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

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Abstract

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 pre-clinical and clinical testing to assess acceptability, safety and effectiveness in preventing HIV infection.

Areas covered—The tenofovir drug development pathway from compound discovery; pre-clinical 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 favoured prevention option for women compared to 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; NtRTI; tenofovir; tenofovir disoproxil fumarate; HIV prevention; microbicide; PMPA – [9-R-(2-Phosphonomethoxypropyl) adenine]

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, whilst 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 generalised epidemic [2]. Hyper- vulnerability of women in fuelling the epidemic is a result of complex convergences of social and biological 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 anti-retroviral (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 randomised 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) analogue with potent activity against retroviruses[10]. Tenofovir is phosphorlyated 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) has been approved for the treatment of HIV since 2001. With tenofovirs' 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 antiretroviral 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 seven candidate products only one 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 pre-clinical 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 1960's as molecules that played an important role in drug development for the treatment of cancers. Being anti-metabolites that would interfere with the growth of tumour 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 two years later resulted in the identification of the acyclic nucleoside analogue (*S*)-9-(2,3-dihydroxypropyl) adenine (DHPA) as a broad-spectrum antiviral agent [16-18].

DHPA, an acyclic nucleoside analogue was clearly distinct from the acyclic guanosine analogue – 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 to 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 analogue 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 analogues 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 analogues. 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 analogues 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 analogues, 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 percent. 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 ug/ml caused a dramatic decrease of virus titre and this reduction amounted to approximately 4 $\log_{10}$ for the virus yields measured at 24 and 48 hours after infection [16]. A key finding was that only the (*S*)-enantiomer of DHPA proved active, whilst 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 to 20 *u*g/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*u*g/ml, whilst for Mycobacterium tuberculosis, Trichophyton mentagrophytes, Candida albicans, and *Aspergillus niger* the minimum inhibitory dose was 50*u*g/ml[16]. An added advantage was that DHPA even at concentrations as high as 200 *u*g/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*u*g/ml maintaining the viability of the uninfected host cells unless extremely high concentrations were employed.

Following the development of the hybrid molecule 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 to the (S) - enantiomeric complement [24, 25]. The antiviral efficacy of (R)-PMPA was comparable to 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 non-proliferating cells when administered daily for 4 weeks at 20 to 30 mg/kg further indicated their potential therapeutic usefulness[25].

2.2 Pre-clinical animal safety data of the precursors of tenofovir

The potential activity of DHPA *in vivo* was assessed in mice inoculated intra-nasally 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 hour and 1, 2, 3, and 4 days after VSV challenge brought about a significant increase in the final number of surviving mice (DHPA = 67% versus control group =37,5%; P <0 .05) with sustained significant protection 9 days post infection. 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 (*S*)-9-(3-hydroxy-2-phosphonylmethoxypropyl) adenine (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 anti-parasitic 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. Non-human primate data on tenofovir (PMPA) in preventing HIV infection

Multiple non-human 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 summarised by route of tenofovir administration.

3.1 Tenofovir administered subcutaneously

One of the first pre-clinical 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 hours prior to intravenous inoculation (dose of tenofovir, 20 mg/kg in five animals, 30 mg/kg in 10 animals), 4 hours after inoculation (dose of tenofovir 30 mg/kg in five animals), or 24 hours 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. In contrast, each of 10 animals receiving placebo 48 hours 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 hours prior and the second, 24 hours post oral SIV inoculation [28]. Even macaques inoculated with the virulent SIV_{mac055} strain, which has a five-fold reduced susceptibility to tenofovir, were partially protected when they received subcutaneous tenofovir 24 hours prior to viral challenge followed by administration for 4 weeks compared to the untreated controls that all became infected[29].

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 post-inoculation. All macaques that were treated with tenofovir for 28 days beginning 24 hours 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 hours post-inoculation or decreasing the duration of treatment, reduced effectiveness in preventing establishment of persistent infection. Only half of the macaques treated for 10 days, and none of those treated for 3 days, were completely protected when treatment was initiated at 24 hours. Despite the reduced efficacy of delayed and shortened 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[30].

Subcutaneous administration of tenofovir has also been shown to be completely protective against HIV-2 infection when given up to 36 hours post-inoculation[31]. However, breakthrough infection in one animal in the 72 hour post HIV exposure group was detected at 16 weeks [31].

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 the gel. In Study 1, a TFV gel dose given 24 hours prior to exposure, at exposure, 24 hours after, and 48 hours after exposure, provided complete protection from SIV infection[32]. 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 minutes prior to viral challenge consistently protected from vaginal SHIV infection[33]. Higher doses of TFV gel (10% weight per weight) administered intravaginally at four timepoints; 24 hours before, 0 hours, 24 hours after and 48 hours post-inoculation, was also fully effective in preventing intravaginal SIV transmission after repeated viral challenge[34]. Even a single dose given 15 minutes before viral inoculation provided partial protection[32]. Most recently, in delayed challenge experiments four out of six macaques were protected from SHIV exposure occurring 30 minutes and 3 days after 1% TFV gel application, compared to 10 placebo treated animals [35]. 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 minutes or 2 hours prior to, or 2 hours after intrarectal challenge. In the 2 control groups of macaques, 4 of 4 untreated macaques and 3 of 4 macaques given placebo gel became infected. Virus was recovered from only 1 of 6 animals receiving TFV gel 15 minutes prior to virus challenge. In one other animal in this group, virus was recovered only at weeks 2 and 6. Two of 3 animals receiving the drug 2 hours 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 hours after virus challenge, 2 out of the 3 animals became infected. Interestingly, gag-specific interferon-gamma secreting T cells were detected by ELISpot in 4 of 7 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[36].

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 breastfeeding, showed that oral tenofovir given daily was partially protective in infant macaques who received 15[37] or 30[38] 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[39]. 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 6 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 to 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 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. These results showed that short but potent intermittent PrEP could provide protection comparable to that of daily PrEP [40].

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 hrs. C_{max} and area under the curve (AUC) values are 0.30 ± 0.09 μg/mL and 2.29 ± 0.69 μg·hr/mL, respectively[41]. 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 mg/kg and 3.0 mg/kg [41] 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 hours of dosing. Following a single oral dose, the elimination half-life of tenofovir is approximately 17 hours and intracellular half-life is >60 hours [42]. 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 hours. Tenofovir is eliminated by a combination of glomerular filtration and active tubular secretion[41].

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

Tenofovir concentrations in the genital tract and in rectal tissues have been assayed in phase 1 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[43]. More recently, tenofovir concentrations in genital secretions was found to be 2.5 fold greater than blood plasma concentrations[44]. The median cervicovaginal fluid concentrations of tenofovir at the end of a 24h dosing interval at steady state was 68 ng/mL [43] Vaginal tissue concentrations at 24 hours are 6.8 ng/g and 50 ng/g in the vaginal and cervical tissue respectively [44]. Rectal tissue exposure of oral tenofovir (C_{24} = 1877 ng/g) is a 100 fold higher than vaginal and cervical tissue content [44].

Following vaginal use of TFV gel, the median C_{max} in cervicovaginal fluid was $1,9 \times 10^6$ ng/ml (range: $1.2 \times 10^4 – 9.9 \times 10^6$ ng/ml) after a single dose and 1.4×10^6 ng/ml (range $8.4 \times 10^4 – 5.8 \times 10^6$ ng/ml) after multiple doses. Blood plasma concentrations were low; Cmax: 4.0 ng/ml 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 hours, demonstrated tenofovir concentrations ranging from $2.1 \times 10^2 – 1.4 \times 10^6$ ng/ml with a median C_{max} of 2.2×10^5 ng/ml [45]. In a crossover study, the tissue concentrations of the active tenofovir diphosphate were found to be 2 $\log_{10}$ higher following vaginal dosing than oral dosing [46].

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 1000ng/ml or more when compared to placebo drug or concentrations <1000ng/ml [47]. 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 to oral TDF.

5. Safety and efficacy of oral and topical formulations of tenofovir in preventing HIV-1 infection in humans (Phase 1-3)

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 1 studies are assessing safety in pregnancy and during lactation, adherence and PK comparing the safety of oral TDF to 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); altered gel formulation characteristics to aid rectal use and resistance screening. Two phase 3 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 1 studies

Topical TFV gel—Two concentrations (0.3% and 1%) of topical tenofovir have been shown to be safe and well tolerated in a phase 1 trial among 84 sexually abstinent HIV-uninfected women. The HPTN 050 study [48], showed that a two 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 [49]. 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 [50] .

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) [51] has shown that high genital tract and cervicovaginal fluid levels, and low blood plasma concentrations were detectable up to 24 hours 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[52]. 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 [53]. A significant increase in anti-HIV activity was detected in cervicovaginal lavage (CVL) from women who applied TFV gel compared to those using the placebo and this activity persisted in thpresence 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 sub-population for gel use is pregnant and lactating women. The Microbicide Trials Network (MTN 002) study was the first phase 1 trial to evaluate the safety and PK of a single dose of 1% TFV gel administered approximately two hours before planned caesarean to 16 pregnant women [54]. 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 non-pregnant women. While TFV does appear to cross the placenta, foetal exposure after vaginal dosing was low, with only trace amounts being passed to the foetus [55].

Collectively these phase 1 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 1 safety studies of TFV gel when used rectally are ongoing. Results of the first phase 1 trial 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 analysed and another trial of rectal safety and acceptability of a new TFV gel formulation (low glycerine concentration) MTN 007 [56] is currently underway in 60 men and women.

A number of new phase 1 studies are also planned. These include a phase 1 study that will expand on the positive results from MTN 002 to provide important PK information of the use of TFV gel daily for seven days in pregnant and lactating women[57] and a phase 1 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 hr before or 1 hr after sex) dose or BAT 24 dosing [58].

Oral TDF—The review of phase 1 studies on the use of oral TDF for HIV prevention are restricted to ongoing trials only as the safety and tolerability of oral TDF for daily use has been well established from extensive experience with this formulation of the drug for HIV treatment.

The ongoing phase 1 studies of oral TDF for prevention focus on drug PK in special populations and participant acceptability with PK assessment of the oral formulation compared to 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 [59] 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 1 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 2 studies

Table 2 provides an overview of oral TDF and topical TFV gel phase 2 and 3 studies, completed and in progress for tenofovir safety and effectiveness determination against HIV-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 vs placebo in preventing HIV in 936 African women [60]. 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 [60]. The use of oral TDF in preventing HIV acquisition was also evaluated by the US CDC in 400 HIV-negative men who have sex with men (MSM) (CDC

4323) [61]. The preliminary analysis (July 2010) suggested no serious safety concerns and no increased risk compensation in men taking a study pill compared to those not taking prophylactic pills [62].

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) to 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 risk of HIV infection [63, 64] .

The CAPRISA 004 study, a phase 2b study compared 1% TFV gel vs placebo (applied intra-vaginally by women, up to 12 hours before sex and as soon as possible within 12 hours after sex but not more than twice in 24 hours-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 antiretroviral agent can protect women from acquiring HIV. The trial showed that TFV gel use reduced HIV infection in women by 39% 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% [65].

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 one week, no drug, washout period between sequences. All 3 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 to the oral tablet but compared to 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 [66, 67].

Currently in the field, the MTN 003 (VOICE) trial is examining the safety and effectiveness of two oral antiretroviral agents (TDF and FTC-TDF) taken daily and 1% TFV gel also administered daily to reduce the risk of HIV acquisition in women. The study enrolled 5,029 African women (approximately 1,000 in each study group) [68]. On 16 September 2011, the TDF tablet component of the VOICE 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 VOICE 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 to 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 [69-71]. Final results are expected in late 2012.

5.3 Phase 3 studies

Three phase three trials, two examining TDF tablets and one evaluating TFV gel are currently underway. The Partners PrEP trial, which was initiated in serodiscordant 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 [72]. 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 to placebo [12, 73]. 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 [74, 75], with results expected in 2012.

The FACTS 001 (Follow on consortium on tenofovir studies) study is evaluating the safety and effectiveness of 1% TFV gel in preventing HIV and HSV-2 infection in young South African women [76]. 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 one year among MTN 003 participants receiving oral TDF and FTC-TDF compared with oral placebo [77]. MTN 003C will explore how community and household factors affect adherence in VOICE participants [78]. MTN 015 is comparing the plasma HIV-1 RNA level twelve months after HIV-1 seroconversion among ART naive participants assigned to an active study product compared to control participants [79]. 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 to that in mothers not exposed to an active study agent during pregnancy [80].

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 [81].

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 antiretroviral drugs can effectively prevent HIV acquisition in men who have sex with men (MSM). The trial was conducted Brazil, Peru, Ecuador, Thailand and South Africa among 2,499 men or transgender women who have sex with men, showed that the daily oral combined FTC-TDF, reduced HIV incidence by 44% (95% CI 15;63) (4). During 3,324 person-years of follow-up, there were 100 HIV infections; 36 in the FTC-TDF group and 64 in the placebo group[13]. Further, evidence for the effectiveness of daily oral pre-exposure prophylaxis (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 PartnersPrEP trial [82], involving 4,758 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 Bostwana TDF2 trial [83], conducted in 1,200 heterosexual men and women from the general population, found that daily oral FTC-TDF reduced HIV incidence by 63% (95% CI 21;48).

In 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 [84].

6. Regulatory pathway for licensure

At present no antiretrovirals 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 becomes available [85]. The FDA had also agreed to consider a combination of CAPRISA 004 and the VOICE trials for licensure of tenofovir gel, despite their differences in dosing. This plan is no longer applicable as the VOICE trial's daily dosing of tenfovir gel did not demonstrated protection against HIV. For both the oral TDF and TFV gel, positive results from confirmatory studies are necessary in order to establish expanded safety and efficacy data that is required for licensure. In addition, safety data in generally excluded populations such as adolescents, pregnant women and Hepatitis B positive individuals would be required by regulators to effectively complete the drug dossier.

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 2, 2b and 3 studies to be protective against HIV infection by sexual transmission, with a tolerable side effect profile and with adequate monitoring, demonstrated low risk for the development of tenofovir resistance. However research, particularly in women, has demonstrated apparently conflicting results in two important studies which 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 18 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 antiretrovirals can potentially change the course of the HIV epidemic, when used as early treatment for prevention [86], as topical [15] or oral pre-exposure prophylaxis[72]. 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 antiretroviral 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, Tenofovir has reinvigorated the HIV prevention field and created new found optimism that it may be possible to control the HIV epidemic.

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[87]. 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 [47, 88]. 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 [88]. 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 only be realised when there is adequate robust evidence of 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 pre-clinical 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 VOICE study (MTN003) of daily TFV gel would show similar or better results. Instead, the VOICE trial did not demonstrate that tenfovoir gel and TDF tablets were 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 VOICE study's unexpected outcome could be due to inadequate or non-use 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 tiresome, lead to poor adherence and possibly product failure [89]. 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.

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.

The PartnersPrEP and TDF2 trials, which have not been published yet, produced the first evidence that antiretroviral tablets can also prevent HIV in serodiscordant couples and the general heterosexual population. In women, however, additional recent data on oral PrEP have not demonstrated consistent results. Two trials – FEMPrEP [84] and VOICE [90] –

found no protection against HIV in women using daily oral FTC-TDF and TDF respectively. These trials are currently being analysed to help determine the reasons the results. Until these data are available, it is unclear whether the mixed results from oral PrEP studies in women is due to differing adherence (behavior) or due to differing efficacy (biology).

In several countries throughout the world, MSM constitute a major sub-group where new HIV infections are taking place, especially in concentrated epidemics. Traditional safe sex messages have only had limited impact on the HIV epidemic in this group. The iPrEX trial produced the first evidence that antiretroviral tablets can prevent HIV in MSM. This introduces a new approach which could be used in combination with condom promotion and educational safe sex programs to prevent HIV in this MSM. Since FTC-TDF, known by its trade name - Truvada®, is already a licensed antiretroviral 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 United States as part of a comprehensive set of HIV prevention services [91]. However, concerns about adherence, drug resistance[92] and behavioural 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 behavioural 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 VOICE 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 sub-groups in generalized epidemics. Large-scale implementation, post-licensure, will require vigilance and surveillance to monitor behaviour changes, adverse events, adherence levels, drug resistance patterns, and the effect of drug resistance on later AIDS treatment.

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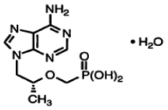
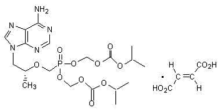
9. Drug summary box

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	Anti-retroviral, nucleotide reverse transcriptase inhibitor	Anti-retroviral, 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]	iPrEX trial - Grant et al [13]
	MTN-003 –VOICE -[93]	Partners Prep Study - [12]

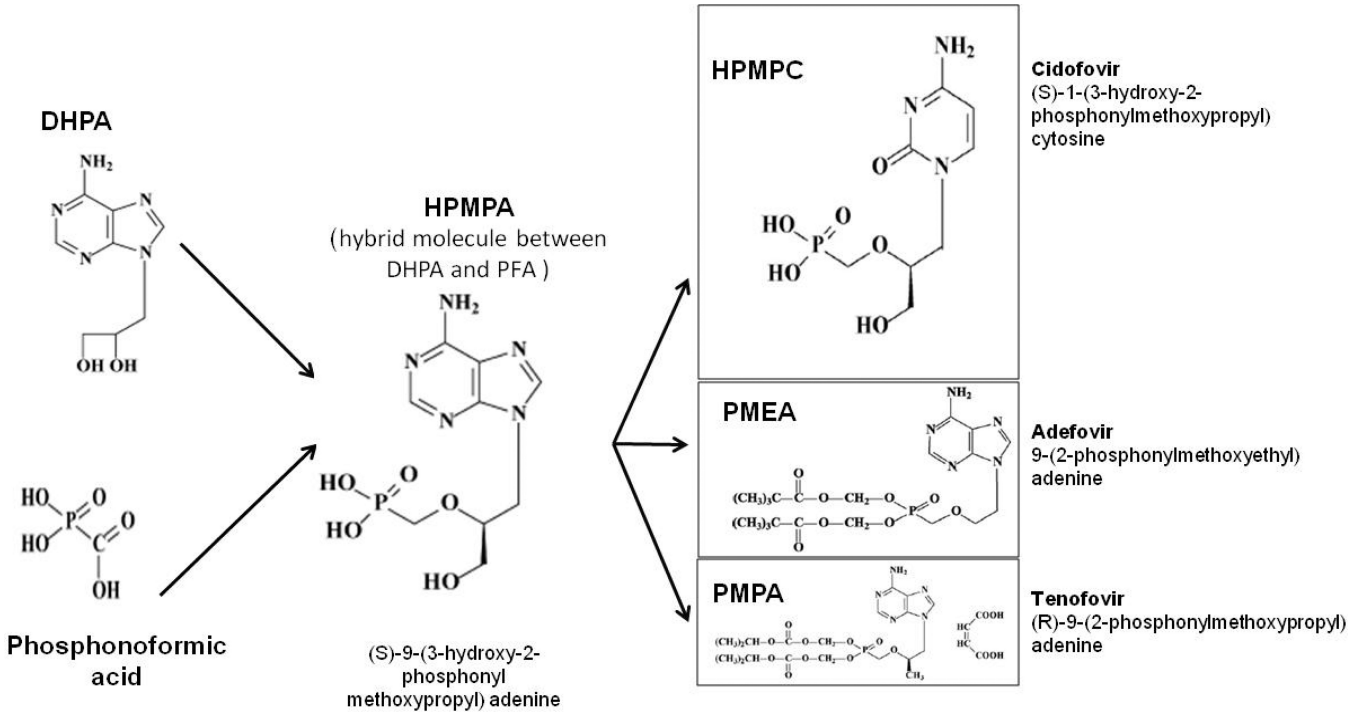


Figure 1.
Development of acyclic nucleoside phosphonate antiviral agents (adapted from De Clercq, 2007[22]

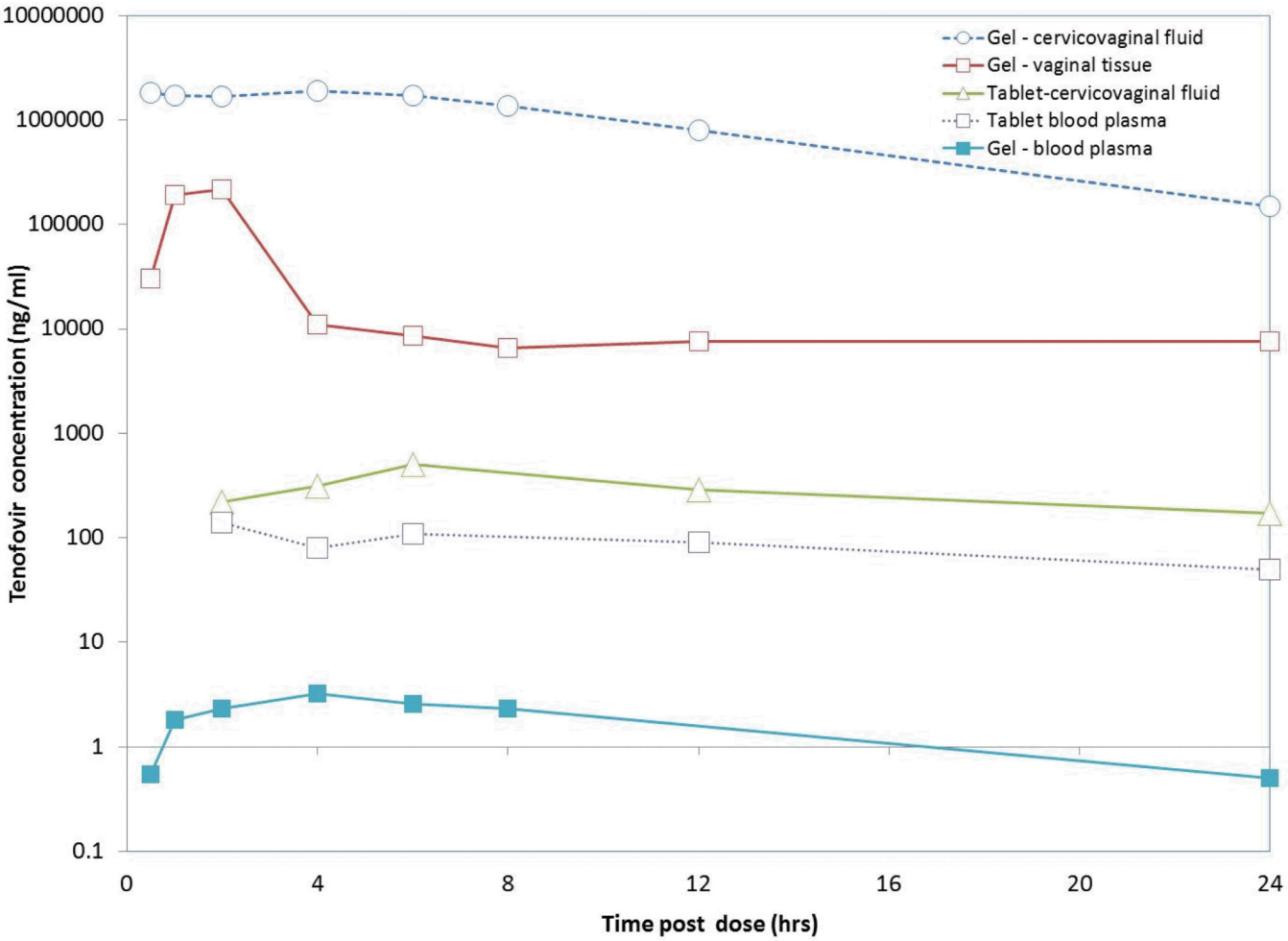


Figure 2.
Tenofovir concentration distribution post single dose oral and topical vaginal administration. Adapted from Dumond et al, 2007 [43], Schwartz et al, 2011 [45]

Table 1

Summary of studies of non-human 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 (20mg/kg) PMPA (30mg/kg) PMPA (30mg/kg) PMPA (30mg/kg) vehicle	-48 h - 48 h 4 h 24 h n/a	0 of 5 0 of 10 0 of 5 0 of 5 10 of 10	100 100 100 100 0
Tsai et al [30] 1998	1	SIV IV inoculation	PMPA (30mg/mL) PMPA (30mg/mL) PMPA (30mg/mL) PMPA (30mg/mL) PMPA (30mg/mL) Untreated control	24h, daily for 28 days 48h, daily for 28 days 72h, daily for 28 days 24h, daily for 10 days 24h, daily for 3 days n/a	0 of 4 2 of 4 2 of 4 2 of 4 4 of 4 4 of 4	100 50 50 50 0 0
Van Rompay et al [28] 1998	1	SIV_{mac} oral inoculation	PMPA SC Untreated controls	-4h, 24h N/a	0 of 4 4 of 4	100 0
*Van Rompay et al [94] 1998	2	SIV_{mac251} oral inoculation and SHIV-SF33 IV	PMPA SC Untreated controls	0h for 14 days n/a	1 of 4 12 of 12	75 0
Van Rompay et al [29] 2000	1	$SIV_{mac055\infty}$	PMPA Untreated control	-24h for 4 weeks n/a	3 of 5 3 of 3	40 0
Otten et al [31] 2000	1	HIV-2_{GB122} inoculated intravaginally	PMPA SC PMPA SC PMPA SC Untreated control	12h 36h 72h n/a	0 of 4 0of 4 1of 4 4 of 4	100 100 75 0
Van Rompay et al [95] 2001	1	SIV_{mac251} oral inoculation	PMPA SC (4mg/kg) PMPA SC (4mg/kg) PMPA SC (30mg/kg) Untreated control	-4h, 20h 1h, 25h 1h n/a	1of 4 2of 4 0 of 1 4 of 4	75 50 100 0
Subbarao et al [39] 2006	14	$SHIV_{SF162P3}$	Tenofovir orally Tenofovir orally Untreated controls	-2, daily for 36 weeks -2, weekly for 36 weeks n/a	4 of 4 by 6 weeks 4 of 4 by 7 weeks 4 of 4 by 1.5 weeks	0 0 0
TFV gel administered intravaginally						
NIH/CDC study 1[32]	2	SIV_{mac251} inoculated intravaginally	10% TFV gel 1 mL vehicle	-24h, 0h, 24h, 48h -24h, 0h, 24h, 48h	0 of 4 2 of 2	100 0
NIH/CDC study 2[32]	1	SIV_{mac251} inoculated intravaginally	10% TFV gel 1% TFV gel 1% TFV gel untreated control	-24h, -15m, -24h -24h, -15m, -24h -15m N/A	1 of 5 1of 5 2of 5 5 of 5	80 80 60 0

Study*	Number of Exposures	Inoculating virus	Treatment	Time of Administration	Number Infected	Protection (%)
NIH/CDC study 3[32]	1	SIV_{mac251} inoculated intravaginally	1% TFV gel 1% TFV gel 1% TFV gel vehicle untreated control	-15m -2h -8h -15m N/A	1 of 5 3 of 5 1 of 5 1 of 5 2 of 5	80 40 80 80 60
NIH/CDC study 4[32]	1	SIV_{mac251} inoculated intravaginally	1% TFV gel 1% TFV gel 1% TFV gel vehicle untreated control	-15m -2h -8h -15 m N/A	1 of 5 1of 5 2of 5 2 of 5 4 of 5	80 80 60 60 20
NIH/CDC study 5[32]	1	SIV_{mac251} inoculated intravaginally	1% TFV gel vehicle untreated control	-2h -2h N/A	0 of 5 2 of 5 2 of 5	100 60 60
NIH/CDC study 6[32]	1	SIV_{mac251} inoculated intravaginally	1% TFV gel 1% TFV gel 1% TFV gel vehicle vehicle untreated control	-12h -24h -72h, -48h, -24h -12h -24h N/A	5of 8 8 of 8 6of 8 8 of 8 8 of 8 8 of 8	38 0 25 0 0 0
*Parikh et al [33] 2009	20	$SHIV_{SF162p3}$	5%FTC and 1% TFV gel Vehicle Untreated control 1% TFV gel vehicle	- 30 min	0 of 6 5 of 6 2of 2 0 of 6 3of 3	100 17 0 100 0
Miller et al [96] 1996	1	SIV_{mac251}	10% TFV gel vehicle	-24, 0h, 24h, 48h -24, 0h, 24h, 48h	0 of 5 5 of 5	100 0
Dobard et al 2011	2	$SHIV_{SF162P3}$	1% TFV gel 2% HEC placebo gel	20m,72h	4of 6 9 of 10	74
TFV gel administered rectally						
Cranage et al [97]2008	1	$SIV_{mac251/32H}$	TFV gel rectally TFV gel rectally Vehicle Untreated control	-2h 2h -2 n/a	3 of 9 1 of 3 3 of4 4of 4	67 67 25 0
Tenofovir administered orally						
Garcia-Lerma et al [98] 2008	14	SHIV rectal inoculation	FTC SC TDF (20mg/kg) and FTC orally TDF (22mg/kg) and FTC SC Intermittent FTC and TDF (22mg/kg) SC Untreated controls	-7-9 days, daily for 28 days -7-9 days, daily for 28 days -7-9 days, daily for 28 days -2h, 24h n/a	4 of 6 2 of 6 0 of 6 0 of 6 17 of 18	33 66 100 100 5
Van Rompay[37] et al 2002	15	SIV_{mac251} oral inoculation 3 times/day	5mL Tenofovir orally 2.5mL Tenofovir orally vehicle	daily daily daily	3 of 4 2of 4 3of 6	50 50 50

Study*	Number of Exposures	Inoculating virus	Treatment	Time of Administration	Number Infected	Protection (%)
Van Rompay et al[99]	30	SIV_{mac251}	10mg/kg Tenofovir orally Topical GS-7340 Untreated controls	Once daily for 7 days 3 times daily for 7 days n/a	8 of 12 4 of 5 29 of 31	33 20 6

*macaques pretreated with 30mg depo-provera 30 days prior to vaginal challenge, ∞virulent isolate that has a five-fold reduced susceptibility to tenofovir, TDF - Tenofovir Disoproxil Fumarate, FTC – emtricitabine, PMPA – [9-R-(2-Phosphonomethoxypropyl)adenine] (tenofovir), SC – subcutaneous, IV – intravenously

Table 2

Summary of key TFV oral and gel phase 2 and 3 safety and effectiveness studies for HIV prevention

Phase 2/2b - Safety and Effectiveness Studies					
FHI West Africa Study (9780) [60] Safety and Preliminary Effectiveness 2007	Ghana, Cameroon, Nigeria	Sexually active HIV-women, (N=936)	**Active:** Daily TDF 300mg 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[64] 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[100] Higher gel adherence 54% effective in preventing HIV
CDC 4323 [61, 62] Extended behavioural safety 2010	US	Sexually active HIV-MSM, (N=400)	**Active:** Daily TDF 300mg 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 to those not taking it.
MTN 001[46, 66] 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-2log$_{10}$ higher after vaginal dosing than oral dosing.
MTN 003 (VOICE)[93] 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 300mg 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 fultility November 2011 No serious safety concerns Results expected in 2013
Phase 3 - Effectiveness Studies					
Partners PrEP study [12] 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 [76] Safety and effectiveness study including HSV-2 infection as a	South Africa	HIV-uninfected sexually active women (N=2200)	**Active:** BAT 24* - TDF 1% Gel **Control:** HEC Placebo Gel	Ongoing	Results expected in 2014

primary endpoint Initiated
October 2011

*BAT24 Regimen - within 12 hours BEFORE coitus, as soon as possible within 12 hours AFTER coitus and up to TWO doses in a 24 hour period