EXPERT OPINION

A drug evaluation of 1% tenofovir gel and tenofovir disoproxil fumarate tablets for the prevention of HIV infection

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Introduction: More than a million people acquire HIV infection annually. Pre-exposure prophylaxis (PrEP) using antiretrovirals is currently being investigated for HIV prevention. Oral and topical formulations of tenofovir have undergone preclinical and clinical testing to assess acceptability, safety and effectiveness in preventing HIV infection.

Areas covered: The tenofovir drug development pathway from compound discovery, preclinical animal model testing and human testing were reviewed for safety, tolerability and efficacy. Tenofovir is well tolerated and safe when used both systemically or applied topically for HIV prevention. High drug concentrations at the site of HIV transmission and concomitant low systemic drug concentrations are achieved with vaginal application. Coitally applied gel may be the favored prevention option for women compared with the tablets, which may be more suitable for prevention in men and sero-discordant couples. However, recent contradictory effectiveness outcomes in women need to be better understood.

Expert opinion: Emerging evidence has brought new hope that antiretrovirals can potentially change the course of the HIV epidemic when used as early treatment for prevention, as topical or oral PrEP. Although some trial results appear conflicting, behavioral factors, adherence to dosing and pharmacokinetic properties of the different tenofovir formulations and dosing approaches offer plausible explanations for most of the variations in effectiveness observed in different trials.

Keywords: ART, HIV prevention, microbicide, NtRTI, PMPA – [9-R-(2-Phosphonomethoxypropyl) adenine], tenofovir, tenofovir disoproxil fumarate

Expert Opin. Investig. Drugs [Early Online]

1. Introduction

Thirty years after the discovery of the human immunodeficiency virus (HIV), the HIV pandemic continues to spread [1]. UNAIDS estimates indicate 2.7 million (2.4 – 2.9 million) newly infected people at the end of 2010, while 34 million (31.6 – 35.2 million) people are living with HIV globally [2]. More than 6.6 million of the approximately 14.2 million people, living with HIV and eligible for treatment, are currently actually receiving HIV treatment. Sub-Saharan Africa contributes 70% of all infections primarily via heterosexual transmission. Women are disproportionally burdened with disease and contribute 60% of all HIV infections in the sub-Saharan African generalized epidemic [2]. Hyper-vulnerability of women in fuelling the epidemic is a result of complex convergences of social and biological

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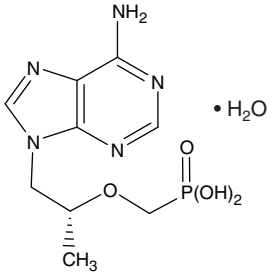
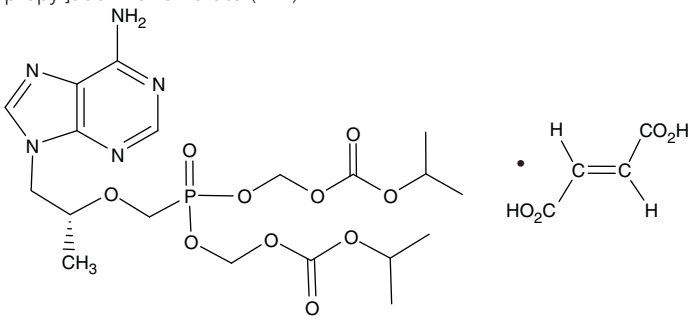
Box 1. Drug summary.

Drug name (generic)	TFV gel (GS-1278)	Tenofovir Disoproxil Fumarate (GS-4331-05)
Phase	IIb	IIb/III
Indication	HIV prevention	HIV prevention
Mechanism of action	Antiretroviral, nucleotide reverse transcriptase inhibitor	Antiretroviral, nucleotide reverse transcriptase inhibitor
Route of administration	Vaginal gel – topical vaginal administration	Oral
Chemical structure	9-[(R)-2-(phosphinyl]methoxy) propyl]adenine monohydrate (PMPA) 	9-[(*R*)-2-[[bis[[(isopropoxycarbonyl)oxy]methoxy]phosphinyl]methoxy] propyl]adenine fumarate (TDF) 
Pivotal trials	CAPRISA 004 – Abdool Karim *et al.* [15] MTN-003 – [78]	iPrEX trial – Grant *et al.* [13] Partners Prep Study – [12]

factors [3]. In this context, women's inability to negotiate mutual monogamy and consistent condom use translates to limited control of existing HIV prevention methods. Therefore, in the absence of an effective vaccine, the search for HIV biomedical prevention modalities that can stem the tide of new infections – the use of which can be controlled by women themselves – is a public health priority [4].

The concept of using orally administered antiretroviral (ARV) drugs to prevent HIV infection is well established and has been successfully used either as a single drug [5] or in a drug combination [6] for prevention of mother to child transmission (PMTCT) and for post-exposure prophylaxis (PEP) following occupational exposure to HIV or sexual assault [7]. Pre-exposure prophylaxis (PrEP) using daily or intermittent oral or topical agents (microbicides) is currently being tested in randomized clinical trials in HIV-uninfected people to assess safety and efficacy in preventing HIV acquisition [8]. Microbicides are products formulated for application on the vaginal or rectal mucosa and contain active ingredients that potentially block HIV and possibly other sexually transmitted infections [9].

Tenofovir, (PMPA, (*R*)-9-[2-(phosphonomethoxy)propyl] adenine monohydrate), is a adenosine nucleoside monophosphate (nucleotide) analog with potent activity against retroviruses [10]. Tenofovir is phosphorylated intracellularly to tenofovir diphosphate, its active metabolite, and competitively inhibits HIV reverse transcriptase resulting in DNA chain termination, thereby preventing further replication [11]. An orally bioavailable form of tenofovir (tenofovir disoproxil fumarate, TDF) (**Box 1**) has been approved for the treatment of HIV since 2001. With tenofovir's efficient intracellular activation, pro-longed intracellular half-life and activity in both active and resting cells and high barrier to resistance this agent is a safe and potent ARV with a proven track record for effective treatment of HIV-1 infection. When used daily for HIV prevention, TDF has shown significant activity in sero-discordant couples [12] and when used in combination with emtricitabine (FTC) was effective in protecting men who have sex with men (MSM) [13] as well as in heterosexual men and women [14]. Over the last 20 years of microbicide research, of the 12 effectiveness trials of 7 candidate products, only 1 candidate product demonstrated proof of concept for significant protection against HIV infection. The CAPRISA 004 Phase IIb safety and effectiveness trial showed that 1% tenofovir, vaginally administered gel (TFV), reduced HIV acquisition in 18-40 year old women, at high risk for HIV infection, by 39% overall and by 54% in women who used the gel consistently [15].

In this drug evaluation, we review available preclinical and clinical data on the tenofovir compound, in both its gel and oral formulations to assess its HIV infection prevention potential for PrEP.

2. Introduction to the PMPA compound and history of the development of tenofovir

Nucleoside derivatives were identified in the early 1960s as molecules that played an important role in drug development for the treatment of cancers. Being antimetabolites that would interfere with the growth of tumor cells, they were invariably

accompanied by host toxicity. However, extensive complex modifications of the nucleosides subsequently enhanced the therapeutic effect of the drugs. Further development of nucleoside derivatives, namely the acyclic nucleoside phosphonates, began in 1976, following a symposium on synthetic nucleosides, nucleotides and polynucleotides at the Max Planck Institute. The collaboration between the two research groups of Erik De Clercq and Antonin Holy 2 years later resulted in the identification of the acyclic nucleoside analog (*S*)-9-(2,3-dihydroxypropyl) adenine (DHPA) as a broad-spectrum antiviral agent [16-18].

DHPA, an acyclic nucleoside analog, was clearly distinct from the acyclic guanosine analog – acyclovir – as a selective anti-herpes simplex virus (HSV) agent, with acyclovir owing its selectivity to the specific phosphorylation by the HSV-induced thymidine kinase (TK). In spite of the broad spectrum of antiviral activity as well as its mode of action, DHPA had a limited appearance on the market as a gel formulation for the treatment of cold sores compared with acyclovir [19,20]. Thus, acyclovir became the 'gold standard' for the treatment of HSV types 1 and 2 infections. A clear disadvantage of DHPA was that it is not metabolized [21], but served as an excellent tool for various subsequent molecular and biological investigations. DHPA behaves as an aliphatic nucleoside analog by occupying the adenosine-binding site of *S*-adenosyl-l-homocysteine hydrolase, an important regulatory enzyme in *S*-adenosyl-l-methionine-mediated methylations [21] with the sugar moieties replaced by the aliphatic chains. DHPA is representative of unusual acyclic nucleoside analogs that have an alkyl group linked to N1 (in pyrimidines) or N9 (in purines) that bear hydroxyl(s) necessary for activation by phosphorylation. Such compounds can readily adopt a conformation appropriate for forming a complex with the active site of the enzyme. Thus, investigations of DHPA as a phosphonate derivative of bioactive nucleoside were aimed at creating catabolically stable, isopolar and, possibly, isosteric nucleotide analogs. The polar nature of nucleotides precludes their crossing the cellular membrane and the phosphonate derivatives of bioactive nucleosides in which the oxygen atom is placed in the nearest position adjacent to the α-carbon to transform the phosphoric ester grouping (= P-O-C-) to its isomeric phosphonomethyl ether (= P-C-O-) are, therefore, catabolically stable [22]. The rationale for the underlying development of acyclic nucleoside phosphonates is that nucleoside analogs generally need to be converted by three phosphorylation steps to their 5′-triphosphate metabolites to show activity. The first phosphorylation step, which affords the 5′-monophosphate, is often the bottleneck in this transformation, and so agents could show poor activity owing to lack of transformation to the monophosphate form by nucleoside kinases. This problem can be circumvented by using phosphorus-modified nucleotide analogs, such as phosphonates [23]. It is important that the linkage is resistant to cleavage by cellular enzymes.

2.1 Preclinical data on the effects of the precursors of tenofovir against viruses

Early experiments provided promising data on the marked antiviral activity of DHPA against several viruses in cell cultures. Cell lines, following inoculation with viruses, were exposed to differing concentrations of DHPA to determine the dose required to suppress viral cytopathogenicity by 50%. DHPA in human skin fibroblast cells demonstrated the inhibition of *in vitro* replication of several DNA and RNA viruses, including vaccinia, HSV 1 and 2, measles and vesicular stomatitis virus (VSV) at concentrations at which cellular DNA and RNA synthesis were not affected. DHPA at a concentration of 100 μg/ml caused a dramatic decrease in virus titer and this reduction amounted to approximately 4 $\log_{10}$ for the virus yields measured at 24 and 48 h after infection [16]. A key finding was that only the (*S*)-enantiomer of DHPA proved active, while the (*R*)-enantiomer was not and the racemic mixture of (*R, S*)-DHPA was also as effective as the (S)-enantiomer. Primary rabbit kidney cells with VSV, vaccinia and herpes simplex 1 (strain KOS), (*S*)-DHPA inhibited the cytopathogenic effects at a concentration of about 10 – 20 μg/ml. However, viruses such as poliovirus, Coxsackievirus and Sindbis were not inhibited by DHPA. DHPA did not exhibit any antibacterial or antifungal activity. *Streptococcus faecalis, Staphylococcus aureus, Pseudomonas aeruginosa, Proteus vulgaris* and *Escherichia coli* were found not to be inhibited at concentrations up to 100 μg/ml, while for *Mycobacterium tuberculosis*, *Trichophyton mentagrophytes, Candida albicans* and *Aspergillus niger,* the minimum inhibitory dose was 50 μg/ml [16]. An added advantage was that DHPA even at concentrations as high as 200 μg/ml did not significantly reduce DNA or RNA synthesis as monitored in HeLa, Vero and primary rabbit kidney cells. Similarly, DHPA did not inhibit protein synthesis when applied to primary rabbit kidney cells at a concentration of 400 μg/ml maintaining the viability of the uninfected host cells unless extremely high concentrations were employed.

Following the development of the hybrid molecule (*S*)-9-(3-hydroxy-2-phosphonylmethoxypropyl) adenine (HPMPA), similar to DHPA, (*S*)-HPMPA was shown to exhibit activity against a range of DNA viruses including vaccinia, HSV-1 and HSV-2, adenovirus and Maloney murine sarcoma retrovirus. Later work also found it to be effective against hepatitis B viruses. The inhibitory effect of the derivatives of adenine (PMPA) and 2,6-diaminopurine (PMPDAP) on HIV replication in several human cell systems, including natural peripheral blood lymphocytes (PBL) and freshly isolated monocyte/macrophages (M/M) demonstrated the (R)-enantiomers of PMPDAP and PMPA to be ~ 10- to 100-fold more effective compared with the (S)-enantiomeric complement [24,25]. The antiviral efficacy of (R)-PMPA was comparable with that of the prototype acyclic nucleoside phosphonate 9-(2-phosphonylmethoxyethyl) adenine (PMEA). Thus, (R)-PMPA and (R)-PMPDAP displayed superior inhibitory effects against HIV-1 when the virus was exposed to

those cells (i.e., peripheral blood lymphocytes and monocyte/macrophages) that in nature are the most important target cells for virus infection. These observations provided the potential of PMPDAP and (R)-PMPA as candidate drugs for the treatment of HIV infection in humans. The virtual lack of toxicity of (R)-PMPA and (R)-PMPDAP for proliferating and nonproliferating cells when administered daily for 4 weeks at 20 – 30 mg/kg further indicated their potential therapeutic usefulness [25].

2.2 Preclinical animal safety data of the precursors of tenofovir

The potential activity of DHPA *in vivo* was assessed in mice inoculated intranasally with VSV. The experimental infection resembled natural transmission, infection and spread from a respiratory tract site in humans. The intranasal VSV model showed that repeated doses of DHPA (2 mg per mouse or – 135 mg/kg) injected intraperitoneally 1 h and 1, 2, 3 and 4 days after VSV challenge brought about a significant increase in the final number of surviving mice (DHPA = 67% vs control group = 37.5%; $P < 0.05$) with sustained significant protection 9 days postinfection. Repeated doses of lower concentrations of DHPA at 0.08 mg per mouse (~ 5.4 mg/kg) did not confer protection, whereas repeated doses at 0.4 mg per mouse (~ 27 mg/kg) gave slightly better protection. No signs of toxicity were noted in the mice injected with DHPA [16].

The hybrid molecule between DHPA and PFA (phosphonoformic acid) (**Figure 1**) [22,23] saw the development of HPMPA and a totally new concept of antimicrobials emerged. HPMPA was subsequently shown to exhibit broad-spectrum activity against a range of DNA viruses, including those that did not induce a specific viral TK such as human cytomegalovirus or strains becoming resistant to acyclovir by a deficiency in their TK, such as the TK^- HSV strains [17,26]. Apart from its antiviral activity, HPMPA demonstrated antiparasitic activity as well. Although HPMPA itself was not further developed as an antiviral drug, it also served as the prototype compound for a series of acyclic nucleoside phosphonates now used for the treatment of viral infections, some in their prodrug form to improve their oral bioavailability. The agents are (*S*)-1-[3-hydroxy-2-(phosphonomethoxy)propyl]cytosine, HPMPC (cidofovir), 9-[2-(phosphonomethoxy)ethyl]adenine, PMEA (adefovir) and (*R*)-9-[2-(phosphonomethoxy)propyl]adenine monohydrate, PMPA (tenofovir) – the featured drug for this review (**Figure 1**) [23].

3. Nonhuman primate data on tenofovir (PMPA) in preventing HIV infection

Multiple nonhuman primate infection models have demonstrated the effectiveness of subcutaneous, gel and oral formulations of PMPA in preventing transmission of simian immunodeficiency virus (SIV) or simian–human immunodeficiency virus (SHIV) (**Table 1**), even when applied several hours after viral challenge. The data are summarized by route of tenofovir administration.

3.1 Tenofovir administered subcutaneously

One of the first preclinical studies demonstrating tenofovir's prevention potential was an investigation of subcutaneous injection of tenofovir daily for 4 weeks in macaques who had been intravenously inoculated with SIV. All 25 of the tenofovir-treated macaques were protected from SIV infection without toxicity whether administration began 48 h prior to intravenous inoculation (dose of tenofovir, 20 mg/kg in five animals, 30 mg/kg in 10 animals), 4 h after inoculation (dose of tenofovir 30 mg/kg in five animals) or 24 h post-inoculation (dose of tenofovir, 30 mg/kg in five animals). Evidence of SIV infection was not present in any of the treated animals monitored for up to 52 weeks, including viral load in plasma and peripheral blood mononucleocytes (PBMCs), SIV DNA in PBMCs, SIV-specific antibody and lymph node biopsies. By contrast, each of 10 animals receiving placebo 48 h prior to inoculation became infected [27].

Complete protection against SIV infection was also observed in a study among infant macaques that received just two subcutaneous doses of tenofovir; one 4 h prior and the second 24 h post oral SIV inoculation [28]. PMPA administered perinatally 2 h prior to cesarean section to pregnant macaques did not protect the newborns following SHIV inoculation shortly after birth. By contrast, of four newborns inoculated simultaneously with SIV and SHIV after birth but started immediately on PMPA treatment for 2 weeks, only one animal became persistently infected [29]. Even macaques inoculated with the virulent SIV_{mac055} strain, which has a fivefold reduced susceptibility to tenofovir, were partially protected when they received subcutaneous tenofovir 24 h prior to viral challenge followed by administration for 4 weeks compared with the untreated controls that all became infected [30].

The ability of tenofovir to prevent the establishment of persistent infection was investigated in a study on cynomolgus macaques (*Macaca fascicularis*). Daily subcutaneous dosing with tenofovir was initiated at varying times, as well as different durations of treatment, following intravenous inoculation with SIV. Twenty-four macaques were studied for 46 weeks after inoculation with SIV. All mock-treated control macaques showed evidence of infection within 2 weeks of post-inoculation. All macaques that were treated with tenofovir for 28 days beginning 24 h post-inoculation showed no evidence of viral replication following discontinuation of tenofovir treatment. However, extending the time to initiation of treatment from 24 to 48 or 72 h post-inoculation or decreasing the duration of treatment reduced effectiveness in preventing establishment of persistent infection. Only 50% of the macaques treated for 10 days, and none of those treated for 3 days, were completely protected when treatment was initiated at 24 h. Despite the reduced efficacy of delayed and shortened

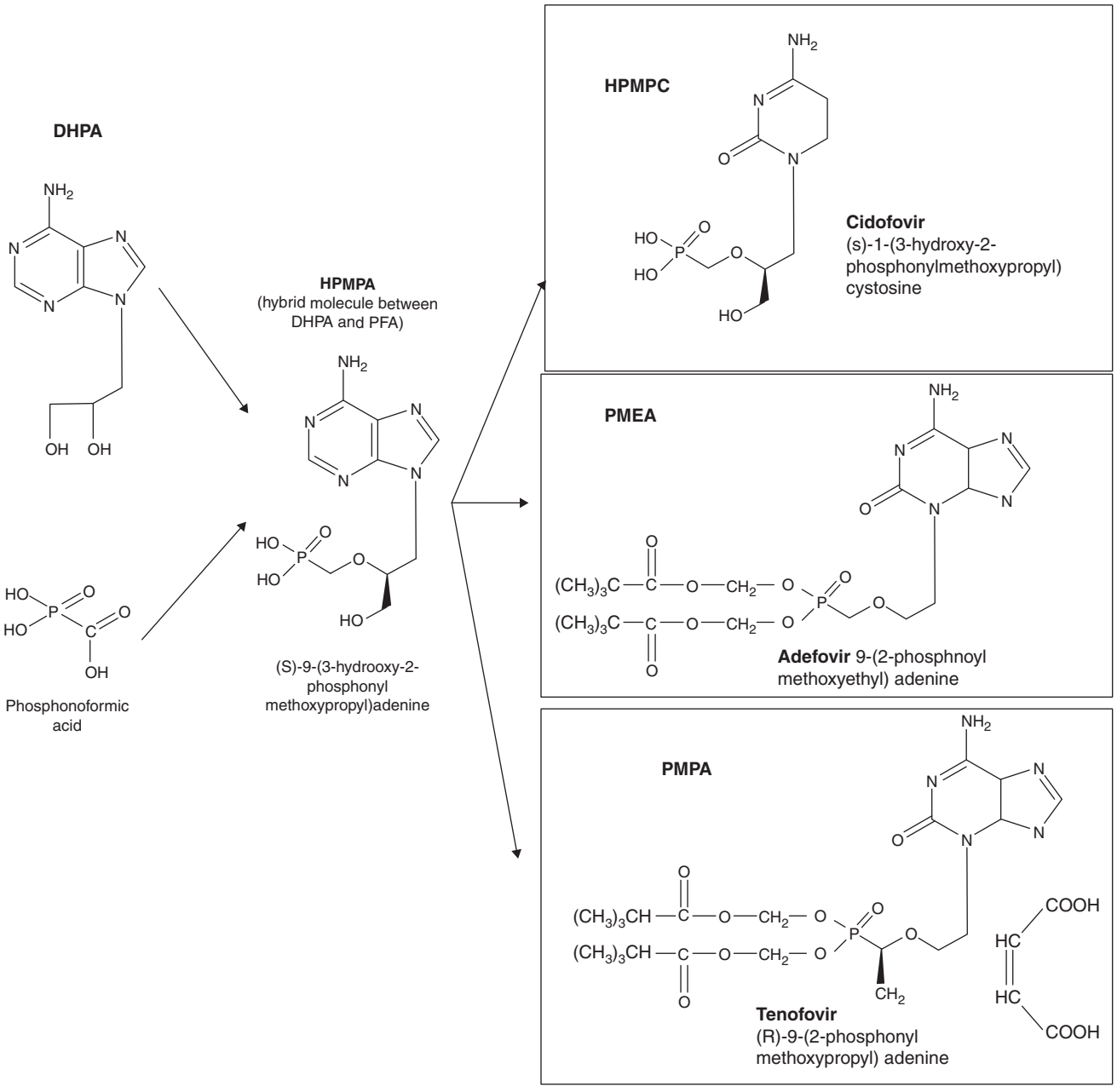


Figure 1. Development of acyclic nucleoside phosphonate antiviral agents.
Adapted from De Clercq, 2007 [22].

treatment, all tenofovir-treated macaques that were not protected showed delays in the onset of cell-associated and plasma viremia and antibody responses compared with mock controls [31].

Subcutaneous administration of tenofovir has also been shown to be completely protective against HIV-2 infection when given up to 36 h post-inoculation [32]. However, breakthrough infection in one animal in the 72 h post HIV exposure group was detected at 16 weeks [32].

Such *in vivo* activity of tenofovir made it a promising agent for prevention of HIV infection.

3.2 TFV gel administered intravaginally

Nine studies, conducted by the NIAID Division of AIDS and the Centers for Disease Control (CDC), have shown that intravaginal administration of TFV gel can prevent the transmission of SIV/SHIV. The studies explored the effectiveness of tenofovir provided at different concentrations and durations. While interpretation of some of these studies remains limited because some control animals remained uninfected and sample sizes were small, they provided proof-of-concept in animals that TFV gel could prevent the transmission of SIV/SHIV when vaginal dosing mimics coital usage of

Table 1. Summary of studies of nonhuman primate models of infection using tenofovir as pre-exposure prophylaxis against simian immunodeficiency virus infection.

Study*	Number of exposures	Inoculating virus	Treatment	Time of administration	Number infected	Protection (%)
Tenofovir administered subcutaneously						
Tsai *et al.* [27] 1995	1	SIV Intravenous (IV) inoculation	PMPA (20 mg/kg)	-48 h	0 of 5	100
			PMPA (30 mg/kg)	-48 h	0 of 10	100
			PMPA (30 mg/kg)	4 h	0 of 5	100
			PMPA (30 mg/kg)	24 h	0 of 5	100
			vehicle	n/a	10 of 10	0
Tsai *et al.* [31] 1998	1	SIV IV inoculation	PMPA (30 mg/ml)	24 h, daily for 28 days	0 of 4	100
			PMPA (30 mg/ml)	48 h, daily for 28 days	2 of 4	50
			PMPA (30 mg/ml)	72 h, daily for 28 days	2 of 4	50
			PMPA (30 mg/ml)	24 h, daily for 10 days	2 of 4	50
			PMPA (30 mg/ml)	24 h, daily for 3 days	4 of 4	0
			Untreated control	n/a	4 of 4	0
Van Rompay *et al.* [28] 1998	1	SIV_{mac} oral inoculation	PMPA SC	-4 h, 24 h	0 of 4	100
			Untreated controls	N/a	4 of 4	0
*Van Rompay *et al.* [29] 1998	2	SIV_{mac251} oral inoculation and SHIV-SF33 IV	PMPA SC	0 h for 14 days	1 of 4	75
			Untreated controls	n/a	12 of 12	0
Van Rompay *et al.* [30] 2000	1	$SIV_{mac055\infty}$	PMPA	-24 h for 4 weeks	3 of 5	40
			Untreated control	n/a	3 of 3	0
Otten *et al.* [32] 2000	1	$HIV\text{-}2_{GB122}$ inoculated intravaginally	PMPA SC	12 h	0 of 4	100
			PMPA SC	36 h	0 of 4	100
			PMPA SC	72 h	1 of 4	75
			Untreated control	n/a	4 of 4	0
Van Rompay *et al.* [36] 2001	1	SIV_{mac251} oral inoculation	PMPA SC (4 mg/kg)	-4 h, 20 h	1 of 4	75
			PMPA SC (4 mg/kg)	1 h, 25 h	2 of 4	50
			PMPA SC (30 mg/kg)	1 h	0 of 1	100
			Untreated control	n/a	4 of 4	0
Subbarao *et al.* [41] 2006	14	$SHIV_{SF162P3}$	Tenofovir orally	-2, daily for 36 weeks	4 of 4 by 6 weeks	0
			Tenofovir orally	-2, weekly for 36 weeks	4 of 4 by 7 weeks	0
			Untreated controls	n/a	4 of 4 by 1.5 weeks	0

*Macaques pretreated with 30 mg Depo-Provera 30 days prior to vaginal challenge.

$_{\infty}$virulent isolate that has a fivefold reduced susceptibility to tenofovir.

FTC: Emtricitabine; IV: Intravenously; PMPA: [9-R-(2-Phosphonomethoxypropyl)adenine] (tenofovir); SC: Subcutaneous; TDF: Tenofovir disoproxil fumarate.

Table 1. Summary of studies of nonhuman primate models of infection using tenofovir as pre-exposure prophylaxis against simian immunodeficiency virus infection (continued).

Study*	Number of exposures	Inoculating virus	Treatment	Time of administration	Number infected	Protection (%)
TFV gel administered intravaginally						
NIH/CDC study 1 [33]	2	SIV_{mac251} inoculated intravaginally	10% TFV gel	-24 h, 0 h, 24 h, 48 h	0 of 4	100
			1 ml vehicle	-24 h, 0 h, 24 h, 48 h	2 of 2	0
NIH/CDC study 2 [33]	1	SIV_{mac251} inoculated intravaginally	10% TFV gel	-24 h, -15 m, -24 h	1 of 5	80
			1% TFV gel	-24 h, -15 m, -24 h	1 of 5	80
			1% TFV gel	-15 m	2 of 5	60
			untreated control	N/A	5 of 5	0
NIH/CDC study 3 [33]	1	SIV_{mac251} inoculated intravaginally	1% TFV gel	-15 m	1 of 5	80
			1% TFV gel	-2 h	3 of 5	40
			1% TFV gel	-8 h	1 of 5	80
			vehicle	-15 m	1 of 5	80
			untreated control	N/A	2 of 5	60
NIH/CDC study 4 [33]	1	SIV_{mac251} inoculated intravaginally	1% TFV gel	-15 m	1 of 5	80
			1% TFV gel	-2 h	1 of 5	80
			1% TFV gel	-8 h	2 of 5	60
			vehicle	-15 m	2 of 5	60
			untreated control	N/A	4 of 5	20
NIH/CDC study 5 [33]	1	SIV_{mac251} inoculated intravaginally	1% TFV gel	-2 h	0 of 5	100
			vehicle	-2 h	2 of 5	60
			untreated control	N/A	2 of 5	60
NIH/CDC study 6 [33]	1	SIV_{mac251} inoculated intravaginally	1% TFV gel	-12 h	5 of 8	38
			1% TFV gel	-24 h	8 of 8	0
			1% TFV gel	-72 h, -48 h, -24 h	6 of 8	25
			vehicle	-12 h	8 of 8	0
			vehicle	-24 h	8 of 8	0
			untreated control	N/A	8 of 8	0
*Parikh *et al.* [34] 2009	20	$SHIV_{SF162p3}$	5% FTC and 1% TFV gel	-30 min	0 of 6	100
			Vehicle		5 of 6	17
			Untreated control		2 of 2	0
			1% TFV gel		0 of 6	100
			vehicle		3 of 3	0
Miller *et al.* [35] 1996	1	SIV_{mac251}	10% TFV gel	-24 h, 0 h, 24 h, 48 h	0 of 5	100

*Macaques pretreated with 30 mg Depo-Provera 30 days prior to vaginal challenge.

$_{\infty}$virulent isolate that has a fivefold reduced susceptibility to tenofovir.

FTC: Emtricitabine; IV: Intravenously; PMPA: [9-R-(2-Phosphonomethoxypropyl)adenine] (tenofovir); SC: Subcutaneous; TDF: Tenofovir disoproxil fumarate.

Table 1. Summary of studies of nonhuman primate models of infection using tenofovir as pre-exposure prophylaxis against simian immunodeficiency virus infection (continued).

Study*	Number of exposures	Inoculating virus	Treatment	Time of administration	Number infected	Protection (%)
			vehicle	-24 h, 0 h, 24 h, 48 h	5 of 5	0
Dobard *et al.* [37] 2012	2	$SHIV_{SF162P3}$	1% TFV gel	20 m,72 h	4 of 6	74
			2% HEC placebo gel		9 of 10	
TFV gel administered rectally						
Cranage *et al.* [38] 2008	1	$SIV_{mac251/32H}$	TFV gel rectally	-2 h	3 of 9	67
			TFV gel rectally	2 h	1 of 3	67
			Vehicle	-2 h	3 of 4	25
			Untreated control	n/a	4 of 4	0
Tenofovir administered orally						
Garcia-Lerma *et al.* [42] 2008	14	SHIV rectal inoculation	FTC SC	-7 to 9 days, daily for 28 days	4 of 6	33
			TDF (20 mg/kg) and FTC orally	-7 to 9 days, daily for 28 days	2 of 6	66
			TDF (22 mg/kg) and FTC SC	-7 to 9 days, daily for 28 days	0 of 6	100
			Intermittent FTC and TDF (22 mg/kg) SC	-2 h, 24 h	0 of 6	100
			Untreated controls	n/a	17 of 18	5
Van Rompay *et al.* [39] 2002	15	SIV_{mac251} oral inoculation 3 times/day	5 ml Tenofovir orally	daily	3 of 4	50
			2.5 ml Tenofovir orally	daily	2 of 4	50
			vehicle	daily	3 of 6	50
Van Rompay *et al.* [40] 2006	30	SIV_{mac251}	10 mg/kg Tenofovir orally	Once daily for 7 days	8 of 12	33
			Topical GS-7340	3 times daily for 7 days	4 of 5	20
			Untreated controls	n/a	29 of 31	6
Garcia-Lerma *et al.* [43] 2010	14	$SHIV_{SF162p}$ rectal inoculation	TDF (22 mg/kg) and FTC (20 mg/kg) orally	-22 h; 2 h	1 of 6	83
				-3 days; 2 h	1 of 6	83
				-7 days, 2 h	2 of 6	67
				-2 h; 22 h	3 of 6	50
				2 h; 26 h	3 of 6	50
			Untreated controls	n/a	9 of 9	0

*Macaques pretreated with 30 mg Depo-Provera 30 days prior to vaginal challenge.

∞virulent isolate that has a fivefold reduced susceptibility to tenofovir.

FTC: Emtricitabine; IV: Intravenously; PMPA: [9-R-(2-Phosphonomethoxypropyl)adenine] (tenofovir); SC: Subcutaneous; TDF: Tenofovir disoproxil fumarate.

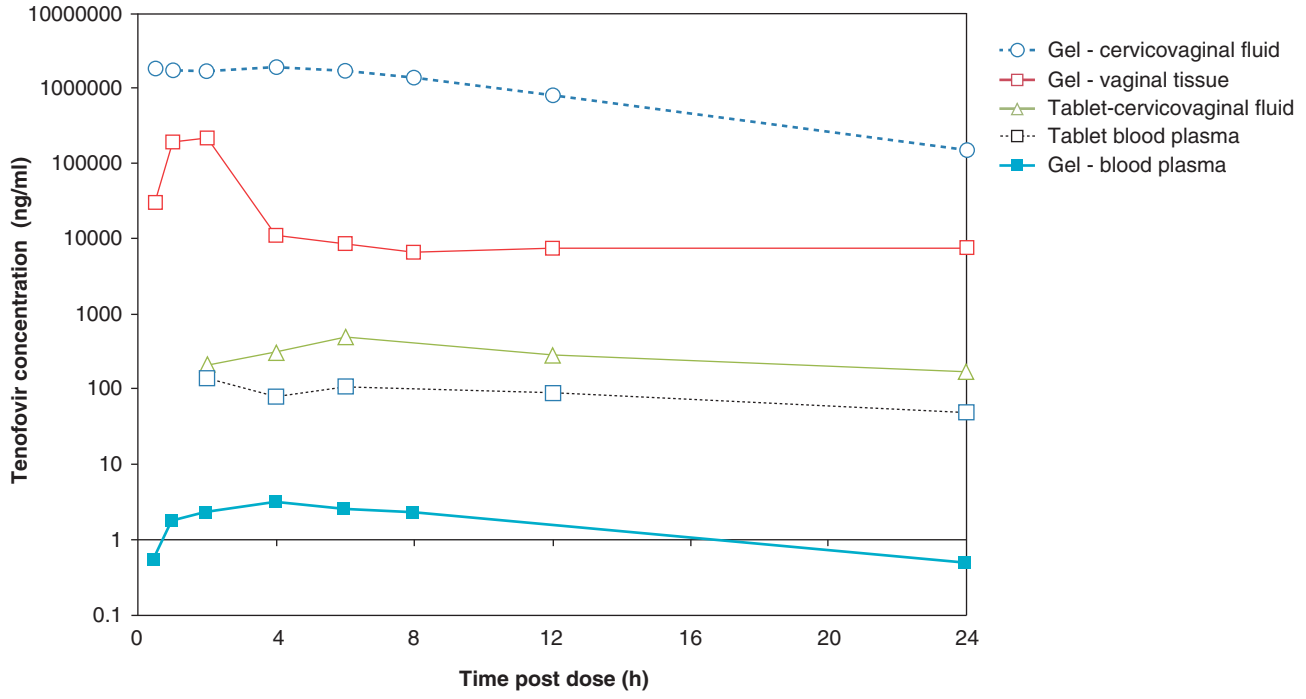


Figure 2. Tenofovir concentration distribution post single dose oral and topical vaginal administration.
Adapted from Dumond *et al.*, 2007 [52], Schwartz *et al.*, 2011 [54].

the gel. In study 1, a TFV gel dose given 24 h prior to exposure, at exposure, 24 h after and 48 h after exposure provided complete protection from SIV infection [33]. A repeat-challenge macaque model with a low-dose SHIV inoculum containing an CCR5-tropic HIV-1 envelope similar to naturally transmitted human viruses (10 TCID50) showed that application of 1% TFV gel just 30 min prior to viral challenge consistently protected from vaginal SHIV infection [34]. Higher doses of TFV gel (10% weight per weight) administered intravaginally at four timepoints, 24 h before, 0 h, 24 h after and 48 h post-inoculation, were also fully effective in preventing intravaginal SIV transmission after repeated viral challenge [35]. Even a single dose given 15 min before viral inoculation provided partial protection [33]. However, lower doses administered at 1 and 25 h after viral exposure were insufficient for protection, suggesting a dose–response effect [36]. Most recently, in delayed challenge experiments, four out of six macaques were protected from SHIV exposure occurring 30 min and 3 days after 1% TFV gel application, compared with 10 placebo-treated animals [37]. Together, these studies provided a scientific rationale for clinical studies of vaginally applied TFV gel in humans.

3.3 TFV gel administered rectally

TFV gel has also been tested in a rectal SIV challenge model. Mucosally applied TFV gel was given rectally as a single dose 15 min or 2 h prior to, or 2 h after intrarectal challenge. In the two control groups of macaques, four of four untreated macaques and three of four macaques given placebo gel became infected. Virus was recovered from only one of six animals receiving TFV gel 15 min prior to virus challenge. In one other animal in this group, virus was recovered only at weeks 2 and 6. Two of three animals receiving the drug 2 h prior to virus challenge showed no evidence of circulating virus and in the third animal virus isolation was delayed until week 12. In the third intervention group where gel was administered 2 h after virus challenge, two out of the three animals became infected. Interestingly, gag-specific interferon-gamma secreting T cells were detected by ELISpot in four of seven animals in which virus were unrecoverable from PBMC. These T-cell responses confirm exposure to challenge virus antigens and suggest that infection did not become established despite the virus having triggered an immune response [38].

3.4 Oral tenofovir administration

Several repeated-challenge macaque models have investigated the use of oral TDF in preventing SIV/SHIV. Two studies, mimicking viral exposure through breast-feeding, showed that oral tenofovir given daily was partially protective in infant macaques who received 15 [39] or 30 [40] low doses of SIV. In another study, tenofovir given daily for 36 weeks or weekly for 36 weeks to Chinese rhesus macaques – inoculated intrarectally with a high-dose SHIVSF162P3 once weekly for 14 weeks or until a macaque became infected – provided only partial protection against SHIV infection [41]. A repeat-exposure macaque model with 14 weekly rectal virus challenges was used to compare the effectiveness of daily versus intermittent

PrEP. The four treatment groups of six macaques each received either a daily subcutaneous dose of FTC (group 1) for 28 day, or a combination of oral FTC and TDF (group 2) for 28 days, or a subcutaneous dose of FTC and in combination with a higher dose of TDF (group 3) for 28 days, or a regimen similar to group 3 but only 2 h before and 24 h after each weekly virus challenge (group 4). Results of these groups were compared with 18 control macaques that did not receive any drug treatment. The risk of infection in macaques treated in groups 1 and 2 was 3.8- and 7.8-fold lower than that in untreated macaques ($p = 0.02$ and $p = 0.008$, respectively). All six macaques in group 3 were protected and all six animals in group 4 that received intermittent PrEP were protected [42]. These results showed that short but potent intermittent PrEP could provide protection comparable with that of daily PrEP. Most recently, the ability of intermittent human equivalent oral dosing of FTC (20 mg/kg) and TDF (22 mg/kg) to prevent infection in a repeated-challenge macaque model (weekly rectal SHIV162p3 exposure for up to 14 weeks) demonstrated best protection when the drug combination was administered 1, 3 or 7 days before virus exposure followed by a second dose 2 h after exposure [43]. This study indicates that pre-exposure drug administration at a fixed interval, and a booster dose within 2 h but not more than 24 h after virus exposure, could be an effective and a more convenient cost-effective dosing strategy for oral PrEP.

GS7340, a new generation tenofovir oral pro-drug, structurally suitable for improved delivery of tenofovir into cells was also studied in the macaque model. Although the authors demonstrated high concentrations in peripheral lymphocytes, lymphoid tissue and rectal tissue, GS7340 was not able to prevent rectal SHIV infection in macaques. The authors postulate that high dATP concentrations and dATP/TFV-DP ratios in activated rectal mononuclear cells may modulate the efficacy of tenofovir. The addition of a second ARV such as FTC may be required for enhanced efficacy against rectal transmission [44].

3.5 Intravaginal ring administration

There is considerable interest in vaginal ring technology for the administration of drugs for HIV prevention because of the ring's potential to provide coitally independent, sustained or controlled drug release with fewer adherence challenges [45]. Tenofovir's hydrophilic nature and poor solubility in silicone matrices make it a challenging compound to disperse in the traditional silicone elastomer that currently licensed vaginal rings are mostly formulated with. Despite these challenges, two *in vivo* studies in sheep and rabbit models have studied tenofovir and the prodrug TDF [46] as well as tenofovir in combination with acyclovir [47] in a modified silicone pod intravaginal device. The encouraging drug release rates from the pod device shows potential for further testing. In addition, polyurethane intravaginal rings, which may be better suited for tenofovir's physiochemical characteristics [48], will be tested in planned Phase I trials [49].

4. Pharmacokinetics and metabolism of tenofovir in humans

The pharmacokinetics of oral TDF has been evaluated in healthy volunteers and HIV-1-infected individuals and was found to be similar. TDF is a water-soluble diester prodrug of the active ingredient tenofovir. Following oral administration of a single dose of TDF 300 mg to HIV-infected fasting subjects, maximum serum concentrations (C_{max}) are achieved in 1.0 ± 0.4 h. C_{max} and area under the curve (AUC) values are 0.30 ± 0.09 μg/ml and 2.29 ± 0.69 μg·h/ml, respectively [50]. The volume of distribution at steady state is 1.3 ± 0.6 l/kg and 1.2 ± 0.4 l/kg, following intravenous administration of tenofovir 1.0 and 3.0 mg/kg [50] and 7.2% of the drug is plasma protein bound. Tenofovir is not a substrate of cytochrome P450 enzymes and following intravenous administration approximately 70 – 80% of the dose is recovered in the urine as unchanged tenofovir within 72 h of dosing. Following a single oral dose, the elimination half-life of tenofovir is approximately 17 h and intracellular half-life is ≥ 60 h [51]. After multiple oral doses of TDF 300 mg once daily (under fed conditions), 32 ± 10% of the administered dose is recovered in urine over 24 h. Tenofovir is eliminated by a combination of glomerular filtration and active tubular secretion [50].

Figure 2 is adapted from published data to illustrate the cervicovaginal fluid, vaginal tissue and blood plasma distribution of tenofovir over a 24-h interval following single dose oral and vaginal tenofovir administration.

Tenofovir concentrations in the genital tract and in rectal tissues have been assayed in Phase I and PK studies. Following oral TDF dosing, female genital tract (cervicovaginal fluid) concentrations of tenofovir relative to blood plasma at first dose achieved a median of 135%, and 75% of that of blood plasma concentrations at steady state [52]. More recently, tenofovir concentrations in genital secretions was found to be 2.5-fold greater than blood plasma concentrations [53]. The median cervicovaginal fluid concentrations of tenofovir at the end of a 24-h dosing interval at steady state was 68 ng/ml [52] Vaginal tissue concentrations at 24 h are 6.8 and 50 ng/g in the vaginal and cervical tissue respectively [53]. Rectal tissue exposure of oral tenofovir (C_{24} = 1877 ng/g) is 100-fold higher than vaginal and cervical tissue content [53].

Following vaginal use of TFV gel, the median C_{max} in cervicovaginal fluid was 1.9×10^6 ng/ml (range: 1.2×10^4 – 9.9×10^6 ng/ml) after a single dose and 1.4×10^6 ng/ml (range 8.4×10^4 – 5.8×10^6 ng/ml) after multiple doses. Blood plasma concentrations were low; C_{max} 4.0 and 3.4 ng/ml after single and multiple doses respectively. Tenofovir vaginal tissue concentrations (assayed from biopsies), and assessed by pooling samples from 1 to 24 h, demonstrated tenofovir concentrations ranging from 2.1×10^2 – 1.4×10^6 ng/ml with a median C_{max} of 2.2×10^5 ng/ml [54]. In a cross-over study, the tissue concentrations of the active tenofovir

Table 2. Summary of key TFV oral and gel Phase II and III safety and effectiveness studies for HIV prevention.

Phase II/IIb – Safety and effectiveness studies	Country	Population	Intervention	Status	Results
FHI West Africa Study (9780) [70] Safety and Preliminary Effectiveness 2007	Ghana, Cameroon, Nigeria	Sexually active HIV- women, (N = 936)	Active: Daily TDF 300 mg tablet Control: Daily placebo tablet	Complete	No clinical or laboratory safety concerns Effectiveness not evaluable due to a small number of HIV infections observed.
HPTN 059 [74] Safety and acceptability 2008	US, India	Sexually active HIV- women, (N = 200)	Active: Daily TFV 1% gel (N = 50) Coital use: TFV 1% gel (N = 50) Control: Daily placebo gel (N = 50) Coital Use – placebo gel (N = 50)	Complete	No safety or acceptability differences or concerns between the arms
CAPRISA 004 [15] Expanded safety and effectiveness study 2010	South Africa	Sexually active HIV- women, (N = 889)	Active: TFV 1% Gel (N = 445) Control: Placebo Gel (N = 444) *BAT 24	Complete	39% (95% CI: 6 – 60%) effective against HIV 51% (95% CI: 22 – 70%) effective against HSV-2[75] Higher gel adherence 54% effective in preventing HIV
CDC 4323 [71,72] Extended behavioral safety 2010	US	Sexually active HIV-MSM, (N = 400)	Active: Daily TDF 300 mg tablet Control: Daily placebo tablet Group 1: Started at enrolment Group 2: Started 9 months after enrolment	Complete	Preliminary analysis in July 2010 No serious safety concerns No increased risk in men taking PrEP compared with those not taking it
MTN 001 [55,76] Adherence and PK study 2011	US, South Africa, Uganda	Sexually active, HIV-women, (N = 144)	Daily TFV 1% gel (N = 144) Daily TDF 300 mg tablet (N = 144) Both (N = 144)	Complete	No safety or acceptability differences Tablet preferred in US women Vaginal tissue concentrations of TFV-DP-2$\log_{10}$ higher after vaginal dosing than oral dosing
MTN 003 (VOICE) [78] Safety and effectiveness study Initiated September 2009	South Africa, Zimbabwe, Uganda	Sexually active, HIV-women, (N = 5000)	Active: Daily TFV 1% gel (N = 1000) Daily TDF 300 mg tablet (N = 1000) Daily TDF/FTC 300/200 mg tablet (N = 1000) Control: Placebo gel (N = 1000) Oral placebo tablet (N = 1000)	Ongoing	Oral TDF arm stopped for futility September 2011 1% TFV gel arm stopped for futility November 2011 No serious safety concerns Results expected in 2013

*BAT24 Regimen – within 12 h *before* coitus, as soon as possible within 12 h *after* coitus and up to *two* doses in a 24-h period.

Table 2. Summary of key TFV oral and gel Phase II and III safety and effectiveness studies for HIV prevention (continued).

Phase II/IIb – Safety and effectiveness studies	Country	Population	Intervention	Status	Results
Phase III - Effectiveness Studies					
Partners PrEP study [84] Safety and effectiveness study Initiated May 2008	Kenya, Uganda	Sero-discordant heterosexual couples (N = 4758)	Active: Daily oral TDF tablet or Daily oral TDF/FTC tablet Control: Placebo tablet	Ongoing	Preliminary analysis: safety concerns - SAEs similar in all arms TDF effectiveness: 62% (95% CI: 34 – 78%) TDF/FTC effectiveness 73% (95% CI: 49 – 85%)
CDC 4370 Bangkok Tenofovir Study Safety and effectiveness study Initiated June 2005	Bangkok	Injection drug users (N = 2400)	Active: Daily oral TDF tablet Control: Placebo tablet	Ongoing	Results expected in 2012
FACTS 001 [87] Safety and effectiveness study including HSV-2 infection as a primary endpoint Initiated October 2011	South Africa	HIV-uninfected sexually active women (N = 2200)	Active: BAT 24* - TDF 1% Gel Control: HEC Placebo Gel	Ongoing	Results expected in 2014

*BAT24 Regimen – within 12 h *before* coitus, as soon as possible within 12 h *after* coitus and up to *two* doses in a 24-h period.

diphosphate were found to be 2 $\log_{10}$ higher following vaginal dosing than oral dosing [55].

Although vaginal fluid and tissue concentration ranges have not been established for protection against HIV infection, data from the CAPRISA 004 study indicate that HIV incidence was considerably lower in women with tenofovir cervicovaginal concentrations of 1000 ng/ml or more when compared with placebo drug or concentrations < 1000 ng/ml [56]. In summary, tenofovir concentrations with oral TDF are approximately 100-fold higher in rectal than vaginal tissues and 2.5-fold higher in the cervicovaginal fluid than blood plasma. TFV gel demonstrates 1000-fold higher concentration in vaginal tissues compared with oral TDF.

5. Safety and efficacy of oral and topical formulations of tenofovir in preventing HIV-1 infection in humans (Phase I – III)

Following the promising anti-HIV results from animal studies, the tenofovir research agenda advanced rapidly to comprehensively testing both oral and topical tenofovir in humans. Studies of topical TFV gel and oral TDF span a decade of research that includes clinical assessment of safety, acceptability, tolerance, pharmacokinetics (PK) and pharmacodynamics (PD) in differing populations and with various patterns of use.

Several ongoing Phase I studies are assessing safety in pregnancy and during lactation, adherence and PK comparing the safety of oral TDF with TFV gel. In addition, these studies are assessing safety, acceptability and PK with rectal use by women and men (including a separate study in young men); low osmolality gel formulation characteristics to aid rectal use and resistance screening. Two Phase III studies, one testing oral TDF as PrEP among injection drug users and the other assessing effectiveness of coitally linked topical tenofovir gel, are also ongoing.

5.1 Phase I studies

5.1.1 Topical TFV gel

Two concentrations (0.3 and 1%) of topical tenofovir have been shown to be safe and well tolerated in a Phase I trial among 84 sexually abstinent HIV-uninfected women. The HPTN 050 study [57] showed that a 2-week course of 1% TFV gel used twice daily was as well tolerated as 0.3% used once daily by all 84 women and 24 male sexual partners. The highest practical dose and frequency (HPDF) was determined to be 1% twice daily. Adverse events (AEs) were mainly those that occurred in the genital tract, with 92% of women reporting at least one AE and 87% of the AEs being mild and short lived. Serum tenofovir levels were low but detectable in 14 of the 25 women who had PK evaluations completed. No new HIV resistance mutations were detected after 2 weeks of twice-daily TFV gel use in the HIV-infected women. Quantitative results indicate that tenofovir vaginal gel was acceptable to almost all users

(94%), while qualitative findings indicate that acceptability is complex – lubrication, leakage, sexual pleasure and the possibilities for covert use being important considerations to women [58]. A male tolerability study of TFV gel has shown that multiple topical gel exposures on the penis were well tolerated by both circumcised and uncircumcised men [59].

A number of pharmacology studies investigating local and systemic absorption, genital tract drug concentrations and the impact of TFV gel on mucosal immune mediators have also been completed. One study among 45 HIV-negative sexually abstinent women (A04-095) [60] has shown that high genital tract and cervicovaginal fluid levels, and low blood plasma concentrations were detectable up to 24 h following a single or multiple doses of 1% TFV gel. The active tenofovir diphosphate concentration was high in endocervical cells and detectable in about 40% of vaginal tissue biopsy samples at exposures similar to or higher than what is seen in PMBCs after oral exposure [54]. Candidate biomarkers of microbicide pharmacodynamics and safety were evaluated in a double-blind, placebo-controlled trial (TFV 010) with 30 women randomized to apply single daily dose of TFV or placebo gel for 14 consecutive days [61]. A significant increase in anti-HIV activity was detected in cervicovaginal lavage (CVL) from women who applied TFV gel compared with those using the placebo and this activity persisted in the presence of virus-infected semen. The gels were well tolerated and AEs were similar in both arms. Repeated vaginal application of TFV gel was not associated with reduction in endogenous antimicrobial activity, loss of protective mediators or pro-inflammatory response.

An important subpopulation for gel use is pregnant and lactating women. The Microbicide Trials Network (MTN 002) study was the first Phase I trial to evaluate the safety and PK of a single dose of 1% TFV gel administered approximately 2 h before planned cesarean to 16 pregnant women [62]. There were no serious AEs among mothers or neonates related to TFV gel exposure. Single application of the gel in term pregnancy produces low overall serum levels consistent with levels reported in nonpregnant women. While TFV does appear to cross the placenta, fetal exposure after vaginal dosing was low, with only trace amounts being passed to the fetus [63].

Collectively these Phase I studies indicate that the 1% TFV gel is safe, well tolerated and acceptable to users. Additionally, there is compelling evidence for anti-HIV activity and availability of high drug concentrations at the site of HIV exposure.

Phase I safety studies of TFV gel when used rectally are ongoing. Results of the first Phase I trial – MTN 006 – assessing the systemic safety of 1% TFV gel when applied rectally in 18 sexually abstinent HIV-uninfected men and women in the US is currently being analyzed. Initial reports indicate that rectal use of the current hyperosmolar 1% TFV gel formulation, although suitable for vaginal use, can cause lower gastrointestinal tract distress and is not appropriate for rectal use [64]. Another trial of rectal safety and acceptability of a new TFV gel formulation (low glycerine concentration) MTN 007 [65] is currently underway in 60 men and women. Application of the hyperosmolar 1% TFV gel to a cervicovaginal explant model demonstrates a transient reduction in epithelial resistance and fracture of the ecto-cervical tissue but no damage to lamina propria. It is noted that explant models appear unable to self-repair and more sensitive to formulation content than with vivo use [66].

A number of new Phase I studies are also planned. These include a Phase I study that will expand on the positive results from MTN 002 to provide important PK information of the use of TFV gel daily for 7 days in pregnant and lactating women [67] and a Phase I study comparing the PK/PD in 100 sexually active US women using either a daily 1% TFV gel dose, a peri-coital (dosed either 1 h before or 1 h after sex) dose or BAT 24 dosing [68].

5.1.2 *Oral TDF*

The review of Phase I studies on the use of oral TDF for HIV prevention is restricted to ongoing trials only as the safety and tolerability of oral TDF for daily use have been well established from extensive experience with this formulation of the drug for HIV treatment.

The ongoing Phase I studies of oral TDF for prevention focus on drug PK in special populations and participant acceptability with PK assessment of the oral formulation compared with the gel formulation. MTN 006 is a safety, acceptability and PK assessment comparing rectally applied TFV gel with oral TDF in 18 HIV-negative US men and women [69] and will provide valuable data to support the vaginal microbicide application, should vaginal efficacy be demonstrated in other trials and additional safety data on rectal microbicides for use by men and women. Another ongoing Phase I trial in Malawi and Brazil, HPTN057, is assessing the safety and PK of oral TDF in 110 HIV-infected women and their infants and will provide data to inform the optimal regimen for a subsequent MTCT efficacy trial, if indicated.

5.2 Phase II studies

Table 2 provides an overview of oral TDF and topical TFV gel Phase II and III studies, completed and in progress for tenofovir safety and effectiveness determination against HIV-1.

5.2.1 *Oral TDF*

The first study to assess oral PrEP in women, under the direction of FHI 360, evaluated the safety and preliminary effectiveness of daily oral TDF versus placebo in preventing HIV in 936 African women [70]. Although this West African study showed that oral TDF was safe, it was unable to assess effectiveness due to the small number of HIV infections observed [70]. The use of oral TDF in preventing HIV acquisition was also evaluated by the US CDC in 400 HIV-negative MSM (CDC 4323) [71]. The preliminary

analysis (July 2010) suggested no serious safety concerns and no increased risk compensation in men taking a study pill compared with those not taking prophylactic pills [72].

5.2.2 *Topical TFV gel*

The evaluation of expanded safety and effectiveness of topical tenofovir includes two completed studies and one ongoing study. The first trial, HPTN 059, which compared 1% TFV gel coitally dependent use (up to twice daily) with daily vaginal use versus a placebo gel over 24 weeks in HIV-1-uninfected women, showed no difference in safety, acceptability and adherence. The gel was widely acceptable and 90% of the women reported that they would use the gel if reduced the risk of HIV infection [73,74].

The CAPRISA 004 study, a Phase IIb study, compared 1% TFV gel versus placebo (applied intravaginally by women, up to 12 h before sex and as soon as possible within 12 h after sex but not more than twice in 24 h – termed BAT 24) in 889 South African women aged 18 – 40 years. It was the first microbicide effectiveness trial to demonstrate proof-of-concept that a gel containing an ARV agent can protect women from acquiring HIV. The trial showed that TFV gel use reduced HIV infection in women by 39% (95% CI 6, 60%) overall [15]. The reduction in HIV risk reached 54% in women who used the gel consistently (> 80% of sex acts were covered by gel). No tenofovir-related resistance mutations were detected and AE rates were similar in the two study arms. The trial also showed that TFV gel reduced the risk of HSV-2 infection by 51% [75].

5.2.3 *Combination of oral TDF tablets and topical TFV gel*

An adherence and PK study has also been completed. The MTN 001 study randomized 144 sexually active HIV-uninfected women at four US and three African sites to a sequence of oral TDF, topical TFV gel and both formulations daily for 6 weeks, with a 1-week no-drug washout period between sequences. All three regimens were well tolerated and acceptable, with high self-reported adherence. Of note, daily use of the vaginal gel achieved a more than 100-fold higher concentration of active drug in vaginal tissue compared with the oral tablet but compared with the gel, the tablet used daily was associated with a 20-fold higher active drug concentration in blood. Women in the US preferred the tablets over the gel, while African women showed a preference for the gel [76,77].

Currently in the field, the MTN 003 (VOICE) trial is examining the safety and effectiveness of two oral ARV agents (TDF and FTC-TDF) taken daily and 1% TFV gel also administered daily to reduce the risk of HIV acquisition in women [78]. The study enrolled 5029 African women (approximately 1000 in each study group). On 16 September 2011, the TDF tablet component of the MTN 003 study was discontinued after interim results showed that it was no better than placebo in preventing HIV in the study women. Two months later, on 17 November 2011, a scheduled review of the MTN 003 study's data by the independent Data Safety and Monitoring Board (DSMB) revealed that the incidence rate of HIV infection in the women assigned to daily TFV gel was 6.0% compared with 6.1% in women assigned to placebo gel. The FTC-TDF tablet arm is continuing to study completion. The DSMB found no safety concerns with any of the products used in the study [79-81]. Final results are expected in late 2012.

5.3 Phase III studies

Three Phase III trials, two examining TDF tablets and one evaluating TFV gel, are currently underway. The Partners PrEP trial, which was initiated in sero-discordant couples in May 2008, is investigating whether once-daily PrEP (TDF or FTC-TDF) taken by an HIV-uninfected person can reduce their risk of acquiring HIV from their infected partner [82]. In July 2011, an interim analysis indicated a strong HIV prevention effect and the placebo arm was discontinued. The preliminary data showed that HIV was reduced by 62% in the TDF arm and by 73% in the FTC/TDF arm when compared with placebo [83,84]. The trial is continuing and will provide additional safety and effectiveness data in 2012 or 2013. Another ongoing trial, the Bangkok tenofovir study (CDC 4370), is assessing the safety and efficacy of daily oral TDF to prevent parenteral HIV infection among injection drug users in Thailand [85,86], with results expected in 2012.

The FACTS 001 (Follow-on African Consortium for Tenofovir Studies) is evaluating the safety and effectiveness of 1% TFV gel in preventing HIV and HSV-2 infection in young South African women [87]. The study enrolled their first participant on 21 October 2011 and results are expected in 2014. This important study will contribute additional data to the CAPRISA 004 results and is critical for potential licensure of 1% TFV gel for HIV prevention in women.

5.4 Observational/ exploratory studies

Several small observational and exploratory studies are underway to provide additional information on tenofovir PrEP. MTN 003B is a sub-study of the MTN 003 trial monitoring changes in bone mineral density after 1 year among MTN 003 participants receiving oral TDF and FTC-TDF compared with oral placebo [88]. MTN 003C will explore how community and household factors affect adherence in MTN 003 participants [89]. MTN 015 is comparing the plasma HIV-1 RNA level 12 months after HIV-1 sero-conversion among ART-naive participants assigned to an active study product compared with control participants [90]. MTN 016 is evaluating the prevalence of spontaneous loss in mothers and congenital abnormalities in infants of mothers exposed to an active study agent during pregnancy as compared with that in mothers not exposed to an active study agent during pregnancy [91].

CAPRISA 009, an open label RCT, will assess the impact of prophylactic exposure to TFV gel on the therapeutic effect of subsequent TFV-containing ART on viral suppression [92].

Together, these studies will provide important information to supplement primary outcome date required for product licensure.

5.5 HIV prevention trials investigating the FTC-TDF combination tablet

Although the scope of this review is limited to the tenofovir compound, we provide here a brief summary of trial outcomes when oral TDF was tested in combination with FTC.

In November 2010, the Pre-exposure Prophylaxis Initiative (iPrEX) trial provided the first evidence that oral ARV drugs can effectively prevent HIV acquisition in MSM. The trial was conducted in Brazil, Peru, Ecuador, Thailand and South Africa among 2499 men or transgender women who have sex with men, which showed that the daily oral combined FTC-TDF reduced HIV incidence by 44% (95% CI 15, 63) (4). During 3324 person-years of follow-up, there were 100 HIV infections: 36 in the FTC-TDF group and 64 in the placebo group [13]. Furthermore, evidence for the effectiveness of daily oral PrEP in heterosexual men (and women) comes from results of two studies presented at the 6th International AIDS Society Conference on HIV Pathogenesis, Treatment and Prevention in Rome in July 2011.

The Partners PrEP trial [84], involving 4758 HIV discordant couples from Kenya and Uganda, found that daily oral TDF and FTC-TDF reduced HIV incidence by 62% (95% CI 34, 78) and 73% (95% CI 49, 85) respectively. The Botswana TDF2 trial [93], conducted in 1200 heterosexual men and women from the general population, found that daily oral FTC-TDF reduced HIV incidence by 63% (95% CI 21, 48).

By contrast, the FEM-PREP study, having enrolled 1952 women to assess the effectiveness of FTC-TDF tablets in preventing HIV infection, was terminated early by its DSMB for futility. A total of 56 new HIV infections had occurred, with an equal number of infections in those participants assigned to FTC-TDF and those assigned to a placebo pill [94].

6. Regulatory pathway for licensure

At present no ARVs are licensed for prevention of sexual transmission of HIV as an indication. In order for candidate agents to be considered for registration, two independent well-conducted trials showing efficacy are generally needed. At a WHO consultation in 2010, the US Food and Drug Administration (FDA) has agreed to review the TFV gel licensing submission under a 'fast track' designation, which also allows for a rolling submission of relevant data that can be reviewed by the regulatory authority as the data become available [95]. The FDA had also agreed to consider a combination of CAPRISA 004 and the MTN 003 trials for licensure of tenofovir gel, despite their differences in dosing. This plan is no longer applicable as the MTN 003 trial's daily dosing of tenofovir gel did not demonstrate protection against HIV. The FDA has agreed that the combination of the CAPRISA 004 trial and the FACTS trial, if it demonstrates protection, are sufficient for consideration of the licensure of coital use of tenofovir gel. In addition, information on the safety of tenofovir gel in adolescents, post-menopausal women and pregnancy will be required.

7. Conclusion

Tenofovir exhibits distinct biological and pharmacologic properties that make this agent an ideal candidate for HIV prevention. Extensive and comprehensive testing of tenofovir in animal models has demonstrated efficacy in this model for advancement in humans for HIV prevention. Both the topical and systemic forms of tenofovir have been shown in Phase II, IIb and III studies to be protective against HIV infection by sexual transmission, with a tolerable side effect profile and with adequate monitoring, and demonstrated low risk for the development of tenofovir resistance. However, research, particularly in women, has demonstrated apparently conflicting results in two important studies that may potentially hamper progress in finding a women-controlled prevention. Once positive confirmatory results from similarly conducted, comparable effectiveness trials become available, tenofovir in its gel and/or oral form should be advanced for licensure for HIV prevention.

8. Expert opinion

In the last 20 months, there has been a sea change in HIV prevention. A series of studies since July 2010 have generated a confluence of new evidence that has brought new hope that ARVs can potentially change the course of the HIV epidemic, when used as early treatment for prevention [96], as topical [15] or oral PrEP [82]. Tenofovir has been central to this achievement; every HIV prophylaxis trial tested tenofovir, either alone or in combination with FTC. Tenofovir is the first-choice ARV for HIV prophylaxis because of its safety profile, efficacy in suppressing viral replication, long half-life, rapid absorption and evidence of efficacy in animal challenge studies. The HIV prevention field has been reinvigorated and exhibits a newfound optimism that it may be possible to control the HIV epidemic.

The barriers to wide-scale PrEP implementation and translating results from controlled clinical trials to public health benefit are challenges that need to be overcome. However, for women who are unable to convince their male partners to be faithful or use condoms, tenofovir represents a completely new approach to HIV prevention. Tenofovir, either as tablets or gel, is the first HIV prevention technology that women can genuinely control themselves and enables women to take charge of their HIV risk. In South Africa alone, this new prevention technology could avert an estimated 1.3 million

new HIV infections and 800,000 AIDS deaths over the next 20 years [97]. Implemented on a broader scale, tenofovir, used as prophylaxis, could save millions of lives.

In addition to its effect on HIV acquisition, the gel formulation of tenofovir also reduced genital herpes infections by 51%, an effect not found with oral TDF, since the oral drug does not achieve the high drug concentrations observed at the site of HIV exposure in the genital tract observed with topical administration [56,98]. A recent study confirmed the mechanism of action of high doses of tenofovir against HSV-2 and provided further evidence from tissue culture and animal models for this effect [98]. Genital herpes infections are the most important global cause of genital ulcer disease with up to a quarter of sexually active adults estimated to be infected with HSV-2. Women who have genital herpes are also significantly more likely to acquire HIV than those who do not. Besides general safe sex practices, there is no known prevention or cure for genital herpes infection, thereby making TFV gel an important breakthrough against HSV-2 infection.

However, this new hope can be realized only when there is adequate robust evidence of safety and efficacy of oral and topical tenofovir to achieve licensure by a medicines regulator, such as the FDA. As at the end of 2011, the available evidence that tenofovir is efficacious is strong. Data from cell culture, tissue explants, mice and monkeys demonstrate consistent results that tenofovir is efficacious in preventing HIV or SIV. Human efficacy trials confirm these preclinical observations: tenofovir gel and oral tenofovir, either singly or in combination with FTC, have demonstrated effectiveness in humans. However, two trials conducted among women have produced apparently conflicting results. It is unclear at this time as to whether adherence or some other reason is responsible for the differences in the outcomes from these studies. A detailed analysis of the study data from these two trials, anticipated in late 2012, will be critical to understanding the reasons for these perplexing results.

Based on the CAPRISA 004 results on coital dosing of tenofovir gel, there was high hope that the MTN 003 study of daily TFV gel would show similar or better results. Instead, the MTN 003 trial did not demonstrate that daily dosing of tenofovir gel and TDF tablets was effective in preventing HIV. As highlighted in previous microbicide studies, to be able to demonstrate effectiveness of a microbicide gel, women have to *consistently* use the *right amount* of the *right drug* to get the *right drug levels* in the *right cells* at the *right time*. At present, it is unclear whether the MTN 003 study's unexpected outcome could be due to inadequate or nonuse of the products by women in the study, to insufficient drug levels in the genital tract of the women at the time of HIV exposure during sex, or to some other reason. Indeed there have been opinions expressed prior to the results that non-coitally dependent daily use of a product may be tedious, lead to poor adherence and possibly product failure [99]. It is critical that the CAPRISA 004 results (although positive has a wide confidence interval around the effectiveness point estimate) be verified in a larger, similarly designed study. The FACTS 001 study, which is currently underway, is designed to confirm the CAPRISA 004 trial's coital use of tenofovir gel in preventing HIV and HSV-2 in order to generate the data needed for licensure.

The Partners PrEP and TDF2 trials, which have not been published yet, produced the first evidence that ARV tablets can also prevent HIV in sero-discordant couples and the general heterosexual population. In women, however, additional recent data on oral PrEP have not demonstrated consistent results. Two trials – FEMPrEP [94] and MTN 003 [100] – found no protection against HIV in women using daily oral FTC-TDF and TDF respectively. These trials are currently being analyzed to help determine the reasons of the results. Until these data are available, it is unclear whether the mixed results from oral PrEP studies in women are due to differing adherence (behavior) or due to differing efficacy (biology). Some clues are evident from the recently released qualitative adherence assessment of the partners PrEP study, which indicated that the desire to protect the uninfected partner and ultimately preserve the relationship among disclosed sero-discordant couples is a strong motivator to adhere to PrEP with the support of a partner who encourages adherence [101].

In several countries throughout the world, MSM constitute a major subgroup where new HIV infections are taking place, especially in concentrated epidemics. Traditional safe sex messages have had only limited impact on the HIV epidemic in this group. The iPrEX trial produced the first evidence that ARV tablets can prevent HIV in MSM. This introduces a new approach that could be used in combination with condom promotion and educational safe sex programs to prevent HIV in this MSM. Since FTC-TDF, is already a licensed ARV drug for AIDS treatment, it is already being prescribed off-label for HIV prevention in MSM. Based on the results of the iPrEX study, the Centers for Disease Control and Prevention and other US Public Health Service agencies have issued guidance on the use of PrEP among MSM in the US as part of a comprehensive set of HIV prevention services [102]. However, concerns about adherence, drug resistance [103] and behavioral disinhibition have hampered the widespread roll out of FTC-TDF for HIV prevention among MSM. Within the iPrEX trial, tolerability to FTC-TDF was not compromised, side effects were minimal and generally well tolerated, no behavioral disinhibition was observed, TDF resistance was not reported and FTC resistance was only reported in patients who were already infected with HIV at entry into the trial.

The new findings on the effectiveness of tenofovir in preventing HIV, despite some conflicting data from the FEM-PrEP and MTN 003 trials, have reinvigorated the HIV prevention field and created new found optimism that it may be possible to impact the spread of HIV in the general heterosexual population through implementation in high-risk subgroups in generalized epidemics. Large-scale implementation, post-licensure, will require vigilance and surveillance to monitor behavior changes, adverse events,

adherence levels, drug resistance patterns and the effect of drug resistance on later AIDS treatment. One of the most crucial challenges in HIV prevention in Africa is reducing the high infection rates among young women. Young women in Africa bear the brunt of the HIV epidemic, with HIV rates up to 8 higher than the rates in their male counterparts. For women unable to negotiate mutual faithfulness and/or consistent condom use with their male partners, microbicides are a critically important technology. The need for a woman-controlled HIV prevention technology remains urgent.

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Declaration of interest

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