



## Research paper

# Single oral dose for HIV pre or post-exposure prophylaxis: user desirability and biological efficacy in macaques



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## ABSTRACT

**Background:** Daily oral pre- or post-exposure prophylaxis (PrEP or PEP) is highly effective in preventing HIV infection. However, many people find it challenging to adhere to a daily oral regimen. Chemoprophylaxis with single oral doses of antiretroviral drugs taken before or after sex may better adapt to changing or unanticipated sexual practices and be a desirable alternative to daily PrEP or PEP. We investigated willingness to use a single oral pill before or after sex among men who have sex with men (MSM) and assessed the biological efficacy of a potent antiretroviral combination containing elvitegravir (EVG), emtricitabine (FTC), and tenofovir alafenamide (TAF).

**Methods:** Data on willingness to use single-dose PrEP or PEP were obtained from the 2017 cycle of the American Men's Internet Survey (AMIS), an annual online behavioral surveillance survey of MSM in the United States. Antiretroviral drug levels were measured in humans and macaques to define drug distribution in rectal tissue and identify clinically relevant doses for macaque modeling studies. The biological efficacy of a single dose of FTC/TAF/EVG as PrEP or PEP was investigated using a repeat-challenge macaque model of rectal HIV infection.

**Findings:** Through pharmacokinetic assessment in humans and macaques we found that EVG penetrates and concentrates in rectal tissues supporting its addition to FTC/TAF to boost and extend chemoprophylactic activity. Efficacy estimates for a single oral dose given to macaques 4h before or 2h after SHIV exposure was 91•7% [35•7%–98•9%] and 100%, respectively, compared to 80•1% [13•9%–95•4%] and 64•6% [–19•4%–89•5%] when single doses were given 6 and 24h post challenge, respectively. A two-dose regimen at 24h and 48h after exposure was also protective [77•1% [1•7%–94•7%]].

**Interpretation:** Informed by user willingness, human and macaque pharmacokinetic data, and preclinical efficacy we show that single-dose prophylaxis before or after sex is a promising HIV prevention strategy. Carefully designed clinical trials are needed to determine if any of these strategies will be effective in humans.

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## 1. Introduction

In 2018, there were 1•7 million people newly infected with HIV and 37•9 million people living with HIV worldwide [1].

According to the latest HIV surveillance report for the United States, there were about 38,000 new diagnoses and 991,000 people living with diagnosed infection in 2017 [2]. Substantial progress has been made in the development of antiretroviral (ARV)-based strategies to prevent HIV transmission including treatment as prevention and pre-exposure prophylaxis (PrEP). PrEP is an established prevention strategy to protect individuals

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at risk of HIV acquisition. Public health organizations including the US Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO) recommend scaling up PrEP around the world. In the U.S. alone, 1•1 million at-risk individuals can benefit from PrEP which is now a critical pillar of the *Ending the HIV Epidemic* plans [3]. Daily oral regimens containing emtricitabine (FTC) in combination with either tenofovir disoproxil fumarate (TDF) or tenofovir alafenamide (TAF) are currently approved for PrEP. When taken as prescribed, PrEP is 99% effective although inadequate adherence reduces efficacy and public health benefit [4,5]. Thus, identifying effective non-daily PrEP modalities that better adapt to different needs among users remains a high priority. An on-demand PrEP option with FTC/TDF is available for men who have sex with men (MSM) [6–9]. However, this regimen requires four FTC/TDF pills, two before sex followed by one pill at 24h and one at 48h post sex (2+1+1 dose schedule). Available post-exposure prophylaxis (PEP) practices recommend PEP with 3 ARV drugs for 28 days after a sexual HIV exposure [10]. PEP, however, is intended for infrequent and accidental exposures, requires rapid clinical provision of ARVs, and is, therefore, of limited public health impact.

We sought to identify single-dose on-demand regimens with flexible dosing windows that can be administered by the end-user either before or after sexually viral exposure. This simple regimen may not necessarily require anticipation of sex and may better adapt to individuals who are unable to take daily pills or have infrequent or changing sexual practices. We reason that an effective single-dose PrEP and PEP regimen would require high potency, favorable dosing of mucosal tissues for local antiviral activity, and a combination of ARVs of different classes that act at early and late stages of the virus replicative cycle to extend the protective window. In macaques, one subcutaneous dose of the reverse transcriptase inhibitors (RT) tenofovir and FTC given 2h before exposure was effective in preventing infection with simian HIV (SHIV). However, the same drug combination failed to protect when given in two doses at 24h and 48h after SHIV exposure [11]. These observations point to a limited window of PEP protection by RT inhibitors unless treatment is initiated before reverse transcription takes place or treatment is extended for 28 days [12,13]. In contrast, integrase strand-transfer inhibitors (INSTIs) may be more suitable for post-coital dosing as they target late steps of the replicative cycle. In time-to-drug addition experiments *in vitro*, the INSTI raltegravir (RAL) showed high post-infection inhibition when added to the culture between 6 and 10 h after virus exposure [14]. *In vivo*, a RAL gel administered vaginally to macaques 3 h after vaginal challenge with SHIV was also protective [14]. Thus, we posit that single-dose combinations of potent RT and INSTIs may potentially confer extended PrEP and PEP protection especially if drug concentrations at the mucosal site of HIV exposure are sufficiently high and persist for several days.

For an optimal selection of an INSTI for prevention we previously conducted pharmacokinetic (PK) studies in macaques comparing oral elvitegravir (EVG), dolutegravir (DTG), and RAL and found that after a single dose, EVG had the highest rectal and vaginal penetration persisting in secretions above the protein-adjusted 95% inhibition concentration for >48h post dosing [15]. Notably, EVG concentrations in rectal and vaginal secretions were 694 and 114-fold higher than in plasma. These data supported the selection and addition of EVG to FTC and TAF for further preclinical evaluation as a potent on-demand regimen for HIV prevention. Here, we provide a comprehensive preclinical assessment of a single-dose prevention strategy that includes additional macaque and human PK studies demonstrating favorable mucosal dosing by EVG, high willingness by MSMs to use single dose PrEP or PEP, and extensive efficacy studies in macaques of different dosing modalities defining windows of high efficacy. We document high biological efficacy of FTC/TAF/EVG

in macaques and high willingness to use single-dose PrEP/PEP with a clear preference for PEP over PrEP but less preference regarding timing of the single-dose regimen.

## 2. Materials and methods

### 2.1. Willingness to use single-dose PrEP or PEP

Data on willingness to use different modalities of single-dose PrEP/PEP were obtained from the 2017 cycle of AMIS, an annual online behavioral surveillance survey of MSM in the United States. The survey methodology has been previously published [16]. Briefly, participants were recruited for the online survey through convenience sampling from a variety of websites using banner advertisements. Participants were eligible to take the survey if they were at least 15 years of age, cisgender male, resided in the United States, and reported that they either had oral or anal sex with a man at least once in the past or identified as gay or bisexual. Data were collected between July and November 2017. The race/ethnicity distribution of the sample was as follows: 8.6% non-Hispanic Black, 21.5% Hispanic/Latino, 61.9% non-Hispanic white and 6.5% other or multiple races. There was a good a representation (at least 15%) from each of the 4 US Census regions. The AMIS study was conducted in compliance with Federal Regulations Governing Protection of Human Subjects and was reviewed and approved by Emory University's Institutional Review Board. Informed consent was obtained from all individual participants in the study.

Participants who did not self-report as being currently HIV-positive were asked about current daily PrEP use and if not current users, they were asked: "Would you be willing to take anti-HIV medicines **every day** to lower your chances of getting HIV?" A subset of AMIS respondents, who did not self-report as being currently HIV-positive but included current PrEP users, were randomized to receive one of four questions: 24 h before sex, 2 h before sex, 2 h after sex or 24 h after sex. The text of the question: "Researchers are also working on a form of PrEP that does not require you taking a daily pill. Instead, you would take only one pill within [2 or 12–24 h] [before or after] you had sex. How likely would you be to use this type of PrEP that is taken within [2 or 12–24 h] [before or after] you had sex?" The response options were very likely, somewhat likely, neither likely nor unlikely, somewhat unlikely and very unlikely. Those who answered that they were either "very likely" or "somewhat likely" were categorized as willing to use that modality of on-demand PrEP/PEP and other response options were categorized as not willing.

The analysis sample (N=1,668) was restricted to unduplicated responses from eligible and consenting participants who provided a valid US ZIP code, reported sex with a man in the past 12 months and responded to one of the randomized questions about willingness to use on-demand PrEP/PEP.

### 2.2. Macaque procedures

All animal procedures were approved by the US Centers for Disease Control and Prevention (CDC) Institutional Animal Care and Use Committee. The work was performed in a United States Department of Agriculture (USDA)-registered, Office of Laboratory Animal Welfare (OLAW)-assured and AAALAC International-accredited animal facility in accordance with The Guide for the Care and Use of Laboratory Animals.

FTC, TAF, EVG and cobicistat (COBI) powder were prepared as a single solution in a vehicle containing 65% of PEG [polyethylene glycol 400 (Sigma-Aldrich)], 20% TPGS [ $\alpha$ -tocopherol polyethylene glycol 1000 succinate (Spectrum)] and 15% of 10nM phosphate buffer pH 8. Macaque doses were adjusted by allometric scaling and administered

orally based on body weight (30 mg/kg EVG, 30 mg/kg COBI, 20 mg/kg FTC, and 1•5 mg/kg TAF).

### 2.3. Drug concentrations in blood and rectal tissues from macaques and humans

Human biopsies were collected from a subset of participants in a clinical trial funded by the CDC and approved by the Emory University and CDC Institutional Review Boards. Written informed consent was obtained from all study participants. This trial is registered at clinicaltrials.gov (NCT02985996) and conforms to the US Federal Policy for the Protection of Human Subjects. Seven HIV-negative men who have sex with men (MSM) between the ages of 18–49 received a single observed oral tablet of FTC/TAF/EVG/COBI (Genvoya®; 200 mg FTC/10 mg TAF /150 mg EVG/150 mg COBI) at the Emory Hope Clinic (Atlanta, GA) and returned 24 h later for biopsy specimen collection. Biopsies were collected from the mucosa approximately 8–10 cm above the external anal aperture using a rigid sigmoidoscope and flexible sigmoidoscopic forceps (Olympus America, Center Valley, Pennsylvania) mounted on a semi-flexible rod. Participants were asked to refrain from receptive anal intercourse during the intervention. All participants tested negative for rectal *Neisseria gonorrhoea* and *Chlamydia trachomatis* by nucleic acid amplification (Hologic, Marlborough, Massachusetts). Blood plasma and PBMCs were collected at each time point.

The pharmacokinetic (PK) profile of FTC/TAF and EVG in macaques was established at first dose. Macaques ( $n=4$ ) received a single oral solution containing 20 mg/kg of FTC, 1•5 mg/kg of TAF, 30 mg/kg of EVG, and 30 mg/kg of the pharmacoenhancer COBI. Blood plasma and PBMCs were collected over time. Drug concentrations in rectal tissues were measured in biopsies collected 24h after oral dosing. Biopsies were collected from the mucosa approximately 5–6 cm above the external anal aperture using biopsy forceps (Radial JawTMBiopsy Forceps, Boston Scientific or equivalent). A total of 12 macaques received FTC, TAF, EVG and COBI and were tested for tissue FTC and EVG levels. Four of these animals were also tested for TFV-DP and FTC-TP.

### 2.4. Measurement of extracellular and intracellular drug concentrations

Concentrations of TFV, FTC, and EVG were measured by high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) (Sciex, Foster City, CA, Shimadzu Scientific, Columbus, MD) [15]. Drug concentrations were estimated from a standard curve with a range of 0•5–2000 ng/mL using Analyst software. The lower limit of quantification (LLOQ) of this assay was 10 ng/mL for each analyte. Standard curves were constructed using normal human plasma as matrix.

Intracellular concentrations of TFV-DP and FTC-TP in PBMC and rectal biopsies were measured by LC-MS-MS. Briefly PBMC samples collected from buffy coat were lysed with 500  $\mu$ l of cold 80% methanol and stored at -80C until further analysis [17]. The LLOQ for TFV-DP was 100 fmol/sample and 500 fmol/sample for FTC-TP. TFV-DP concentrations in rectal biopsies were measured using methods previously described [18].

### 2.5. Efficacy of FTC/TAF/boosted EVG against rectal SHIV infection in macaques

The efficacy of FTC/TAF/boosted EVG in preventing rectal infection was investigated using an established macaque model consisting of repeated exposures to 10 tissue culture infectious doses (TCID<sub>50</sub>) of an R5-tropic SHIV<sub>162P3</sub> isolate. Macaques were exposed rectally to SHIV weekly (PrEP regimens) or biweekly (PEP regimens) for up to 8 weeks or until an animal became SHIV RNA positive. Animals received FTC/TAF/boosted EVG (200 mg FTC/1.5 mg TAF/30 mg EVG/30 mg COBI) at different times before or after each SHIV exposure:

-24h ( $n=6$ ), -4h ( $n=6$ ), +2h ( $n=5$ ), +6h ( $n=6$ ), +24h ( $n=6$ ), or +24h/+48h ( $n=6$ ). Infection outcome was compared to that seen in 18 untreated macaques (8 exposed to SHIV weekly and used as control for PrEP, and 10 exposed every two weeks and used as controls for PEP). Animals were considered protected if they remained seronegative for SHIV antibodies and negative for SHIV RNA and DNA during the 8 weekly virus challenges and the following 16 weeks of drug washout. SHIV RNA in plasma was quantified by an RT-PCR assay with a sensitivity of 50 RNA copies/ml [18]. Cell-associated SHIV DNA was quantified in PBMCs using a double-stranded primer assay [21]. SHIV DNA was also measured in lymph node biopsies (inguinal and mesenteric) collected 26–28 weeks after the last virus challenge to exclude the possibility of an occult infection. Virus-specific serologic responses were measured using a synthetic peptide enzyme immunoassay assay (BioRad, Genetic Systems HIV-1/HIV-2, Redmond, WA) [18].

### 2.6. Statistical analysis

Differences in willingness to use single-dose PrEP/PEP between current users and non-users of daily PrEP were assessed. Log-binomial regression models (one for each regimen of single-dose PrEP/PEP) were used to estimate prevalence ratios and 95% confidence intervals (CI) for the associations between current daily PrEP status (non-user vs. user) and willingness to use single-dose PrEP/PEP. Among those who were not current daily PrEP users, similar models were used to calculate PR and 95% CI for the associations between modality (single-dose PrEP or PEP vs. daily PrEP) and willingness. SAS 9•4 (SAS Institute, Cary, NC) was used for these statistical analyses.

Median and range for time to infection and drug concentrations (for treatment groups) were calculated for each of the study groups. Drug concentrations in macaques versus humans were compared using the Wilcoxon rank sum test.

To compare the number of animals protected in the different treatment groups relative to untreated controls, we used Fisher's exact test due to the small sample sizes. Efficacy was calculated as 1-relative risk of infection where the risk was the number of infections divided by the total number of challenges. Ninety-five percent confidence intervals were calculated around efficacy estimates using the bootstrap method. Survival analysis was used to compare time to infection in treated animals relative to the placebo group. The log rank test was used to compare survival distributions.

## 3. Results

### 3.1. Willingness to use single-dose PrEP or PEP

We investigated willingness to use single-dose PrEP or PEP at various time points through a 2017 cross-sectional online survey among sexually active MSM in the United States 15+ years of age – The American Men's Internet Survey (AMIS). Participants were recruited for the online survey through convenience sampling from a variety of websites using banner advertisements. Among 1668 MSM participants who answered questions about willingness to use single-dose PrEP/PEP, 1032 (61•9%) were non-Hispanic white, 359 (21•5%) were Hispanic and 108 (6•5%) were non-Hispanic black. The mean age of participants was 38•2 years old (standard deviation=17•0) and the median age was 33 years old. Over two-thirds of the sample participants (68•9%) reported condomless anal intercourse in the past 12 months and 13•2% were currently taking daily PrEP. There were no statistically significant differences in the sample characteristics by the four randomly assigned questions concerning timing options for single-dose PrEP/PEP (24 h before sex, 2 h before sex, 2 h after sex or 24 h after sex). There were no statistically significant differences in participant characteristics (age, race/ethnicity, past-year prevalence of condomless anal intercourse, current PrEP use) and randomized group.

Willingness was highest (83•4%) for PEP 24 h after sex, and lowest (67•1%) for PrEP 24 h before sex (Table 1). In general, willingness was also higher for single-dose PEP than single-dose PrEP, regardless of timing. When examining differences in willingness to use single-dose PrEP/PEP by whether participants were or were not already taking daily PrEP, those not currently taking daily PrEP were more willing to use single dose PrEP 24 h before sex than those currently taking daily PrEP (PR=1•34, 95%CI: 1•06, 1•70). Other timing options had a relative prevalence of willingness ranging from 0•99 to 1•16 and were not statistically significant. Among those not currently taking daily PrEP, willingness to use single-dose PrEP/PEP was higher than willingness to use daily PrEP for all modalities (Table 2). Willingness to use daily PrEP (asked of all participants) was also not substantially different across groups randomized to the four single-dose PrEP/PEP questions.

### 3.2. Selection of clinically-relevant macaque doses of FTC, TAF, and EVG

Four adult rhesus macaques were administered a single oral solution containing FTC, TAF, and EVG. The clinically-relevant doses of TAF (1•5 mg/kg) and FTC (20 mg/kg) in macaques have been previously defined [17,19]. Table 3 shows that median maximum drug concentration in plasma ( $C_{max}$ ) and area under the curve values over 24h ( $AUC_{0-24h}$ ) for FTC (1328 [range=341–5093] ng/ml and 16,937 [7147–62,064] ngxh/ml, respectively), and median plasma  $C_{max}$  and  $AUC_{0-24h}$  values for TFV (29 [range=24–96] ng/ml and 494 [371–1,591] ngxh/ml, respectively) were within the range of those seen in humans treated with 25 mg of TAF and 200 mg of FTC [20].

The pharmacoenhancer COBI was co-administered since high (50 mg/kg) doses of EVG in macaques without a booster results in low plasma EVG concentrations ( $C_{max}$  of 280 ng/ml and  $AUC_{0-24h}$  of 4000 ngxh/ml) [15] and supplementary Fig. 1). Co-administration of a reduced 30 mg/kg EVG dose with 30 mg/kg of COBI increased plasma  $C_{max}$  and  $AUC_{0-24h}$  values about 4-fold (1101 [268–3495] ng/ml and 14,033 [2895–41,969] ngxh/ml, respectively) (Table 3 and supplementary Fig. 1). These results support the selection of 30 mg/kg doses of EVG and COBI.

**Table 3**

Drug concentrations in plasma from macaques after a single oral dose of FTC, TAF and boosted EVG.

	$C_{max}$ (ng/ml)	$AUC_{0-24h}$ (ngxh/ml)
FTC	1,328 (341–5,093)*	16,937 (7,147–62,064)
EVG	1,101 (268–3,495)	14,033 (2,895–41,969)
TFV	29 (24–96)	494 (376–1,591)

\* median (range)

### 3.3. Comparative analysis of blood and tissue drug concentrations in macaques and humans

We next compared drugs concentrations in PBMCs and rectal tissues between macaques receiving 20 mg/kg FTC, 1•5 mg/kg TAF, and 30 mg/kg EVG/COBI and humans treated with a single fixed-dose combination FTC/TAF/EVG/COBI tablet (Genvoya®; 200 mg FTC/10 mg TAF/150 mg EVG/150 mg COBI). In human PBMCs, TFV-DP and FTC-TP peaked at 4h (299 [121–523] and 4215 [3630–7,150] fmols/10<sup>6</sup> cells, respectively). In macaques, TFV-DP and FTC-TP in PBMCs peaked at 24h (746 [509–1828] and 1737 [1153–3523] fmols/10<sup>6</sup> cells, respectively) (Fig 1).

Fig 2A shows median intracellular TFV-DP and FTC-TP levels achieved in rectal tissues 24h after dosing. Levels of TFV-DP in humans (17,100 fmols/mg of tissue) were similar to those observed in macaques (10,600 fmols/mg;  $p=1$ ); FTC-TP was mostly undetectable in humans and macaques. Tissue EVG, FTC, and TFV concentrations were also similar between macaques and humans ( $p=0•43$ ,  $p=0•073$ , and  $p=1$ , respectively) (Fig 2B). These findings support the selection of the doses of FTC, TAF, EVG and COBI for preclinical assessment of biological efficacy in macaques.

### 3.4. Protection by FTC/TAF/boosted EVG as PrEP in macaques

To investigate protection of FTC/TAF/boosted EVG as PrEP, we administered a single oral dose to two groups of macaques either 24h ( $n=6$ ) or 4h ( $n=6$ ) before rectal SHIV exposure. Virus exposures were

**Table 1**

Willingness of AMIS participants to use single-dose PrEP/PEP, either 2 h or 12–24 h before/after sex, stratified by current daily PrEP usage.

Randomized question subset	TOTAL		Daily PrEP non-users		Current daily PrEP users		PR (95% CI)
	N	n (%)	N	n (%)	N	n (%)	
Group 1: PrEP,12-24h	432	290 (67•1)	363	254 (70•0)	69	36 (52•2)	1•34 (1•06, 1•70)
Group 2: PrEP, 2h	385	281 (73•0)	338	251 (74•3)	47	30 (63•8)	1•16 (0•93, 1•46)
Group 3: PEP, 2h	442	360 (81•4)	394	324 (82•2)	48	36 (75•0)	1•10 (0•93, 1•30)
Group 4: PEP, 12-24h	409	341 (83•4)	353	294 (83•3)	56	47 (83•9)	0•99 (0•88, 1•12)

PR: Prevalence Ratio; CI: Confidence Interval, PrEP: pre-exposure prophylaxis, PEP: post-exposure prophylaxis  
n represents the number of AMIS participants that reported willingness to use that type of PrEP/PEP strategy. These are provided for all participants combined (total) and stratified by whether they were current daily PrEP users or not.

AMIS: The American Men's Internet Survey

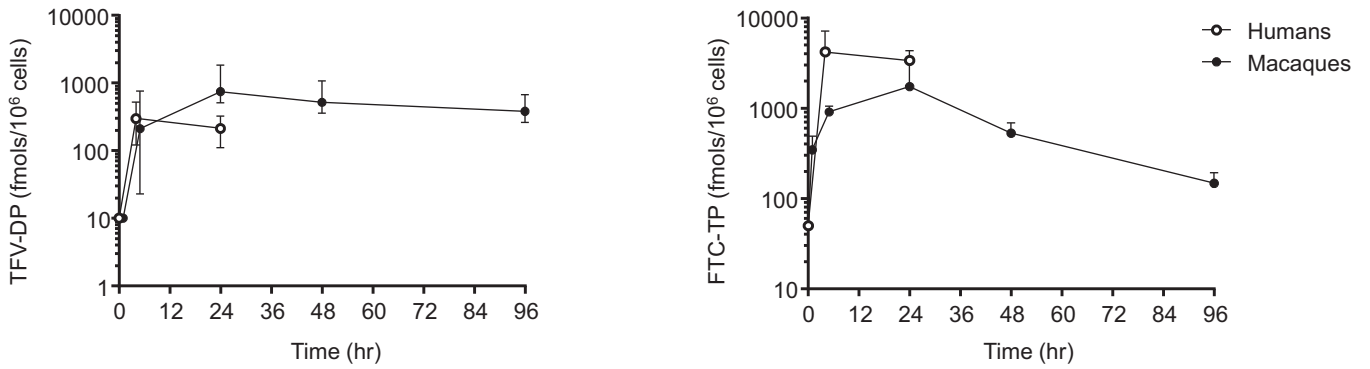
**Table 2**

Comparisons of willingness to use single-dose PrEP/PEP vs. daily PrEP, among AMIS participants not currently taking daily PrEP.

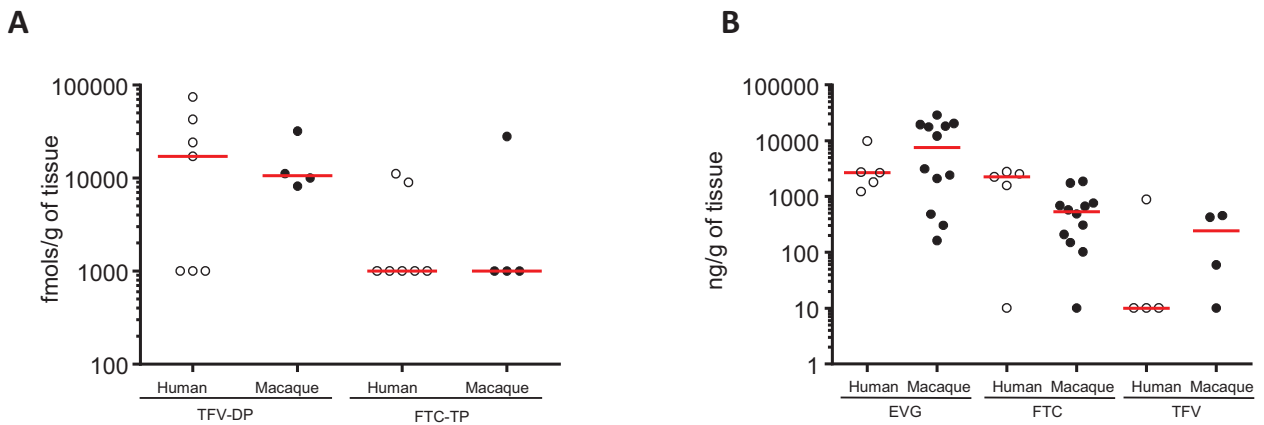
Randomized question subset	N	Willing to use single-dose PrEP/PEP, n (%)	Willing to use daily PrEP, n (%)	PR (95% CI) <sup>a</sup>
Group 1: PrEP,12- 24h	363	254 (70•0)	195 (53•7)	1•30 (1•16, 1•46)
Group 2: PrEP, 2h	338	251 (74•3)	198 (58•6)	1•27 (1•14, 1•41)
Group 3: PEP, 2h	394	324 (82•2)	222 (56•4)	1•46 (1•32, 1•61)
Group 4: PEP, 12-24h	353	294 (83•3)	213 (60•3)	1•38 (1•25, 1•52)

PR: Prevalence Ratio; CI: Confidence Interval

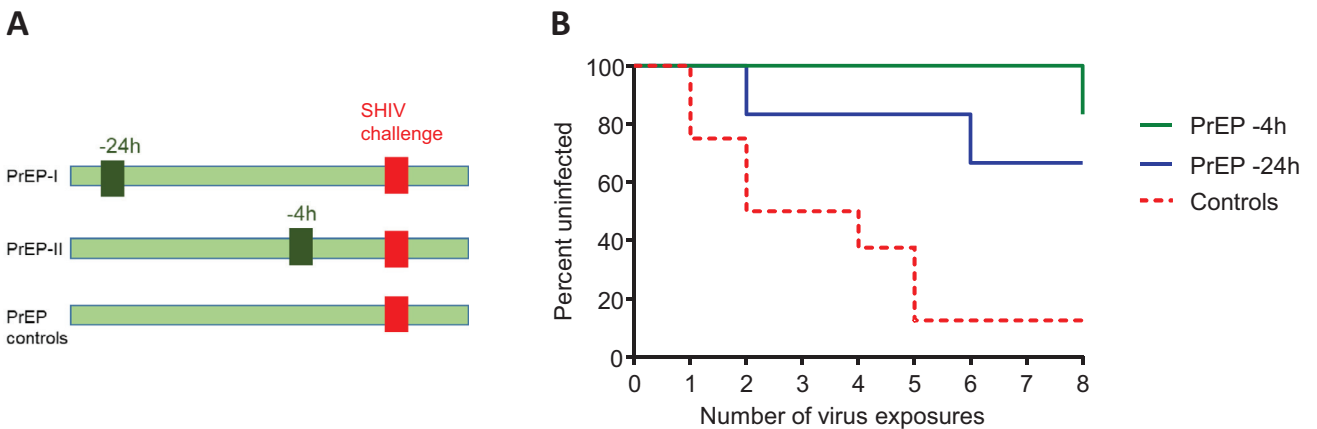
n represents the number of AMIS participants that reported willingness to use that type of PrEP/PEP strategy.



**Fig 1. Concentrations of TFV-DP and FTC-TP in PBMCs in macaques and humans.** Macaques ( $n=4$ ) received FTC (20 mg/kg), TAF (1•5 mg/k), EVG (30 mg/kg), and COBI (30 mg/kg) orally by gavage. Humans ( $n=7$ ) received a single oral dose of co-formulated FTC/TAF/EVG/COBI tablet (200 mg of FTC, 10 mg of TAF, 150 mg EVG and 150 mg COBI). Values represent median (range) intracellular levels. Values below the LLOQ were assigned a value of 10 (TFV-DP) or 50 (FTC-TP) fmol/10<sup>6</sup> cells or half of the LLOQ.



**Fig 2. Drug concentrations in rectal tissues in macaques and humans.** A) Concentrations of intracellular TFV-DP and FTC-TP. B) Concentrations of EVG, FTC, TFV. Macaques received a single oral dose of EVG (30 mg/kg), COBI (30 mg/kg), FTC (20 mg/kg) and TAF (1•5 mg/kg). HIV-negative men received a single oral dose of co-formulated FTC/TAF/EVG/COBI tablet (200 mg of FTC, 10 mg of TAF, 150 mg EVG and 150 mg COBI). Testing was done in 5 of the 7 participants. Horizontal red lines denote median concentrations observed in tissues at 24h. Undetectable levels were given an arbitrary value of 1000 fmols/g or 10 ng/g. None of the macaques to human comparisons reached statistical significance at the  $p<0•05$  value. Median weight of macaque and human biopsies for intracellular drug level testing were 0•042 g and 0•020 g, respectively. Likewise, median weight of macaque and human biopsies for extracellular drug level testing were 0•037 g and 0•020 g, respectively.



**Fig 3. Efficacy of single oral doses of FTC, TAF and boosted EVG as PrEP in macaques.** A) Study design. B) Macaques received FTC, TAF and boosted EVG orally by gavage at the indicated time points relative to the time of rectal SHIV exposure. Survival curve shows the cumulative percentage of uninfected macaques as a function of the number of rectal SHIV exposures. Time to infection was delayed in animals receiving single-dose PrEP 4h or 24h before exposure ( $p=0•005$  and  $p=0•027$ , respectively).

done once a week for up to 8 weeks or until an animal became infected (Fig 3A). Infection outcome was compared with that seen in eight untreated control animals. Fig 3B shows the relative proportion of uninfected macaques as a function of the number of virus exposures.

The proportion of macaques in the -4h dose group that became infected with SHIV was significantly lower than the proportion of macaques infected in the untreated control group (1/6 and 7/8, respectively;  $p=0•026$ ). Based on the number of infections per total

number of challenges, we calculated that the efficacy of a single -4h dose of 91.7% (95%CI=35.7%, 98.9%). Time to infection in these animals was also delayed compared to controls ( $p=0.005$ ).

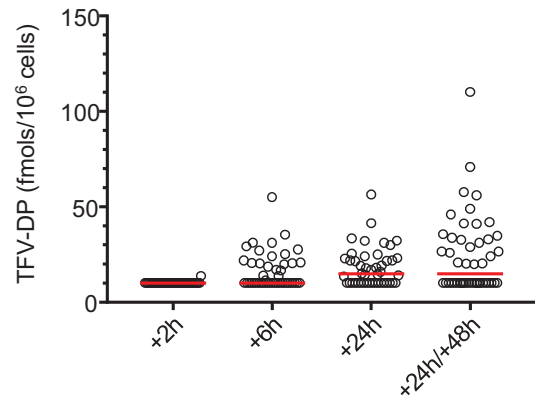
When the macaques received the PrEP dose 24h before virus exposure, the proportion of infected animals did not differ statistically from that seen in untreated control animals (2/6 compared to 7/8 controls;  $p=0.091$ ). However, efficacy of the -24h dose was also high (80.0% [95%CI=10.8%, 95.5%]). Time to infection in these animals was also delayed compared to controls ( $p=0.027$ ) (Fig 3). Overall, these results document an 80-92% efficacy achieved with a single oral dose of FTC/TAF/boosted EVG administered to macaques within 24h prior to SHIV exposure.

### 3.5. Protection by FTC/TAF/boosted EVG as PEP in macaques

We next investigated the efficacy of combination FTC/TAF/boosted EVG as PEP. We modeled single-dose PEP regimens given +2h ( $n=5$ ), +6h ( $n=6$ ), or +24h ( $n=6$ ) after virus exposure, and a two-dose PEP regimen given +24h/+48h after exposure (Fig 4A). Drug dosing and SHIV exposures were done every two weeks to minimize residual drug activity from previous doses.

The proportion of macaques that became infected in the +2h (0/5) and +6h (2/6 infections) groups was significantly lower than the proportion of macaques infected in the untreated control group (10/10 controls infected;  $p=0.001$  and  $p=0.028$  respectively). The efficacy of the +2h and +6h dose was 100% and 80.1% (95%CI=13.9%, 95.4%), respectively. Time to infection in these two groups was also delayed compared to untreated controls ( $p<0.001$  and  $p=0.011$ , respectively) (Fig 4B). Protection by a +24h dose was not significant with only 3 of the 6 PEP treated animals remaining uninfected after 8 exposures ( $p=0.099$  and a calculated efficacy of 64.6% [95%CI=-19.4%, 89.5%]). Time to infection was also not statistically different from that seen in control animals ( $p=0.057$ ).

The results with the +24h dose suggest that PEP with a single dose initiated after SHIV integration into the host genome has likely occurred may not be sufficient to block local virus expansion and systemic infection. We therefore investigated if the addition of a second PEP dose 24h later could improve efficacy. Fig. 4B shows that four of the six macaques that received a first PEP dose +24h and a second PEP dose +48h were protected against infection ( $p=0.028$ ), with a calculated efficacy of 77.1% (95%CI=1.7%, 94.7%). Infection in these animals was also delayed compared to untreated controls ( $p=0.013$ ) (Fig 4B). Supplementary Fig 2 shows that none of the animals that were protected by PEP had detectable SHIV DNA in axillary or inguinal lymph nodes collected 26-28 weeks after the last virus challenge.



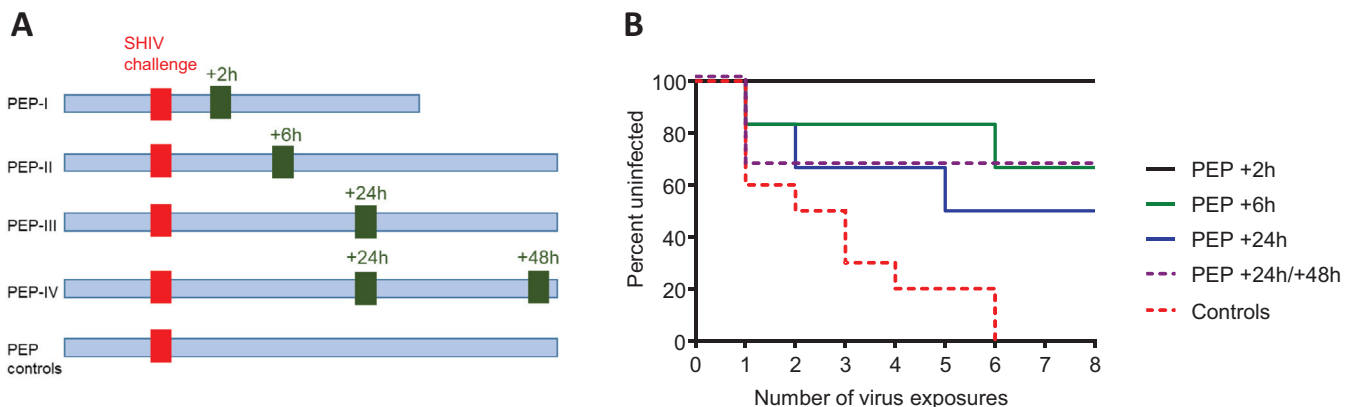
**Fig 5. Concentrations of TFV-DP in PBMCs at the time of SHIV challenge in PEP-treated animals.** Animals received one or two doses of FTC/TAF/EVG/COBI at the indicated time after virus exposure. Values reflect median TFV-DP levels seen 2 weeks later. Median levels (red lines) were below the limit of detection for the +2h, and +6h arms and 14.9 fmols/ $10^6$  cells for the +24h arm and the +24h/+48h arm. Values below the LLOQ were assigned an arbitrary value of 10 fmol TFV-DP/ $10^6$  cells or half of the LLOQ.

To better understand the protection achieved with PEP we measured the amount of residual TFV-DP and FTC-TP in PBMCs at the time of each SHIV exposure (Fig 5). Overall, TFV-DP was detected in 1/40 (2.5%), 22/45 (49%), 26/45 (58%), and 24/48 (50%) of the challenges done in the +2h, +6h, +24h, and +24h/+48h groups, respectively. However, median levels were generally low (<LLOQ in the +2h and +6h arms, and 14.9 fmols/ $10^6$  cells in the +24h arm, and +24h/+48h arm) and unlikely to contribute to any protection (Fig 5). We did not detect any residual FTC-TP at the time of virus challenges (not shown).

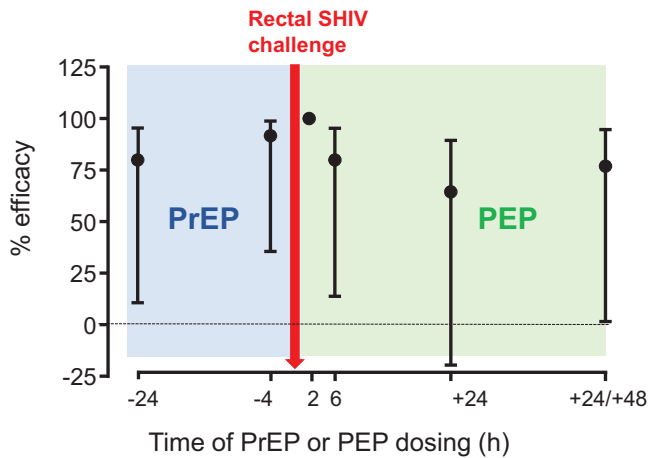
The efficacy estimates achieved with the different PrEP and PEP modalities relative to the time of drug administration are summarized in Fig 6. Overall, single oral doses of FTC, TAF and boosted EVG given within 24h before or 2-6h after SHIV exposure conferred high (80-100%) and significant protection against infection. However, a delayed PEP dose given 24h after exposure required the addition of a second dose 24h later to increase effectiveness.

## 4. Discussion

As efforts to scale up PrEP around the world accelerate, simple non-daily modalities may benefit users who may not require or prefer daily PrEP [6-8]. The at-risk populations targeted for PrEP



**Fig 4. Efficacy of single oral doses of FTC, TAF and boosted EVG as PEP in macaques.** A) Study design. B) Macaques received FTC, TAF and boosted EVG orally by gavage at the indicated time points relative to the time of rectal SHIV exposure. Survival curve shows the cumulative percentage of uninfected macaques as a function of the number of rectal SHIV exposures. Time to infection was delayed in animals receiving single-dose PEP 2h or 6h after exposure ( $p<0.001$  and  $p=0.011$ , respectively). Time to infection was also delayed in animals receiving two PEP doses at 24 and 48h after exposure but not with a single +24h dose ( $p=0.013$  and  $p=0.057$ , respectively).



**Fig 6. Efficacy estimates for PrEP/PEP with oral FTC/TAF/EVG/COBI.** Values reflect point estimates achieved with single doses as PrEP or PEP, or two doses as PEP. Horizontal lines denote the 95% confidence intervals. The closer the administration of the PrEP or PEP doses to the time of SHIV exposure the higher the efficacy.

implementation around the world are diverse and large and will likely exceed the number of HIV-infected persons. Thus, it is critical to identify PrEP modalities that adapt to the different needs to maximize coverage and public health benefit. In this study we provide a comprehensive assessment of a single-dose prophylaxis approach that includes user willingness, macaque and human PK, and biological efficacy measurements in a validated preclinical macaque model.

There was substantial willingness of MSM to consider use of single-dose PrEP/PEP regimens with a clear preference for PEP over PrEP but less preference regarding timing of the single-dose regimen. Single-dose PrEP/PEP in any of the proposed approaches was substantially preferred over daily PrEP among those who were not currently taking it. This is consistent with previous studies that have compared theoretical willingness for daily PrEP versus on-demand PrEP regimens [21,22], but hasn't been previously reported for PEP in a standard 28-day regimen. Willingness to use single-dose PrEP/PEP did also not seem to differ substantially between those who were already taking daily PrEP and those who were not, except for PrEP users being less willing to use single-dose PrEP taken 24 h before sex. This could be due to differences in sexual practices between the two groups or could be due to PrEP users' awareness of the difficulty of timing a dose of PrEP at least 24 h before sex. Regardless, a substantial majority of MSM were willing to use single-dose PEP, even if it needed to be taken within a couple of hours of having sex.

The biological efficacy of a prototype single-dose PrEP or PEP dose containing a combination FTC/TAF/boosted EVG was tested using a validated macaque model of rectal SHIV infection that has previously predicted the clinical PrEP efficacy of FTC/TDF and FTC/TAF [17,19]. The selection of TAF was based on the high intracellular TFV-DP concentrations achieved in PBMCs and sought to provide long intracellular drug persistence and potential for extended prophylactic activity [23,24]. FTC was added given the high efficacy of FTC/TAF combination as daily PrEP in humans and macaques and its rapid penetration in rectal tissues [5,19]. The addition of an integrase inhibitor to FTC/TAF was critical for PEP since integration occurs several hours after reverse transcription [14]. This unique mechanism of action extends the window for intervention beyond what is afforded by RT inhibitors. *In vitro* testing in highly activated cell lines can define the timing of reverse transcription and integration and together with PK data on mucosal drug distribution can help guide *in vivo* single dose modalities. However, timing

data from *in vitro* infections may not fully reflect the duration of these steps that occur mucosally in less activated lymphocytes which typically take longer to complete [25]. Therefore, challenge studies are critical for identifying protective doses and defining efficacy windows of single-dose regimens.

We modeled the efficacy of EVG added to the approved TAF/FTC PrEP regimen. EVG dose finding was facilitated by COBI administration which we show is active in macaques as in humans and is able to boost plasma EVG levels. We tested this potent ARV combination representing a peri-coital modality to be taken shortly before or after sex. We show high protection (91-100%) against rectal SHIV infection when the regimen is given 4h before or 2h after exposure. This efficacy level is similar to daily TDF/FTC or TAF/FTC among MSM [4,5]. The high efficacy seen in the model and the willingness to use on-demand PrEP or PEP among MSM support the clinical development of this single-dose modality for HIV prevention.

Our data defining the PrEP and PEP protection windows are also important. We show that this regimen maintained efficacy (80%) when given 24h before or 6h after challenge providing flexible dosing options. Importantly, we show that delayed PEP initiation at +24h requires a second +48h dose to increase effectiveness possibly through a better control of local virus expansion and virus dissemination [26]. These data clearly highlight the added value of EVG as our previous results with subcutaneous TFV and FTC combination failed to protect when given as PEP at 24h and 48h [11]. Operationalizing these efficacy data for a clinical trial design would suggest a preferred single dose between 4h before to 2h after sex. If this option was not feasible a less preferred option of 2 doses 24h apart may be considered if treatment is initiated 24h before or 2-24h after exposure.

The addition of the pharmacoenhancer COBI was necessary to achieve clinically relevant concentrations of EVG in plasma. However, COBI has the potential to increase adverse reactions associated with other medications that are metabolized by CYP3A [27]. Our previous PK studies in macaques showed EVG concentrations in rectal fluids without COBI were several orders of magnitude above the PA-IC<sub>90</sub> for up to 48h and sufficient to block SHIV infection *in vitro* [15]. These findings suggest that it may be possible to find an unboosted EVG dose that retains protective efficacy. More PK and challenge studies may be needed to support this dose selection. Alternatively, newer integrase inhibitors that do not require boosting such as cabotegravir or bicitegravir may be other options to circumvent the limitations of pharmacoenhancers. It will be important to define the penetration of such drugs in rectal and vaginal tissues as mucosal exposures, as our EVG data suggest, are important for protection.

This study has limitations. The desirability data in MSM collected through the online survey may not be generalizable since we did not analyze the data based on risk criteria. In addition, willingness to use single dose PrEP or PEP may not necessarily reflect true uptake, which will be influenced by other factors including implementation and access. The efficacy data are against rectal SHIV acquisition and may not reflect vaginal or penile protection. However, the favorable EVG penetration in vaginal tissues shown in macaques may predict efficacy especially since the TAF/FTC combination has been found to protect against vaginal SHIV acquisition [15,28]. Lastly, in some cases variances for our efficacy estimates in macaques were large due to the small sample sizes.

In summary, using user willingness, human and macaque PK data, and virus challenge studies in macaques we identify a single-dose before or after sex HIV prevention regimen that may better adapt to different needs among PrEP users. Clinical trials in humans are needed to confirm this encouraging preclinical efficacy data.

## RESEARCH IN CONTEXT

### Evidence before this study

Daily oral regimens with emtricitabine (FTC) in combination with either tenofovir disoproxil fumarate (TDF) or tenofovir alafenamide (TAF) are currently approved for pre-exposure prophylaxis (PrEP) against HIV infection. When taken as prescribed, PrEP is highly effective although inadequate adherence reduces efficacy and public health benefit. An on-demand PrEP option with FTC/TDF is also available for men who have sex with men (MSM) but requires 4 doses within three days (2+1+1). Post-exposure regimens include 3 antiretroviral drugs for 28 days and are intended for infrequent and accidental exposures thus limiting public health impact. We investigated willingness to use a single-dose PrEP or PEP regimen among MSM in the US and assessed biological efficacy in a validated macaque model of rectal exposure to simian HIV (SHIV).

### Added value of this study

We posit that for a single-dose PrEP or PEP regimen to be effective would require a combination of drugs from different classes that act at early and late stages of the virus replicative cycle and achieve high mucosal exposures. We document high willingness of MSM to consider use of a single-dose PrEP or PEP regimen with a clear preference for PEP over PrEP but less preference regarding timing of the single-dose regimen. We show high biological efficacy of a prototype combination containing FTC/TAF and boosted elvitegravir and defined windows of PrEP and PEP protection.

### Implications of all the available evidence

We provide preclinical proof-of-concept for a “before or after sex” HIV prevention pill that may better adapt to different needs among PrEP and PEP users. Clinical trials in humans are needed to determine if any of these strategies will be effective.

M.K.: study design, data acquisition and analysis, interpretation of results. R.H.: study design, data acquisition and analysis, interpretation of results. P. M. veterinary procedures. M.C.: data acquisition and analysis. K.K.: animal tech procedures. R.J.: animal tech procedures. A.H.: data acquisition and analysis. C.D.: data acquisition and analysis. G.K.: statistical analysis. Y.P.: statistical analysis. C.F.K.: study design, data acquisition and analysis. T.S.: study design, data acquisition and analysis, interpretation of results. W.H.: study design, data acquisition and analysis, interpretation of results, and manuscript preparation. J.G.G-L.: study design, data acquisition and analysis, interpretation of results, and manuscript preparation.

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## Supplementary materials

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## Declaration of Competing Interest

J.G.G-L and W.H. are named in US. Government patents on “Inhibition of HIV infection through chemoprophylaxis”. I.M, W.H. and J.G.G-L are named in US. Government patent applications on “HIV post-exposure prophylaxis” and “HIV pre-exposure prophylaxis”. The findings and conclusions of this manuscript are those of the authors and do not necessarily represent the official views of the Centers for Disease Control and Prevention.

## Author contributions

I.M.: study design, data acquisition and analysis, interpretation of results, and manuscript preparation. S.R.: data acquisition and analysis.



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