Title: Once-weekly Oral Dosing of MK-8591 Protects Male Rhesus Macaques from Intrarectal SHIV109CP3 Challenge

Authors: Martin Markowitz[1], Agegnehu Gettie[1], Leslie St. Bernard[1], Chasity D. Andrews[1], Hiroshi Mohri[1], Amir Horowitz[1]†, Brooke F. Grasperge[2], James L. Blanchard,[2] Tao Niu[3], Li Sun[3], Kerry Fillgrove[3], Daria J. Hazuda[4], Jay A. Grobler[4].

Affiliations:

[1]Aaron Diamond AIDS Research Center, an affiliate of the Rockefeller University, 455 First Avenue 7th Floor, New York, NY 10016.

[2]Tulane National Primate Research Center, Covington LA 70433

[3] Merck Research Laboratories, Merck and Co., Inc. West Point, PA 19486

[4] Merck and Co., Inc., Kenilworth, NJ 07033

1. Corresponding author:

Martin Markowitz MD

Aaron Diamond AIDS Research Center

455 First Avenue 7th Floor

New York, NY 10016

Tel: 212-448-5020 Fax: 212-725-1126 email: mmarkowitz@adarc.org

2. † Current address: Precision Immunology Institute / Tisch Cancer Institute

Icahn School of Medicine at Mount Sinai

1425 Madison Avenue / New York, NY 10029

One Sentence Summary: MK-8591 (EFdA) prevents SHIV infection in the rhesus macaque/SHIV model when administered orally once-weekly in increasingly decreased doses, demonstrating its potential for low-dose, extended duration HIV prophylaxis. (27 words)

Footnote page:

3. The following authors do not have a commercial or other association that may pose a conflict of interest: Agegnehu Gettie, Leslie St. Bernard, Chasity D. Andrews, Hiroshi Mohri[1], Amir Horowitz[1], Brooke F. Grasperge, and James L. Blanchard

   The following authors are employees of Merck Laboratories: Tao Niu, Li Sun, Kerry Fillgrove, Daria J. Hazuda, and Jay A. Grobler.

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Abstract:

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

Methods: We assessed MK-8591 as pre-exposure prophylaxis (PrEP) in the rhesus macaque (RM)-simian/human immunodeficiency virus (SHIV) intrarectal challenge model. In Study 1, 8 RMs received 3.9 mg/kg of MK-8591 orally on day 0 and weekly for 14 weeks. Eight controls were treated with vehicle. All RMs 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 QW and animals challenged 4 times once a week with SHIV109CP3.

Results: Control macaques were infected after a median of one challenge (range 1-4). All treated animals in Studies 1 and 2 were protected, consistent with a 41.5-fold lower risk of infection ($P<0.0001$, log-rank test). In Study 3, at the 0.1 mg/kg dosing

level, two RMs became infected consistent with a 7.2-fold lower risk of infection (P=0.0003, log-rank test). The 0.025 mg/kg dose offered no protection.

Conclusions: These data support MK-8591's potential as a PrEP agent.

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Key words: MK-8591 (EFdA); pre-exposure prophylaxis; SHIV intrarectal challenge; rhesus macaques

Background:

Antiretroviral agents are efficacious when taken as prescribed for pre-exposure prophylaxis (PrEP) against 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 due to futility, likely due to the observation that less than 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 adherent, 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 (NRTTI). Its unique mechanisms of action and distinct pharmacology 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 (RT). Finally, the 2-fluoro on the adenine base ring renders the drug less susceptible to deamination by adenosine deaminase

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 potency and unique pharmacology making low-dose, infrequent dosing 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 (PBMC) [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 plasma SIV RNA. The 3.9 mg/kg/week dose, provided a mean MK-8591-TP trough level at 168 hours of 0.53 pmol/10^6 PBMC, and was on the plateau of treatment efficacy. This informed the initial proof of concept experiments using MK-8591 dosing once weekly (QW) in prophylaxis as 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 $t_{1/2}$ (78.5 to 128 hours in PBMC) [23]. Single oral doses of MK-8591 of 0.5 mg to 30 mg reduced plasma HIV-1 levels from 1.2 to 1.8 log_{10} at day 7 to 10 in HIV-1 infected individuals [24]. The MK-8591-TP

trough level required for virologic efficacy was consistent with that observed in SIV-infected RMs. At the 10 mg dose, which was well on the plateau of virologic response, 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 SHIV swarm raised from a SHIV clone, SHIVC109.PB4 which 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 rapidly progressing RM. 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 pre-clinical studies of MK-8591 as a potential PrEP agent in the RM-SHIV low dose intrarectal challenge model, 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 RMs ranging in age from 4.3 to 9.3 years and weighing 10.0 kg on average were evaluated in Study 1 (Fig. 1a). Animals were given 5 mL/kg 10% Tween 80 with or without 3.9 mg/kg MK-8591 by oral gavage on days 0, 7, and weekly thereafter for a total of 14 treatments or until infection was documented. Phlebotomy was performed at day 0, day 6, and weekly thereafter for virologic, immunologic, and when indicated pharmacokinetic analyses. SHIV109CP3 challenge was performed post-phlebotomy on day 6, 13, and weekly thereafter as described below.

In Study 2 (Fig. 2a), the remaining uninfected animals (N=8) from Study 1 were used and the MK-8591 dose reduced step-wise, first to 1.3 mg/kg/week and then to 0.43 mg/kg/week. As control animals became infected within 4 challenges, here we treated the 8 animals with MK-8591 six times at each dose and conducted 4 weekly intrarectal challenges with SHIV109CP3. As 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 between the 1.3 and 0.43 mg/kg dosing intervals of MK-8591 was

deemed adequate (Supplemental data Table 1). Challenges with the same viral stock were initiated on day 6, 13, and weekly for 4 challenges after phlebotomy.

Finally, in Study 3 we again used the remaining uninfected animals from Study 1 and 2 (N=8), reduced the MK-8591 dose step-wise to 0.1 and 0.025 mg/kg/week, and conducted challenges and dosing as was described in Study 2 (Fig. 3a). In Study 3, the washout between the two dosing levels was reduced to 3 weeks given 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 RT-PCR assay with a sensitivity of 40 copies/mL plasma and by proviral DNA amplification from PBMC as previously described [26]. Virus-specific antibody responses were measured using a commercial immunoassay as per manufacturer's instructions (Genetic Systems HIV-1/HIV-2 Plus O; Bio-Rad). Animals were considered infected and virus

challenges were stopped after two consecutive positive plasma viral RNA results or in the case of treated animals either two consecutive plasma RNA levels or one plasma RNA positive result followed by at least 2 wells positive for proviral DNA.

To assess for MK-8591 resistant virus, we amplified and sequenced the reverse transcriptase gene subregion (332 bp) in plasma from treated animals that became viremic. The RT coding region of plasma viral RNA was reverse transcribed with Superscript III (Invitrogen, MA) using reverse primer, macRT.R1, followed by RNase H treatment. cDNA was subjected to nested PCR with Platinum Taq High Fidelity DNA polymerase (Invitrogen). The amplification condition for the first PCR was 94 °C, 2 min, 30 cycles of 94 °C, 15 sec, 55 °C, 30 sec and 68 °C, 1 min, followed by 68 °C, 10 min. The amplification condition for the second PCR was 94 °C, 2 min, 35 cycles of 94 °C, 15 sec, 55 °C, 30 sec and 68 °C, 30 sec, followed by 68 °C, 10 min. The primer set for the first PCR are macRT-F1: 5'-AGCCAGGAAAACGATACATTTATAAGGTTCT-3' (3267-3297 in SIVmac239) and macRT-R1: 5'-TTTCCTCATATTCTGCTTCTGCCATCT-3' (3767-3741). The primer set for the second PCR are macRT-F2: 5'- CCTCAGGGATGGAAGGGGTCAC-3' (3299-3320) and

macRT-R2: 5'- AACTTCTGTATATCATTCACTGTCCAGGTCTC-3' (3630-3599). The second PCR product was purified prior to sequencing. To increase the coverage of the sequences of viral RNA in plasma, we amplified 4 PCR reactions and combined them for sequencing. The sequences were analyzed with software package, Lasergene (DNASTAR, WI).

Virus stock and challenge: The SHIV109CP3 viral stock was expanded and titrated in RM PBMC. The virus infectious titer of the challenge stock was calculated at 1,580 $TCID_{50}$ 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 day 6, 13 and weekly thereafter at a time of lowest intracellular level of MK-8591-TP. 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 spectrometric (LC-MS/MS) assays following protein precipitation. Aliquots of plasma (150 µL) were precipitated by addition of 600 µL of acetonitrile, followed by centrifugation at 4,000 rpm 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 analysis was performed on a Waters Acquity UPLC system interfaced to an API-5000 mass spectrometer utilizing the turbo ionspray interface (AB Sciex, Framingham, MA). Chromatography was performed on a Waters XSelect HSS T3 column (50 x 2.1 mm, 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/min. The chromatography was run using a gradient as follows: column was equilibrated with 10% B, after sample injection, solvent B was maintained at 10% for 0.5 minute before being increased linearly to 90% of solvent B over a 1.25 minutes 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 of *m/z* 294 to *m/z* 154 for MK-8591 using [$^{13}C^{15}N_3$] MK-8591 as 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 utilizing the turbo ionspray interface operated under negative ionization mode (AB Sciex, Framingham, Massachusetts). Separation of MK-8591-DP and MK-8591-TP was achieved by ion exchange chromatography on an Thermo Basic AX column (50 x 1.0 mm, 5 µm) using a gradient elution with mobile phases consisting of 70% water:30% acetonitrile containing 10 mM ammonium acetate and 0.5% dimethyl sulfone (solvent A) and 70% water:30% acetonitrile containing 20 mM ammonium acetate,1.5% ammonium hydroxide and 0.5% dimethyl sulfone (solvent B) at a flow rate of 0.12 mL/min. Separation was achieved using the following conditions: column was equilibrated with 2% solvent B, and after sample injection, solvent B was maintained at 2% for 0.1 minute 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 min. The total run time was 3.5 minutes. Quantification was performed by monitoring transition of *m/z* 452 to *m/z* 159 for MK-8591-DP and *m/z* 532 to *m/z* 159 for MK-8591-TP using [$^{13}C^{15}N_3$] MK-8591-DP as 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 the challenges would result in infection in control animals, the study had 99% power to detect a 90% effective PrEP agent using Fisher's exact t test with a p-value of 0.05. Studies 2 and 3 were single arm, open-label, and performed with as many as 8 and as few as 6 uninfected RMs, respectively, with results compared to the control animals from Study 1, as all animals were treated with the identical viral stock in an identical manner. In Studies 2 and 3, having 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 Fisher's exact t test with a p-value of 0.05. 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 low-dose rectal challenge experiment, Study 1, (Fig. 1a), 6 of 8 control macaques became infected after 1 challenge, one after 2 challenges, and the last after 4 challenges (Fig.1b). One control was euthanized on day 63 (56 days after confirming infection) due to weight loss, diarrhea, and gastrointestinal bleeding. Plasma SHIV levels and CD4+ T cell counts were not consistent with simian AIDS-related mortality and post- mortem gastrointestinal pathology 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, no detectable proviral DNA in circulating PBMCs, and no seroconversion out to 24-weeks of study (Fig. 1c). This corresponds to a 41.5-fold lower risk of infection (95% C.I. = 7.3, 237.9; $P<0.0001$ log-rank test) when compared to control animals. Intracellular levels of MK-8591-TP at the time of challenge ranged from 0.45 to 1.04 pmol/10^6 PBMC (mean 0.81 pmol/10^6 PBMC)(Fig. 1d). To facilitate comparison of MK-8591 plasma concentrations with MK-8591-TP levels in PBMCs, the amount of MK-8591-TP in PBMCs is converted to units of µM assuming a PBMC cell volume of 200 fL/cell. Mean MK-8591 plasma concentrations at the time of challenge (2.8 nM) were

approximately 2,000-fold lower on average than mean intracellular concentrations of the active triphosphate moiety at the time of challenge (4.1 μM) consistent with the pharmacology and mechanisms of action of MK-8591.

In Study 2, all animals were protected at the 1.3 and 0.43 mg/kg/week dose levels, translating respectively into a 41.5-fold lower risk of infection (95% C.I. = 7.3, 237.9; P<0.0001 log-rank test) when compared to Study 1 controls (Fig. 2b and 2c). Mean levels of MK-8591-TP at the time of challenge at these dosing levels were 0.28 (range: 0.20-0.33) and 0.10 (range: 0.08-0.12) pmol/10^6 respectively (Fig. 2d).

Six of 8 animals were protected at the 0.1 mg/kg dose level (Fig. 3b). Unlike that seen in untreated control animals in which there was a clear eclipse phase of one week between infection and the appearance of viremia, plasma viremia and proviral DNA results in animal JT33 support 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 KF34 was infected after 2 challenges and JT33, after 4 challenges. This translates into a 7.2-fold lower risk of infection (95% C.I. = 2.0, 26.2; P=0.003 log-rank test). At the lowest dose, 4 of the 6

remaining animals became infected after one to three challenges (Fig. 3c). This was comparable to controls and was not statistically consistent with any degree of protection (HR 3.37; 95% C.I. = 0.8, 13.7; P=0.089). Study 3 was concluded 2 weeks prior to the end of the planned washout period, 14 weeks as opposed to 16 weeks, as the drug proved ineffective at the lowest dosing level tested (Fig. 3a).

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 Fig. 1d and Fig 2d. Mean levels of MK-8591-TP were approximately linear as the MK-8591 dose is reduced from 3.9 to 1.3 to 0.43 mg/kg. At the 0.1 mg/kg dose level, 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 total of 29 of 32 challenges and controls were infected by one 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

Peak plasma levels of SHIV109CP3 in treated animals with breakthrough infections were lower than untreated animals, $\log_{10}$ 3.89±4.22 versus $\log_{10}$ 6.86±7.00 copies/mL (P=0.0007, Mann Whitney). We believe the presence of active drug is responsible for this difference. Time to seroconversion between treated animals and controls was not statistically different, 3.6 weeks versus 2.5 weeks (P=0.26 Mann Whitney). The two animals that remained aviremic had negative qualitative proviral DNA determinations 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 sub-inhibitory 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 after 2 weeks

on average from 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 were detected.

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 at or above 102 fmol/10^6 PBMC resulted in complete protection in this model. The EC_{90} is estimated to be 24 fmol/10^6 PBMC 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 PBMC in RMs treated with oral TDF/FTC and 16 fmol/10^6 PBMC in men participating in the iPrEx study [28]. The dose of MK-8591 required to obtain a target concentration of 24 fmol/10^6 PBMC in humans, assuming dose proportionality below 0.5 mg (the lowest dose for which human pharmacokinetic data are available) is approximately 0.25 mg weekly and less than 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 hrs [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 QW 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 both men who have sex with men and high-risk women [31]. It is administered intramuscularly every 8 weeks after an every 4 week loading dose. Long acting injectable formulations have complicated clinical development plans. Currently, oral dosing is required prior to the

administration of the first injection to rule out drug hypersensitivity as once injected the drug cannot be easily removed. There are also concerns regarding the pharmacokinetic "tail", the circulating levels of CAB that persist below efficacious levels in individuals when they cease treatment, which may increase the risk of resistance should infection occur [32]. This risk has prompted current clinical trials to include the use daily oral TDF/FTC for a year after the last CAB injection. If dosed orally, the projected pharmacologic "tail" of MK-8591 is predictably shorter than intramuscularly administered CAB, however, this remains hypothetical as final decisions regarding dosing and 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 non-human 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 as well as injectables 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. MK-8591, because it has a substantially lower dose requirement may have longer durations of release from implants and lower frequency of dosing than for other agents (e.g. 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/SHIV intrarectal challenge model. The SHIV/RM low dose intrarectal challenge model has successfully predicted the clinical activity of TDF/FTC as PrEP in high risk MSM [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.

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References:

1. Baeten JM, Donnell D, Ndase P, et al. Antiretroviral prophylaxis for HIV prevention in heterosexual men and women. The New England journal of medicine **2012**; 367:399-410.

2. Grant RM, Lama JR, Anderson PL, et al. Preexposure chemoprophylaxis for HIV prevention in men who have sex with men. The New England journal of medicine **2010**; 363:2587-99.

3. Centers for Disease Control. Guidelines: Preexposure prophylaxis for the prevention of HIV infection in the United States. , **2014**.

4. Molina JM, Capitant C, Spire B, et al. On-Demand Preexposure Prophylaxis in Men at High Risk for HIV-1 Infection. N Engl J Med **2015**; 373:2237-46.

5. Celum C, Baeten JM. Tenofovir-based pre-exposure prophylaxis for HIV prevention: evolving evidence. Curr Opin Infect Dis **2012**; 25:51-7.

6. Celum C, Baeten JM. Antiretroviral-based HIV-1 prevention: antiretroviral treatment and pre-exposure prophylaxis. Antiviral therapy **2012**; 17:1483-93.

7. Gandhi M, Glidden DV, Liu A, et al. Strong Correlation Between Concentrations of Tenofovir (TFV) Emtricitabine (FTC) in Hair and TFV Diphosphate and FTC Triphosphate in Dried Blood Spots in the iPrEx Open Label Extension: Implications for Pre-exposure Prophylaxis Adherence Monitoring. J Infect Dis **2015**; 212:1402-6.

8. Grant RM, Anderson PL, McMahan V, et al. Uptake of pre-exposure prophylaxis, sexual practices, and HIV incidence in men and transgender women who have sex with men: a cohort study. The Lancet Infectious diseases **2014**; 14:820-9.

9. Liu AY, Yang Q, Huang Y, et al. Strong relationship between oral dose and tenofovir hair levels in a randomized trial: hair as a potential adherence measure for pre-exposure prophylaxis (PrEP). PLoS One **2014**; 9:e83736.

10. Van Damme L, Corneli A, Ahmed K, et al. Preexposure prophylaxis for HIV infection among African women. The New England journal of medicine **2012**; 367:411-22.

11. Marrazzo JM, Ramjee G, Richardson BA, et al. Tenofovir-based preexposure prophylaxis for HIV infection among African women. The New England journal of medicine **2015**; 372:509-18.

12. Markowitz M, Sarafianos SG. 4'-Ethynyl-2-fluoro-2'-deoxyadenosine, MK-8591: a novel HIV-1 reverse transcriptase translocation inhibitor. Curr Opin HIV AIDS **2018**; 13:294-9.

13. Kawamoto A, Kodama E, Sarafianos SG, et al. 2'-deoxy-4'-C-ethynyl-2-halo-adenosines active against drug-resistant human immunodeficiency virus type 1 variants. Int J Biochem Cell Biol **2008**; 40:2410-20.

14. Kodama EI, Kohgo S, Kitano K, et al. 4'-Ethynyl nucleoside analogs: potent inhibitors of multidrug-resistant human immunodeficiency virus variants in vitro. Antimicrob Agents Chemother **2001**; 45:1539-46.

15. Ohrui H. 2'-deoxy-4'-C-ethynyl-2-fluoroadenosine, a nucleoside reverse transcriptase inhibitor, is highly potent against all human immunodeficiency viruses type 1 and has low toxicity. Chem Rec **2006**; 6:133-43.

16. Ohrui H, Kohgo S, Hayakawa H, et al. 2'-Deoxy-4'-C-ethynyl-2-fluoroadenosine: a nucleoside reverse transcriptase inhibitor with highly potent activity against all HIV-1 strains, favorable toxic profiles and stability in plasma. Nucleic Acids Symp Ser (Oxf) **2006**:1-2.

17. Michailidis E, Huber AD, Ryan EM, et al. 4'-Ethynyl-2-fluoro-2'-deoxyadenosine (EFdA) inhibits HIV-1 reverse transcriptase with multiple mechanisms. J Biol Chem **2014**; 289:24533-48.

18. Michailidis E, Marchand B, Kodama EN, et al. Mechanism of inhibition of HIV-1 reverse transcriptase by 4'-Ethynyl-2-fluoro-2'-deoxyadenosine triphosphate, a translocation-defective reverse transcriptase inhibitor. J Biol Chem **2009**; 284:35681-91.

19. Salie ZL, Kirby KA, Michailidis E, et al. Structural basis of HIV inhibition by translocation-defective RT inhibitor 4'-ethynyl-2-fluoro-2'-deoxyadenosine (EFdA). Proc Natl Acad Sci U S A **2016**; 113:9274-9.

20. Kirby KA, Michailidis E, Fetterly TL, et al. Effects of substitutions at the 4' and 2 positions on the bioactivity of 4'-ethynyl-2-fluoro-2'-deoxyadenosine. Antimicrob Agents Chemother **2013**; 57:6254-64.

21. Nakata H, Amano M, Koh Y, et al. Activity against human immunodeficiency virus type 1, intracellular metabolism, and effects on human DNA polymerases of 4'-ethynyl-2-fluoro-2'-deoxyadenosine. Antimicrob Agents Chemother **2007**; 51:2701-8.

22. J.A. Grobler, Nicoll-Griffith D, Lai M-T, et al. Efficacy of once-weekly MK-8591 in SIV infected rhesus macaques. In: 17th International Workshop on Clinical Pharmacology of HIV and Hepatitis Therapy (Washington DC), **2016**.Abstract O_13.

23. Friedman E, Scheurmann D, Rudd DJ, et al. A Single Monotherapy Dose of MK-8591, A Novel NRTI, Suppresses HIV for 10 Days. In: Conference on Retroviruses and Opportunistic Infections. (Boston, MA), **2016**.Abstract 437 LB

24. Matthews R, Schuermann D, Rudd DJ, et al. Single doses as low as 0.5 mg of the novel NRTTI MK-8591 suppress HIV for at least 7 days. In: 9th International Conference on HIV Science. (Paris, FR), **2017**. Abstract 5525.

25. Ren W, Mumbauer A, Gettie A, et al. Generation of lineage-related, mucosally transmissible subtype C R5 simian-human immunodeficiency viruses capable of AIDS development, induction of neurological disease, and coreceptor switching in rhesus macaques. J Virol **2013**; 87:6137-49.

26. Andrews CD, Spreen WR, Mohri H, et al. Long-acting integrase inhibitor protects macaques from intrarectal simian/human immunodeficiency virus. Science **2014**; 343:1151-4.

27. Reed LJ, Meunch H. A simple method of estimating fifty percent endpoints. Am J Hyg **1938**:493-7.

28. Anderson PL, Glidden DV, Bushman LR, Heneine W, Garcia-Lerma JG. Tenofovir diphosphate concentrations and prophylactic effect in a macaque model of rectal simian HIV transmission. J Antimicrob Chemother **2014**; 69:2470-6.

29. Oliveira M, Brenner BG, Xu H, Ibanescu RI, Mesplede T, Wainberg MA. M184I/V substitutions and E138K/M184I/V double substitutions in HIV reverse transcriptase do not significantly affect the antiviral activity of EFdA. J Antimicrob Chemother **2017**; 72:3008-11.

30. Grobler JA, Friedman E, Barrett SE, et al. Long-Acting Oral and Parenteral Dosing of MK-8591 for HIV Treatment or Prophylaxis. In: Conference on Retroviruses and Opportunistic Infections. (Boston, MA), **2016**. Abstract 98.

31. McPherson TD, Sobieszczyk ME, Markowitz M. Cabotegravir in the treatment and prevention of Human Immunodeficiency Virus-1. Expert Opin Investig Drugs **2018**; 27:413-20.

32. Markowitz M, Frank I, Grant RM, et al. Safety and tolerability of long-acting cabotegravir injections in HIV-uninfected men (ECLAIR): a multicentre, double-blind, randomised, placebo-controlled, phase 2a trial. Lancet HIV **2017**; 4:e331-e40.

33. Barrett SE, Teller RS, Forster SP, et al. Extended-Duration MK-8591-Eluting Implant as a Candidate for HIV Treatment and Prevention. Antimicrob Agents Chemother **2018**; 62.

34. Gunawardana M, Remedios-Chan M, Miller CS, et al. Pharmacokinetics of long-acting tenofovir alafenamide (GS-7340) subdermal implant for HIV prophylaxis. Antimicrob Agents Chemother **2015**; 59:3913-9.

35. Garcia-Lerma J G, Cong M, Mitchell J, et al. Intermittent Prophylaxis with Oral Truvada Protects Macaques from Rectal SHIV Infection. Sci Transl Med **2010**; 2:14 14ra4.

Figure Legend

Fig. 1. Weekly oral gavages of 3.9 mg/kg MK-8591 protects macaques against repeated SHIV rectal exposures.

a. Study design- Step 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 $TCID_{50}$ 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 the presence of plasma viremia, proviral DNA or seroconversion.

b. Plasma viral loads of individual control macaques. 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) expressed as picomol/10^6 PBMC at the time of challenge .

Fig. 2. Weekly oral gavages of 1.3 and 0.43 mg/kg MK-8591 protect macaques against repeated SHIV rectal exposures.

a. Study design- Step 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 $TCID_{50}$ 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 the presence 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 challenges.

c. Kaplan-Meir plot of macaques treated with 0.43 mg/kg MK-8591 and macaques remaining uninfected after serial SHIV challenges.

d. Mean intracellular levels of 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).

Fig. 3. Weekly oral gavages of MK-8591 at 0.1 mg/kg protect macaques against repeated SHIV rectal exposures however doses of 0.025 mg/kg weekly fail to protect animals against repeated SHIV rectal challenges.

a. Study design- Step 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 $TCID_{50}$ 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 the presence 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 challenges.

c. Kaplan-Meir plot of macaques treated with 0.025 mg/kg MK-8591 and macaques remaining uninfected after serial SHIV challenges.

Table 1. Mean MK-8591-triphosphate levels at the times of challenge at various MK-8591 dosing levels.

MK-8591 Dose (mg/kg)	MK-8591 Dose Ratio to Index[1]	Mean MK-8591-TP Levels at Challenge (fmol per 10^6 PBMC) Mean (range)	Ratio of Mean MK-8591-TP Level to Index
3.9	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[2]	0.029

1. Index refers to the 3.9 mg/kg dosing level
2. Estimated

d.

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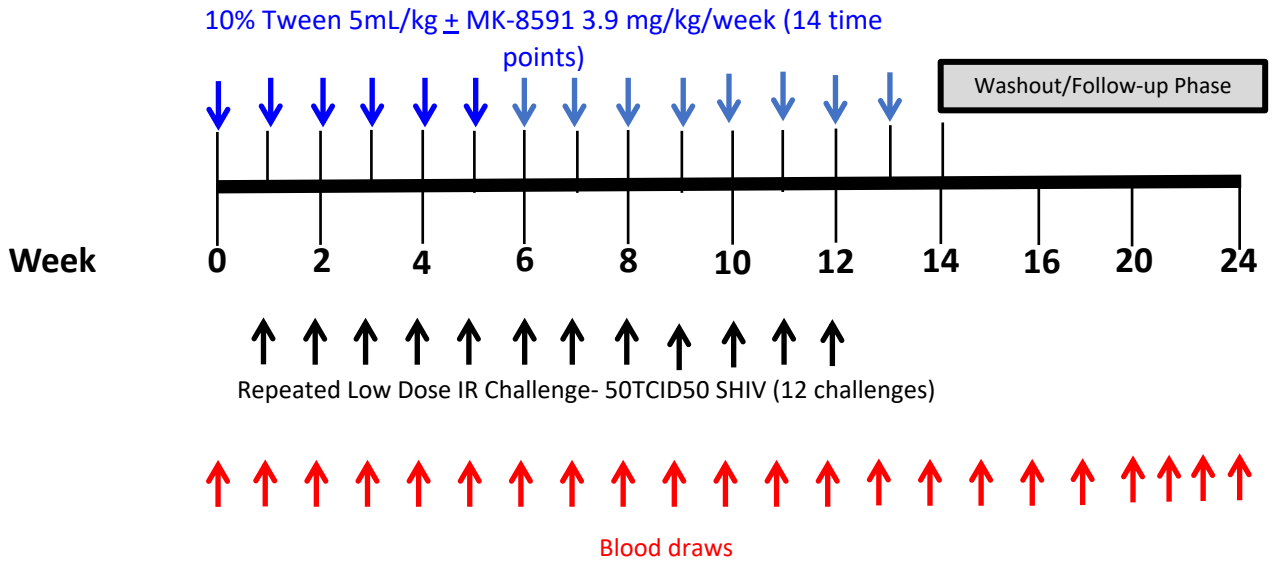

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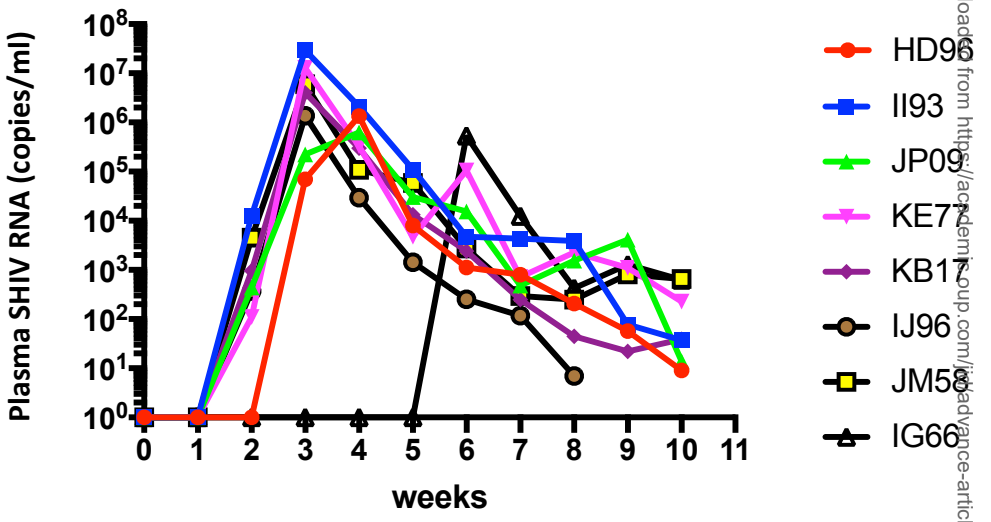

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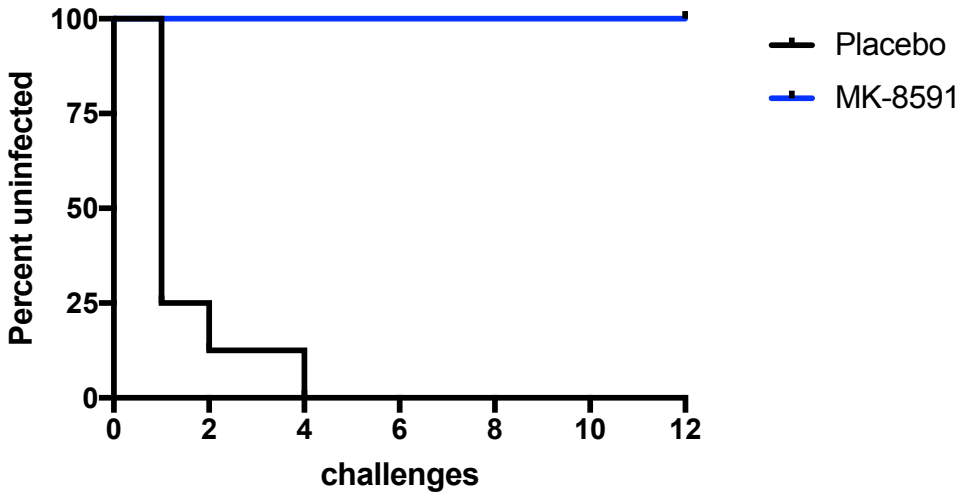

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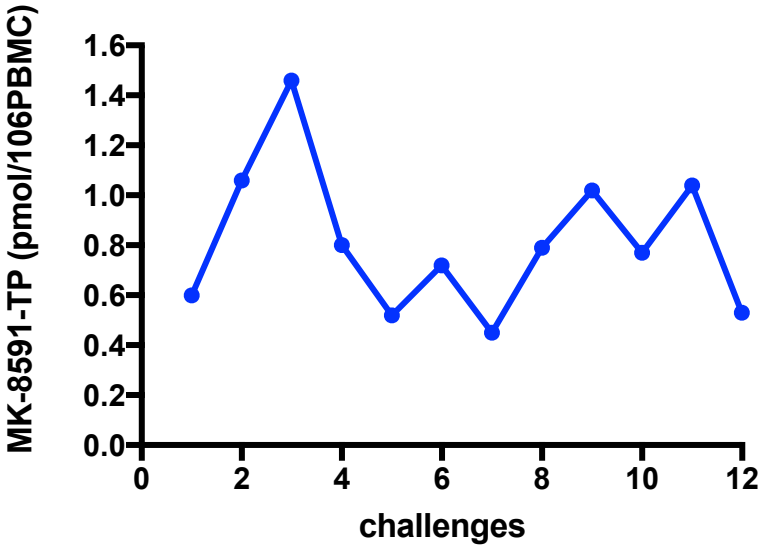

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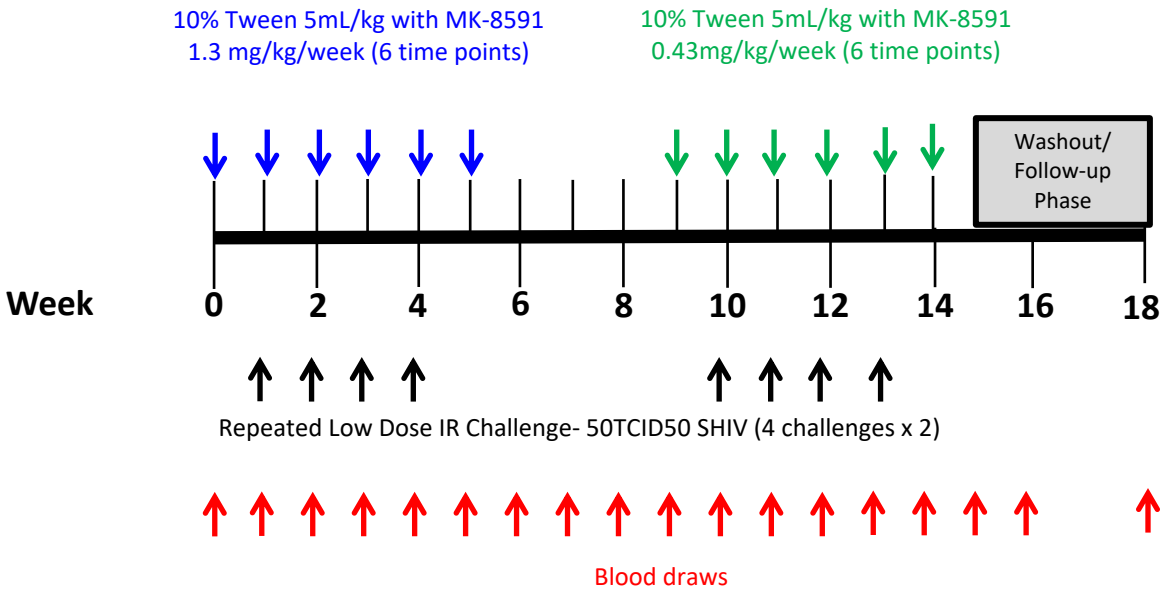

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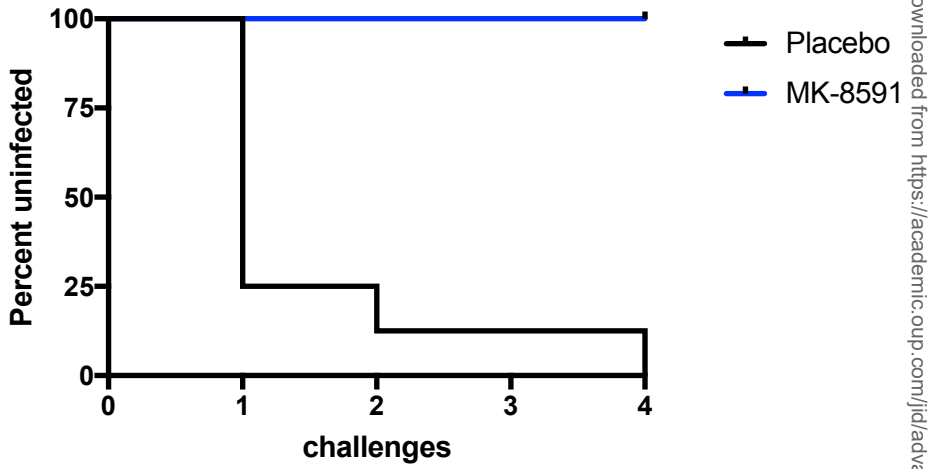

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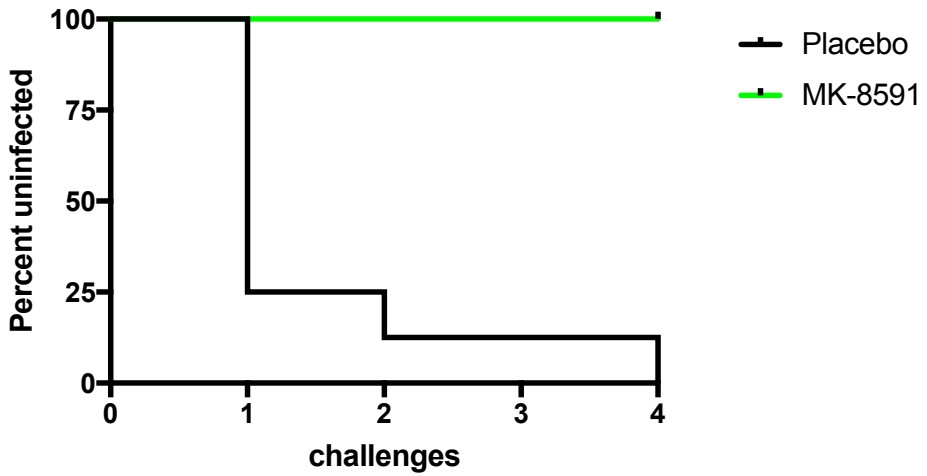

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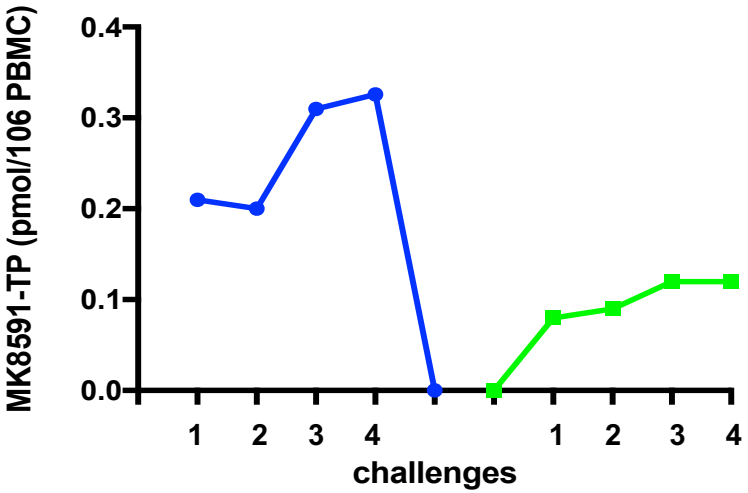

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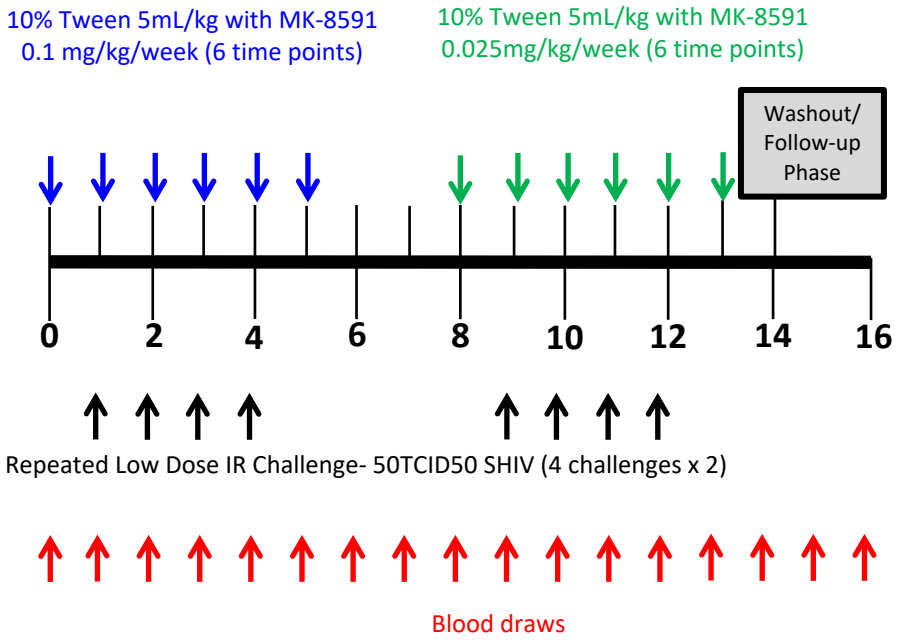

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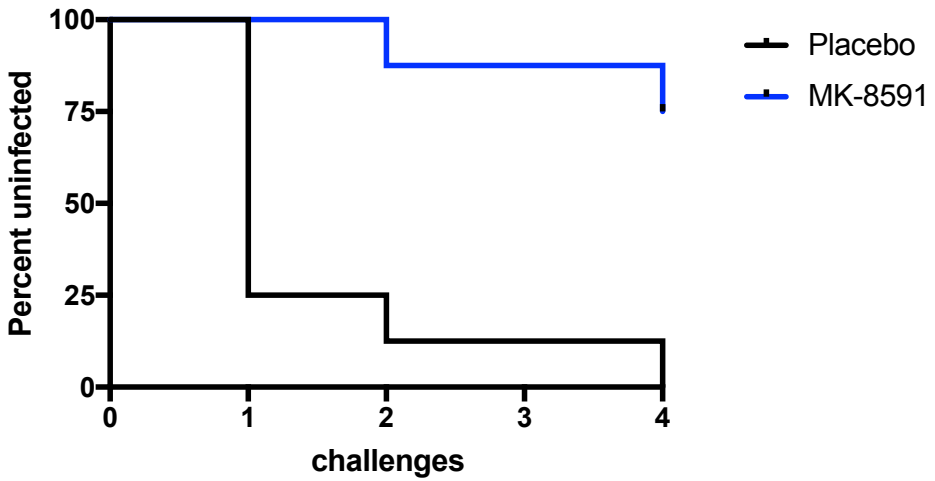

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