HIV

The long-acting integrase inhibitor GSK744 protects macaques from repeated intravaginal SHIV challenge

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Daily preexposure prophylaxis (PrEP) with Truvada is a proven HIV prevention strategy; however, its effectiveness is limited by low adherence. Antiretroviral drug formulations that require infrequent dosing may increase adherence and thus PrEP effectiveness. We investigated whether monthly injections of a long-acting formulation of the HIV integrase inhibitor GSK1265744 (GSK744 LA) prevented simian/human immunodeficiency virus (SHIV) infection by vaginal challenge in macaques. Female pigtail macaques ($n = 12$) were exposed to intravaginal inoculations of SHIV twice a week for up to 11 weeks. Half of the animals received a GSK744 LA injection every 4 weeks, and half received placebo. GSK744 LA, at plasma concentrations achievable with quarterly injections in humans, protected all six macaques from infection. Placebo controls were all infected after a median of 4 (range, 2 to 20) vaginal challenges with SHIV. Efficacy was related to high and sustained vaginal and plasma drug concentrations that remained above the protein-adjusted 90% inhibitory concentration during the dosing cycles. These data support advancement of GSK744 LA as a potential PrEP candidate for women.

INTRODUCTION

HIV type 1 (HIV-1) continues to spread globally with an estimated 2.3 million new infections in 2012 (*1*), highlighting the need for efficacious biomedical interventions to limit its transmission. HIV continues to be driven by gender inequalities with a majority burden on women and particularly girls. Globally, women comprise 52% of all people living with HIV in low- and middle-income countries. In sub-Saharan Africa, women account for about 57% of all people living with HIV (*1*). In addition to vaccine development, there are several antiretroviral (ARV) drug–based intervention strategies currently being advanced, including oral or topical preexposure prophylaxis (PrEP) and treatment as prevention. Recent clinical trials have shown that daily PrEP with tenofovir disoproxil fumarate (TDF), alone or in combination with emtricitabine (FTC) (Truvada), can reduce the risk of sexually acquired HIV infection by 44 to 75% (*2*–*4*). However, these studies also highlight the difficulty that participants have with adhering to the daily oral regimen because only ~50 to 80% had consistently detectable tenofovir, a marker of compliance. Better adherence to the PrEP regimen increased efficacy estimates to ~74 to 92% because the risk of HIV acquisition was found to be substantially lower among participants with detectable drug in peripheral blood mononuclear cells (PBMCs) compared to those with undetectable drug (*2*–*4*). Likewise, very low adherence (<30%) was also the likely reason why two other clinical trials (VOICE and FEM-PrEP) failed to show any efficacy of either daily TDF, Truvada, or vaginal gel with tenofovir (*5*, *6*). Low adherence also reduced the efficacy of non-daily regimens in the CAPRISA 004 study of coital-dependent tenofovir gels (*7*). Recent data from an open-label study with daily Truvada also noted inconsistent adherence among participants who were made aware of the safety and efficacy of the PrEP regimen (*8*).

In women, barriers to PrEP adherence include the stigma of HIV infection, misperceptions of risk, inappropriate partner and community support, and the lack of multiple PrEP formulation choices that address different circumstances and preferences (*9*). For family planning, the availability of different contraceptive methods (oral pills, rings, injections, or implants) is known to increase uptake and adherence (*10*). New long-acting ARV formulations that are not dependent on daily dosing are attractive PrEP modalities that obviate the need for adherence between medication visits, and may be preferred by some women. Interest in new PrEP products has recently focused on the development of intravaginal rings and injectable long-acting ARV formulations that provide sustained drug exposures but require infrequent dosing (*11*–*14*). Two long-acting injectable ARVs currently under evaluation for both treatment and HIV prevention include the HIV reverse transcriptase inhibitor TMC278 (rilpivirine) and GSK1265744 (GSK744) (*13*–*15*). GSK744 is an analog of the marketed integrase inhibitor dolutegravir, which is formulated as a long-acting nanosuspension for parenteral administration (GSK744 LA). GSK744 is potent with a 50% inhibitory concentration (IC_{50}) of around 0.22 nM (*14*). GSK744 is also highly protein-bound and has an IC_{50} of around 100 nM in the presence of human serum (*14*). The high potency of GSK744, low water solubility, and other characteristics allow its formulation as a 200 mg/ml injectable formulation. Two phase 1 studies in humans demonstrated that GSK744 LA is safe and well tolerated and that monthly or quarterly intramuscular injections maintained plasma drug concentrations that exceeded four times the protein-adjusted 90% inhibitory concentration ($PA\text{-}IC_{90}$) for GSK744 (*14*, *16*, *17*). Plans are under way for two phase 2a studies (HPTN 077 and ViiV-sponsored NCT02120352) to assess safety, tolerability, and acceptability of GSK744 LA for PrEP in HIV-1 uninfected men and women.

Animal models are invaluable preclinical tools to provide proof-of-concept data on efficacy and to inform dose selection. Recently, GSK744 LA was found to be highly protective in rhesus macaques challenged repeatedly rectally with simian/human immunodeficiency virus (SHIV) (*13*). This study also identified drug concentrations that correlated with rectal protection and suggested that plasma GSK744 concentrations >3× $PA\text{-}IC_{90}$ conferred 100% protection, whereas concentrations ≥1× $PA\text{-}IC_{90}$ provided ~97% protection (*13*). Here, we used a repeat,

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low-dose virus challenge macaque model to evaluate the efficacy of GSK744 LA against vaginal infection. This well-established model has several advantages, including the use of pigtail macaques, which have a menstrual cycle similar to women, a SHIV inoculum dose similar to the physiological HIV-1 RNA range found in semen, twice-weekly virus challenges to mimic high-risk human exposure, and a $SHIV_{SF162p3}$ isolate that uses an R5-tropic envelope similar to that of most transmitted HIVs (*18*). In previous studies, this model has accurately predicted efficacy of oral Truvada PrEP in women (*2*, *4*, *19*). Our study and a companion article on rhesus macaques exposed to a higher dose of $SHIV_{SF162p3}$ (*20*) investigated whether the long-acting integrase inhibitor GSK744 at concentrations achievable in humans confers protection against vaginal SHIV infection.

RESULTS

Pharmacokinetics of GSK744 in pigtail macaques reproduces clinical drug exposures in humans

We conducted two pharmacokinetic studies to measure drug distribution in tissues and secretions to better inform dose selection in pigtail macaques and understand the relationship between drug concentrations and protection. In rhesus macaques, monthly intramuscular injections of GSK744 LA (50 mg/kg) recapitulated plasma drug concentrations achieved in humans with a single 800-mg intramuscular injection (*13*). We first investigated if the same dose could also reproduce human drug concentrations in pigtail macaques in blood and secretions. Female macaques ($n = 6$) received two GSK744 LA doses (50 mg/kg) 4 weeks apart, followed by longitudinal drug concentration measurements in blood plasma, and secretions from the vaginal and rectal cavities. Figure 1A shows that plasma GSK744 concentrations during the first 4 weeks were well above the PA-IC_{90} (166 ng/ml), with peak concentrations (3753 ng/ml; range, 2488 to 9903 ng/ml) that approximated those seen in humans receiving the 800-mg dose (*14*, *16*, *17*). Interanimal variability was also within the range seen in humans (*13*). A second GSK744 injection at week 4 further maintained plasma drug concentrations near the PA-IC_{90} for an additional 4 to 8 weeks (Fig. 1A). After the second and final dose, plasma GSK744 concentrations declined with a terminal elimination half-life of 8.4 days (range, 3.4 to 11.9), which is shorter than the half-life of 21 to 50 days described in humans (Fig. 1A) (*14*). Although a shorter half-life is not surprising given the generally faster metabolism of drugs in macaques (*21*), this finding underscores the need for more frequent dosing in macaques to sustain human drug exposure.

To evaluate local concentrations at the site of virus entry, we also measured GSK744 concentrations in vaginal and, for comparative purposes, rectal secretions. GSK744 was consistently detected in both vaginal and rectal secretions throughout the time course (Fig. 1B). At first dose, peak GSK744 concentrations in vaginal secretions (median, 911 ng/ml; minimum to maximum, 427 to 1877) were similar to those seen in rectal secretions (median, 2215 ng/ml; minimum to maximum, 647 to 2680) ($P = 0.240$), albeit at concentrations significantly lower than in plasma ($P = 0.002$) (Fig. 1B). As a measure of the overall drug exposure, we calculated the area under the curve values over 28 days (AUC_{0-28d}) in vaginal secretions and compared the values with those seen in plasma or rectal secretions. The AUC_{0-28d} values in vaginal secretions (median, 11,511 ng × day/ml; minimum to maximum, 3956 to 14,011) were lower than those in rectal secretions (median, 26,717 ng × day/ml; minimum to maximum, 10,120 to 39,989), although the difference was not statistically significant ($P = 0.065$) (Fig. 1B). In contrast, AUC_{0-28d} values in vaginal secretions were lower than those in plasma (median, 70,333 ng × day/ml; minimum to maximum, 40,265 to 169,341) ($P = 0.002$) (Fig. 1B). Despite the lower GSK744 concentrations in vaginal secretions, concentrations remained above the PA-IC_{90} throughout the entire 4-week period after each dose (Fig. 1B).

We also conducted a second pharmacokinetic study on six SHIV-infected female macaques to measure GSK744 distribution in systemic and mucosal tissues as well as tissue-to-plasma ratios (Fig. 2). Macaques were given a single GSK744 LA dose (50 mg/kg) into the quadriceps muscle, followed by blood plasma and mucosal secretion sampling. At

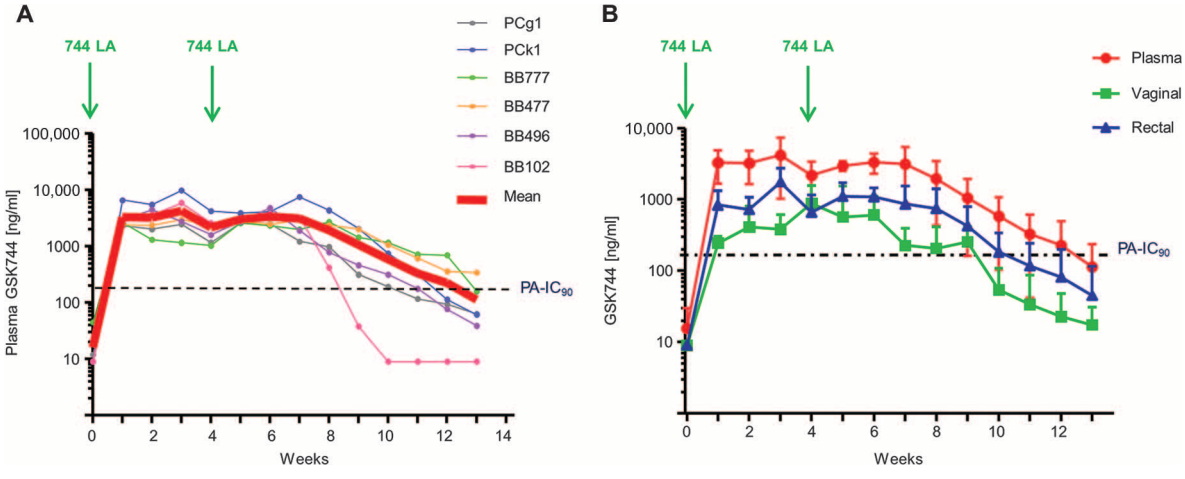

Fig. 1. Pharmacokinetic profile of GSK744 LA in pigtail macaques. Female macaques ($n = 6$; 11 years old and 6 kg on average) received two GSK744 LA doses (50 mg/kg) 4 weeks apart, followed by weekly measurements of drug concentrations in blood and in vaginal and rectal secretions. (**A**) GSK744 concentrations in each macaque. Red line denotes the mean plasma drug concentrations. (**B**). GSK744 concentrations in blood plasma and in rectal and vaginal secretions. Values denote means ± SEM. GSK744 concentrations were measured by high-performance liquid chromatography (HPLC) tandem mass spectrometry (MS/MS) as described (*13*). This assay has a limit of quantification of 10 ng/ml. Horizontal dotted lines denote the PA-IC_{90}.

euthanasia, vaginal, cervical, rectal, and lymph node tissues (mesenteric, axillary, inguinal, and internal iliac) were harvested at days 7 ($n = 2$), 14 ($n = 2$), 21 ($n = 1$), and 28 ($n = 1$) after dosing. GSK744 concentrations in vaginal secretions highly correlated with the concentrations seen in blood plasma in both pharmacokinetic evaluations (Fig. 2A; Pearson $r = 0.6397$, $P < 0.0001$). Likewise, concentrations of GSK744 remained high in rectal, vaginal, cervical, and lymph node tissues through day 28 after a single injection (Fig. 2B). In vaginal tissues, GSK744 concentrations were 719 ng/g at day 7, 273 ng/g at day 14, 278 ng/g at day 21, and 146 ng/g at day 28. In cervical tissues, GSK744 concentrations were 401 ng/g at day 7, 331 ng/g at day 14, 195 ng/g at day 21, and 192 ng/g at day 28. Among the different lymph nodes, iliac lymph nodes had the highest drug concentrations through the entire observation period (Fig. 2B). For instance, GSK744 concentrations in iliac lymph nodes at day 7 were 1350 ng/g compared to 652 ng/g in inguinal lymph nodes, 424 ng/g in mesenteric lymph nodes, and 456 ng/g in axillary lymph nodes (Fig. 2B). These values at day 14 were 848 ng/g (iliac lymph nodes), 218 ng/g (inguinal lymph nodes), 221 ng/g (mesenteric lymph nodes), and 220 ng/g (axillary lymph nodes) (Fig. 2B).

The mean tissue-to-plasma ratios were stable over time and similar across the rectum (0.13; range, 0.05 to 0.31), vagina (0.15; range, 0.06 to 0.23), cervix (0.14; range, 0.08 to 0.30), and the mesenteric, axillary, and inguinal lymph nodes (0.12; range, 0.03 to 0.25). Comparatively, drug concentrations in iliac lymph nodes were highest among all tissues, resulting in a tissue-to-plasma ratio of 0.43 (range, 0.24 to 0.70) (Fig. 2B). Overall, these results show that GSK744 LA (50 mg/kg) administered every 4 weeks in pigtail macaques resulted in plasma drug concentrations that mimic those seen in humans receiving a single 800-mg intramuscular dose of GSK744 LA. These data also document the presence of GSK744 in vaginal secretions at concentrations lower than in plasma but above the PA-IC_{90} for GSK744. We also demonstrated sustained drug coverage of genital tissues over the course of 4 weeks and GSK744 partitioning in vaginal, cervical, and rectal tissues that is within the range seen in humans (*17*).

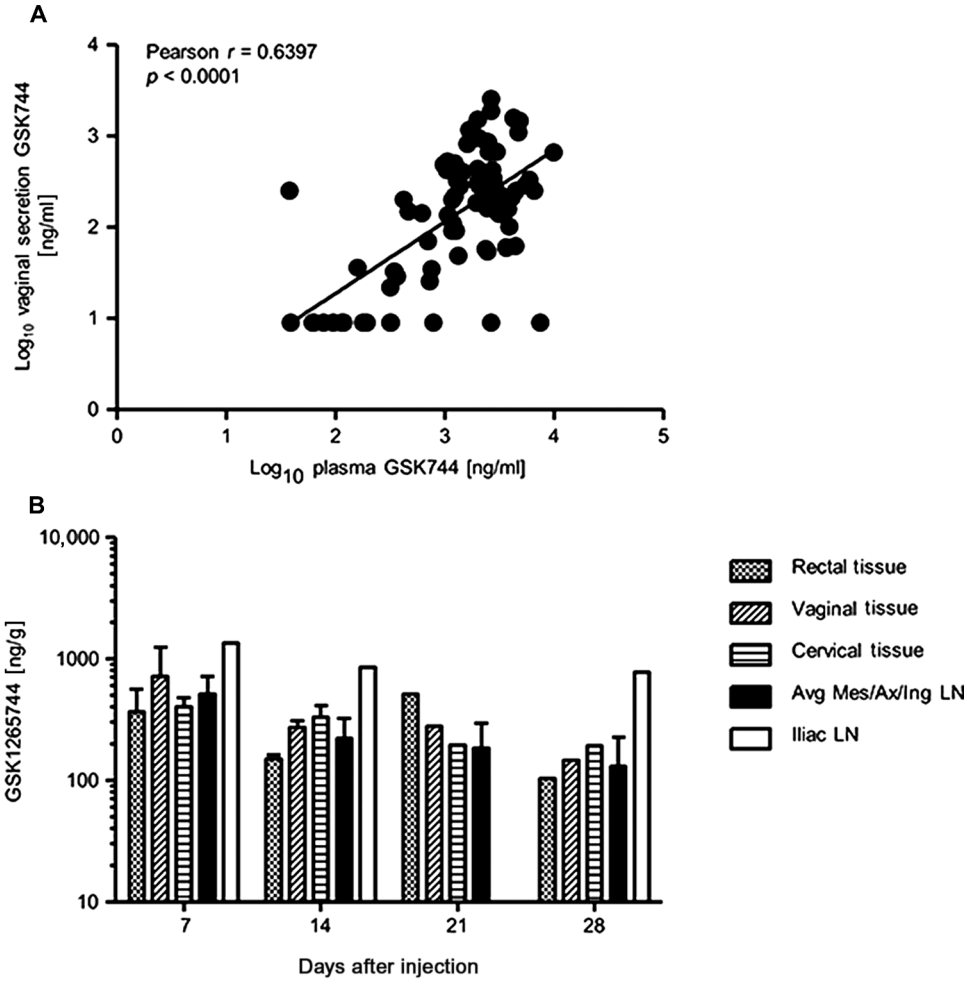

Fig. 2. Relationship between GSK744 concentrations in plasma and vaginal fluids and tissue drug exposure. (**A**) Correlation between the concentrations of GSK744 in plasma and in vaginal secretions. The concentrations of GSK744 in plasma and vaginal secretions were significantly correlated ($P < 0.0001$, two-tailed test). (**B**) Concentrations of GSK744 in tissues. Rectal, vaginal, cervical, and lymphoid tissues [mesenteric (Mes), axillary (Ax), inguinal (Ing), and internal iliac lymph nodes (LNs)] were collected from six female macaques at four time points after one GSK744 LA dose (50 mg/kg). Tissues were harvested at 7 ($n = 2$), 14 ($n = 2$), 21 ($n = 1$), and 28 days ($n = 1$) after dosing. Avg Mes/Ax/Ing LN denotes average GSK744 concentrations in these three LNs. Histogram values denote means ± SEM. GSK744 concentrations were measured by HPLC MS/MS as described (*13*).

Prophylactic efficacy of GSK744 LA

The efficacy of GSK744 LA in preventing vaginal infection was evaluated using an established pigtail macaque model consisting of repeated vaginal exposures to low [50 $TCID_{50}$ (median tissue culture infectious dose)] doses of $SHIV_{162p3}$. The study design is shown in Fig. 3A. Six of the 12 pigtail macaques received a GSK744 LA regimen consisting of one injection 7 days before the start of the challenge series, followed by an injection every 4 weeks, for a total of three injections (Fig. 3A). Six additional animals received placebo injections. The average age and weight were similar in the two study arms (7.3 years and 6.2 kg in the placebo group and 8.0 years and 6.1 kg in the treated group).

Animals were considered protected from infection if they remained consistently seronegative for SHIV-specific antibodies and negative by polymerase chain reaction (PCR) for SHIV plasma RNA and SHIV DNA in PBMCs during the 22 virus challenges and a drug washout and follow-up period of 34 weeks after the final virus challenge. Figure 3B shows the cumulative percentage of uninfected macaques as a function of the number of vaginal exposures. All six macaques that received placebo were infected after a median of 4 (range, 2 to 20) virus exposures ($P = 0.0005$, log-rank test). In contrast, the six animals receiving GSK744 LA remained seronegative and viral RNA– and DNA–

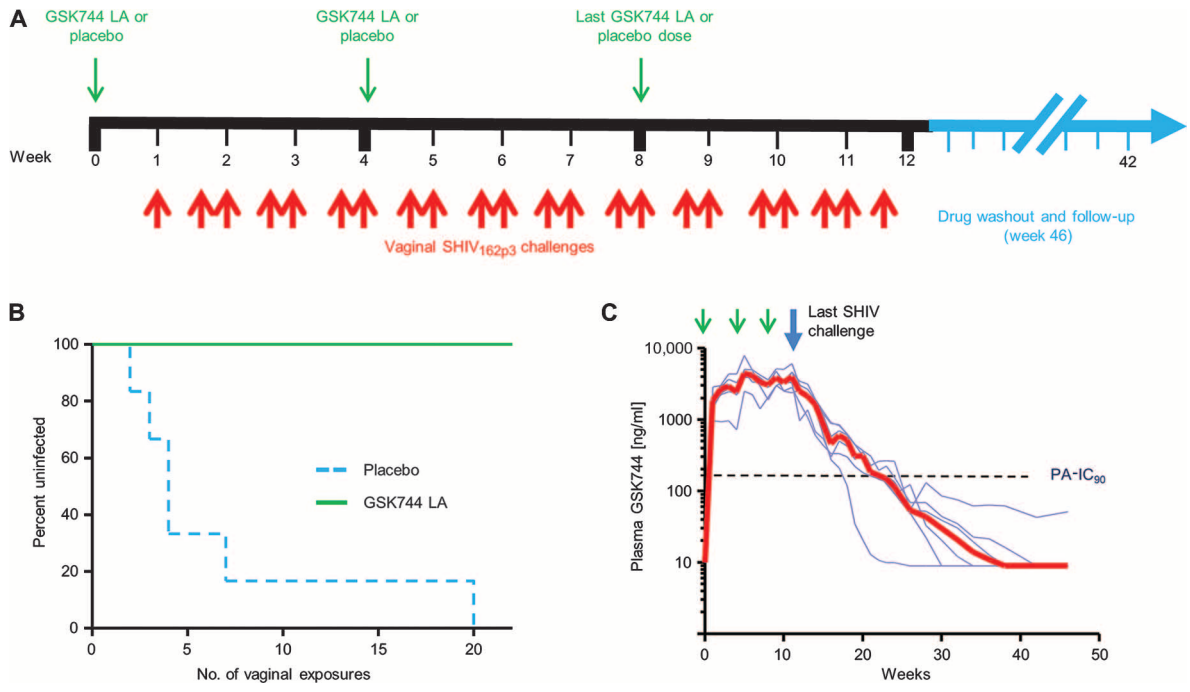

Fig. 3. Protection against vaginal SHIV infection with GSK744 LA. (**A**) Experimental design. Female pigtail macaques received a GSK744 LA ($n = 6$) or placebo ($n = 6$) regimen consisting of one injection 7 days before the start of the challenge series (initiated at week 1), followed by an injection every 4 weeks, for a total of three injections. Animals were exposed vaginally twice a week for up to 11 weeks. (**B**) Kaplan-Meier plot. Each survival curve represents the cumulative percentage of uninfected macaques as a function of the number of vaginal SHIV exposures. (**C**) Plasma GSK744 concentrations during the virus challenge phase (weeks 1 to 11) and the drug washout and follow-up period. Each line represents an individual macaque. Red line denotes the mean plasma drug concentrations. The horizontal dotted line denotes the PA-IC_{90} for GSK744.

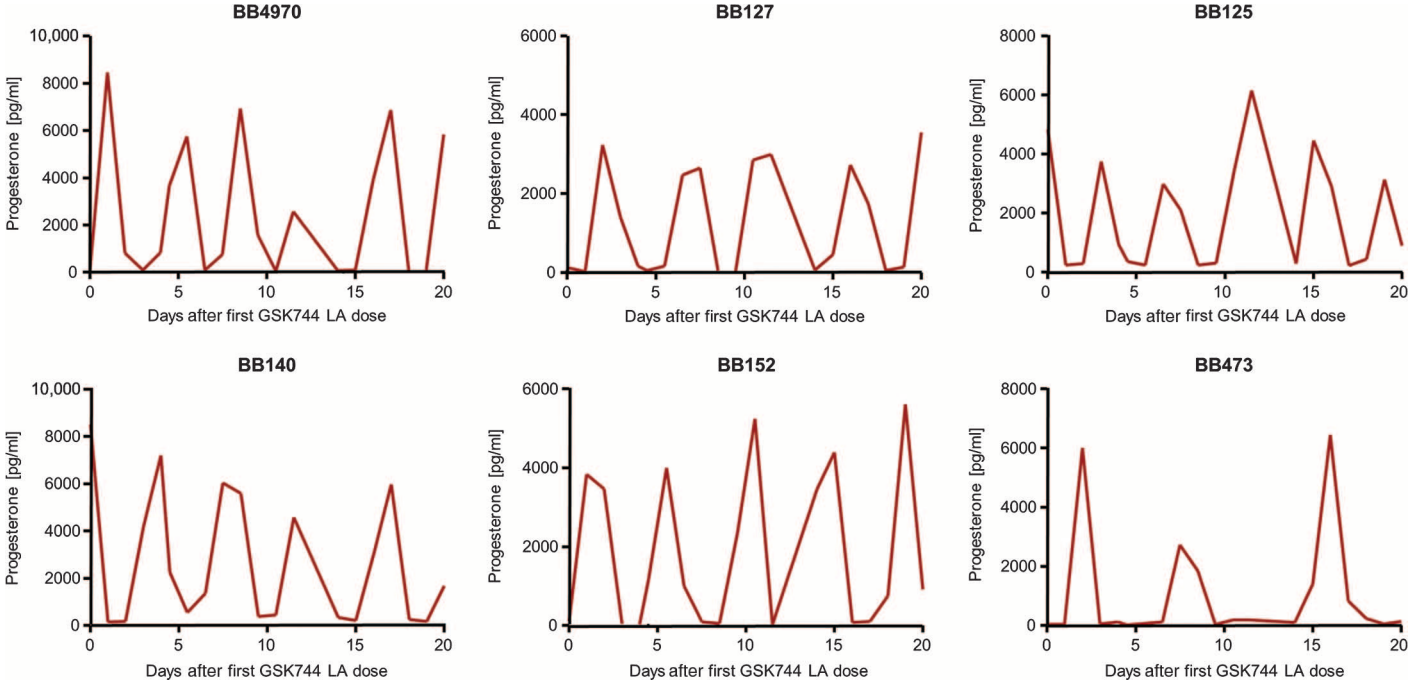

Fig. 4. Plasma progesterone concentrations in GSK744 LA–treated macaques. Progesterone was measured by liquid chromatography/mass spectrometry as previously described (*19*). Animals remained protected from infection during several complete menstrual cycles as indicated by the fluctuations in progesterone over time.

negative during the 22 virus challenges (weeks 0 to 11) and the follow-up period comprising a drug washout phase (weeks 11 to 46). Figure 4 shows that the six GSK744 LA–treated macaques were protected during several full menstrual cycles.

We also monitored drug concentrations during the challenge and follow-up periods (Fig. 3C). This analysis showed that plasma GSK744 concentrations throughout the 12-week challenge period were about 1.5 $\log_{10}$ higher than the PA-IC_{90} (166 ng/ml) in all animals and closely paralleled plasma concentrations detected in humans given a single 800-mg GSK744 LA intramuscular dose. After week 11, the GSK744 plasma concentrations fell slowly to concentrations below PA-IC_{90} between weeks 18 and 26 in the six animals and reached undetectable or very low concentrations at the end of the follow-up at week 46. Figure 5 illustrates the dynamics of acute infection in the six placebo controls and also shows that none of the GSK744 LA–treated animals had detectable SHIV RNA or DNA during the study, including the period with very low or undetectable GSK744 in plasma. In the placebo controls, median peak RNA concentrations were 6.8 $\log_{10}$ RNA copies/ml (minimum to maximum, 6.1 to 7.3), and median peak DNA concentrations were 2.6 $\log_{10}$ DNA copies per 10^6 PBMCs (minimum to maximum, 1.5 to 3.0).

DISCUSSION

Vaginal transmission is the most common route of HIV acquisition worldwide (*1*). Thus, safe, effective, and well-tolerated PrEP modalities that increase adherence and effectiveness of PrEP among women are critical. Long-acting drug formulations that require infrequent dosing may be preferred by some women and have the potential to increase adherence. Here, we used a pigtail macaque model to demonstrate that the long-acting integrase inhibitor GSK744 at concentrations achievable in humans affords protection against repeated vaginal SHIV challenges. These data establish the proof of concept of vaginal efficacy, and, together with findings of protection against rectal infection in macaques (*13*), they support the clinical development of GSK744 LA as a next-generation PrEP agent.

The pigtail macaque model used in this study was designed to recapitulate both the pharmacological and the virological exposures observed in humans. Previously, using the same approach and conditions, this model accurately predicted the high efficacy of oral TDF/FTC combination in women (*2*, *4*, *19*). We showed that the same formulation used clinically administered monthly to pigtail macaques resulted in sustained GSK744 concentrations in plasma, similar to those achieved with 800-mg quarterly doses in humans. In addition, this dose recapitulated the human vaginal exposure of GSK744 by showing similar vaginal-to-plasma ratios, providing further support for the pharmacokinetic similarities between humans and pigtail macaques (*17*).

Our repeated vaginal SHIV challenges also included lower and more physiological SHIV doses than conventional single high-dose challenges that risk underestimating protection. The SHIV challenges were all done in animals with normal menstrual cycles in the absence of high doses of Depo-Provera that alter susceptibility to SHIV, and they were all given twice weekly for 11 weeks to mimic repeated HIV exposures that might occur with humans under therapeutic GSK744 LA conditions. The finding of complete protection in the GSK744 LA–treated animals despite receiving 22 challenges compared to a median of 4 SHIV challenges that resulted in infection of controls reflected the robustness and durability of the protection afforded by GSK744 LA. The robustness of this intervention is further supported by the high protection achieved in rhesus macaques treated with Depo-Provera and exposed to a higher dose of $SHIV_{SF162p3}$ as reported in the companion paper (*20*). Sustained drug exposure in the vaginal compartment demonstrated through GSK744 concentrations above the PA-IC_{90} in the secretions as well as sustained drug exposure in the cervical and vaginal tissues may have contributed to the observed protection. The high concentrations in the iliac lymph nodes may be vital to protection at the mucosa because these are known to be functional inductive sites for CD4 T cells, which preferentially home to the rectal, cervical, and vaginal mucosa (*22*).

Although these data support the selection of the 800-mg GSK744 LA dose administered every 3 months for PrEP in women, follow-up experiments in macaques may help to define the minimal GSK744 LA dose required for protection. These experiments may also inform whether a lower GSK744 LA dose or a less frequent administration can be equally efficacious against virus acquisition. For instance, on the basis of single-dose pharmacokinetic data in humans and given the long half-life of GSK744 LA in plasma (21 to 50 days), low-

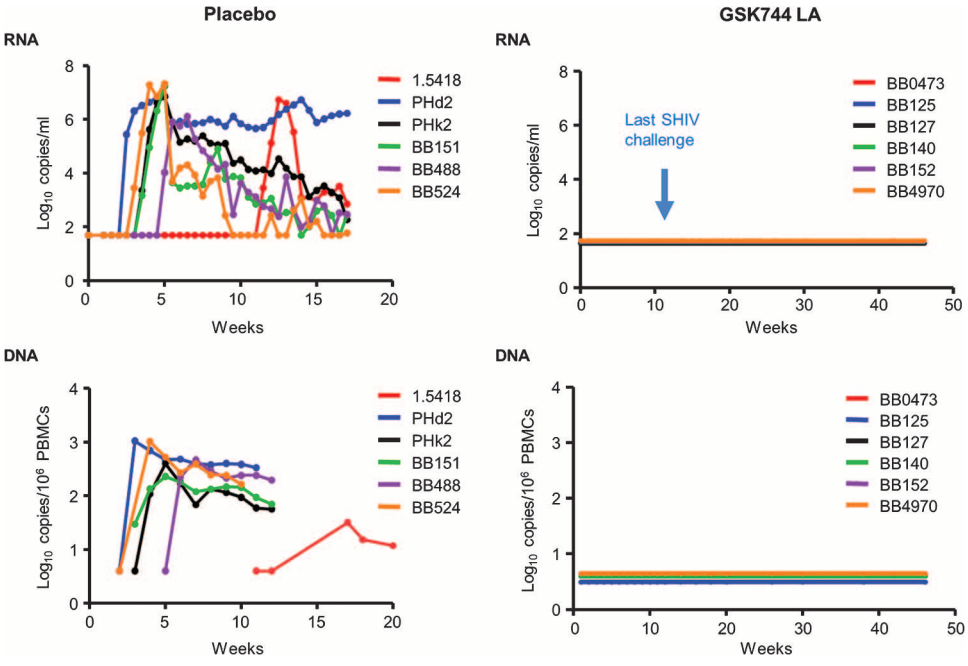

Fig. 5. Plasma RNA and proviral DNA in macaques receiving GSK744 LA or placebo. Animals were exposed vaginally to SHIV for up to 22 vaginal challenges or until they became SHIV RNA–positive. All six animals receiving GSK744 LA remained seronegative and viral RNA– and DNA–negative during the 22 virus challenges and the additional drug washout and follow-up period.

ering the plasma target concentration for PrEP from 4× PA-IC_{90} to 1× PA-IC_{90} could potentially extend the injection interval of 800-mg GSK744 LA from 12 to 36 weeks (*13*). However, selection of the most appropriate dose and frequency of GSK744 LA requires additional clinical data from treated patients and larger studies in macaques and should carefully consider pharmacokinetic variability among persons. Also, although our findings are encouraging, they are subject to several limitations. For instance, our design used atraumatic virus inoculations that were done in the absence of cofactors that may increase HIV transmission risk such as sexually transmitted infections. It will be important to see if efficacy of GSK744 LA is also maintained in macaques co-infected with other sexually transmitted infections such as *Chlamydia trachomatis* and *Trichomonas vaginalis* (*23*).

Drug resistance emergence in PrEP trials with FTC and TDF has been rare and mostly limited to participants that initiated PrEP during acute infection. Concerns about long-acting formulations for PrEP include managing drug discontinuation if HIV is acquired because prolonged subtherapeutic drug concentrations may potentially select for resistant viruses. It would be informative to investigate in macaques if GSK744 LA treatment during acute SIV or SHIV infection may rapidly select for resistant viruses. These studies can also define the window for resistance emergence and test potential mitigation strategies that may, for instance, include the addition of other drugs during the tail of declining drug concentrations to prevent drug resistance emergence.

Our results in a validated vaginal transmission model highlight the promise of GSK744 LA as a next-generation PrEP candidate to prevent HIV infection in women and, therefore, support its clinical development. If proven safe and effective in human clinical trials, GSK744 LA may usher in a new way for PrEP delivery that parallels that of long-acting contraceptives, providing opportunities for synergy between reproductive health and HIV prevention.

MATERIALS AND METHODS

PrEP efficacy study design

Twelve pigtail macaques were used to measure GSK744 LA efficacy. All 12 animals were confirmed to have normal menstrual cycles before the study based on progesterone measurements. Six of 12 pigtail macaques received a GSK744 LA regimen consisting of one injection 7 days before the start of the virus challenge series, followed by an injection every 4 weeks, for a total of three injections. The six untreated animals were given a placebo-matched injection using the same schedule. Vaginal SHIV exposures were performed twice a week for up to 11 weeks by nontraumatic inoculation of 1 ml of $SHIV_{162p3}$ into the vaginal vault via a sterile gastric feeding tube of adjusted length. Anesthetized macaques remained recumbent for at least 15 min after each intravaginal inoculation. Blood was collected twice weekly to monitor for infection. Exposures were stopped when a macaque became SHIV RNA–positive. All experiments were done under highly controlled conditions by the same personnel and using aliquots of the same virus stock stored in liquid nitrogen.

Infection of macaques was monitored by serologic and molecular testing. Virus-specific serologic responses were measured using a synthetic-peptide enzyme immunoassay (Genetic Systems HIV-1/HIV-2, Bio-Rad). SHIV RNA was quantified using a reverse transcription PCR assay with a detection limit of 50 copies/ml (*19*). Proviral DNA was quantified using a double-stranded primer assay with a detection limit of three DNA copies per million PBMCs (*19*). Animals were considered protected from infection if they remained seronegative and negative for SHIV plasma RNA and SHIV DNA in PBMCs during the 22 virus challenges and a drug washout and follow-up period of an additional 34 weeks.

Animal care

All the animal procedures performed in this study were approved by the Centers for Disease Control and Prevention (CDC) Institutional Animal Care and Use Committee. Macaques were housed at the CDC under the full care of CDC veterinarians in accordance with the standards incorporated in the *Guide for the Care and Use of Laboratory Animals* (National Research Council of the National Academies, 2010). SHIV-infected macaques were humanely euthanized in accordance with the American Veterinary Medical Association Guidelines on Euthanasia, 2013. All procedures were performed under anesthesia using ketamine, and all efforts were made to minimize suffering, improve housing conditions, and provide enrichment opportunities (for example, objects to manipulate in cage, varied food supplements, foraging and task-oriented feeding methods, and interaction with caregivers and research staff).

Drug and virus stock

GSK744 LA (200 mg/ml injectable stock aqueous suspension) was given at a dose of 50 mg/kg under anesthesia by intramuscular injection into the quadriceps muscle. Volumes greater than 1.0 ml were divided into two injection sites. $SHIV_{162p3}$ was obtained from the National Institutes of Health AIDS Research repository and expanded in pigtail macaque PBMCs before this study.

GSK744 LA pharmacokinetic analysis

For the longitudinal pharmacokinetic evaluation, female macaques (n = 6) received two doses 4 weeks apart, followed by drug level measurements in blood plasma and secretions (rectal and vaginal). In the cross-sectional study, $SHIV^+$ female macaques (n = 6) were given a single dose of GSK744 LA, followed by collection of blood plasma and secretions. Vaginal, rectal, cervical, and iliac lymph node tissues were harvested at necropsy at 7 (n = 2), 14 (n = 2), 21 (n = 1), or 28 (n = 1) days after injection. Secretions were collected in Weck-Cel Surgical Spears (Medtronic Ophthalmic) using a well-established protocol (*24*). Tissue preparations, blood plasma, and eluted secretions were frozen at −80°C before analysis. GSK744 concentrations were determined by Worldwide Bioanalysis (GlaxoSmithKline) using a validated LC-MS method with a lower limit of quantification of 10 ng/ml.

Statistical analysis

Peak drug concentrations and AUC values in blood and secretions were compared using a two-tailed Wilcoxon rank sum test. The log-rank test was used to compare the survival distribution between animals receiving GSK744 LA or placebo. All statistical analyses were done using GraphPad Prism for Windows (version 5.04).

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The long-acting integrase inhibitor GSK744 protects macaques from repeated intravaginal SHIV challenge

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A Step-Up for PrEP

Daily preexposure prophylaxis (PrEP) is effective in preventing HIV acquisition among men and women, but many are not able to adhere to the daily pill schedule and cannot fully benefit from PrEP. New long-acting antiretroviral formulations provide sustained drug delivery for many weeks and do not require adherence between medication visits. Here, Radzio *et al.* showed in macaques that the long-acting integrase inhibitor GSK744, at concentrations achievable in humans by quarterly injections, afforded protection against repeated vaginal simian HIV challenges. These findings support the clinical development of GSK744 LA as a PrEP agent for HIV prevention in women.