INTRODUCTION

Two decades ago Tsai et al. [1] demonstrated that subcutaneous tenofovir could protect macaques from intravenous simian immunodeficiency virus (SIV) infection, providing the first proof-of-concept of the efficacy of antiretroviral (ARV) prophylaxis as a strategy for HIV-1 prevention. Subsequent animal modeling with clinically relevant regimens and doses showed that the marketed oral formulation of tenofovir, tenofovir disoproxil fumarate (TDF), in combination with oral emtricitabine (Truvada, Gilead Sciences), protected macaques from rectal and vaginal infection providing further support for clinical trials of preexposure prophylaxis (PrEP) for sexual HIV-1 prevention [2–4]. In the United States, the results of these oral PrEP studies led to Truvada being licensed in 2012 for HIV-1 prevention [5]. Because an effective HIV-1 vaccine remains elusive, the licensure of Truvada for PrEP ushered in the promise of a new biomedical intervention to address the global spread of HIV-1, estimated to 2.1 million new infections in 2013 acquired primarily through sex [6]. Women bear the greatest burden of infections in sub-Saharan Africa, whereas HIV-1 spread among MSM dominates in resource-rich countries [6,7].

Although clinical PrEP trials with daily TDF or Truvada reduce the risk of HIV-1 infection by 44–75%, they also highlight the difficulty of participants to adhere to the daily oral regimen as only approximately 50–80% had consistently detectable tenofovir, a marker of compliance [8–10]. Better adherence to the PrEP regimen increased efficacy estimates to approximately 74–92% as the risk of HIV-1 acquisition was found to be substantially lower among participants with detectable drugs than those with undetectable drug [8–10]. Very low adherence (<30%) was also the likely reason why two clinical
KEY POINTS

- Cabotegravir long-acting is a potent integrase inhibitor with a pharmacokinetic profile in HIV-1-uninfected individuals that makes it amenable for dosing every 3 months, offering an alternative to daily PrEP regimens.
- Compared with plasma cabotegravir concentrations, low mucosal tissue penetration was observed in humans and macaques.
- Protection from rectal and vaginal SHIV transmission was observed in macaques treated with a dose of cabotegravir long-acting that provides plasma concentrations similar to those observed in humans administered 800 mg of cabotegravir long-acting quarterly.
- Current data support the clinical development of cabotegravir long-acting for HIV-1 prevention.

Trials among young African women (VOICE and FEM-PrEP) failed to show any efficacy of either daily TDF or Truvada [11,12]. 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 [13]. Although many factors contribute to poor PrEP adherence, long-acting ARV drug formulations are being advanced to improve adherence because they are not dependent on daily dosing and only require user compliance for infrequent medical visits. Interest in long-acting PrEP products has recently focused on the development of intravaginal rings and injectable long-acting ARV formulations that provide sustained drug exposures over many weeks [14,15,16**,17]. Two long-acting injectable ARVs currently under evaluation for both HIV-1 treatment and prevention, include the HIV-1 reverse transcriptase inhibitor TMC278 (rilpivirine) and the integrase inhibitor cabotegravir, also known as GSK1265744 or GSK744 [15,17,18**]. This article reviews data on the development of injectable long-acting ARVs for HIV-1 prevention and focuses on cabotegravir long-acting, while a companion article will focus on TMC278.

Cabotegravir is an analogue of the marketed integrase inhibitor, dolutegravir, formulated as a long-lasting nanosuspension for parenteral administration (cabotegravir long-acting). Cabotegravir is very potent and has a 50% inhibitory concentration (IC_{50}) of around 0.22 nmol/l [17] with activity against various clades of HIV-1 [19]. Cabotegravir is also highly protein-bound and has a higher IC_{50} of around 100 nmol/l in the presence of human serum [17]. Cabotegravir dosed orally at 5 or 30 mg/day reduced HIV-1 RNA in plasma by 2.2–2.3 log_{10} copies per milliliter during a 10-day monotherapy trial in HIV-1-infected persons [20]. The high potency of cabotegravir, low water solubility, low metabolic clearance, and other pharmaceutical properties enabled cabotegravir to be formulated in a 200-mg/ml suspension as a long-acting injectable. Two phase 1 studies in humans demonstrated that cabotegravir long-acting is well tolerated, and that monthly or quarterly intramuscular injection maintains plasma drug levels that exceed four times the protein-adjusted 90% inhibitory concentration (PAIC_{90}) for cabotegravir [17,18**,21**]. A study of repeated cabotegravir long-acting injections demonstrated that these injections were generally well tolerated and no grade 3 or 4 adverse events were reported [18**]. Two phase 2a studies (ViIV Healthcare sponsored protocol 201120 and HPTN 077) are underway to assess safety, tolerability, and acceptability of cabotegravir long-acting for PrEP in HIV-1 uninfected men and women.

PHARMACOKINETICS OF CABOTEGRAVIR LONG-ACTING IN NONHUMAN PRIMATES

Animal models are invaluable preclinical tools to provide proof-of-concept data on efficacy and inform dose selection. The pharmacokinetic profile of cabotegravir long-acting was evaluated in macaques to determine a cabotegravir long-acting dosing regimen that provides similar plasma concentrations to those achieved in humans. Pharmacokinetic studies were performed in male Indian rhesus macaques (Macaca mulatta) comparing plasma concentrations from macaques dosed with 10, 30, or 50 mg/kg of cabotegravir long-acting [22**]. On a weight basis, 10 mg/kg cabotegravir long-acting is similar to an 800-mg dose in an approximately 70 kg human; however, this dose provided lower plasma concentrations in macaques than observed in humans [22**]. Although 30 mg/kg of cabotegravir long-acting provided comparable peak concentrations as observed in humans, the trough concentrations were significantly lower than in humans [22**]. The lower plasma concentrations observed when macaques were dosed with 10 or 30 mg/kg of cabotegravir long-acting were not unexpected, as smaller mammals often eliminate drug more rapidly than humans [23]. A dose of 50 mg/kg of cabotegravir long-acting administered every 4 weeks maintained plasma concentrations similar to those observed in humans dosed with 800 mg cabotegravir long-acting every 12 weeks [22**]. Similar plasma pharmacokinetic results were observed when administering 50 mg/kg of cabotegravir long-acting to female pigtail macaques (Macaca nemestrina) [24**] and slightly lower trough concentrations were observed in female rhesus
macaques pretreated with Depo-Provera [25**]. The average terminal elimination half-life of cabotegravir long-acting was much shorter in macaques, 8.4–9.6 days [22**,24**,25**], compared with 21–50 days observed humans [17], requiring more frequent dosing in the macaque to sustain clinical drug exposures.

**MUCOSAL TISSUE PENETRATION OF CABOTEGRAVIR LONG-ACTING**

Penetration of ARVs to mucosal tissues and secretions is a property that is considered important for PrEP agents utilized against sexual HIV-1 transmission; however, mucosal distribution differs widely among ARVs [26]. Penetration of cabotegravir to the mucosal site of virus entry was evaluated following various dosing regimens at various time points in nonhuman primates and was compared with the limited data available from humans (Table 1). A linear correlation between plasma and tissue concentration was observed in humans and nonhuman primates; however, rectal, vaginal, and cervical tissue:plasma ratios were generally low in all studies [21**,22**,24**,25**]. The low tissue penetration of cabotegravir may be related to its high protein binding similar to what has been observed with dolutegravir in human studies [27,28]. Despite the lower cabotegravir concentrations in mucosal tissues, levels remained above the PAIC90 in vaginal secretions from pigtail macaques during dosing cycles with the clinically relevant 50 mg/kg regimen [24**]. Overall, the similarities in the distribution of cabotegravir in plasma and mucosal tissues between macaques and humans provide further support for using these macaque models to test efficacy under pharmacologically relevant conditions and inform dose selection for human trials.

**Efficacy of Cabotegravir Long-Acting Against Rectal Transmission**

The efficacy of cabotegravir long-acting was first evaluated in a repeat low-dose intrarectal challenge macaque model that was developed to more closely mimic HIV-1 transmission in humans [2,3,29–31]. Repeated challenge with SHIV162p3 containing an R5-tropic subtype B HIV-1 envelope (SF162) at a lower and more physiologically relevant inoculum is performed to evaluate protection against multiple transmission events [2,3,29–31]. To evaluate the efficacy of cabotegravir long-acting as PrEP in this model, eight male rhesus macaques were administered 50 mg/kg of cabotegravir long-acting on weeks 0 and 4, and an additional eight male rhesus macaques remained untreated as controls [22**]. All macaques were challenged intrarectally beginning on week 1 with 50 TCID50 SHIV162p3 weekly for up to eight challenges or until infection was detected [22**]. All control macaques became infected after a median of 2 (range 1–7) challenges, whereas all cabotegravir long-acting-treated macaques remained aviremic and seronegative during the challenge and drug washout phase of the study [22**].

An additional study was conducted using the same model to determine plasma cabotegravir concentrations that correlate with protection from SHIV infection. In this study, 12 male rhesus macaques were administered a single dose of 50 mg/kg of cabotegravir long-acting 1 week prior to beginning repeated weekly SHIV challenges until infection was detected [22**]. A single dose of cabotegravir long-acting delayed infection by five to 10 challenges compared with control macaques [22**]. When plasma concentrations were more than three times PAIC90, viremia was not detected during 59 collective challenges confirming results of the initial challenge study that showed complete protection at high (>3x PAIC90) plasma concentrations [22**]. However, as plasma cabotegravir concentrations waned, one macaque became viremic at plasma concentrations between one to three times PAIC90, whereas the remaining 11 macaques became viremic at plasma concentrations less than one times PAIC90 [22**]. Overall, these studies demonstrated that cabotegravir long-acting is an effective PrEP agent against intrarectal SHIV challenge. Plasma concentrations correlating with protection

**Table 1. Comparison of cabotegravir tissue penetration in nonhuman primates and humans**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Tissue:plasma ratio (range)</th>
<th>Non human primates</th>
<th>Humans</th>
<th>Tissue:plasma ratio (range)</th>
<th>Non human primates</th>
<th>Humans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rectum</td>
<td>0.21 (0.08–0.54)</td>
<td>0.08 (NQ–0.2)</td>
<td></td>
<td>0.13 (0.05–0.31)</td>
<td>0.19 (NQ–0.7)</td>
<td></td>
</tr>
<tr>
<td>Vagina</td>
<td>0.15 (0.06–0.23)</td>
<td>0.09 (NQ–0.20)</td>
<td>0.16</td>
<td>0.14 (0.08–0.30)</td>
<td>0.16 (NQ–0.4)</td>
<td></td>
</tr>
<tr>
<td>Cervix</td>
<td>0.09 (NQ–0.20)</td>
<td>0.16 (NQ–0.4)</td>
<td></td>
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</tr>
</tbody>
</table>

NQ, nonquantifiable concentration measured as below the lower limit of quantitation.

*Mean ratio.

**Median ratio.

†Single 400 mg intramuscular split [2 × 200 mg] cabotegravir long-acting dose: n = 4 females; n = 4 males [21**].

‡Repeat 10 mg/kg (n = 8) or 30 mg/kg (n = 4) intramuscular cabotegravir long-acting dosages in male rhesus macaques [22**].

§Repeat 50 mg/kg intramuscular cabotegravir long-acting dose; n = 8 female pigtail macaques, Depo [25**].

‖Single 50 mg/kg intramuscular cabotegravir long-acting dose; n = 6 female pigtail macaques, no Depo [24**].
in macaques can be achieved in humans dosed with 800 mg of cabotegravir long-acting every 3 months.

**Efficacy of Cabotegravir Long-Acting Against Vaginal Transmission**

Because of the physiological and pharmacological differences between vaginal and rectal tissues, the efficacy of cabotegravir long-acting against vaginal transmission cannot be predicted from rectal prevention data. The efficacy of cabotegravir long-acting against vaginal transmission was evaluated in both rhesus and pigtail macaques. Female rhesus and pigtail macaques exhibit different susceptibilities to SIV/SHIV infection [32], which influence the challenge design in each species. The barriers to infection are more substantial in the vagina compared with the rectum. Although untreated rhesus macaques can be infected vaginally with large challenge doses of SIV, pretreatment with progesterone thins the vaginal epithelium increasing susceptibility of the macaque to vaginal infection [33]. This rhesus macaque model utilizes a progestin-based contraceptive, Depo-Provera (depot medroxyprogesterone acetate, Pfizer), and has been employed to assess the efficacy of vaccines, neutralizing antibodies, ARVs, and vaginal microbicides against SIV/SHIV infection [34–44]. This model is limited by the use of a challenge dose exceeding physiological relevance and excessive thinning of the vaginal epithelium by a high dose of Depo-Provera, both of which can potentially underestimate the efficacy [45]. A vaginal efficacy study was recently reported using this rhesus macaque model [25**]. Depo-Provera pretreated rhesus macaques were dosed with 50 mg/kg of cabotegravir long-acting on weeks 0 and 4, and macaques were challenged intravaginally with 300 TCID$_{50}$ SHIV162p3 on weeks 1, 5, and 7 [25**]. As expected in this model, all control macaques became infected with viremia detected 1–2 weeks after the challenge, whereas viremia was detected in two of the cabotegravir long-acting-treated macaques at weeks 10 and 14 [25**]. In the cabotegravir long-acting-treated infected macaques, sequencing analysis indicated that infection was established by virus encoding wild-type integrase [25**]. Six of eight cabotegravir long-acting-treated macaques remained protected from three intravaginal challenges during the challenge phase and drug washout phase [25**]. Although there are some limitations with model that may underestimate the protective efficacy of an agent against intravaginal transmission, cabotegravir long-acting was at least 90% protective in this model.

Vaginal efficacy was also evaluated in the repeat-low dose model in pigtail macaques [24**]. This well-established model has several advantages, including the use of pigtail macaques, which have a menstrual cycle similar to women, a low SHIV162p3 inoculum dose (50 TCID$_{50}$) similar to the physiologic HIV-1 RNA range found in semen, and twice-weekly virus challenges to mimic high-risk human exposure [4]. In previous studies, this model has accurately predicted the efficacy of oral Truvada PrEP in women [4,9,10]. This study reported complete protection in cycling animals treated with 50 mg/kg of cabotegravir long-acting every 4 weeks that recapitulates the quarterly 800 mg dose in humans [24**]. Protected animals remained uninfected despite receiving 22 SHIV challenges over three cabotegravir long-acting cycles compared with a median of four SHIV challenges required to infect controls reflecting the robustness and durability of the protection [24**].

Additional studies in rhesus macaques challenged longitudinally with intravaginal high-dose SIV$_{mac251}$ (1000 TCID$_{50}$) also provided important information on the correlation of plasma cabotegravir concentrations and efficacy [46]. In this study, cabotegravir long-acting was given at three dose levels (10, 30, and 50 mg/kg; n = 9) at days -7 and -1 prior to initiation of weekly intravaginal challenges. Significant protection from virus acquisition was noted in macaques dosed with 30 and 50 mg/kg cabotegravir long-acting, whereas no protection was observed at the 10 mg/kg dose [46]. Analysis of plasma cabotegravir concentrations suggested that targeting four times PAIC$_{90}$ may be sufficient to protect against vaginal SIV transmission [46]. Therefore, data from all macaque studies to date demonstrate high efficacy of cabotegravir long-acting against vaginal infection and support the selection of an 800 mg dose administered every 3 months for PrEP in women.

**Clinical Evaluation of Cabotegravir Long-Acting**

Preclinical studies (Table 2) have supported the advancement of cabotegravir long-acting into clinical trials for HIV-1 prevention. Two phase 2a studies, a ViiV Healthcare-sponsored study (ÉCLAIR;...
CONCLUSION

As PrEP with daily Truvada is now a proven prevention strategy against HIV-1 acquisition, next-generation PrEP agents are being developed to provide sustained drug delivery to alleviate poor adherence associated with daily regimens. Such products include novel intravaginal rings for women and long-acting injectable drugs such as cabotegravir long-acting. Preclinical data in macaque models that have previously predicted Truvada efficacy in humans show that cabotegravir long-acting was highly protective against both rectal and vaginal SHIV transmission. Efficacy was seen at cabotegravir plasma concentrations achievable clinically by quarterly dosing of 800 mg of cabotegravir long-acting supporting clinical evaluation of this regimen as PrEP. Although long-acting ARVs provides hope for improving PrEP adherence, the clinical experience, gained once phase 2a trials are completed, will better address the safety, tolerability, and acceptability of cabotegravir long-acting for repeated dosing. Ultimately the efficacy of cabotegravir long-acting must be addressed in phase 2b/3 clinical trials to understand the full potential of this agent.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

* of outstanding interest


Authors describe the pharmacokinetic profiles of repeated cabotegravir long-acting injections with or without rilpivirine long-acting injections including repeat dose injections with 800 mg of cabotegravir long-acting in humans.


In addition to single pharmacokinetics of cabotegravir long-acting, the authors describe cervicovaginal or rectal tissue penetration in humans.


In this study, complete protection against repeated rectal SHIV transmission was observed in macaques treated with cabotegravir long-acting as PrEP and plasma cabotegravir concentrations were correlated with protection.


In this study, complete protection against repeated low dose SHIV challenge was observed in pigtail macaques in the absence of Depo-Provera treatment.


Cabotegravir long-acting was highly protective against vaginal transmission in a rhesus model utilizing Depo-Provera pretreatment and high-dose virus challenge.


