (*Macaca nemestrina*) were utilized for this study. Macaques were cared for by CDC veterinarians in compliance with the Guide for the Care and Use of Laboratory Animals 8th Ed. All procedures were performed under anesthesia ($10\ \text{mg/kg}$ ketamine or $2\text{--}6\ \text{mg/kg}$ telazol; intramuscular) and all efforts were made to minimize suffering, improve housing conditions, and provide enrichment opportunities. Animals had access to water at all times and were fed a commercial diet specifically formulated to meet all dietary requirements. Compatible macaques were pair-housed and animals that were single-housed were separated with grated dividers that, at minimum, allowed visual contact.

Role of funding source

Work at CDC was funded by intramural funds and an Interagency Agreement between USAID and the Centers for Disease Control and Prevention (USAID/CDC IAA AID-GH-T-15-00002). Work related to the insert formulation was funded by U.S. PEPFAR through USAID under a Cooperative Agreement (AID-OAA-A-14-00010) with CONRAD/Eastern Virginia Medical School. The funders of this study had no role in study design, data collection, data analysis, interpretation, or writing of the manuscript.

Results

PK profile of TAF/EVG vaginal insert (dose-ranging study)

To determine tissue and fluid concentrations as well as dose proportionality, we investigated the PK profile of inserts formulated with three different fixed-dose combinations of TAF and EVG (10/8 mg, 20/16 mg, 40/24 mg, respectively) in 3 groups of pigtailed macaques (Fig. 1). The median concentrations of EVG, TAF, TFV, and TFV-DP in plasma, vaginal fluids, or vaginal tissues over a 24 h period are shown in Supplementary Table S2 and the corresponding concentration-versus-time curves are presented in Figs. 2 and 3. Overall, the frequency of drug detection in plasma was low. Of the total plasma specimens collected over the 24 h period post-dosing, only 4.3% (4/92), 7.6% (7/92), and 14.1% (13/92) had measurable EVG, TAF, or TFV, respectively. With the exception of TFV at the 4 h time point in group 2 animals that received the mid-dose TAF/EVG (20/16 mg) inserts, the median EVG, TAF, and TFV levels in plasma

were below the limit of quantification (BLOQ) across all dosage groups and time points (Fig. 2a).

The PK parameters (T_{max} , C_{max} and AUC_{2-24h}) for each drug in vaginal fluids were similar across groups and showed no clear dose proportionality (Fig. 2b and Table 1). The median concentrations of TFV in vaginal fluids in all 3 dosage groups were high at 2 h (90, 133, and 98 $\mu\text{g}/\text{mL}$) and remained more than 2 logs above the TFV *in vitro* IC_{50} (0.516 $\mu\text{g}/\text{mL}$) at 24 h post dosing (155, 158, and 140 $\mu\text{g}/\text{mL}$, respectively). TAF concentrations in vaginal fluids were also high at 2 h (161, 217, and 433 $\mu\text{g}/\text{mL}$, respectively) but precipitously declined by more than 1-log by 4 h (2.1, 11.2, and 13.8 $\mu\text{g}/\text{mL}$, respectively), suggesting rapid conversion of TAF to TFV (Fig. 2b). Consistent with rapid disintegration and drug release properties of this insert formulation, EVG also reached peak levels in vaginal fluids within 2 h (453, 281, and 835 $\mu\text{g}/\text{mL}$, respectively) and remained about 3 orders of magnitude above the *in vitro* EVG protein-adjusted IC_{95} (45 ng/mL^{23}) at 24 h post dosing (53, 45, and 126 $\mu\text{g}/\text{mL}$, respectively).

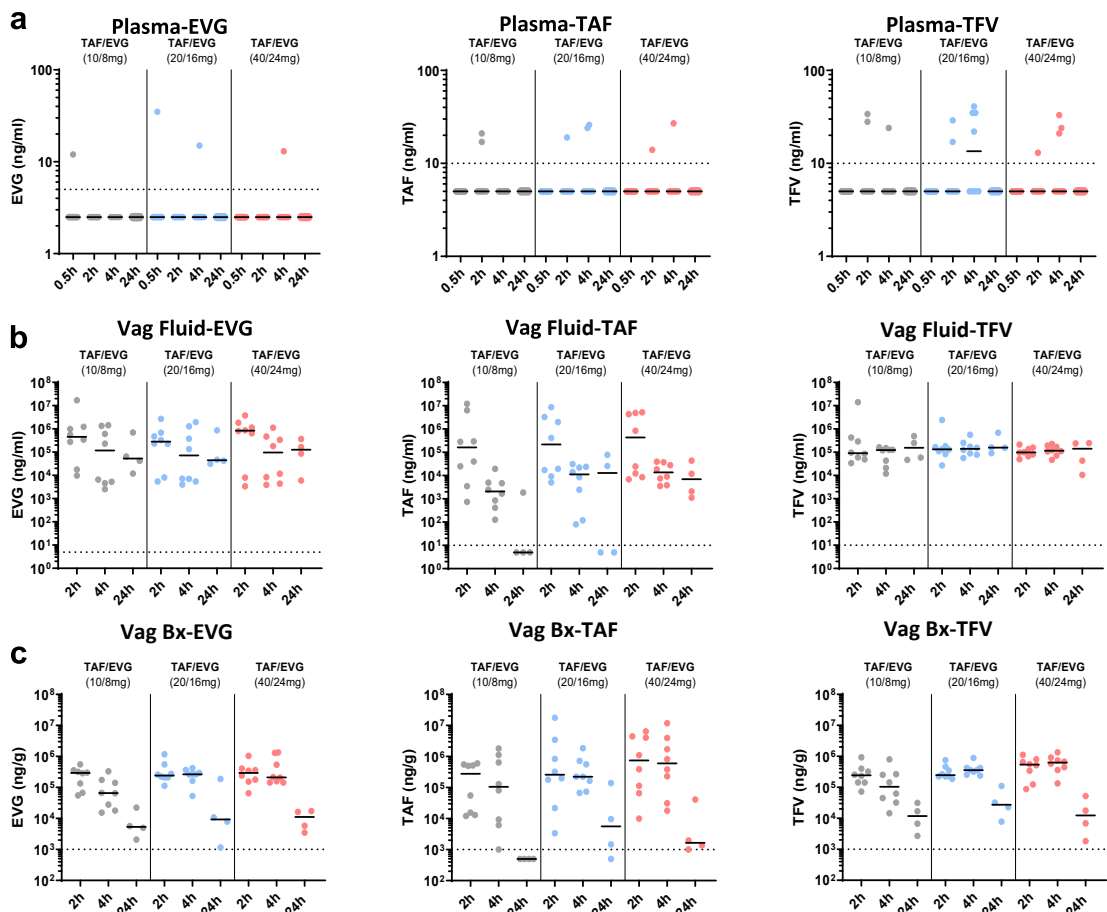


Fig. 2: Pharmacokinetic profiles of EVG, TAF, and TFV in plasma, vaginal fluids and vaginal tissues after vaginal administration of TAF/ EVG inserts. Scatter-plot analysis of drug concentrations in plasma (a), vaginal fluids (b), and vaginal biopsies (c) over time. Solid line indicates the median and dashed line the LOQ.

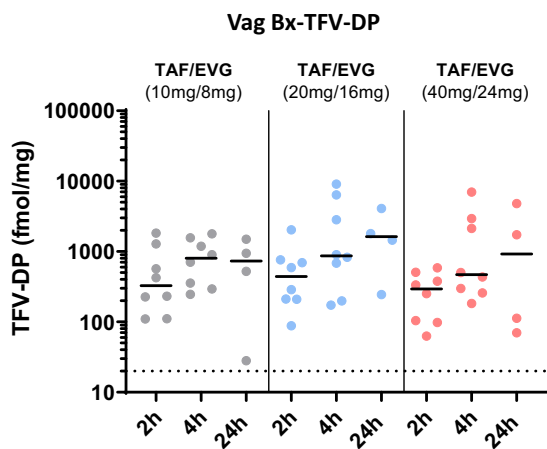


Fig. 3: Pharmacokinetic profiles of TFV-DP in vaginal tissues following vaginal administration of TAF/EVG inserts. Vaginal biopsies were collected at 2, 4, and 24 h post insert dosing. Solid line indicates the median and dashed line the LOQ.

In vaginal biopsies, peak EVG levels were detected at 2 h and peak TAF and TFV levels at 2–4 h, demonstrating rapid tissue drug penetration. The C_{max} values for EVG in vaginal tissues were similar across groups with inserts containing 8, 16, or 24 mg of EVG (294, 264, and 292 ng/mg, respectively) (Table 2). The median C_{max} concentrations of TAF and TFV in vaginal tissues were also similar in groups with inserts containing 10, 20, or 40 mg TAF, albeit a trend for higher levels in the high-dose group [(TAF = 277, 260, and 747 ng/mg) and (TFV = 245, 359, 625 ng/mg), respectively]. In contrast to vaginal fluids, analysis of $AUC_{2-24 h}$ values in vaginal tissues showed semi-dose proportionality between the low-, mid- and high-dose groups for TAF (1443, 2755, 7386 ng·h/mg, respectively) and TFV (1503, 4465, 7537 ng h/mL, respectively) but not for EVG, which yielded the highest AUC values with the mid-dose inserts (1072, 3246, and 2719 ng·h/mg) (Table 2).

We also measured the pharmacologically active metabolite, TFV-DP, in vaginal tissues. TFV-DP levels in vaginal tissues peaked at 4 h with the insert containing 10 mg of TAF (803 fmol/mg of tissue), and at 24 h with inserts containing 20 and 40 mg of TAF (1627 and 925 fmol/mg of tissue, respectively) (Fig. 3 and Supplementary Table S2). The $AUC_{2-24 h}$ values for TFV-DP in vaginal tissues did not significantly increase with dose, and were highest with the mid-dose inserts. Overall, the PK profiles in plasma, vaginal fluids, and tissues show that increases in doses of TAF (10–40 mg) and EVG (8–24 mg) generally did not translate into dose dependent increases in drug concentrations. However, vaginal tissue concentrations (AUCs) were highest for EVG and TFV-DP with the mid-dose TAF/EVG (20/16 mg) inserts leading to selection of these inserts, which also appear to show less data dispersion, to assess *in vivo* efficacy. Moreover, from a PK perspective, the high-dose inserts did not appear to show clear advantages.

Efficacy of inserts as PrEP or PEP

We evaluated the efficacy of the mid-dose TAF/EVG (20/16 mg) inserts (Fig. 4a) as PrEP or PEP by exposing macaques to SHIV 4 h before or 4 h after insert application. The study design included 12 control macaques that received placebo inserts; 7 were used in the PrEP arm and 5 in the PEP arm (Fig. 4b). Overall, 6 of the 7 placebo treated animals in the PrEP arm and 4 of the 5 in the PEP arm were infected with SHIV after a median (range) of 3 (2–13) and 4 (2–13) challenges, respectively. Analysis of infection outcome between the two groups of placebo controls showed no difference in infection rate by number of challenges ($p = 0.6174$; [Wilcoxon rank-sum exact test]) or time to infection ($p = 1.0$; [Log-rank test]). We thus combined all animals in the two placebo groups in our analysis of PrEP and PEP efficacy in order to increase sample size in the control group.

Analyte	T_{max} (h)	C_{max} ($\mu\text{g/mL}$)	$AUC_{2-24 h}$ ($\mu\text{g}\cdot\text{h/mL}$)
EVG			
TAF/EVG (10 mg/8 mg)	2	453 (10–16,992)	2285
TAF/EVG (20 mg/16 mg)	2	281 (6–2704)	1514
TAF/EVG (40 mg/24 mg)	2	835 (3–3750)	3161
TAF			
TAF/EVG (10 mg/8 mg)	2	161 (1–12,087)	184
TAF/EVG (20 mg/16 mg)	2	217 (5–8630)	469
TAF/EVG (40 mg/24 mg)	2	433 (7–5220)	655
TFV			
TAF/EVG (10 mg/8 mg)	24	155 (47–490)	2990
TAF/EVG (20 mg/16 mg)	24	158 (91–689)	3251
TAF/EVG (40 mg/24 mg)	24	140 (11–244)	2780

Table 1: Pharmacokinetic summary in vaginal fluids following vaginal administration of TAF/EVG inserts.

Analyte	T _{max} (h)	C _{max} (ng/mg) or (fmol/mg)	AUC _{2-24 h} (ng-h/mL) or (fmol-h/mg)
EVG			
TAF/EVG (10 mg/8 mg)	2	294 (55-553)	1072
TAF/EVG (20 mg/16 mg)	4	264 (53-419)	3246
TAF/EVG (40 mg/24 mg)	2	292 (65-1046)	2719
TAF			
TAF/EVG (10 mg/8 mg)	2	277 (12-600)	1443
TAF/EVG (20 mg/16 mg)	2	260 (3-17,642)	2755
TAF/EVG (40 mg/24 mg)	2	747 (10-6416)	7386
TFV			
TAF/EVG (10 mg/8 mg)	2	245.4 (73-925)	1503
TAF/EVG (20 mg/16 mg)	4	358.6 (239-888)	4465
TAF/EVG (40 mg/24 mg)	4	625 (134-1343)	7537
TFV-DP			
TAF/EVG (10 mg/8 mg)	4	803 (246-1780)	16,511
TAF/EVG (20 mg/16 mg)	24	1627 (245-4091)	26,253
TAF/EVG (40 mg/24 mg)	24	925 (70-4789)	14,703

Table 2: Pharmacokinetic summary in vaginal tissues following vaginal administration of TAF/EVG inserts.

Five of the 6 animals that received TAF/EVG inserts 4 h before SHIV challenge remained uninfected after 13 weekly SHIV exposures and the 16 week no-drug washout period (Fig. 4c). The proportion of infected macaques was lower than the proportion among the placebo treated control group (1/6 compared to 10/12

controls; p = 0.0088; Fisher’s exact test). The estimated efficacy of TAF/EVG as PrEP was 91.1% (95% CI = 32.2%–98.8%). Survival analysis demonstrated at least an 8.7-fold reduction in risk of infection in macaques receiving TAF/EVG compared to placebo inserts (p = 0.0410; [log-rank test]). Fig. 4c also shows that all 6 macaques that received TAF/EVG inserts as PEP 4 h after SHIV exposure remained uninfected after 13 challenges and the 16 week no-drug washout period, resulting in an estimated efficacy of 100% (95% CI = undefined). Fig. 4d shows the SHIV RNA levels during acute infection in the one PrEP breakthrough, demonstrating that viral load levels were within the range of those observed in the placebo treated animals, albeit in most timepoints lower than the median in these animals.

We also defined the concentrations of TFV-DP in PBMCs in PrEP treated animals at the time of each SHIV challenge, which represent a 4 h time point after insert application. Fig. 5a shows high frequency of detection of TFV-DP in PBMCs in 4 of the 6 animals with median (range) levels between individual animals that were 287.5 (23.5–487) fmol/10⁶ cells. The concentrations of TFV-DP in PBMCs in the two remaining animals, including the PrEP breakthrough infection (BB127), were low or undetectable. Assessment of drug levels in corresponding plasma specimens from all treated animals showed lack of detectable EVG and TAF. In contrast, TFV was detected in some plasma

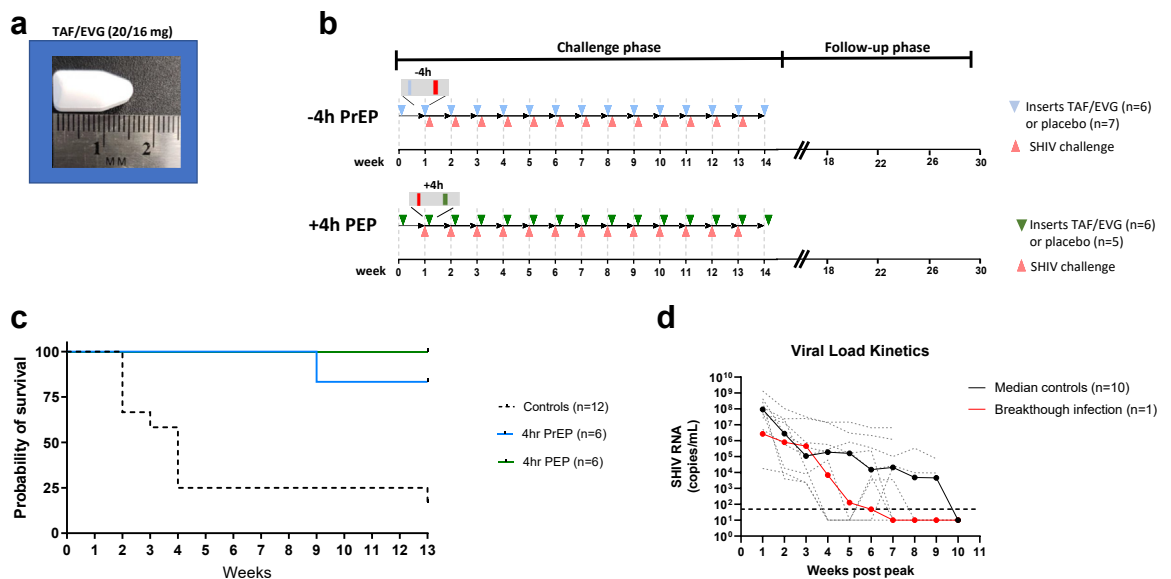


Fig. 4: PrEP and PEP efficacy of TAF/EVG inserts. (a) Image of TAF/EVG (20/16 mg) insert. (b) Study design. Macaques received placebo or TAF/EVG inserts 4 h before (–4 h PrEP) or 4 h after (+4 h PEP) vaginal SHIV challenge. To maintain consistency between challenges, animals received an insert dose one week before and one week after the challenge period (c) Efficacy of TAF/EVG inserts in preventing vaginal SHIV infection. Survival curves show the cumulative percentage of uninfected macaques as a function of the number of weekly vaginal SHIV exposures. Macaques received placebo (n = 12) or TAF/EVG inserts 4 h before (n = 6) or 4 h after (n = 6) SHIV exposure. (d) Plasma SHIV RNA levels in the TAF/EVG breakthrough infection and in untreated controls. The dotted lines represent the viral loads in individual control animals and solid black line the median values. The macaque infected with SHIV during TAF/EVG PrEP is shown in red.

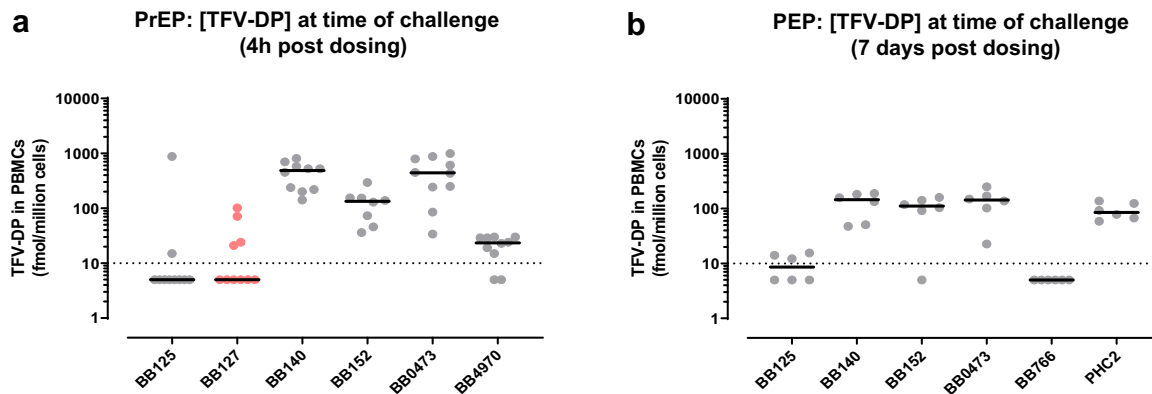


Fig. 5: Intracellular TFV-DP in macaque PBMCs at time of SHIV challenge. Concentrations of TFV-DP in PBMCs from individual macaques collected at time of SHIV challenge. (a) TFV-DP levels in animals in PrEP arm collected 4 h post dosing. Shaded red circles indicate TFV-DP concentrations in the infected animal. (b) TFV-DP levels in animals in PEP arm collected at baseline (7 days post dosing). Solid and dotted line indicates the median and LOQ for TFV-DP, respectively.

specimens from 2/6 animals; 24 [BLOQ-38] ng/mL in animal BB140 and 28 [BLOQ-56] ng/mL in animal BB0473 (Supplementary Table S3).

In the case of the PEP study, TFV-DP levels were measured in PBMCs collected between weeks 1 and 6. These samples were collected at the time of SHIV challenge which by design reflects residual drug concentrations from the previous insert administered 7 days earlier. Overall, 72% (26/36) of the samples had residual TFV-DP in PBMCs from the previous insert application (Fig. 5b). TFV-DP levels were consistently detected in 4 of the 6 animals with median levels that ranged between 85.3 and 146.7 fmol/ 10^6 cells. In the remaining 2 animals, TFV-DP was not detected (BB766) or was detected at low frequency and levels (BB125; median = 6.1 fmol/ 10^6 cells [range: BLOQ-15.5]). As expected, none of the corresponding plasma specimens had detectable EVG, TAF or TFV (Supplementary Table S4).

Discussion

On-demand PrEP and PEP with TAF/EVG inserts can be a discreet and user-friendly form of topical prophylaxis against vaginal transmission of HIV and HSV in women. Using a macaque model, we showed that inserts containing a combination of TAF and EVG rapidly distributed drugs in vaginal tissues, with EVG levels that were 2–3 orders of magnitude above concentrations needed for *in vitro* inhibition and TFV-DP concentrations within known protective levels in macaques and humans.^{18,24} We demonstrated high protection and efficacy against vaginal SHIV infection under PrEP and PEP modalities with estimates ranging from 91% to 100%, respectively. A Phase I study in women (NCT03762772) using these same inserts has recently showed that TAF/EVG inserts are safe and acceptable,

and significantly increase antiviral activity in cervicovaginal fluid.²⁵ Our PK and efficacy results are in agreement and support further clinical advancement of TAF/EVG inserts for on-demand PrEP and PEP in women.

The addition of the integrase inhibitor EVG to the NtRTI TAF was designed to boost PEP efficacy and to address limitations seen in previous macaque studies with single-agent TFV gel losing efficacy when administered rectally in as little as 2 h after SIV challenge.¹⁹ The finding of complete PEP protection with TAF/EVG inserts suggests an added value of EVG in the formulation and expands the potential application of this product for on-demand PEP against sexual HIV acquisition.

The dose ranging PK studies with three different TAF/EVG insert formulations containing increasing doses of TAF/EVG showed high and sustained levels of active drugs in fluids and tissues, with little or no dose proportionality. The lack of dose proportionality is consistent with findings reported in macaques dosed vaginally with escalating doses of TFV gel and may be due to relatively small differences in dosages or drug saturation in the vaginal compartment.^{26,27} TFV-DP and EVG concentrations in vaginal tissues were orders of magnitude higher than those required to inhibit the virus with all doses, but comparing the AUC and C_{max}, the mid-dose insert stood out as delivering actives in higher amounts and more consistently. We therefore selected this insert dose to assess efficacy in validated macaque models of vaginal transmission. The same insert formulation and dose was also evaluated in two Phase I clinical studies following vaginal and rectal administration.^{11,25,28}

TAF/EVG (20/16 mg) inserts showed TFV-DP levels starting at a median of 440 fmol/mg in vaginal tissues at 2 h and reaching a median of 1627 fmol/mg at 24 h post dosing, levels that have been associated with a high

degree of protection (>75%).¹⁸ Although correlates of protection with topical TFV are well established *in vivo*, less is known about concentrations of EVG that are needed for vaginal protection. The median EVG concentration in vaginal tissue 4 h after dosing was 264 ng/mg, which is ~6 times higher than the clinically relevant protein-adjusted IC₉₅ (45 ng/mL²³). Furthermore, EVG and TFV have been shown to display synergistic antiviral activity.²⁹ The PEP protection seen in our study suggests that EVG concentrations achieved *in vivo* with inserts containing 16 mg of EVG may be sufficient to block HIV integration. These results are also in line with early studies with the INSTI raltegravir that showed high level PEP protection associated with similar drug concentrations in vaginal tissues.^{16,17}

The detection of TFV-DP in PBMCs from 4 of the 6 PrEP treated animals was unexpected and to our knowledge has not been previously observed with other topical TFV or TDF products.³⁰ We noted a transient detection of TFV, and to lesser degree TAF, in plasma within 4 h suggesting that plasma TFV or TAF might be the main source of the intracellular TFV-DP in PBMCs. Interestingly, median levels of TFV-DP in PBMCs in the 4 animals exceeded those observed with an oral 1.5 mg/kg macaque TAF dose that is equivalent to 25 mg of TAF in humans (~288 fmol/10⁶ cells compared to 137 fmol/10⁶ cells with oral TAF).³¹ Assuming the average weight of a female pigtail macaque is ~7 kg, then the 1.5 mg/kg oral dose contains about 10 mg of TAF, which is half the TAF dose formulated in topical TAF/EVG (20/16 mg) inserts. Also surprising, the median TFV-DP concentration in PBMCs 7 days after a single insert dose was similar to that observed in PBMCs after a single oral dose of TAF/FTC in women, significantly exceeding levels seen with a single oral dose of TDF/FTC.³² This observation points to a very efficient and rapid absorption of TAF when administered through the vaginal mucosa. It will be important to investigate if the high TFV-DP concentrations detected in macaque PBMCs translate to humans given the approximately 12-fold difference in total blood volume, which may dilute TFV or TAF in plasma if drug absorption from the vaginal mucosa is similar in both species.³³

It seems desirable for a topical product to provide some systemic drug coverage that could avert infection in case virus is actively transported or penetrates across the female genital tract to other tissues. In rhesus macaques infected with SIV, viral RNA can be detected in axillary lymph nodes as early as 24 h post inoculation, suggesting that viral particles may rapidly gain access to systemic lymphoid tissues.³⁴ In this scenario, systemic drug distribution might play a secondary role by protecting other potential sites from initial infection and virus dissemination. It is tempting to hypothesize that the low frequency of detection of TFV-DP in PBMCs in the TAF/EVG breakthrough infection could have played a role in the PrEP failure in this animal. Alternatively,

the lower systemic exposures in this animal may reflect lower drug absorption and dosing of vaginal tissues following insert application, which may have contributed to the breakthrough infection.

Our study has some potential limitations. Although the use of human sized inserts was justified by the need to evaluate a clinically relevant product, it also raises some questions about drug distribution in tissues given the differences in surface area between the macaque and human vaginal cavities. We noted that the concentrations of EVG, TAF and TFV in vaginal tissues were higher in macaques than in humans at 4 h but more similar at 24 h, suggesting that differences in surface area may alter the kinetics of drug absorption.²⁵ Notably, higher concentrations of TAF and TFV in macaque tissues relative to humans did not result in higher tissue TFV-DP concentrations,²⁵ suggesting similar drug activity in both species. A second potential limitation of our study is the use of a macaque model that does not include seminal fluid or co-infection with STIs that might increase inflammation and cell activation state and thus potentially reduce drug activity. However, recent studies in macaques co-infected with *Chlamydia trachomatis* (CT) and *Trichomonas vaginalis* (TV) and administered vaginal TFV gel showed higher TFV absorption and TFV-DP concentrations in vaginal tissues compared to STI-naïve animals.³⁵ More importantly, efficacy of TFV gel was maintained in animals co-infected with STIs suggesting that the pharmacokinetic shift in drug permeability in the presence of STIs was sufficient to overcome the increased susceptibility to SHIV infection associated with co-infection with other STIs.³⁵ It will be important to investigate if these co-infections or other STIs will result in a similar pharmacokinetic shift with TAF/EVG inserts and if the high efficacy of TAF/EVG inserts is maintained in the presence of STIs. Regarding HSV-2, the TAF/EVG inserts show antiviral activity both *in vitro* and *in vivo*²³, confirming clinically observed HSV-2 protection in women^{36,37} and providing the foundation for an on-demand, pre- and post-exposure, multipurpose prevention technology.

There are several other limitations related to our experimental design. Although inserts were administered once a week, the study did not model the efficacy of a weekly dosing schedule given that SHIV challenges were only done within 4 h of product application, which aligns with an on-demand product use. It is for instance unknown if TAF/EVG inserts would also be effective against exposures occurring several days after product use as might be the case with a weekly dosing schedule. Also, long-lasting levels of TFV-DP in PBMC and possibly tissues might have had an impact on the efficacy of subsequent weekly doses, however the design of the study does not allow us to verify or rule this out. We detected some residual TFV-DP in PBMCs at the time of virus challenge in 72% (26/36) of the samples from PEP treated animals. This residual TFV-DP originated from inserts administered 7 days prior and could have

potentially contributed to some of the protection seen in the PEP-treated and possibly some of the PrEP treated animals. However, it is important to note that protection was observed in all PEP-treated animals including those that did not have residual TFV-DP consistently detected. Additional SHIV challenge experiments that incorporate drug dosing and virus exposures spaced more than a week apart would help to address this question.

Vaginal inserts provide a convenient dosage form that is well suited for on-demand prophylaxis, particularly for women who may engage in episodic sex and do not wish to take daily PrEP or desire a more user-controlled option. In this study we show the potential of TAF/EVG inserts for single-dose, on-demand PrEP or PEP using a stringent macaque model of repeated vaginal exposures to mimic women at high risk of HIV infection. Together with encouraging results from Phase I studies, our findings support the clinical advancement of this product for on-demand prophylaxis against HIV acquisition in women.

Contributors

C.W.D.: study design, data acquisition, analysis, verification, interpretation of results, and manuscript preparation. M.M.P.: study design, drug product supplies and coordination, interpretation of results, manuscript preparation. K.N.: data acquisition, verification, and analysis. A.H.: data acquisition and analysis. C.D.: data acquisition and analysis. J.M.: animal tech procedures. G.K.: statistical analysis. Y.P.: statistical analysis. O.N.S.: formulation development, drug product supplies, manuscript review. T.J.M.: drug product supplies. V.A.: drug product supplies, interpretation of results, manuscript review. P.G.: drug product supplies. S.J.: drug product supplies. W.H.: study design, data acquisition and analysis, interpretation of results, and manuscript preparation. M.R.C.: study design, interpretation of results, and manuscript preparation. J.G.G.-L.: study design, data acquisition and analysis, interpretation of results, and manuscript preparation. G.F.D.: study design, interpretation of results, and manuscript preparation. All authors read and approved the final version of the manuscript.

Data sharing statement

Access to data generated and analyzed in this study will be provided upon reasonable request to the corresponding author.

Declaration of interests

J.G.G.-L. and W.H. are named in US Government (USG) patents on “Inhibition of HIV infection through chemoprophylaxis” and in US Government patent applications on “HIV postexposure prophylaxis” and “HIV pre-exposure prophylaxis”. W.H. and J.G.G.-L. report royalties or licenses from Mylan, Laurus Generics, TAD Pharma, and CIPLA Limited. M.M.P., O.N.S., T.J.C., V.A., P.G., S.J., G.F.D., and M.R.C. are named in patent applications on “Pharmaceutical compositions and methods of making on demand solid dosage formulations,” inventions that were developed under USAID-funded cooperative agreements. C.W.D., K.N., A.H., C.D., J.M., G.K., and Y.P. report no conflicts. The findings and conclusions of this manuscript are those of the authors and do not necessarily represent the official views of CDC, USAID, PEPFAR, EVMS, or the USG.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.ebiom.2022.104361>.

References

- UNAIDS. UNAIDS data 2021. 2021.
- Karim SSA, Baxter C. HIV incidence rates in adolescent girls and young women in sub-Saharan Africa. *Lancet Glob Health*. 2019;7(11):e1470–e1471.
- Baeten JM, Donnell D, Ndase P, et al. Antiretroviral prophylaxis for HIV prevention in heterosexual men and women. *N Engl J Med*. 2012;367:399–410.
- Thigpen MC, Kebaabetswe PM, Paxton LA, et al. Antiretroviral preexposure prophylaxis for heterosexual HIV transmission in Botswana. *N Engl J Med*. 2012;367:423–434.
- Van Damme L, Corneli A, Ahmed K, et al. Preexposure prophylaxis for HIV infection among African women. *N Engl J Med*. 2012;367(5):411–422.
- Marrazzo JM, Ramjee G, Richardson BA, et al. Tenofovir-based preexposure prophylaxis for HIV infection among African women. *N Engl J Med*. 2015;372(6):509–518.
- Delany-Moretwe S, Hughes JP, Bock P, et al. Cabotegravir for the prevention of HIV-1 in women: results from HPTN 084, a phase 3, randomised clinical trial. *Lancet*. 2022;399(10337):1779–1789.
- Baeten JM, Palanee Phillips T, Brown ER, et al. Use of a vaginal ring containing dapivirine for HIV-1 prevention in women. *N Engl J Med*. 2016;375(22):2121–2132.
- Nel A, van Niekerk N, Kapiga S, et al. Safety and efficacy of a dapivirine vaginal ring for HIV prevention in women. *N Engl J Med*. 2016;375(22):2133–2143.
- Molina JM, Capitán C, Spire B, et al. On-demand preexposure prophylaxis in men at high risk for HIV-1 infection. *N Engl J Med*. 2015;373(23):2237–2246.
- Peet MM, Agrahari V, Anderson SM, et al. Topical inserts: a versatile delivery form for HIV prevention. *Pharmaceutics*. 2019;11(8):374.
- Palanee-Phillips T, Baeten JM. Topical delivery of long-acting antiretrovirals to prevent HIV acquisition. *Curr Opin HIV AIDS*. 2020;15(1):42–48.
- Hussain A, Ahsan F. The vagina as a route for systemic drug delivery. *J Control Release*. 2005;103(2):301–313.
- Callebaut C, Stepan G, Tian Y, Miller MD. In vitro virology profile of tenofovir alafenamide, a novel oral prodrug of tenofovir with improved antiviral activity compared to that of tenofovir disoproxil fumarate. *Antimicrob Agents Chemother*. 2015;59(10):5909–5916.
- Andreï G, Lisco A, Vanpouille C, et al. Topical tenofovir, a microbicide effective against HIV, inhibits herpes simplex virus-2 replication. *Cell Host Microbe*. 2011;10(4):379–389.
- Dobard C, Sharma S, Parikh UM, et al. Postexposure protection of macaques from vaginal SHIV infection by topical integrase inhibitors. *Sci Transl Med*. 2014;6(227):227ra35.
- Nishiura K, Sharma S, Sterling M, et al. Pharmacokinetics of vaginally applied integrase inhibitors in macaques. *J Antimicrob Chemother*. 2021;76(11):2894–2901.
- Dobard C, Sharma S, Martin A, et al. Durable protection from vaginal simian-human immunodeficiency virus infection in macaques by tenofovir gel and its relationship to drug levels in tissue. *J Virol*. 2012;86:718–725.
- Cranage M, Sharpe S, Herrera C, et al. Prevention of SIV rectal transmission and priming of T cell responses in macaques after local pre-exposure application of tenofovir gel. *PLoS Med*. 2008;5(8):e157. discussion e.
- Radzio J, Aung W, Holder A, et al. Prevention of vaginal SHIV transmission in macaques by a coitally-dependent Truvada regimen. *PLoS One*. 2012;7(12):e50632.
- García-Lerma JG, Otten RA, Qari SH, et al. Prevention of rectal SHIV transmission in macaques by daily or intermittent prophylaxis with emtricitabine and tenofovir. *PLoS Med*. 2008;5:e28.

- 22 Kuklenyik Z, Martin A, Pau CP, et al. On-line coupling of anion exchange and ion pair chromatography for measurement of intracellular triphosphate metabolites of reverse transcriptase inhibitors. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2009;877:3659–3666.
- 23 Ramanathan S, Mathias AA, German P, Kearney BP. Clinical pharmacokinetic and pharmacodynamic profile of the HIV integrase inhibitor elvitegravir. *Clin Pharmacokinet.* 2011;50(4):229–244.
- 24 Schwartz JL, Rountree W, Kashuba AD, et al. A multi-compartment, single and multiple dose pharmacokinetic study of the vaginal candidate microbicide 1% tenofovir gel. *PLoS One.* 2011;6(10):e25974.
- 25 Andrea T. *Multipurpose prevention technologies (MPTs) for prevention of HIV and pregnancy.* HIV R4P; 2021:2021.
- 26 Hummel J, McKendrick S, Brindley C, French R. Exploratory assessment of dose proportionality: review of current approaches and proposal for a practical criterion. *Pharm Stat.* 2009;8(1):38–49.
- 27 Nuttall J, Kashuba A, Wang R, et al. Pharmacokinetics of tenofovir following intravaginal and intrarectal administration of tenofovir gel to rhesus macaques. *Antimicrob Agents Chemother.* 2012;56:103–109.
- 28 Thurman A, Ouattara AL, Yousefieh N, et al. *A phase I study to assess safety, pharmacokinetics and pharmacodynamics of a topical multipurpose prevention insert containing tenofovir alafenamide fumarate and elvitegravir dosed vaginally.* HIV Research for Prevention (HIVR4P); 2021:OA06.05.
- 29 Kulkarni R, Hluhanich R, McColl DM, Miller MD, White KL. The combined anti-HIV-1 activities of emtricitabine and tenofovir plus the integrase inhibitor elvitegravir or raltegravir show high levels of synergy in vitro. *Antimicrob Agents Chemother.* 2014;58(10):6145–6150.
- 30 Dobard CW, Makarova N, West-Deadwyler R, et al. Efficacy of vaginally administered gel containing emtricitabine and tenofovir against repeated rectal simian human immunodeficiency virus exposures in macaques. *J Infect Dis.* 2018;218(8):1284–1290.
- 31 Massud I, Cong ME, Ruone S, et al. Efficacy of oral tenofovir alafenamide/emtricitabine combination or single-agent tenofovir alafenamide against vaginal simian human immunodeficiency virus infection in macaques. *J Infect Dis.* 2019;220(11):1826–1833.
- 32 Thurman AR, Schwartz JL, Cottrell ML, et al. Safety and pharmacokinetics of a tenofovir alafenamide fumarate-emtricitabine based oral antiretroviral regimen for prevention of HIV acquisition in women: a randomized controlled trial. *EClinicalMedicine.* 2021;36:100893.
- 33 Salvato MS, Rater M, Pauza CD. Tracking of dye-labeled lymphocytes in rhesus macaques. *J Med Primatol.* 1996;25(2):112–121.
- 34 Miller CJ, Li Q, Abel K, et al. Propagation and dissemination of infection after vaginal transmission of simian immunodeficiency virus. *J Virol.* 2005;79:9217–9227.
- 35 Makarova N, Henning T, Taylor A, et al. Topical tenofovir protects against vaginal simian HIV infection in macaques coinfecting with *Chlamydia trachomatis* and *Trichomonas vaginalis*. *AIDS.* 2017;31(6):745–752.
- 36 Abdool Karim SS, Abdool Karim Q, Kharsany AB, et al. Tenofovir gel for the prevention of herpes simplex virus type 2 infection. *N Engl J Med.* 2015;373(6):530–539.
- 37 Abdool Karim SS, Abdool Karim Q, Kharsany AB, et al. Tenofovir gel for prevention of herpes simplex virus type 2 acquisition: findings from the VOICE trial. *J Infect Dis.* 2019;219(12):1940–1947.