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Once-Weekly Oral Dosing of MK-8591 Protects Male Rhesus Macaques From Intrarectal Challenge With SHIV109CP3

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(See the Editorial Commentary by Mugwanya and Baeten, on pages 1387–9.)

Background. MK-8591 (4′-ethynyl-2-fluoro-2′-deoxyadenosine [EFdA]) is a novel reverse transcriptase–translocation inhibitor.

Methods. We assessed MK-8591 as preexposure prophylaxis in the rhesus macaque model of intrarectal challenge with simian/human immunodeficiency virus (SHIV). In study 1, 8 rhesus macaques received 3.9 mg/kg of MK-8591 orally on day 0 and once weekly for the next 14 weeks. Eight controls were treated with vehicle. All rhesus macaques were challenged with SHIV109CP3 on day 6 and weekly for up to 12 challenges or until infection was confirmed. The dose of MK-8591 was reduced to 1.3 and 0.43 mg/kg/week in study 2 and further to 0.1 and 0.025 mg/kg/week in study 3. In studies 2 and 3, each dose was given up to 6 times once weekly, and animals were challenged 4 times once weekly with SHIV109CP3.

Results. Control macaques were infected after a median of 1 challenge (range, 1–4 challenges). All treated animals in studies 1 and 2 were protected, consistent with a 41.5-fold lower risk of infection ($P < .0001$, by the log-rank test). In study 3, at a 0.1-mg/kg dose, 2 rhesus macaques became infected, consistent with a 7.2-fold lower risk of infection ($P = .0003$, by the log-rank test). The 0.025-mg/kg dose offered no protection.

Conclusions. These data support MK-8591's potential as a preexposure prophylaxis agent.

Keywords. MK-8591 (EFdA); preexposure prophylaxis; SHIV intrarectal challenge; rhesus macaques.

Antiretroviral agents are efficacious when taken as prescribed for preexposure prophylaxis (PrEP) against human immunodeficiency virus type 1 (HIV-1) infection [1, 2]. Fixed-dose combination tenofovir disoproxil fumarate and emtricitabine (TDF/FTC) prevents HIV-1 infection in high-risk individuals when administered daily [3]. Efficacy has also been demonstrated with "on-demand" dosing [4]. In clinical trials, outcomes have been closely linked to adherence [5–9]. In high-risk heterosexual women [10, 11], TDF/FTC was ineffective, and studies were halted owing to futility, likely because <30% of participants were adherent to the treatment regimen, based on plasma drug concentration determinations. In the initial studies of men who have sex with men, overall efficacy was 44%, with a clear gap between those who were adherent (for whom efficacy was 90%) and those who were not [2]. Regimens that improve adherence are likely to represent important advances in the field. Identifying alternatives to daily oral PrEP has become a research priority, and MK-8591 is one such compound [12].

MK-8591 (4′-ethynyl-2-fluoro-2′-deoxyadenosine [EFdA]) is a novel nucleoside reverse transcriptase–translocation inhibitor. Its unique mechanisms of action and distinct pharmacologic characteristics distinguish MK-8591 from approved antiretroviral agents [13–16]. MK-8591 has structural features that contribute to its pharmacologic attributes: 4′-ethynyl, 3′-hydroxyl, and 2-fluoro groups. The 4′-ethynyl group binds tightly to a conserved hydrophobic pocket in HIV-1 reverse transcriptase and interferes with translocation of the extended primer resulting in immediate chain termination [17–19]. The 3′-hydroxyl group, found in naturally occurring nucleotides, contributes to very high binding affinity for reverse transcriptase. Finally, the 2-fluoro on the adenine base ring renders the drug less susceptible to deamination by adenosine deiminase, contributing to its long intracellular half-life ($t_{1/2}$) [20]. These unique structural elements and mechanisms of action confer MK-8591 with high antiviral

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potency and unique pharmacologic characteristics, making low-dose, infrequent administration feasible.

The potential for extended-duration dosing with MK-8591 was first demonstrated in rhesus macaques (RMs). MK-8591 triphosphate (MK-8591–TP), the active metabolite of MK-8591 [21], exhibited a 50-hour intracellular $t_{1/2}$ in peripheral blood mononuclear cells (PBMCs) [22]. When administered to $SIVmac_{251}$-infected RMs, 2 once-weekly doses ranging from 3.9 to 18.2 mg/kg resulted in a 1.8-log_{10} reduction in the plasma SIV RNA load. The 3.9-mg/kg/week dose provided a mean MK-8591–TP trough level at 168 hours of 0.53 pmol/10^6 PBMCs and was on the plateau of treatment efficacy. This informed the initial proof-of-concept experiments of once-weekly PrEP doses of MK-8591 described here [22].

Efficacy with weekly MK-8591 dosing has also been demonstrated in the clinic. In humans, MK-8591-TP has a long intracellular $t_{1/2}$ (range, 78.5–128 hours in PBMCs) [23]. Single oral doses of MK-8591 of 0.5–30 mg reduced plasma HIV-1 levels by 1.2 to 1.8 log_{10} copies/mL over 7–10 days in HIV-1 infected individuals [24]. The MK-8591–TP trough level required for virologic efficacy was consistent with that observed in simian immunodeficiency virus (SIV)–infected RMs. At the 10-mg dose, which was well on the plateau of virologic response, the MK-8591–TP trough level was approximately 1 pmol/10^6 PBMCs. At steady state, daily oral dosing of 0.25 mg MK-8591 provides approximately the same PBMC MK-8591–TP trough level as after a single 10-mg dose [24].

SHIV109CP3 [25], a pathogenic clade C simian/human immunodeficiency virus (SHIV) swarm raised from a SHIV clone, SHIVC109.PB4, that contains an HIV-1 envelope from a recently infected Zambian individual was used as the challenge stock for these studies. This SHIV is CCR5 tropic and readily transmissible by the mucosal route. SHIV109CP3 was recovered from the third passage in a RM with rapidly progressing infection. This virus replicates to high levels in vivo and, during acute infection, depletes $CD4^+$ T cells in the peripheral blood and the gastrointestinal lymphoid tissue of infected macaques [25].

Here we present the first preclinical studies of MK-8591 as a potential PrEP agent in the RM model of intrarectal challenge with SHIV, using doses of MK-8591 administered weekly.

MATERIALS AND METHODS

Study Design

The efficacy of MK-8591 in preventing intrarectal SHIV transmission was assessed in 3 sequential studies. Sixteen male

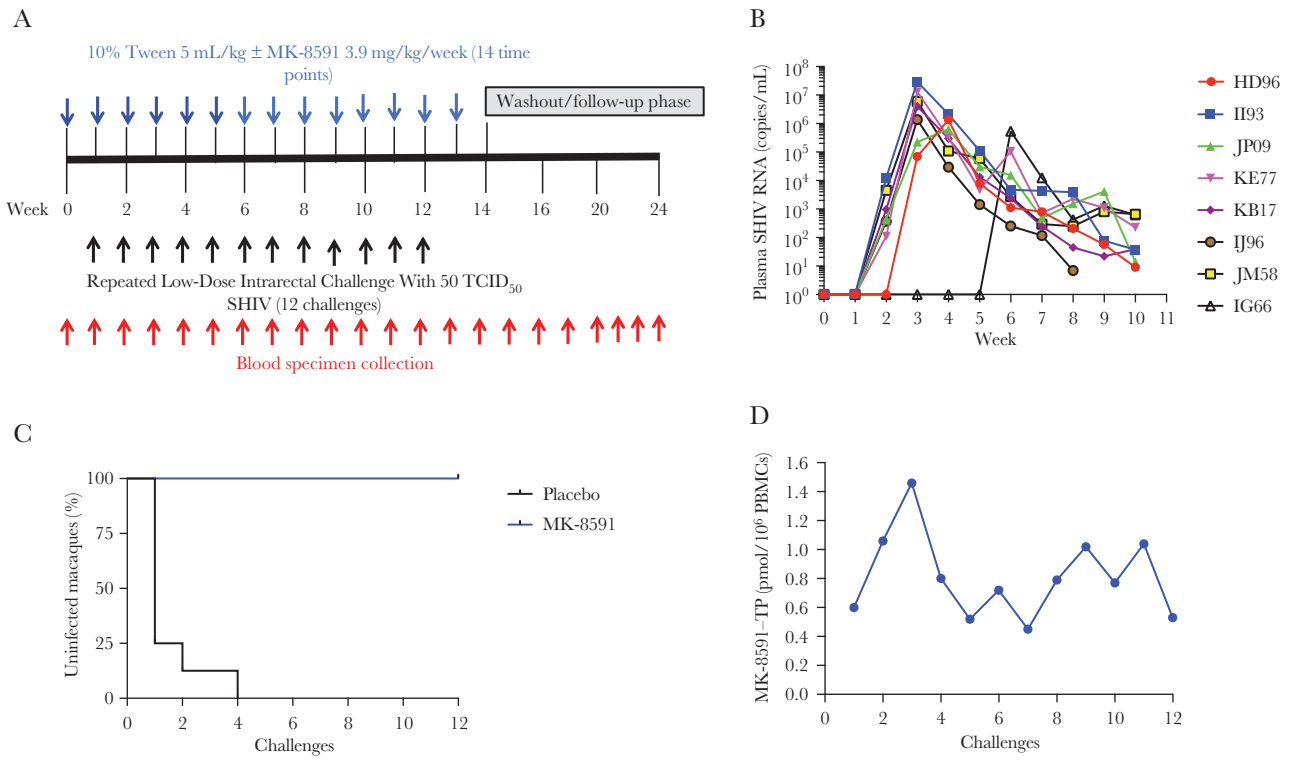

Figure 1. Weekly oral gavage of 3.9 mg/kg MK-8591 protects macaques against repeated rectal exposure to simian/human immunodeficiency virus (SHIV). *A*, Design for study 1. Eight male Indian rhesus macaques were given 5 mL/kg 10% Tween 80 with 3.9 mg/kg MK-8591 by oral gavage on day 0 and weekly thereafter for a total of 14 treatments. Eight were given 5 mL/kg 10% Tween 80 and served as controls. Animals were challenged with 50 median tissue culture infectious doses ($TCID_{50}$) of SHIV109CP3 for up to 12 exposures or until infection occurred. Animals were followed for a maximum of 24 weeks and assessed weekly for the presence of SHIV infection, as determined by detection of plasma viremia, proviral DNA, or seroconversion. *B*, Plasma viral loads for individual control macaques. The lower limit of detection of the assay is 40 SHIV copies/mL plasma. *C*, Kaplan-Meir plot of macaques treated with 3.9 mg/kg MK-8591 and macaques remaining uninfected after serial SHIV challenges. *D*, Mean levels of intracellular MK-8591 triphosphate (MK-8591–TP) at the time of challenge. PBMC, peripheral blood mononuclear cell.

RMs ranging in age from 4.3 to 9.3 years and weighing 10.0 kg on average were evaluated in study 1 (Figure 1*A*). Animals were given 5 mL/kg 10% Tween 80 with or without 3.9 mg/kg MK-8591 by oral gavage on days 0 and 7 and weekly thereafter for a total of 14 treatments or until infection was documented. Phlebotomy was performed on days 0 and 6 and weekly thereafter for virologic, immunologic, and, when indicated, pharmacokinetic analyses. SHIV109CP3 challenge was performed after phlebotomy on days 6 and 13 and weekly thereafter as described below.

In study 2 (Figure 2*A*), the remaining uninfected animals (n = 8) from study 1 were used, and the MK-8591 dose was reduced in a stepwise fashion, first to 1.3 mg/kg/week and then to 0.43 mg/kg/week. Because control animals became infected after ≤4 challenges, we treated the 8 animals with MK-8591 six times at each dose and conducted 4 weekly intrarectal challenges with SHIV109CP3. Because intracellular levels of MK-8591–TP were undetectable in 7 of 8 animals 3 weeks after the last 3.9-mg/kg dose in study 1, a 4-week washout period between the 1.3- and 0.43-mg/kg dosing intervals of MK-8591 was deemed adequate (Supplementary Table 1). Challenges with the same viral stock were initiated on days 6 and 13 and weekly for 4 challenges after phlebotomy.

Finally, in study 3, we again used the remaining uninfected animals from studies 1 and 2 (n = 8), reduced the MK-8591 dose in a stepwise manner to 0.1 and 0.025 mg/kg/week, and conducted challenges and dosing as described for study 2 (Figure 3*A*). In study 3, the washout period between the 2 dosing levels was reduced to 3 weeks because of the predicted pharmacokinetics of MK-8591.

Studies were approved by the Institutional Animal Care and Use Committee of the Tulane National Primate Research Center. During all procedures, including phlebotomy, intrarectal challenge, and oral gavage, animals were anesthetized with tiletamine/zolazepam (Telazol; 8 mg/kg) or ketamine (10 mg/kg).

Virologic and Immunologic Monitoring

Animals were monitored using a real-time reverse transcription–polymerase chain reaction (PCR) assay with a sensitivity of 40 copies/mL plasma and by proviral DNA amplification from PBMCs as previously described [26]. Virus-specific antibody

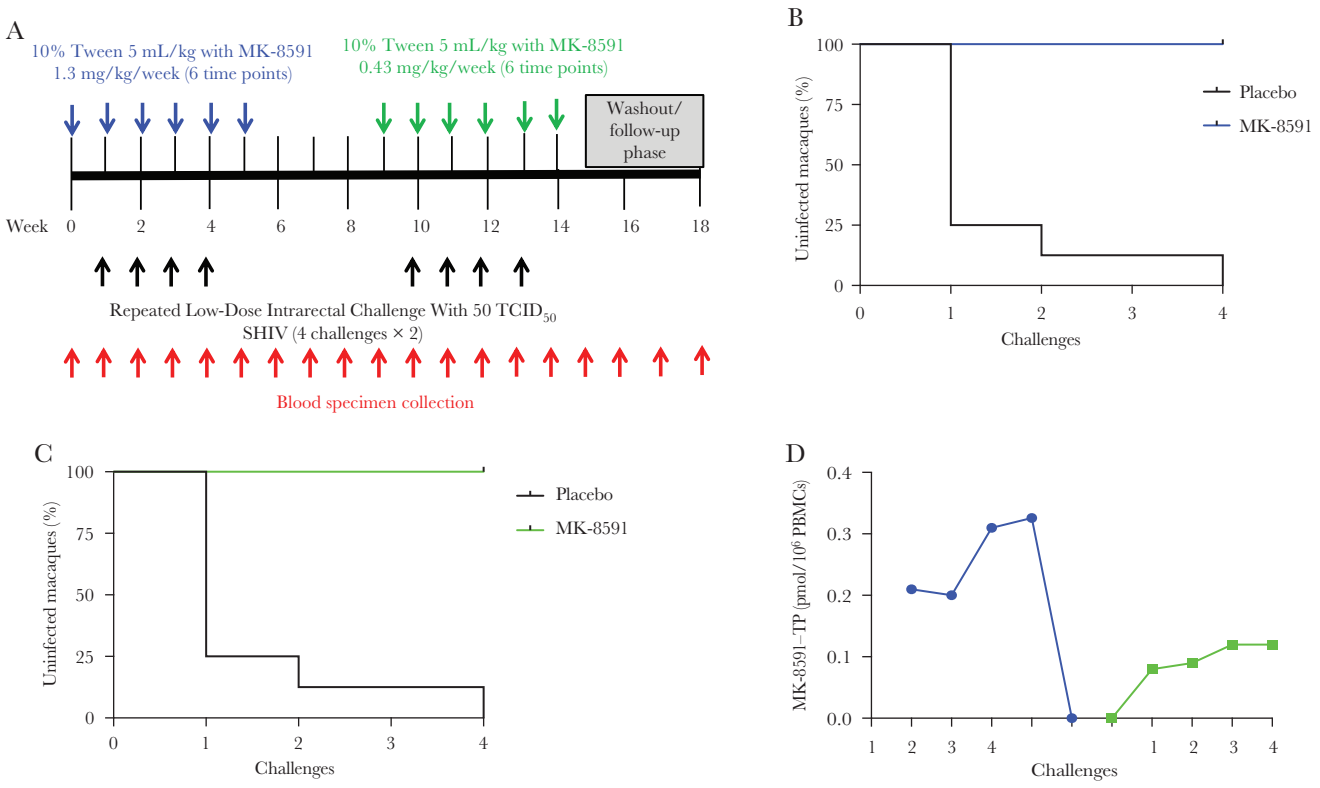

Figure 2. Weekly oral gavage of 1.3 and 0.43 mg/kg MK-8591 protects macaques against repeated rectal exposure to simian/human immunodeficiency virus (SHIV). *A*, Design for study 2. Eight male Indian rhesus macaques were given 5 mL/kg 10% Tween 80 with 1.3 and 0.43 mg/kg MK-8591 by oral gavage on day 0 and weekly thereafter for a total of 6 treatments or until infection was documented. Animals were challenged with 50 median tissue culture infectious doses ($TCID_{50}$) of SHIV109CP3 for 4 exposures. Animals were followed for a maximum of 18 weeks and assessed weekly for the presence of SHIV infection as determined by detection of plasma viremia, proviral DNA, or seroconversion. *B*, Kaplan-Meir plot of macaques treated with 1.3 mg/kg MK-8591 and macaques remaining uninfected after serial SHIV challenge. *C*, Kaplan-Meir plot of macaques treated with 0.43 mg/kg MK-8591 and macaques remaining uninfected after serial SHIV challenge. *D*, Mean intracellular levels of MK-8591 triphosphate (MK-8591–TP) at the time of challenge in the 1.3-mg/kg dosing group (blue) and the 0.43-mg/kg dosing group (green). PBMC, peripheral blood mononuclear cell.

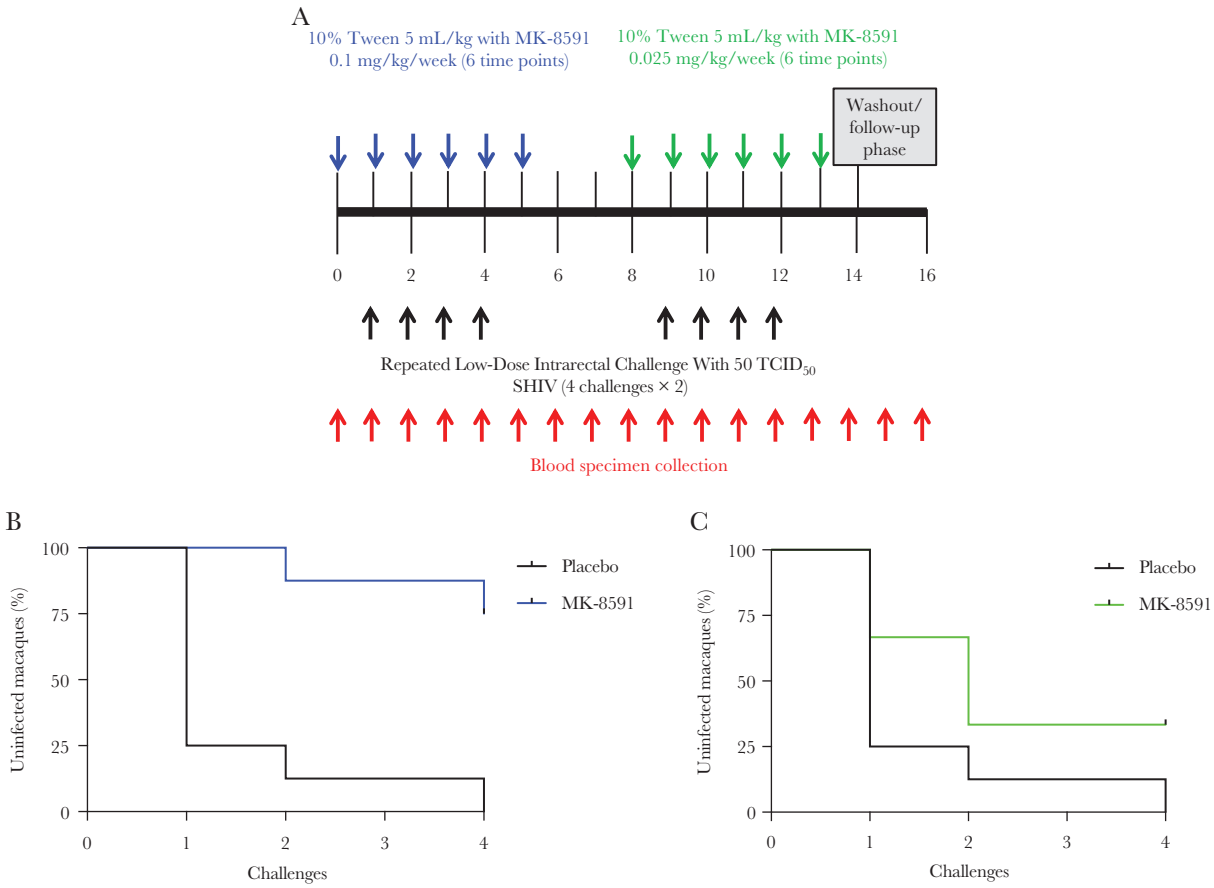

Figure 3. Weekly oral gavage of 0.1 mg/kg MK-8591 protects macaques against repeated rectal exposure to simian/human immunodeficiency virus (SHIV), but doses of 0.025 mg/kg weekly fail to protect animals against repeated SHIV rectal challenge. *A*, Design for study 3. Eight male Indian rhesus macaques were given 5 mL/kg 10% Tween 80 with 0.1 and 0.025 mg/kg MK-8591 by oral gavage on day 0 and weekly thereafter for a total of 6 treatments or until infection was documented. Animals were challenged with 50 median tissue culture infectious doses ($TCID_{50}$) of SHIV109CP3 for up to 4 exposures or until infection occurred. Animals were followed for a maximum of 14 weeks and assessed weekly for the presence of SHIV infection, as determined by detection of plasma viremia, proviral DNA, or seroconversion. *B*, Kaplan-Meir plot of macaques treated with 0.1 mg/kg MK-8591 and macaques remaining uninfected after serial SHIV challenge. *C*, Kaplan-Meir plot of macaques treated with 0.025 mg/kg MK-8591 and macaques remaining uninfected after serial SHIV challenge.

responses were measured using a commercial immunoassay as per the manufacturer's instructions (Genetic Systems HIV-1/HIV-2 Plus O; Bio-Rad). Animals were considered infected and virus challenges stopped after viral RNA was detected in 2 consecutive plasma specimens or, in the case of treated animals, either viral RNA was detected in 2 consecutive plasma specimens or viral RNA was detected in 1 plasma specimen followed by detection of proviral DNA in ≥2 wells.

To assess for MK-8591–resistant virus, we amplified and sequenced the reverse transcriptase gene subregion (332 bp) in plasma specimens from treated animals that became viremic. The reverse transcriptase coding region of plasma viral RNA was reverse transcribed with Superscript III (Invitrogen, MA), using the reverse primer macRT.R1, followed by RNase H treatment. Complementary DNA was subjected to nested PCR analysis with the Platinum Taq High Fidelity DNA polymerase (Invitrogen). The amplification conditions for the first PCR assay were 1 cycle at 94°C for 2 minutes; 30 cycles at 94°C for 15 seconds, 55°C for 30 seconds, and 68°C for 1 minute; and 1 cycle at 68°C for 10 minutes. The amplification conditions for the second PCR assay were 1 cycle at 94°C for 2 minutes; 35 cycles at 94°C for 15 seconds, 55°C for 30 seconds, and 68°C for 30 seconds; and 1 cycle at 68°C for 10 minutes. The primer set for the first PCR assay was macRT-F1, with a sequence of 5′-AGC CAGGAAAACGATACATTTATAAGGTTCT-3′ (base numbers 3267–3297 in SIVmac239), and macRT-R1, with a sequence of 5′-TTTCCTCATATTCTGCTTCTGCCATCT-3′ (base numbers 3767–3741). The primer set for the second PCR assay was macRT-F2, with a sequence of 5′-CCTCAG GGATGGAAGGGGTCAC-3′ (base numbers 3299–3320), and macRT-R2, with a sequence of 5′-AACTTCTGTATATC ATTCACTGTCCAGGTCTC-3′ (base numbers 3630–3599). The second PCR product was purified before sequencing. To increase the coverage of the sequences of viral RNA in plasma, we amplified products from 4 PCR assays and combined

them for sequencing. The sequences were analyzed with the software package Lasergene (DNAStar, WI).

Virus Stock and Challenge

The SHIV109CP3 viral stock was expanded and titrated in RM PBMCs. The virus infectious titer of the challenge stock was calculated at 1580 median tissue culture infectious doses ($TCID_{50}$) by the method of Reed and Meunch [27]. Challenges were performed with 1.0 mL of 50 $TCID_{50}$ of viral supernatant appropriately diluted and inoculated atraumatically on days 6 and 13 and weekly thereafter at the time when the intracellular level of MK-8591–TP was lowest. Challenges were performed on the same day with the same virus stock and inoculation method.

Pharmacokinetic Studies

The concentrations of MK-8591 in plasma were determined by liquid chromatography tandem mass spectrometry (LC-MS/MS) following protein precipitation. Aliquots of plasma (150 µL) were precipitated by addition of 600 µL of acetonitrile, followed by centrifugation at 3000 g for 5 minutes. A 450-µL aliquot of supernatant was evaporated to dryness, reconstituted with 100 µL of 50/50 methanol/water, and injected for analysis. LC-MS/MS was performed on a Waters Acquity ultraperformance liquid chromatography (UPLC) system interfaced to an API-5000 mass spectrometer, using the turbo ionspray interface (AB Sciex, Framingham, MA). Chromatography was performed on a Waters XSelect high-strength silica T3 column (50 × 2.1 mm; particle size, 2.5 µm), using mobile phases consisting of 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B) at a flow rate of 0.45 mL/minute. Chromatography was performed using a gradient, as follows: the column was equilibrated with 10% solvent B; after sample injection, solvent B was maintained at 10% for 0.5 minutes before being increased linearly to 90% over a 1.25-minute period; and solvent B was then returned to initial conditions and held for an additional 1 minute. The total run time was 2.8 minutes. Quantification was done by monitoring the transition from a mass-to-charge ratio of 294 to a ratio of 154 for MK-8591, using [$^{13}C^{15}N_3$] MK-8591 as an internal standard. Analyte concentrations were determined by weighted ($1/x^2$) linear regression, and the linear calibration range was from 0.1 to 100 ng/mL.

Concentrations of MK-8591–DP and MK-8591–TP in PBMCs were determined by LC-MS/MS, using a Waters Acquity UPLC interfaced to an API-5500 mass spectrometer, using the turbo ionspray interface operated under negative ionization mode (AB Sciex). Separation of MK-8591–DP and MK-8591–TP was achieved by ion exchange chromatography on a Thermo Basic AX column (50 × 1.0 mm; particle size, 5 µm), using gradient elution with mobile phases consisting of 70% water and 30% acetonitrile solution containing 10 mM ammonium acetate and 0.5% dimethyl sulfone (solvent A) and of 70% water and 30% acetonitrile solution containing 20 mM ammonium acetate, 1.5% ammonium hydroxide, and 0.5% dimethyl sulfone (solvent B), at a flow rate of 0.12 mL/minute. Separation was achieved using the following conditions: the column was equilibrated with 2% solvent B, and after sample injection, solvent B was maintained at 2% for 0.1 minutes before being increased linearly to 95% over a 2.0-minute period. Solvent B was then returned to initial conditions and held for an additional 1.45 minutes. The total run time was 3.5 minutes. Quantification was performed by monitoring the transition from a mass-to-charge ratio of 452 to a ratio of 159 for MK-8591–DP and from a mass-to-charge ratio of 532 to a ratio of 159 for MK-8591–TP, using [$^{13}C^{15}N_3$] MK-8591–DP as an internal standard. Analyte concentrations in PBMCs were determined by weighted ($1/x^2$) linear regression, and the linear calibration range was from 0.1 to 40 ng/mL.

Statistical Considerations and Analysis

In study 1, with the assumption that 50% of challenges would result in infection in control animals, the study had 99% power to detect a 90% effective PrEP agent, using the Fisher exact *t* test with a *P* value of .05. Studies 2 and 3 were single arm, open label, and performed with as many as 8 and as few as 6 uninfected RMs, respectively. Results in both studies were compared to results for the control animals from study 1 because all animals were treated with the identical viral stock in an identical manner. In studies 2 and 3, because was established that 100% of the challenges would result in infection in control animals (median value), the studies had 100% power to detect a 90% effective PrEP agent, using the Fisher exact *t* test with a *P* value of .05. The log-rank test was used to calculate statistical differences between MK-8591–treated RMs at all dosing levels and control animals. The hazard ratios presented were estimated by the log-rank model. All analyses were performed using Graph Pad Prism (version 7.0).

RESULTS

In the first experiment involving rectal challenge with low-dose SHIV, study 1 (Figure 1*A*), 6 of 8 control macaques became infected after 1 challenge, 1 became infected after 2 challenges, and the last became infected after 4 challenges (Figure 1*B*). One control animal was euthanized on day 63 (56 days after infection was confirmed), owing to weight loss, diarrhea, and gastrointestinal bleeding. Plasma SHIV levels and $CD4^+$ T-cell counts were not consistent with simian AIDS–related mortality and postmortem gastrointestinal pathology findings suggested concomitant infection with another viral pathogen. At this dosing level, all MK-8591–treated animals remained uninfected after 12 challenges, based on the absence of plasma viremia, of detectable proviral DNA in circulating PBMCs, and of seroconversion out to week 24 of the study (Figure 1*C*). This corresponded

to a 41.5-fold lower risk of infection (95% confidence interval [CI], 7.3–237.9; $P < .0001$, by the log-rank test), compared with control animals. Intracellular levels of MK-8591–TP at the time of challenge ranged from 0.45 to 1.04 pmol/10^6 PBMCs (mean, 0.81 pmol/10^6 PBMCs; Figure 1*D*). To facilitate comparison of MK-8591 plasma concentrations to MK-8591–TP levels in PBMCs, the amount of MK-8591–TP in PBMCs was converted to micromoles/liter, assuming a volume of 200 fL/PBMC. Mean MK-8591 plasma concentrations at the time of challenge (2.8 nM) were approximately 2000-fold lower on average than mean intracellular concentrations of the active triphosphate moiety at the time of challenge (4.1 μM), consistent with the pharmacologic characteristics and mechanisms of action of MK-8591.

In study 2, all animals were protected at doses of 1.3 and 0.43 mg/kg/week, translating into a 41.5-fold (95% CI, 7.3–237.9-fold) lower risk of infection ($P < .0001$, by the log-rank test) when compared to study 1 controls (Figure 2*B* and 2*C*). Mean levels of MK-8591–TP at the time of challenge at these dosing levels were 0.28 pmol/10^6 (range, 0.20–0.33 pmol/10^6) and 0.10 pmol/10^6 (range, 0.08–0.12 pmol/10^6), respectively (Figure 2*D*).

Six of 8 animals were protected at the 0.1-mg/kg dose (Figure 3*B*). Unlike findings in untreated control animals, in which there was a clear eclipse phase of 1 week between infection and the appearance of viremia, plasma viremia and proviral DNA results in animal JT33 supported an eclipse phase of 2 weeks between infection and the appearance of viremia (Supplementary Table 2). Assuming a 2-week eclipse phase in these treated animals, we conclude that animal KF34 was infected after 2 challenges and animal JT33 was infected after 4 challenges. This translates into a 7.2-fold (95% CI, 2.0–26.2-fold) lower risk of infection ($P = .003$, by the log-rank test). At the lowest dose, 4 of 6 remaining animals became infected after 1–3 challenges (Figure 3*C*). This was comparable to findings for controls and was not statistically consistent with any degree of protection (hazard ratio, 3.37; 95% CI, .8–13.7; $P = .089$). Study 3 was concluded 2 weeks before the end of the planned washout period, 14 weeks as opposed to 16 weeks, because the drug proved to be ineffective at the lowest dosing level tested (Figure 3*A*).

Intracellular Concentrations of MK-8591–TP at the Times of Challenge

Mean intracellular levels of MK-8591–TP at the times of challenge for the various doses are shown in Table 1 and Figures 1*D* and 2*D*. Mean levels of MK-8591–TP were approximately linear as the MK-8591 dose decreased from 3.9 to 1.3 to 0.43 mg/kg. At the 0.1-mg/kg dose, the intracellular level of MK-8591–TP could not be reliably quantified. Therefore, based on the observed dose proportionality of intracellular MK-8591–TP levels at the time of challenge for the higher doses, the intracellular levels at the time of challenge for the 0.1-mg/kg weekly dose was estimated to be 24 fmol/10^6 PBMCs (0.13 μM). Given that this estimated level of MK-8591–TP protected 8 animals against a 29 of 32 challenges and that controls were infected by 1 challenge on average, this would translate to a prophylactic 90% effective concentration (EC_{90}) of approximately 24 fmol/10^6 PBMCs, comparable to that estimated for tenofovir diphosphate in RMs when dosed daily at 22 mg/kg [28].

Characterization of Breakthrough Viruses and Seroconversion

Mean peak plasma levels (±SD) of SHIV109CP3 in treated animals with breakthrough infections were lower than in untreated animals ($\log_{10}$ 3.89 ± 4.22 vs $\log_{10}$ 6.86 ± 7.00 copies/mL; $P = .0007$, by the Mann-Whitney test). We believe that the presence of active drug was responsible for this difference. The time to seroconversion between treated animals and controls was not statistically different (3.6 and 2.5 weeks, respectively; $P = .26$, by the Mann-Whitney test). The 2 animals that remained aviremic had negative results of qualitative proviral DNA analyses at all time points and did not seroconvert.

Hypothetically, when antiretroviral agents are used to prevent infection, it is possible for drug-resistant variants to emerge and establish infection if there are subinhibitory drug concentrations. In vitro–passage experiments demonstrated that the main resistance-conferring mutations that reduce susceptibility to MK-8591 are M184V and M184I [13, 29]. We performed consensus sequencing of the reverse transcriptase coding region in all 6 MK-8591–treated animals with evidence of viremia 2 weeks on average after the first quantifiable plasma viral load determination, when mean plasma SHIV-1 RNA levels were 3.1 $\log_{10}$ copies/mL plasma. Neither M184V nor M184I was detected.

Table 1. MK-8591 Triphosphate (MK-8591–TP) Levels at the Time of Intrarectal Simian/Human Immunodeficiency Virus Challenge, by MK-8591 Dose

MK-8591 Dose, mg/kg	Ratio of Specified MK-8591 Dose to Index MK-8591 Dose	MK-8591–TP Level at Challenge, fmol per 10^6 PBMCs, Mean (Range)	Ratio of MK-8591–TP Level to Index MK-8591 Dose
3.9 (index)	1	810 (339–1616)	1
1.3	0.33	282 (161–399)	0.35
0.43	0.11	102 (68–159)	0.125
0.10	0.025	24[a]	0.029

[a]Estimated value.

DISCUSSION

The protection against SHIV109CP3 infection provided by low-dose, weekly administration of MK-8591 demonstrates its potential as a next-generation PrEP agent. Intracellular levels of MK-8591–TP of ≥102 fmol/10^6 PBMCs resulted in complete protection in this model. The EC_{90} is estimated to be 24 fmol/10^6 PBMCs, which was achieved with a 0.1-mg/kg oral weekly dose. For comparison, it has been estimated that the EC_{90} of TDF is 22.6 fmol/10^6 PBMCs in RMs treated with oral TDF/FTC and 16 fmol/10^6 PBMCs in men participating in the iPrEx study [28]. The dose of MK-8591 required to obtain a target concentration of 24 fmol/10^6 PBMCs in humans, assuming a dose proportionality of <0.5 mg (the lowest dose for which human pharmacokinetic data are available), is approximately 0.25 mg weekly and <0.01 mg daily. The projected dose required to achieve efficacious drug levels for prophylaxis against HIV infection are therefore approximately 30 000-fold lower than that demonstrated for TDF. The low daily dose requirement, coupled with the long $t_{1/2}$ of MK-8591–TP in humans, provides the opportunity for flexibility with regard to both dosing frequency and, potentially, route of administration.

MK-8591 has been evaluated in clinical trials in HIV-1–infected and uninfected individuals. MK-8591–TP exhibited an intracellular half-life of approximately 100 hours [23, 24]. In these studies, MK-8591–TP levels were well above the predicted EC_{90} for prophylaxis in excess of a month and suggests the potential for use with oral dosing regimens that are less frequent than once weekly and perhaps may couple efficacy with forgiveness for late or missed dosing [30].

There is current enthusiasm for developing long-acting formulations as PrEP. Cabotegravir (CAB), a potent integrase strand–transfer inhibitor, has been formulated as an injectable nanosuspension and is in phase 3 efficacy testing in men who have sex with men and in high-risk women [31]. It is administered intramuscularly every 8 weeks after a loading dose of 2 injections given 4 weeks apart. Long-acting injectable formulations have complicated clinical development plans. Currently, oral dosing is required before the administration of the first injection, to rule out drug hypersensitivity, because once injected the drug cannot be easily removed. There are also concerns regarding the pharmacokinetic tail (ie, the period during which circulating levels of CAB persist below efficacious levels in individuals after they cease treatment), which may increase the risk of resistance should infection occur [32]. This risk has prompted current clinical trials to use daily oral TDF/FTC treatment for a year after the last CAB injection. If dosed orally, MK-8591 has a projected pharmacologic tail that is predictably shorter than that of intramuscularly administered CAB. However, this remains hypothetical because final decisions regarding dosing and the route of MK-8591 have yet to be made, as the drug is amenable to dosing in an extended-release formulation.

Extended release of MK-8591 from implants formulated using a drug-eluting polymeric matrix has been demonstrated in both rats and nonhuman primates [33] MK-8591 release from these formulations is driven by dissolution and diffusion out of the matrix. Implants have some advantages over oral therapies and injectable agents in that they are potentially much longer lasting, can be removed in the event of untoward adverse events, and, once removed, do not have the pharmacokinetic tail associated with injectable formulations. Because MK-8591 has a substantially lower dose requirement, it may have longer durations of release from implants and a lower frequency of dosing than other agents (eg, tenofovir alafenamide) for which these types of drug-delivery systems are being pursued [34].

In summary, MK-8591 demonstrates robust efficacy as prevention in the RM model of intrarectal challenge with SHIV. The RM model of low-dose, intrarectal SHIV challenge has successfully predicted the clinical activity of TDF/FTC as PrEP in high-risk men who have sex with men [35], and our findings are encouraging. MK-8591 combines antiviral potency and pharmacokinetics that translate to flexibility in dosing level, route, and frequency of administration. Given these favorable attributes, clinical development of MK-8591 is highly anticipated, and much will be learned in the near future regarding its efficacy and safety as a potential addition to the armamentarium in both HIV-1 therapy and prevention.

SUPPLEMENTARY DATA

Supplementary materials are available at The *Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Potential conflicts of interest. T. N., L. S., K. F., D. J. H., and J. A. G. are employees of Merck Laboratories. M. M. is a paid consultant to Merck and receives grant support from Merck Laboratories, which is paid directly to the Aaron Diamond AIDS Research Center. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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