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Two-dose emtricitabine/tenofovir alafenamide plus bictegravir prophylaxis protects macaques against SHIV infection

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Objectives: Current prophylaxis options for people at risk for HIV infection include two US FDA-approved daily pre-exposure prophylaxis (PrEP) regimens and guidelines for a 2-1-1 event-driven course specifically for men who have sex with men. Despite this, PrEP use rates remain suboptimal, and additional PrEP options may help to improve uptake among diverse populations. Here, we evaluated protective efficacy of two-dose PrEP and two-dose postexposure prophylaxis (PEP) schedules with emtricitabine (FTC)/tenofovir alafenamide (TAF) with or without bictegravir (BIC) in an SHIV macaque model.

Methods: Macaques received one oral dose of 200 mg emtricitabine, 25 mg tenofovir alafenamide and 25-100 mg of bictegravir to establish pharmacokinetic profiles of each drug either in the plasma or the peripheral blood mononuclear cells. Protective efficacy of multiple two-dose PrEP and PEP schedules with FTC/TAF with or without bictegravir was then assessed in two repeat low-dose rectal SHIV challenge studies.

Results: The data revealed over 95% per-exposure risk reduction with FTC/TAF PrEP initiated 2 h before the exposure, but a loss of significant protection with treatment initiation postexposure. In contrast, FTC/TAF plus BIC offered complete protection as PrEP and greater than 80% per-exposure risk reduction with treatment initiation up to 24 h postexposure.

Conclusions: Together, these results demonstrate that two-dose schedules can protect macaques against SHIV acquisition and highlight the protective advantage of adding the integrase inhibitor bictegravir to the reverse transcriptase inhibitors emtricitabine and tenofovir alafenamide as part of event-driven prophylaxis.

Introduction

HIV infection remains one of the top public health challenges around the globe. An estimated 1.7 million individuals worldwide became infected with HIV in 2018.[1] In the USA, which recorded over 37000 new infections in 2018, the government has recently spearheaded an initiative to reduce new domestic HIV infections by 90% by 2030.[2,3] One of the cornerstones of HIV infection prevention is the use of antiretroviral medicines as prophylaxis. Combinations of the nucleo(t)side reverse transcriptase inhibitors (NRTIs) emtricitabine (FTC) and either tenofovir disoproxil fumarate (TDF) or tenofovir alafenamide (TAF) are two oral regimens with demonstrated efficacy as HIV pre-exposure prophylaxis (PrEP).[4–7] Studies have shown that daily PrEP reduces the risk of contracting HIV from sexual intercourse by about 99% and among people who inject drugs by 74%.[8] Importantly, real-world data has shown a reduced incidence of HIV infection among PrEP users.[9–12] However, the number of people using daily PrEP still accounts for only a fraction of the people at risk for HIV who may benefit from PrEP. Surveys on the barriers to and preferences for HIV prevention modalities among individuals with diverse sociodemographic characteristics and sexual behaviours have documented varying responses among different groups on the available and next-generation prevention options.[13–17] Taken together, research suggests that continued innovation of prophylaxis options can offer additional choice for those individuals who are unable to accommodate daily PrEP into their lives, and thus improve the overall uptake.

Intermittent, event-driven prophylaxis represents one alternative to daily PrEP. Preclinical research evaluating prophylaxis of event-driven NRTI regimens demonstrated a range of

protection with treatment initiation before or after the exposure in a non-human primate (NHP) model for HIV acquisition.[18–21] Macaques challenged rectally or vaginally with a chimeric simian/human immunodeficiency virus (SHIV) revealed the greatest protection with dosing schedules initiated within 24 h pre-exposure. These findings demonstrated that drug loading into cells closest to the time of virus exposure yields the greatest protection, consistent with the mechanism of action of NRTIs, which inhibit the early steps in the viral life cycle.[22] Furthermore, these animal studies informed the design of IPERGAY, an event-driven clinical study conducted in MSM.[23] In that study the active group was instructed to take a double FTC/TDF dose 2 to 24 h prior to sex followed by a once daily dose for 2 days postexposure (2–1-1 schedule) and demonstrated an 86% infection risk reduction relative to placebo.[23] These results formed the basis of the current recommendation by the WHO for oral PrEP to include an option of event-driven dosing for MSM.[24] It should be noted, however, that the high frequency of sexual events in the population enrolled in IPERGAY resulted in a median of 16 pills taken per month, possibly confounding a true estimate of protection with an isolated exposure event. This warrants further investigation of the event-driven prophylaxis approach and any efforts to augment the level of protection it can offer.

In the present preclinical study, we relied on a previously established repeat low-dose rectal challenge model in NHPs to evaluate event-driven schedules with increased convenience and flexibility relative to the IPERGAY model.[20] To do so we reduced the number of drug doses per exposure and tested postexposure only schedules, which conceptually reduce the need for advanced planning of sexual activity. Specifically, we assessed dosing schedules with just two administrations of either FTC/TAF alone or FTC/TAF plus an integrase strand transfer inhibitor (INSTI), bictegravir (BIC), initiated either pre-exposure or at various times postexposure.

Bictegravir is a potent unboosted INSTI, which was evaluated and found to be well tolerated at doses up to 600 mg in human volunteers and subsequently approved for HIV treatment as a 50 mg fixed-dose single-tablet-regimen with 200 mg FTC and 25 mg TAF.[25,26] Published models estimate strand transfer and integration of the HIV proviral DNA into the human genome to occur about 5 h following the completion of reverse transcription which, depending on the experimental conditions, happens over the course of 6 to 48 h post entry.[22,27] Thus, we hypothesized that the addition of a late-stage inhibitor such as the INSTI bictegravir, to an NRTI regimen would broaden the window of opportunity for a successful prophylactic intervention administered postexposure. Our assessment corroborated previous findings showing the efficacy of NRTI-only combinations for event-driven PrEP but demonstrated a loss of protection with treatment initiation postexposure. In contrast, the triple regimen containing 100 mg bictegravir demonstrated efficacy both as short-course PrEP and early postexposure prophylaxis (PEP), highlighting the advantage of adding a late-stage integrase inhibitor to NRTIs for event-driven prophylaxis.

Materials and methods

Animals/procedures

Outbred, naive, Indian-origin, adult male rhesus macaques (*Macaca mulatta*) weighing an average of 6.0 kg (2.9-13.3 kg range) were housed and handled at BIOQUAL Inc., Rockville, MD. Procedures including intrarectal challenges, ART dosing and blood collections were performed on animals anaesthetized via the intramuscular route with 10 mg/kg to 25 mg/kg of ketamine. Cage-side monitoring occurred daily, and routine haematology and clinical chemistry evaluations were performed monthly to ensure animal health. Procedures, sample collections and analyses were conducted on the same day across study groups to minimize technical variability. The studies were non-blinded. Animal stratification into groups was done to balance average animal weight per group and prior study participation in the case of Study-2. Study-2 was initiated 8 weeks after completion of the last dose on Study-1.

Study approval

All the procedures described herein were approved by the appropriate Institutional Animal Care and Use Committee at Bioqual, Inc (Protocol #18-035P).

Intrarectal virus infection

Intrarectal challenges were done with 10 $TCID_{50}$ SHIV.SF162P3 administered in a 1.0 mL inoculum following 1:100 dilution of stock material in RPMI medium. For this procedure, the animal was placed in ventral recumbency with its head down and hindquarters elevated to ensure that the inoculum remains in the rectum. A slip tip syringe was inserted slowly into the rectum to approximately half of the syringe's length, the inoculum was injected slowly and the syringe then carefully retracted to ensure optimum uptake.

Drug dosing and sample collection

Drug formulation was prepared fresh on the day of administration for each animal by dissolving a single crushed tablet of 200 mg emtricitabine and 25 mg tenofovir alafenamide, and 25–100 mg sodium salt of bictegravir (when applicable) in 15 mL of sterile PBS. The suspension was administered via oral gavage at the timepoints indicated.

Whole blood was collected and processed into plasma and PBMCs as necessary for the assessment of routine haematology and clinical chemistry, viral load analysis, and bioanalysis of drug levels. Animals exhibiting viraemia were monitored for an additional 6 months, followed by initiation of standard daily ART to achieve suppression. The formulated ART cocktail (Gilead Sciences, Inc.) contained tenofovir disoproxil fumarate (5.1 mg/mL) and emtricitabine (40 mg/mL), and dolutegravir (2.5 mg/mL) and was administered subcutaneously once daily at 1 mL/kg body weight.

Drug pharmacokinetics

TFV-DP and FTC-TP in PBMCs

Approximately 8 mL of whole blood was collected into sodium citrate CPT Vacutainer tubes and PBMCs isolated according to manufacturer's instructions (BD Biosciences). Cells were washed using 0.9% NaCl and subjected to red blood cell lysis using ammonium salt solution. Cell pellets were flash frozen and stored at −80°C until bioanalysis. Tenofovir-diphosphate (TFV-DP) and emtricitabine-triphosphate (FTC-TP) concentrations were quantified using liquid chromatography (LC) coupled with positive-ion-mode tandem mass spectrometry (MS/MS) methods, essentially as described previously.[28]

Bictegravir in plasma

Macaque whole blood was collected into EDTA-treated anticoagulant tubes (ThermoFisher) and centrifuged for 20 min at 2000 **g** at 20°C for cell removal. The resulting supernatant, designated as plasma, was subjected to an LC-MS/MS method to measure the concentration of bictegravir. A protein

precipitation procedure was followed to prepare plasma samples for bioanalysis. 450 μL of 500 ng/mL internal standard in acetonitrile (ACN) was added to a 50 μL aliquot of each plasma sample with the exception of the matrix blanks. The matrix blank samples received 450 μL of ACN only. The precipitated proteins were removed by centrifugation and 50 μL of supernatant was transferred into a clean 96 deep-well plate containing 200 μL aliquots of water. 750 μL of methanol: water (50:50) was added to each well. An aliquot of 2–4 μL was injected into the LC-MS/MS system. The standard curve and quality control (QC) samples were prepared by spiking an appropriate amount of bictegravir solution, prepared in ACN: DMSO, into blank (undosed) macaque plasma, then further diluting in blank macaque plasma to complete the calibration line. Standards and QC samples were processed as described above. The lower limit of quantification for the assay was 10 ng/mL.

Plasma viral load quantification

SHIV copy number in plasma was determined by TAQMAN quantitative real-time PCR assay. Viral RNA was extracted from 200 μL of plasma using QIAamp MiniElute Virus Spin Kit (57704, Qiagen) and amplified using the following primer/probe set: SIV Fwd, GTCTGCGTCATCTGGTGCATTC; SIV Rev, CACTAGGTGTCTCTGCACTATCTGTTTTG; probe, 6FAM-CTTCCTCAGTGTGTTTCA CTTTCTCTTCTGCG-TAMRA. All samples were amplified in triplicate in an Applied Biosystems 7500 Sequence detector using the following program: 48°C for 30 min, 95°C for 10 min followed by 40 cycles at 95°C for 15 s and 60°C for 1 min. Values above 50 copies/mL limit of detection were extrapolated from a standard curve.

Statistical analysis

Protection against acquisition of infection was analysed using a Cox proportional hazard regression model based on the exact partial likelihood for discrete time. The number of challenges was used as a discrete time scale rather that an actual time variable. The hazard ratios (HRs) with 95% CIs for the per-exposure relative reductions of acquisition risk were calculated for the drug regimens as compared with the placebo group. No animals or timepoints were excluded from the final analysis. *P* values were also reported. Comparisons were considered statistically significant at a two-sided alpha level of 0.05. Analyses were performed using GraphPad Prism version 8.1.2 and SAS version 9.4.

Results

Single-dose pharmacokinetic profile of antiretrovirals in macaques

To establish the pharmacokinetic (PK) profile of emtricitabine, tenofovir alafenamide and bictegravir in rhesus macaques we administered a single-dose drug regimen by oral gavage and measured exposure to each drug in the plasma or the PBMCs. We tested a fixed dose of 200 mg emtricitabine and 25 mg tenofovir alafenamide administered as a crushed FTC/TAF tablet resuspended in PBS alone or in combination with 25, 50 or 100 mg of bictegravir. Each regimen was tested in three male macaques ranging from 6.2 to 10 kg in weight.

Because emtricitabine and tenofovir alafenamide have short half-lives in the plasma and their active metabolites, FTC-TP and TFV-DP, are only present intracellularly, we quantified the levels of FTC-TP and TFV-DP in PBMCs as measures of emtricitabine and tenofovir alafenamide exposure. FTC-TP concentrations peaked at the 6 h timepoint with an average of 2876 fmol/million PBMCs and had a half-life close to 11 h (Table 1). In contrast, TFV-DP half-life extended beyond the 48 h observation period and reached a steady-state concentration of 733 fmol/million PBMCs over the course of 2 days post dose (Table 1). Overall, FTC-TP and TFV-DP levels measured in macaque PBMCs were within the range of exposures achieved in humans dosed with emtricitabine and tenofovir alafenamide in the clinic.[29–32]

Table 1. Pharmacokinetic profiles of emtricitabine (FTC) and tenofovir alafenamide (TAF) active metabolites in rhesus PBMCs[a]

PK parameter (single-dose)	FTC 200 mg	TAF 25 mg
Mean FTC-TP C_{6h}, fmol/10^6 PBMCs (%CV)	2876 (9.0)	—
Mean TFV-DP C_{ss}, fmol/10^6 PBMCs (%CV)	—	733 (30.3)
Median $t_{1/2}$, h	10.9	>48

Abbreviations: %CV, % coefficient of variation; C_{ss}, steady-state concentration; C_{6h}, concentration at 6 hours; DP, diphosphate; $t_{1/2}$, half-life; TP, triphosphate.
[a]Intracellular phosphometabolite analysis is semiquantitative.

To characterize bictegravir exposure, the level of drug in plasma was measured. Table 2 shows mean maximum concentration (C_{max}) and total area under the curve (AUC_{∞}) values (3030–10 000 ng/mL and 37 900–158 000 ng·h/mL, respectively), as well as median half-life ($T_{1/2}$) and time of maximum concentration (T_{max}) values (5.67–8.28 h and 2.67–4.33 h, respectively) at the three bictegravir dose levels with percentage variance per group. Table 2 also summarizes the corresponding values measured in humans receiving a single 50 mg dose of bictegravir, to allow for comparison between the macaque and human PK profiles. These data reveal a dose-proportional increase in the plasma AUC and C_{max} between 25 and 50 mg and in the half-life between 25 and 100 mg. Additionally, the results highlight a distinct dose level versus exposure relationship between the macaques and humans, cautioning against a direct comparison of efficacy between species at a given dose.

Efficacy of PrEP and PEP schedules

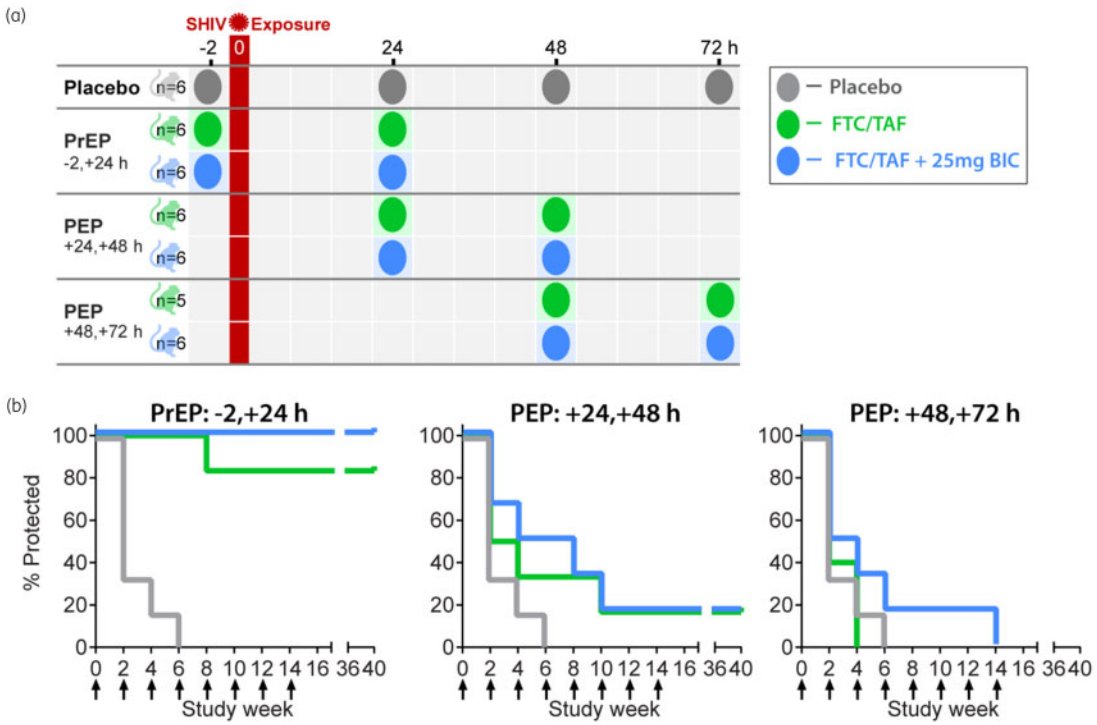
We next evaluated the protective efficacy of the 200 mg emtricitabine, 25 mg tenofovir alafenamide regimen alone or with the addition of a lower dose of 25 mg of bictegravir in the efficacy Study-1. A cohort of 41 naive Indian rhesus male macaques was stratified into seven treatment groups (Figure 1a). Animals were challenged intrarectally with a low dose of SHIV.SF162P3 every other week for up to 8 repeat cycles. FTC/TAF, FTC/TAF+BIC or placebo control were administered either as PrEP with the first dose 2 h prior to each challenge and the second dose 24 h post challenge, or as PEP with two doses 24 h apart starting either at 24 or 48 h after each challenge (Figure 1a). The rates of infection were monitored via qRT-PCR plasma viral load measurements every other week through 6 months after the last challenge. Cox proportional hazard analysis was conducted at the end of the study to compute the per-exposure risk reduction.

All (6/6) placebo control animals became infected within three cycles of challenge, while the three active groups displayed varying degrees of protection at the end of study (Figure 1b and c). The PrEP group (−2 and +24 h) demonstrated complete protection with the regimen containing FTC/TAF + 25 mg BIC, while the FTC/TAF regimen protected 5/6 animals. The −2 and +24 h dosing

Table 2. Pharmacokinetic profile of bictegravir (BIC) in plasma

	Rhesus			Human[a]
BIC PK Parameter (single-dose)	25 mg dose	50 mg dose	100 mg dose	50 mg dose
Mean AUC_{∞}, ng·h/mL (%CV)	37 900 (27.4)	158 000 (56.6)	147 000 (30.1)	78 399 (29.7)
Mean C_{max}, ng/mL (%CV)	3030 (45.2)	10 000 (38.8)	7550 (28.1)	3965 (40.1)
Median $t_{1/2}$, h	5.7	7.0	8.3	16.7
Median $t_{max,}$ h	2.7	4.3	4.3	3.0

%CV, % coefficient of variation; AUC_{∞}, area under plasma-concentration curve from time 0 to infinity; C_{max}, maximal concentration; $t_{1/2}$, half-life; t_{max}, time to C_{max}.
[a]Data from study GS-US-141-1218.



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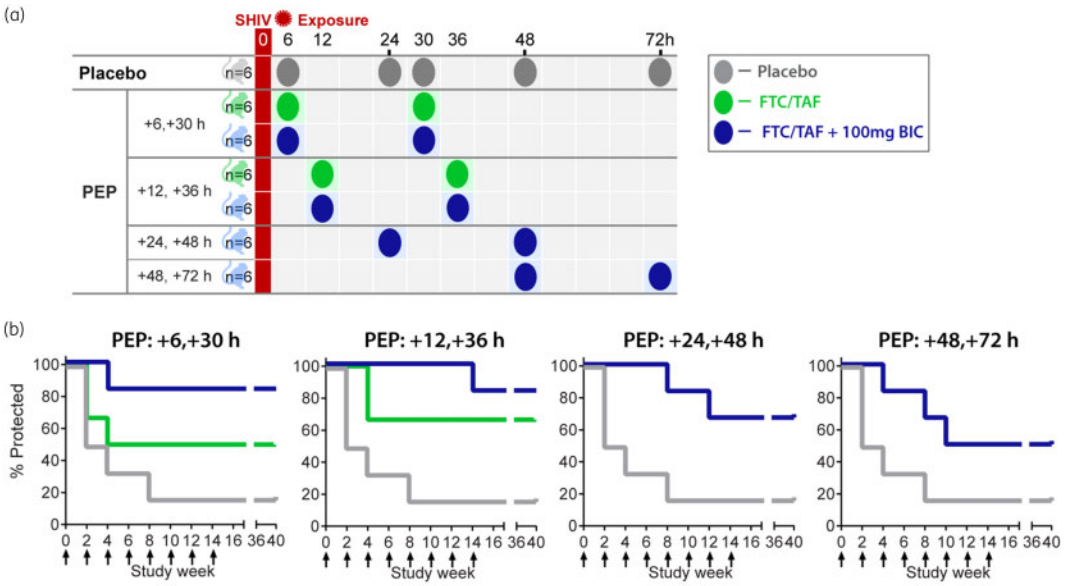
		-2,+24h		+24,+48h		+48,+72h	
	Placebo	FTC/TAF	FTC/TAF+BIC	FTC/TAF	FTC/TAF+BIC	FTC/TAF	FTC/TAF+BIC
Fraction protected	0/6 (0%)	5/6 (83%)	6/6 (100%)	1/6 (17%)	1/6 (17%)	0/5 (0%)	0/6 (0%)
Hazard ratio (95% Conf. Interval)	1	0.048 (0.005-0.43)	N/A	0.484 (0.14-1.67)	0.416 (0.12-1.45)	1.027 (0.31-3.38)	0.669 (0.21-2.13)
Per-exposure risk reduction		95.2%	N/A	51.6%	58.4%	2.7%	33.1%
p-value vs placebo		**0.007**	**<0.001**	0.251	0.169	0.965	0.497

Figure 1. Study-1: emtricitabine/tenofovir alafenamide (FTC/TAF) and FTC/TAF + 25 mg bictegravir (BIC) two-dose regimens are effective as PrEP, but not PEP in macaques. (a) Efficacy Study-1 design. Negative hour values represent timing of drug dosing before SHIV exposure, positive time values represent drug dosing post exposure. SHIV challenge is denoted in red at time = 0. Six placebo control animals were split into three subgroups ($n = 2$ each) dosed at: −2 and +24; +24 and +48; or +48 and +72 h relative to SHIV challenge (b) Percentage of protected animals following eight repeat challenge/drug dosing cycles and a 6 month follow-up as determined by undetectable plasma viral load qRT-PCR read-out. SHIV challenges are denoted by black arrows under the *x*-axis. FTC/TAF and FTC/TAF + 25 mg BIC protect or delay time to infection relative to placebo control as shown. Each of three dosing schedules is plotted against the same group of six placebo control animals, all evaluated in parallel. (c) Summary of the fraction of animals protected, the resulting hazard ratios with 95% CIs and the per-exposure risk reduction rates at the end of study. *P* value determined using Cox proportional hazard regression model. Statistically significant values below a two-sided alpha level of 0.05 are shown in bold.

schedule significantly protected the animals, with both regimens providing 95.2% ($P = 0.007$, Cox proportional hazard model) or greater per exposure risk reduction relative to placebo (6/6 complete protection precluded estimation of risk reduction). Prophylaxis initiation postexposure led to a loss of protection, with only one animal per group remaining protected in the +24 and +48 h schedule and no animals protected in the +48 and +72 h schedule (Figure 1b and c). Accordingly, the FTC/TAF regimen resulted in 51.6% ($P = 0.251$) and 2.7% ($P = 0.965$) per-exposure risk reduction when initiated at 24 and 48 h postexposure, respectively. Comparatively, the FTC/TAF+BIC regimen resulted in 58.4% ($P = 0.169$) and 33.1% ($P = 0.497$) per-exposure risk reduction when initiated at 24 and 48 h postexposure, respectively. Together, these results suggested that either regimen is effective at reducing the risk of infection with treatment initiation shortly before the exposure. However, the protection is reduced or lost with both the two- and the three-drug regimen at these doses when initiated 24 h postexposure or later.

Because a comparison of the four PEP groups with the placebo control in Study-1 did not reveal significant differences, we designed a follow up, Study-2, to determine whether a higher bictegravir dose and/or earlier postexposure treatment initiation could offer protection. Thirty-one naive male rhesus macaques and 11 uninfected animals from Study-1 were distributed into seven new treatment groups for Study-2 (Figure 2a). The three-drug regimen groups now received a higher bictegravir dose of 100 mg along with 200 mg FTC and 25 mg TAF, while the FTC/TAF regimen dose remained unchanged from the previous study. In addition to comparing PEP schedules initiated 24 or 48 h postexposure with the new FTC/TAF+BIC regimen, we evaluated treatment initiation at 6 and 12 h postexposure with both FTC/TAF and FTC/TAF+BIC (100 mg).

Following eight challenge cycles, 5/6 placebo control animals became infected (Figure 2b and c). The FTC/TAF regimen resulted in 3/6 and 4/6 uninfected animals in the +6 and +30 h group and +12 and +36 h group, respectively. This result signified an improvement over the rate of protection seen with FTC/TAF in Study-1 at the 24 and 48 h treatment initiation, but overall still did not significantly reduce the per-exposure risk relative to placebo. In contrast, FTC/TAF + 100 mg BIC provided 90.1% (5/6 protected,



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	Placebo	+6,+30h FTC/TAF	+6,+30h FTC/TAF+BIC	+12,+36h FTC/TAF	+12,+36h FTC/TAF+BIC	+24,+48h FTC/TAF+BIC	+48,+72h FTC/TAF+BIC
Fraction protected	1/6 (17%)	3/6 (50%)	5/6 (83%)	4/6 (67%)	4/6 (67%)	4/6 (67%)	3/6 (50%)
Hazard ratio (95% Conf. Interval)	1	0.422 (0.1-1.79)	0.099 (0.01-0.86)	0.218 (0.04-1.14)	0.183 (0.04-0.97)	0.197 (0.04-1.04)	0.331 (0.08-1.41)
Per-exposure risk reduction		57.8%	90.1%	78.2%	81.7%	80.3%	66.9%
p-value vs placebo		0.241	**0.036**	0.071	**0.046**	0.055	0.134

Figure 2. Study-2: emtricitabine/tenofovir alafenamide (FTC/TAF) + 100 mg bictegravir (BIC), but not FTC/TAF alone, is effective as PEP in macaques. (a) Efficacy Study-2 design. Negative hour values represent timing of drug dosing before SHIV exposure, positive time values represent drug dosing post exposure. SHIV challenge is denoted in red at time = 0. Six placebo control animals were split into three subgroups ($n = 2$ each) dosed at: +6 and +30; +24 and +48; or +48 and +72 h relative to SHIV challenge (b) Percentage of protected animals following eight repeat challenge/drug dosing cycles and a 6 month follow-up as determined by undetectable plasma viral load qRT-PCR read-out. SHIV challenges are denoted by black arrows under the *x*-axis. FTC/TAF + 100 mg BIC protects or delays time to infection relative to placebo control as shown. Each of four dosing schedules is plotted against the same group of six placebo control animals all evaluated in parallel. (c) Summary of the fraction of animals protected, the resulting hazard ratios with 95% CIs and the per-exposure risk reduction rates at the end of study. *P* value determined using Cox proportional hazard regression model. Statistically significant values below a two-sided alpha level of 0.05 are shown in bold.

$P = 0.036$) per-exposure risk reduction when initiated 6 h postexposure and 81.7% (4/6 protected, $P = 0.046$) when initiated 12 h postexposure. Later treatment initiation yielded better protection with the 100 mg bictegravir dose as compared with the 25 mg dose assessed in Study-1, although given the small sample size, this did not reach the 5% significance cutoff relative to placebo. Specifically, the +24 and +48 h group and the +48 and +72 h group offered 80.3% (4/6 protected, $P = 0.055$) and 66.9% (3/6 protected, $P = 0.134$) per-exposure risk reduction, respectively. Overall, these results demonstrate that the three-drug regimen containing the higher dose of bictegravir (100 mg) can reduce the risk of infection postexposure, but that the level of protection also correlates with the time to treatment.

The animals exhibiting viraemia in both studies were monitored for an additional 6 months to track viral load kinetics (Figure S1 and Figure S2, available as Supplementary data at *JAC* Online). Thereafter, the animals that did not spontaneously control the virus to below 50 copies/mL detection limit were placed on standard daily combination ART regimen consisting of tenofovir disoproxil fumarate, emtricitabine and dolutegravir.[33] All ART-treated animals responded to therapy by exhibiting a reduction in plasma viral load, which reached undetectable levels for those that completed a 6 month treatment course to date (Figure S1 and Figure S2).

Discussion

Together, the data from these two NHP studies support prior preclinical and clinical reports demonstrating the effectiveness of combination ART for HIV prophylaxis, but also highlight the advantage of incorporating an INSTI into the short-course event-driven prophylaxis. Distinct timing of action of NRTIs and INSTIs, combined with the specific drug PK properties determine each regimen's protective potential for event-driven prophylaxis. As shown in prior reports, there is a discrepancy between the timing of viral life cycle events *in vitro* versus *in vivo*, thus animal studies are critical in evaluating the efficacy of event-driven HIV prophylaxis schedules.[22] In our animal model, two doses of 200 mg emtricitabine and 25 mg tenofovir alafenamide (both NRTIs) with or without bictegravir (an INSTI) were protective as PrEP. However, for short-course PEP, the FTC/TAF-only regimen was not significantly protective with any dosing schedule tested, as was the three-drug combination containing 25 mg bictegravir. In contrast, the addition of a 100 mg dose of bictegravir to FTC/TAF offered significant protection up to 12 h post challenge. These data are in agreement with the viral genome integration timing, which occurs at a later stage of the lifecycle relative to reverse transcription, and encourage the efforts to extend prophylaxis options from the established short-course PrEP to an early PEP. However, because the pharmacokinetic profiles of FTC/TAF and bictegravir differ between species (e.g. shorter bictegravir half-life in macaques, distinct plasma protein binding) and the actual inhibitory drug concentrations may vary somewhat between HIV and SHIV, the actual human protective dose of each drug component cannot be directly inferred. Notably, a recent report examining one- and two-dose schedules of FTC/TAF in combination with a cobicistat-boosted integrase inhibitor, elvitegravir, similarly demonstrated that an NRTI plus an INSTI regimen provides protective benefit as both PrEP and PEP, where the time to treatment initiation postexposure correlates with the level of protection.[34]

Given the past predictive value of the macaque mucosal challenge model for HIV prevention,[35] the results presented here support further exploration of two-dose PrEP and PEP modalities for individuals at risk for HIV infection. A single regimen such as FTC/TAF+BIC has the potential to offer protection as both PrEP and PEP, thus simplifying the solutions for event-driven prophylaxis. A fixed-dose BIC/FTC/TAF single-tablet-regimen has already demonstrated safety and tolerability both as daily treatment and a 28-course PEP regimen in the clinic.[36,37] The safety and efficacy of the two-dose prophylaxis schedules containing bictegravir, emtricitabine and tenofovir alafenamide remain to be established in appropriately powered clinical studies. The preclinical studies presented here can guide the design of such clinical studies.

Though appealing for individuals who experience challenges with daily adherence, event-driven approaches may have lower adherence forgiveness than daily PrEP. Thus, daily PrEP may continue to provide the highest level of protection for those who are able to take it as directed. Nonetheless, innovation of prevention modalities will provide additional options to those who seek them. If proven effective, alternative options will help broaden the reach of HIV prophylaxis and meet the HIV eradication goals.

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Transparency declarations

The following authors are employees of Gilead Sciences and own shares of the company: E.B., S.C., D.B., F.C., M.D., T.C. and C.C.

Supplementary data

Figures S1 and S2 are available as Supplementary data at *JAC* Online.

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