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Review

Tenofovir alafenamide: A novel prodrug of tenofovir for the treatment of Human Immunodeficiency Virus

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ABSTRACT

Despite substantial progress in the development of antiretroviral regimens that durably suppress Human Immunodeficiency Virus (HIV) infection, new agents that maintain high efficacy while further optimizing the safety of lifelong, chronic therapy are needed. Tenofovir alafenamide (TAF; formerly known as GS-7340) is a novel prodrug of the antiviral acyclic nucleoside phosphonate tenofovir (TFV) with improved properties relative to tenofovir disoproxil fumarate (TDF). Although potent and generally well tolerated, TDF therapy has been associated with changes in markers of renal function, decreases in bone mineral density and a rare occurrence of serious renal adverse events, including Fanconi's Syndrome. The renal and bone toxicity observed with TDF is associated with high circulating plasma levels of TFV. TAF was discovered to be a more efficient prodrug able to further refine HIV therapy and better address life-long therapy in an older and increasingly comorbid HIV infected population. By enhancing stability in biological matrices while being rapidly activated in cells, TAF produces higher levels of intracellular TFV diphosphate, the pharmacologically active metabolite, in HIV-target cells at substantially reduced oral doses of TFV equivalents. All TFV released in the body is eventually eliminated renally; therefore, lowering the TFV equivalents administered reduces off-target kidney exposure. Effective therapy is thus achieved at approximately 90% lower systemic exposure to TFV, translating to statistically and clinically significant improvement in safety parameters associated with bone mineral density and markers of renal function.

Contents

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1. Introduction

1.1. Frontiers in HIV therapy

Highly active antiretroviral therapy (HAART) has greatly reduced morbidity and mortality in patients living with HIV (The Antiretroviral Therapy Cohort Collaboration, 2008; Kitahata et al., 2009; Mocroft et al., 1998; Palella et al., 1998). Despite the impact of HAART, mortality in successfully treated HIV infected patients remains higher than in the general uninfected population (Bhaskaran et al., 2008; Losina et al., 2009; Nakagawa et al., 2012). The effects of persistent inflammation and drug toxicity on comorbidities that are considered non-HIV related, including metabolic, cardiovascular and renal disease, contribute to these differences in the health of infected individuals. Even in the face of successful viral suppression, markers of inflammation (e.g., interleukin 6, C-reactive protein) are elevated in HIV infected patients and have been linked to an increase in type-2 diabetes and hyperlipidemia resulting in a higher prevalence of cardiovascular and kidney disease (De Wit et al., 2008; El-Sadr et al., 2006; Gupta et al., 2015a; McComsey et al., 2014; Samaras, 2012). Further, a number of the drugs used as part of HAART, particularly those associated with dyslipidemia and mitochondrial toxicity, have been found to increase the risk of non-HIV related disease (De Wit et al., 2008; Friis-Moller et al., 2003). In order to further advance therapy, new agents are needed that have minimal impact on comorbidities and maximize long-term tolerability in the context of earlier diagnosis, earlier initiation and longer duration of treatment, and older age.

1.2. Tenofovir

The anti-HIV activity of the acyclic nucleoside phosphonate tenofovir (TFV; structures of TFV and its prodrugs are presented in Fig. 1) was reported in 1993 (Balzarini et al., 1993). Subsequent studies showed that the pharmacologically active diphosphate metabolite (TFV-DP; an analog of 2′-deoxyadenosine-triphosphate) is a potent inhibitor of HIV reverse transcriptase with an inhibition constant (K_i) in biochemical assays with an RNA template of 0.022 μM (Cherrington et al., 1995), and remained active against drug resistant variants including the observation of hypersensitivity by the methionine to valine mutation at 184 (M184V) that is resistant to lamivudine and emtricitabine (Wainberg et al., 1999). Moreover, TFV-DP is an exceedingly poor substrate and inhibitor of the mitochondrial DNA polymerase γ with an incorporation efficiency 11,400-fold less than the natural substrate 2′-deoxyadenosine triphosphate and a K_i of 59.5 μM (Cherrington et al., 1995; Johnson et al., 2001). Consistent with biochemical results, TFV did not selectively deplete mitochondrial DNA when incubated with cells at up to 300 μM for up to 3 weeks *in vitro* (Birkus et al., 2002; Venhoff et al., 2007). TFV-DP also has a long intracellular half-life measured to be 150 h in peripheral blood mononuclear cells (PBMC) isolated from patients (Hawkins et al., 2005; Pruvost et al., 2005). Despite these favorable properties, TFV in parent form could never be an orally administered drug. TFV is a dianion at physiological pH and suffers from poor membrane permeability, as reflected in its poor *in vitro* anti-HIV activity in cell-based assays, and low oral bioavailability (Shaw et al., 1997).

1.3. Tenofovir disoproxil fumarate

The disoproxil prodrug was found to have substantially improved cell permeability and anti-HIV activity *in vitro* (Robbins et al., 1998), increased oral bioavailability in animals (Shaw et al., 1997), and more efficient loading of PBMC relative to parenteral TFV observed *in vivo* (Durand-Gasselin et al., 2009; Lee and Martin, 2006). Based on its improved properties, TFV disoproxil formulated as the fumarate salt (TDF) was the first selected for clinical development and was ultimately approved by the US Food and Drug Administration in 2001, the European Medicines Evaluation Agency in 2002, and was subsequently approved in other countries around the world. TDF administered at a dose of 300 mg has been used extensively (over 9 million patient years) as the preferred backbone of HIV combination therapy. Integral to the clinical success of TDF has been the low rates of discontinuations due to TFV-related viral resistance or toxicity. However, while TDF therapy is generally well tolerated it has been associated with effects on renal function and bone mineral density.

1.4. Goal of this review

Other prodrugs of TFV have been assessed in the interest of further refining long-term therapy and to allow for use in the broadest population of those infected with HIV to determine if more efficient delivery of TFV to HIV-target tissues could be achieved while reducing off-target exposure, particularly to the kidney. One of these prodrugs, tenofovir alafenamide (TAF) was selected for further study based on its favorable properties and will be the

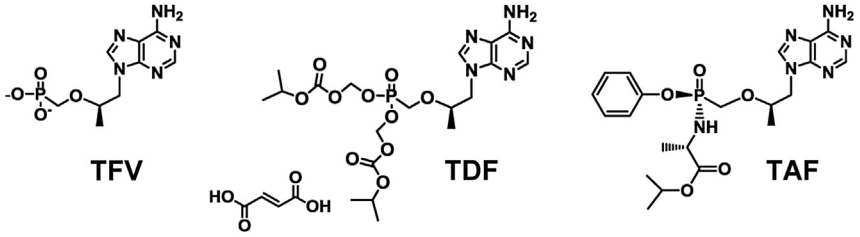

Fig. 1. Structures of acyclic nucleoside phosphonate tenofovir (TFV) and its lipophilic prodrugs tenofovir disoproxil administered as its fumarate salt (TDF) and tenofovir alafenamide (TAF).

subject of this review. The goal of this manuscript is to provide a clear and concise review of the mechanism of action and unique pharmacology of TAF, and how it has translated into clinical proof of concept for efficacy and reduced off-target TFV-related effects in clinical studies conducted to date.

2. Tenofovir disoproxil fumarate associated toxicity

2.1. Effects on bone and kidney

Nonclinical studies in mice, rats, dogs and monkeys identified the kidney (increased serum creatinine, blood urea nitrogen, hypophosphatemia, glucosuria, proteinuria, phosphaturia and calciuria at exposure of 2–20 times those observed in the clinic) and bone (osteomalacia and decreased bone mineral density at exposures greater than or equal to 6-fold those observed in patients) as target organs (Viread prescribing information; http://www.gilead.com/~/media/files/pdfs/medicines/hiv/viread/viread_pi.pdf?la=en). The clinical safety profile of TDF with respect to renal function and bone health has been the subject of comprehensive reviews by Sax (Sax et al., 2007) and Powderly (Powderly, 2012). Briefly, clinical trials have identified an impact on renal function and bone turnover in treatment arms with TDF-containing regimens. Prospective studies have demonstrated greater loss of kidney function and a higher risk of acute renal failure in patients receiving TDF-based therapies versus non-TDF regimens (Cooper et al., 2010). For example, in a combined analysis of Studies 903 and 934, comparing TDF therapy to a thymidine analog, the TDF containing regimen was found to have a small but statistically significant greater decrease in the estimated glomerular filtration rate (Gallant et al., 2008). In post-marketing experience from the Viread Expanded Access Program renal failure, Fanconi's Syndrome or tubular dysfunction, and elevated serum creatinine were rare accounting for 0.3%, 0.05% and 0.1%, respectively, of patients (Nelson et al., 2007). Importantly, increased levels of serum creatinine and the risk of renal adverse events have been associated with the higher levels of plasma TFV observed when TDF is given with HIV protease inhibitors or pharmacoenhancers, inhibitors of the intestinal efflux of TDF (Tong et al., 2007), suggesting a link between plasma TFV exposure and the effect on proximal tubule function (Gallant and Moore, 2009; Nelson et al., 2007). In a large cohort of HIV-1 infected veterans, TFV exposure was independently associated with proteinuria, more rapid eGFR decline, and the development of eGFR <60 ml/min (Scherzer et al., 2012). TDF-related nephrotoxicity has identified risk factors, including older age, underlying renal disease and other co-morbidities, and regimens containing ritonavir-boosted protease inhibitors (Morlat et al., 2013; Nelson et al., 2007).

While the increased prevalence of osteopenia and osteoporosis in patients with HIV infection is multifactorial (Brown and Qaqish, 2006; Grund et al., 2009), initiation of TDF-containing antiretroviral therapy leads to a larger reduction in bone mineral density than regimens not containing TDF (Mateo et al., 2014; Bernardino et al., 2015). In Study 903, TDF resulted in slightly greater decreases in bone mineral density at the lumbar spine but not hip relative to stavudine and these changes correlated with biochemical markers of bone turnover including elevated levels of parathyroid hormone and 1,25 vitamin D (Gallant et al., 2004; Viread Prescribing Information). The etiological mechanism for the effects on bone has not been established but an *in vitro* study found no evidence for a direct effect of TFV on osteoblasts (Liu et al., 2013). While a definitive relationship with TDF use and increased clinical events has not been established, a large observational study done with the Veterans Affairs' Clinical Case Registry from 1988 to 2009 found that cumulative TDF exposure was associated with an increased rate of fractures (Bedimo et al., 2012). These findings could lead to concern in prescribing TDF containing regimens to those with preexisting or who are at increased risk for bone conditions (e.g., osteoporosis).

2.2. Mechanism for tenofovir renal accumulation

TFV is renally eliminated by the combined action of active tubular secretion in the proximal tubule and passive glomerular filtration. Active tubular secretion is mediated by uptake by the organic anion transporters (OAT) 1 and 3 and efflux by the multidrug resistance-related protein (MRP) 4 (Cihlar et al., 2001; Ray et al., 2006). Renal accumulation of TFV and the resulting effects on the function of the proximal tubule are caused by highly efficient uptake from plasma and less rapid efflux into urine. The relationship of counterbalancing transport processes on intracellular accumulation of TFV and toxicity has been nicely illustrated *in vitro* by experiments showing that overexpression of OAT1 and OAT3 increases cytotoxicity while co-transfection of MRP4 causes an incremental decrease in the effects (Stray et al., 2013).

3. Mechanism of action

3.1. In vitro studies

TAF was identified as an alternate TFV prodrug to TDF that more efficiently loads HIV-target cells (Lee et al., 2005). As summarized in Table 1, TAF is 1000- and 10-fold more active against HIV *in vitro* than TFV or TDF, respectively. Reflecting the release of the same pharmacologically active metabolite, TAF has the same resistance profile as TFV and TDF *in vitro* but the higher PBMC levels achieved after TAF administration relative to TDF in patients (discussed further below) suggest better coverage of resistance mutations in the clinic (Margot et al., 2015). Stability in biological matrices, including plasma, and selective intracellular cleavage of TAF allows for prolonged systemic exposure to intact prodrug and the accumulation of higher intracellular levels of the pharmacologically active metabolite TFV-DP relative to TDF. The mechanism of cell loading by TAF has been extensively studied *in vitro* and is summarized in Fig. 2. In whole blood, TAF was found to preferentially load PBMC over red blood cells and formation of TFV and its phosphorylated metabolites was preceded by the intracellular formation of the key intermediate TFV alanine (Eisenberg et al., 2001). Studies in isolated cells have shown efficient TAF activation and potent antiviral activity in the HIV-target cells CD4+ T-cells and monocyte derived macrophages (Bam et al., 2014a). The lysosomal carboxypeptidase cathepsin A (CatA) was identified as the primary hydrolase catalyzing the initial step in intracellular activation of TAF in HIV-target cells (Birkus et al., 2007). CatA is a ubiquitously expressed enzyme including high levels in lymphoid cells (Satake et al., 1994). With the exception of the covalent protease inhibitors of hepatitis C virus telaprevir and boceprevir that nonspecifically inhibit CatA, TAF intracellular activation and antiviral activity is not adversely affected by concomitant agents including HIV protease inhibitors and other HCV protease inhibitors (Birkus et al., 2015; Callebaut et al., 2015). The rapid initial

Table 1
In vitro activity and stability of TFV and its prodrugs TDF and TAF.

	TFV	TDF	TAF
EC_{50} HIV-1 (μM)	5.0	0.05	0.005
Half-life (min)	stable	0.41	90

Results summarized from Lee et al. (Lee et al., 2005).

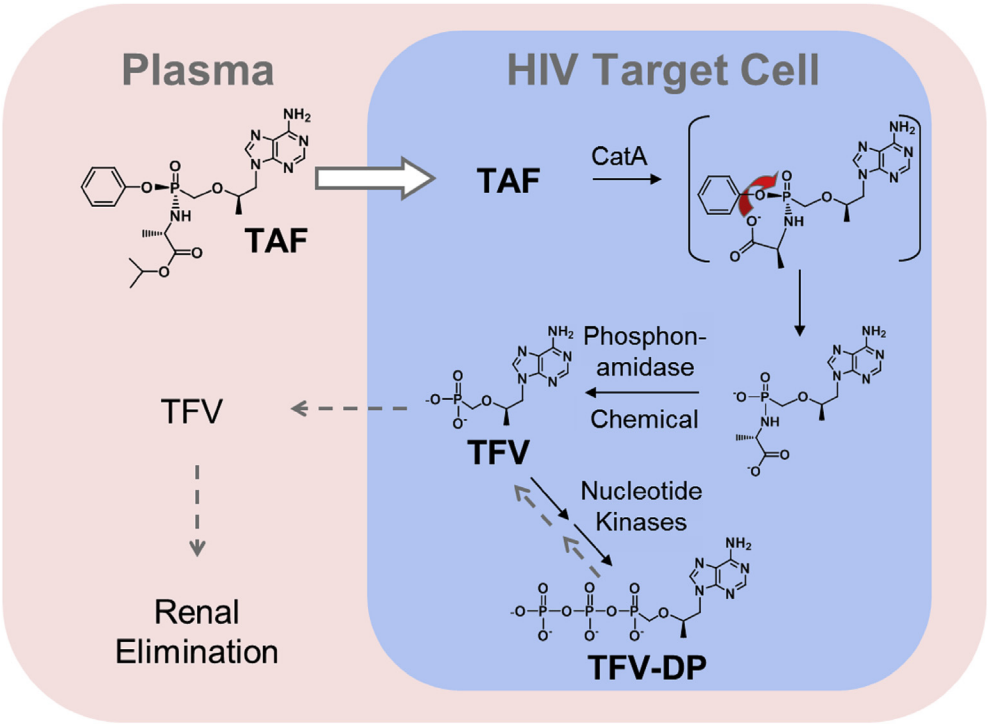

Fig. 2. Mechanism of HIV-target cell loading by TAF. TAF enters cells passively where it is subject to ester hydrolysis by the lysosomal carboxypeptidase cathepsin A (CatA). Following chemical release of phenol from an unstable metabolite, a key intermediate metabolite is formed with alanine conjugated to TFV. Alanine is released either by enzymatic or chemical degradation to release TFV that is subsequently phosphorylated to the pharmacologically active metabolite TFV-DP. TFV is slowly released from cells into plasma where it is eliminated from the body renally.

intracellular cleavage step catalyzed by CatA in HIV-target cells creates a sink causing marked loading during TAF exposure and, coupled with the formation of poorly permeable metabolites that are effectively trapped in cells, allows for substantial accumulation of the pharmacologically active metabolite TFV-DP.

3.2. Nonclinical pharmacokinetics and distribution

In order to establish the effect of the cellular pharmacology described above *in vivo*, pharmacokinetic studies assessing plasma and PBMC levels were completed in dogs (Babusis et al., 2013; Lee et al., 2005). Unlike TDF, these studies found measurable levels of circulating TAF in plasma. While TAF was only transiently present in plasma ($t_{1/2}$ ~30 min), the exposure was sufficient to drive high and persistent levels of TFV-DP in PBMCs while only resulting in low levels of TFV in plasma. The relative efficiency of cell loading by TAF was assessed following administration of equivalent doses of subcutaneous TFV, oral TDF or oral TAF in dogs (Lee et al., 2005). The PBMC to plasma ratio were approximately 1, 5 and 140 for TFV, TDF and TAF, respectively, illustrating that TAF is highly efficient at concentrating TFV and its metabolites in PBMC. More broadly, oral TAF administration also resulted in higher levels relative to TDF in on-target tissues including lymph nodes (iliac, axillary, inguinal and mesenteric; 5.7- to 15-fold) and spleen (12.8-fold) in dogs. In contrast, TAF did not more efficiently load the kidney and, subsequently, has been shown not to be a substrate for renal uptake transporters (Bam et al., 2014b). Taken together, these results suggested that lower doses of TAF could be administered clinically, resulting in similar or even increased levels of TFV and its metabolites in HIV-target cells while markedly reducing off-target exposure to the kidney.

4. Proof of concept

4.1. Antiviral potency

Monotherapy studies were completed in treatment naïve HIV infected subjects to establish clinical proof of concept that TAF achieves enhanced antiviral potency at a greatly reduced dose relative to TDF. An initial study reported by Markowitz et al. assessed doses of 40 and 120 mg of TAF relative to TDF following 14 days of administration (Markowitz et al., 2014). These doses of TAF proved to be substantially more potent than the clinically used dose of 300 mg TDF with mean changes in HIV RNA of −0.94, −1.57, and −1.71 $\log_{10}$ copies/mL in the 300 mg TDF, 40 mg TAF and 120 mg TAF groups, respectively. Therefore, a subsequent study assessed the antiviral activity of TAF at 8, 25 and 40 mg relative to 300 mg TDF following 10 days of monotherapy (Ruane et al., 2013). It was found that 8 mg of TAF showed similar anti-HIV activity to 300 mg of TDF and at 25 mg, approximately 1/10th the TFV equivalents administered with the clinical dose of TDF, a statistically significant greater anti-HIV activity was achieved compared to TDF (−1.46 versus −0.97 $\log_{10}$ copies/mL; $P = 0.024$) at a dose resulting in 86% lower plasma TFV. Consistent with more potent HIV activity, PBMC levels were 7-fold higher at 25 mg TAF relative to 300 mg TDF. The pharmacokinetic profile in patients was consistent with that observed nonclinically in dogs. These studies established the dose of 25 mg TAF for further clinical development. Table 2 summarizes how TFV administration has been refined from the original study of 1 mg/kg intravenous TFV, reported in 1998 by Deeks et al. (Deeks et al., 1998), to the approved dose of 300 mg TDF, and, finally, to 25 mg TAF. In short, increasing antiviral activity has been achieved with lower plasma TFV through the administration of increasingly more efficient prodrugs. The improved stability of

Table 2
Mean Pharmacokinetic and antiviral parameters Following 7 (TFV) or 10 days (TDF and TAF) of monotherapy in HIV-1 infected patients.

	Dose (mg)	Plasma TFV		ΔViral load from baseline (log_{10} copies/ml)
		C_{max} (ng/ml)	AUC_{tau} (ng●h/ml)	
TFV (intravenous)	1 per kg	2500	4800	−0.60
TDF (oral)	300	250	1900	−0.97
TAF (oral)	25	16	270	−1.46

Results reported previously (Deeks et al., 1998; Ruane et al., 2013).

TAF coupled with rapid intracellular activation allows for a markedly lower dose, with correspondingly reduced levels of plasma TFV, while maintaining high levels of TFV-DP in HIV-target tissues and, resulting, potent antiviral activity (Fig. 3).

4.2. Efficacy

The efficacy of low dose TAF has been confirmed in 4 separate patient populations: treatment naive adults, treatment naive adolescents, treatment experienced adults following switch, and renal impairment. For example, in phase 2 and 3 clinical studies where 10 mg TAF (dose of TAF when administered with a pharmacoenhancer that inhibits intestinal efflux and increase TAF oral absorption as reported by Lepist et al. (Lepist et al., 2012)) showed similar viral response as 300 mg TDF when coformulated with emtricitabine/elvitegravir/cobicistat (E/C/F/TAF) (Sax et al., 2015, 2014). The E/C/F/TAF fixed dosed combination has demonstrated potent and durable anti-retroviral activity in two Phase 2 studies (Sax et al., 2014; Mills et al., 2015b), and six Phase 3 studies (Gupta et al., 2015b; Kizito et al., 2015; Mills et al., 2015a; Pozniak et al., 2015; Sax et al., 2015). Using the FDA-defined snapshot methodology at Week 48, E/C/F/TAF was noninferior to standard of care based regimens in the active comparator studies. Results were consistently strong across multiple treatment populations. While resistance to study treatment was not observed in E/C/F/TAF arm of the Phase 2 trial, in the two larger studies a small percentage of patients (<1% in both arms) did develop drug resistance to some of the treatments, most commonly the M184V mutation selected by emtricitabine. The virologic success rates at Week 48 for E/C/F/TAF in naive subjects were greater than 90%, among the highest seen in clinical trials in naive HIV-1 infected adult subjects, demonstrating the potent antiviral efficacy of the E/C/F/TAF fixed dose combination against a highly active comparator. Similar response rates between TAF and TDF arms were also observed in protease inhibitor based single tablet regimen study of TAF coformulated with darunavir/cobicistat/emtricitabine (D/C/F/TAF) (Mills et al., 2015b).

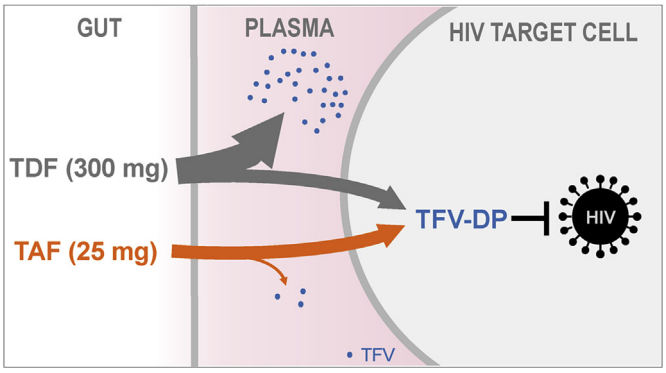

Fig. 3. Comparison of the efficiency of HIV-target cell delivery following oral administration of tenofovir prodrugs. Oral administration of TAF at 25 mg, 1/10th the molar equivalents of TFV present in 300 mg TDF, results in 90% lower systemic levels of TFV while maintaining intracellular levels of the pharmacologically active metabolite TFV-DP in HIV-target cells.

4.3. Safety

TFV is cleared entirely by renal elimination. Therefore, reducing the TFV-equivalents administered and, resulting, circulating levels of TFV to approximately 1/10th those observed with TDF results in a corresponding reduction in TFV exposure to the kidney. The availability of 48 week results from >1000 patients receiving TAF in controlled, head-to-head with TDF, Phase 2 and 3 studies allows for the first assessment of whether the reduced off-target exposure to TFV impacts safety and tolerability in a clinically meaningful way (Gupta et al., 2015b; Mills et al., 2015a,b; Pozniak et al., 2015; Sax et al., 2015, 2014). These studies consistently showed TAF to have a reduced impact on bone mineral density and markers of renal function.

In the phase 3 studies, E/C/F/TAF had statistically significantly smaller increases in serum creatinine and corresponding decreases in estimated glomerular filtration rate, less proteinuria and albuminuria, smaller decreases in bone mineral density in treatment naïve patients and increased bone mineral density in treatment experienced patients switching from a TDF-containing regimen. More specifically with regard to bone, TAF was found to have statistically significant less decrease in mean bone mineral density than TDF at 48 wk at the spine (−1.30 versus −2.86; $p < 0{\cdot}0001$) and hip (−0.66 versus −2.95; $p < 0{\cdot}0001$). Furthermore, changes in bone mineral density in the TAF group were numerically similar to those generally reported after initiation of antiretroviral therapy including with non-TDF containing regimens (Grund et al., 2009; Sax et al., 2015). Consistent with the phase 3 findings, phase 2 studies with both integrase and protease containing regimens found a highly statistically significant difference ($P < 0.001$) between TAF and TDF on their impact on markers for bone formation (procollagen Type 1 N-terminal propeptide) and reabsorption (C-terminal telopeptide) (Mills et al., 2015b; Sax et al., 2014). Highly statistically significant differences ($P < 0.01$) were observed across all studies for markers of proximal tubule function, β2 microglobulin and retinol binding protein. Furthermore, patients switching from a TDF containing regimen to a TAF containing regimen had significant improvements in urinary biomarkers and bone mineral density (Gupta et al., 2015b; Mills et al., 2015a,b). Impressively, patients with renal impairment switching from a TDF containing regimen to a TAF containing regimen had statistically significant increases in bone mineral density at the spine (2.95%) and hip (1.85%) after 48 weeks (Gupta et al., 2015b).

Increases in low-density lipo-protein (LDL), high-density lipo-protein (HDL) and triglycerides were greater with TAF than TDF in Phase 2 and 3 studies suggesting that TAF does not have the same cholesterol lowering effect as has been reported for TDF in HIV infected patients and uninfected subjects receiving pre-exposure prophylaxis (e.g., ACTG5206 study (Tungsiripat et al., 2010) and iPrEx (Mulligan et al., 2013)). These results support the conclusion that lipid effects are off-target and caused by an as yet unknown

mechanism related to higher systemic levels of TFV. However, the total cholesterol to HDL ratio was not different between the groups indicating no effect on overall cardiovascular risk. No new TFV-related adverse effects were identified.

5. Conclusion

TAF is more efficient than TDF in delivering TFV into HIV-target cells. Nonclinical mechanistic studies were successfully translated into the clinic where TAF, at approximately 1/10th the TFV-equivalent dose and plasma TFV exposure, resulted in higher TFV-DP levels in HIV target cells, leading to greater monotherapy activity. When co-formulated as E/C/F/TAF in a single tablet regimen, high clinical efficacy was demonstrated in a broad phase 3 program in which treatment-naïve adults and adolescents, and treatment-suppressed adults with normal kidney function and renal impairment, were evaluated. Most importantly TAF had a reduced impact on renal function and bone mineralization as consistently demonstrated by multiple parameters during clinical studies. The improved safety profile of TAF allows for a broader population of HIV-infected individuals to potentially benefit from TFV-based regimens. This includes patients at risk for renal and bone disease, and adolescents who have not yet reached peak bone mass. The low dose and improved safety profile of TAF also have implications for the effective treatment of HIV in resource limited settings where regular safety monitoring is not possible. Further studies are also under way to assess the potential of TAF in other indications where TDF-based therapy has been approved including pre-exposure prophylaxis and hepatitis B virus (HBV) infection. A long-acting subdermal implant of TAF has been assessed in dogs and discussed by the authors of the article as a potential modality for pre-exposure prophylaxis (Gunawardana et al., 2015). In the context of HBV therapy, nonclinical studies have shown TAF is also more effective at delivering TFV-DP into the liver than TDF (Murakami et al., 2015), a clinical study has established potent anti-HBV activity at doses as low as 8 mg in a Phase 1b clinical study (Agarwal et al., 2015), and TAF is currently in Phase 3 clinical trials for the treatment of HBV. In conclusion, TAF is a promising new therapeutic agent for the lifelong treatment of HIV that maintains high potency while significantly improving the safety profile of TDF.

Conflicts of interest

Authors are employees of Gilead Sciences, Inc.

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