CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-356

MICROBIOLOGY REVIEW

DIVISION OF ANTIVIRAL DRUG PRODUCTS - HFD-530
MICROBIOLOGY REVIEW
NDA#: 21-356 SN00 DATE REVIEWED: October 18, 2001

Reviewer: N. Battula

Date submitted: April 30, 2001
Date assigned: May 5, 2001
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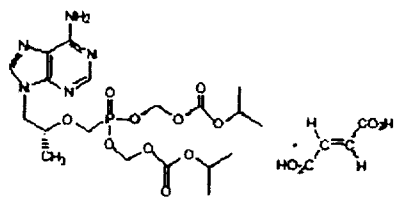
Sponsor: Gilead Sciences.
333 Lakeside Drive
Foster City, Ca 94404

Product name(s): Proprietary: VIREAD™
Nonproprietary: Tenofovir DF
Code: GS-4331-05; PMPA Prodrug and bis-POC PMPA fumarate

Chemical name: 9-[(R)-[[bis[[isopropoxycarbonyl)oxy]methoxyphosphoinoyl] methoxy]-propyl] adenine fumarate (1:1)

Structural Formula:



Molecular Formula: $C_{23}H_{34}N_5O_{14}P$

Molecular Weight: 635.32

Dosage form/route of administration: Tablets (300mg) / Oral

Drug category: HIV reverse transcriptase inhibitor (Nucleotide analogue)

Indication: Treatment of HIV infection

Related Documents: IND [redacted]

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BACKGROUND

In this NDA the applicant, Gilead Sciences, is requesting approval of VIREAD™ (tenofovir disoproxil fumarate tablets), for the treatment of HIV infection in adult patients. The applicant requested a priority review of this application and is also seeking the endorsement of the FDA under the Accelerated Approval of New Drugs for Serious or Life-threatening Illness (21 CFR 314.510). The applicant is requesting the indication that "VIREAD™ is indicated in combination with other antiretroviral agents for the treatment of HIV infection." The proposed indication is based on analyses of plasma HIV-1 RNA levels and CD4 cell counts in a controlled study of VIREAD™ of 24 weeks duration and in a controlled, dose ranging study of VIREAD™ of 48 weeks duration. Both studies were conducted in treatment experienced adults with evidence of HIV-1 replication despite ongoing antiretroviral therapy. Studies in antiretroviral naïve patients are ongoing: consequently, the risk-benefit ratio for this population has yet to be determined.

Human Immunodeficiency Virus (HIV*) the etiological agent of the acquired immunodeficiency syndrome is an RNA virus that replicates through a DNA intermediate. The DNA copy of the viral RNA (proviral DNA) integrates with the cellular DNA (forming the provirus); this establishes the viral infection. Transcription of the proviral DNA and translation of the viral transcripts results in the production of the progeny HIV. In HIV infection of susceptible CD4+ cells, the RNA genome of HIV is copied into the proviral DNA by the virus-coded reverse transcriptase an enzyme that also comes prepackaged with the infecting virus. Inhibition of the viral reverse transcriptase (RT) blocks infection and also viral spread by blocking new rounds of infection. Thus, the viral RT plays a pivotal role in HIV infection and viral spread.

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* Please see Appenxix-1 for glossary of abbreviations

However, RT inhibitors, have no effect on already established infections (chronic infection) from which HIV replication can continue.

To date there are nine FDA approved RT inhibitors (6 nucleoside analogue inhibitors and 3 nonnucleoside analogue inhibitors) for the treatment of HIV infection. As such these drugs can only effect one aspect of the virus life cycle mediated by viral RT i.e., prevent virus spread by blocking new rounds of infections only. Therefore, RT inhibitors can reduce the virus load incompletely but fail to effect the virus production from the large reservoir of already infected cells in HIV positive subjects. Chronic treatment with RT inhibitors to keep virus load down can lead to mechanistic toxicity since these drugs also inhibit cellular DNA polymerases albeit to different degrees. Therefore, there is a great need for additional drugs that repress viral replication and minimize side effects.

An apparently unavoidable consequence of treatment of HIV infections with anti-HIV agents is the development of resistance to the challenging drug due to variants emerging in the presence of the drug and due to preexisting variants (natural polymorphism). HIV replication is remarkably inaccurate both by virtue of it being an RNA virus and because of the greater infidelity of DNA synthesis mediated by the viral RT. The higher rates of replication errors (10^{-4}) result in the production of progeny virions, of which the genomic RNA of each is molecularly different from each other. Combinations of this inherent variability and prolonged treatment with currently available antiviral agents results in the emergence and selection of viruses with reduced susceptibility to the challenging drug. The finite therapeutic effectiveness of the anti-HIV drugs may be due to the emergence of resistance to these drugs. Therefore, novel approaches to seek new therapeutic agents that could stall the emergence of resistance and the resurgence of virus production to provide a greater and prolonged benefit in HIV-infected individuals is awaited.

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VIREAD™ is the brand name for the drug product, tenofovir disoproxil fumarate (a prodrug of tenofovir) tablets. Tenofovir disoproxil fumarate (tenofovir DF) is a fumaric acid salt of *bis*-isopropoxycarbonyloxymethyl ester derivative of tenofovir. In clinical trials VIREAD™ tablets were administered to patients and in in vitro studies as well as in phenotypic and genotypic analysis the drug substance, tenofovir DF or the parent drug tenofovir were used.

Tenofovir is an acyclic nucleoside phosphate analogue and thus shares some of the structural and mechanistic properties with other nucleoside analogues approved for the treatment of HIV infection but also differs from them in several respects. The shared properties include its prodrug status requiring metabolic activation by cellular enzymes to form the pharmacologically active metabolite, the triphosphate form. The triphosphate form competes with the physiological substrate dATP for incorporation into nascent DNA, and causes chain termination due to the lack of sugar moiety. The distinguishable property of tenofovir is its status as a nucleotide analogue and therefore it does not require the initial phosphorylation by the nucleoside kinase of the host cells, one of the enzymes needed by all of the currently approved nucleoside analogues.

SUMMARY

Mechanism of action of Tenofovir DF: The presumed mechanism of action of nucleoside and nucleotide analogues is that they are initially metabolized to their respective triphosphates by cellular nucleoside and nucleotide kinases. Tenofovir disoproxil fumarate (Tenofovir-DF) is an acyclic deoxynucleoside methylphosphonate diester analogue of the natural nucleoside monophosphate, the deoxyadenosine monophosphate. In order for tenofovir-DF to function as an inhibitor of HIV-DNA polymerase (or reverse transcriptase) it needs to undergo two ester hydrolytic steps by the host esterases to form tenofovir and subsequently two phosphorylations by cellular phosphokinase enzymes to

form the active metabolite, the tenofovir diphosphate. The active metabolite, tenofovir diphosphate inhibits the viral reverse transcriptase (RT) activity by binding competition with the natural substrate deoxyadenosine monophosphate and by DNA chain termination after its incorporation into the viral DNA. Tenofovir diphosphate could also be an inhibitor of the cellular DNA polymerases but its preferential inhibition of the HIV-specific DNA polymerase over the cellular DNA polymerases makes it a selective inhibitor of HIV-RT.

Table 1. Tenofovir and its mono and diphosphate levels in PBMC Incubated with 1.0 and 10.0 μM [^{3}H] tenofovir

Cell type	Drug	Incubation time (h)	Intracellular concentration (μM) of:		
			TNV	TNV_P	TNV_{PP}
R-PBMC	TNV-DF 1.0 μM	6	4,960	720	1,660
		12	4,885	915	1,960
		24	4,305	900	2,010
	TNV 10.0 μM	6	1.0	0.1	0.35
		12	1.5	0.15	0.50
		24	1.7	0.45	1.24
S-PBMC	TNV-DF 1.0 μM	8	1,789	645	1,511
		12	1,837	816	1,562
		24	1,875	657	1,608
	TNV 10.0 μM	6	0.4	0.11	0.14
		12	0.7	0.10	0.19
		24	0.9	0.21	0.40

PBMC = Peripheral blood mononuclear cells; R = Resting; S = PHA stimulated; TNV-DF = Tenofovir disoproxil fumarate; TNV = Tenofovir;. TNV_P= Tenofovir monophosphate; TNV_{PP} = Tenofovir diphosphate; R-and S-PBMC (10^7 cells) were incubated with 10.0 μM [^{3}H] tenofovir or with 1.0 μM [^{3}H] tenofovir-DF for the times indicated. Nucleoside analogues from the cells were extracted with 70% methanol and quantified by [redacted] Data presented are the means of two separate experiments.

In vitro intracellular metabolism of tenofovir and tenofovir DF: The applicant reported the time course of the intracellular uptake and metabolism of tenofovir and tenofovir-DF in resting human peripheral blood mononuclear cells (PBMC) and also in activated (to divide by the addition of phytohemagglutinin) PBMC. In these studies [(1)] cells were exposed to 10.0 μM [^{3}H] tenofovir or 1.0 μM [^{3}H] tenofovir-DF in parallel experiments.

Nucleoside analogues from the cells were extracted with 70% methanol and quantified by [redacted]

Table 1 shows the accumulation of tenofovir and its mono and diphosphates in resting and activated PBMC. When 1.0 μM [^{3}H] tenofovir-DF was used there was a rapid accumulation of tenofovir and its metabolites in resting and stimulated PBMC. The intracellular concentration of tenofovir, its mono and diphosphates were approximately 4305, 900 and 2010 μM in resting PBMC and 1875, 675, and 1608 μM in activated PBMC after 24 hours of incubation. Kinetic studies [(1)] on the intracellular metabolism indicate that the active metabolite, tenofovir diphosphate, accumulated in the millimolar range with in one hour of incubation. Tenofovir monophosphate and diphosphates accumulated steadily for 8.0 hours reaching a plateau at a total concentration of approximately 2,800 and 2,000 μM in the resting and stimulated PBMC, respectively. It is note worthy that tenofovir DF, the diester or its monoester was not detected in stimulated PBMC within the 0.5 to 24-hour time points examined indicating that tenofovir-DF is rapidly hydrolyzed to form tenofovir.

In contrast to tenofovir-DF, the uptake and intracellular metabolism of 10.0 μM [^{3}H] tenofovir was quite slow in both resting and activated PBMC. The accumulation of tenofovir and its metabolites were barely detectable during the first 6 hours of incubation. The intracellular concentration of tenofovir, its mono and diphosphate were: 1.7, 0.45 and 1.2 μM in resting PBMC and 0.9, 0.21, and 0.4 μM in activated PBMC after 24 hours of incubation. These results indicate that tenofovir is more poorly transported and poorly phosphorylated than tenofovir-DF and that the latter would be a better inhibitor of HIV replication than the former in HIV infected cells.

To investigate the intracellular decay of tenofovir metabolites, PBMC were exposed to 1.0 μM [^{3}H] tenofovir-DF or 10.0 μM [^{3}H] tenofovir for 24 hours and the intracellular

levels of tenofovir metabolites determined at different intervals after drug removal. The results [1] indicate that the tenofovir metabolites decayed with linear kinetics in PBMC. In PHA stimulated PBMC treated with either tenofovir or tenofovir-DF, the tenofovir mono and diphosphates decayed with half-lives of 15 and 11 hours, respectively. However, in resting PBMC treated with tenofovir the mono and diphosphate metabolites of tenofovir decayed with half-lives of 33 and 49 hours, respectively. In resting PBMC treated with tenofovir-DF the metabolites of tenofovir mono and diphosphates showed very little clearance and the half-life could not be estimated.

The data derived from the intracellular metabolism and their decay studies indicates that tenofovir-DF is taken up and metabolized rapidly by cells providing significantly greater intracellular concentrations of tenofovir and the active metabolites. In contrast, tenofovir is taken up and metabolized slowly providing significantly lower concentrations of the metabolites than tenofovir-DF. Tenofovir has limited oral bioavailability and cellular permeability because of the presence of two negative charges on the phosphonyl group. It is believed that tenofovir is internalized by endocytosis and thus accumulates rather slowly within cells. The orally bioavailable tenofovir-DF is rapidly hydrolyzed to form tenofovir. Therefore, tenofovir and not tenofovir-DF is the prodrug form that is available to the host cells for uptake, metabolism and antiviral activity. Tenofovir-DF was used for the purpose of oral bioavailability.

Inhibition of HIV-RT by tenofovir diphosphate: The course of HIV infection involves the conversion of the viral RNA into DNA in infected cells. The conversion reactions are catalyzed by the RNA-dependent and DNA dependent DNA polymerase activities of HIV DNA polymerase that is also called as reverse transcriptase. To further define the mechanism of action, and selectivity of inhibition of viral DNA synthesis compared to cellular DNA synthesis, the applicant investigated the inhibitory effects of tenofovir diphosphate on the viral and cellular DNA polymerases.

Table 2. Kinetic inhibition constants of tenofovir diphosphate on the RNA- and DNA- dependent DNA polymerase activities of HIV-RT.

Template	K_i (μM)	K_m dATP (μM)	K_i / K_m
RNA template	0.02	0.05	0.40
DNA template	1.6	4.6	0.35

Table 2 shows the kinetic inhibition (K_i) constants for the inhibitor, tenofovir diphosphate, compared to the K_m of the natural (physiological) substrate, deoxyadenosine triphosphate, against HIV- RT on both RNA and DNA templates. Tenofovir was a more potent inhibitor of RNA-dependent DNA polymerase activity (Ki = 0.02 μM) than DNA-dependent DNA polymerase activity (K_i = 1.6 μm) of HIV-RT. These data on the kinetics of inhibition suggests that tenofovir diphosphate inhibition is competitive with respect to the physiological substrate dATP.

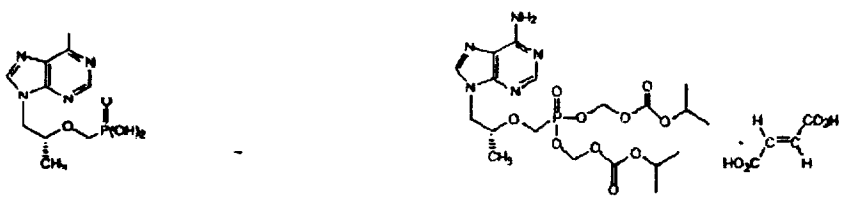
Effect of tenofovir diphosphate on cellular DNA polymerases: In order for tenofovir-DF to be useful in the clinic its metabolically active form, tenofovir diphosphate, should be active not only against the viral DNA polymerase but should ideally show little or no effect on the cellular DNA polymerases. The applicant examined whether tenofovir diphosphate is an inhibitor of, or a substrate for cellular DNA polymerases. The relative inhibitory effect of tenofovir diphosphate on DNA synthesis catalyzed by Human DNA polymerases α, β and γ and by the rat DNA polymerases δ and ε are presented in Table 3.

Table 3. Inhibition constants (Ki) of tenofovir diphosphate and the polymerization rate constant (Km) for the physiological substrate dATP against DNA polymerases α, β, γ, δ and ε

Enzyme	K_i (μM)	K_m dATP (μM)	K_i / K_m
Human DNA pol α	5.2	2.7	1.92
Human DNA pol β	81.7	5.6	14.6
Human DNA pol γ	59.5	0.7	85.3
Rat DNA pol δ	7.1	0.7	10.2
Rat DNA pol ε	95.2	6.1	15.6

Data presented in Table 3 indicates that tenofovir diphosphate has low affinity for DNA polymerases β, γ, δ and ε (K_i / K_m ratio of >10). However, human DNA polymerase α may be inhibited to some extent. The results suggest potential cytotoxicity mediated by incorporation of tenofovir into cellular DNA. The DNA polymerase enzyme studies suggest that tenofovir diphosphate can exert inhibitory effects on DNA polymerase α and some what weaker effects on the DNA repair enzyme, the DNA polymerase β, and mitochondrial DNA polymerase γ.

In the initial nonclinical in vitro anti-viral activity studies the applicant used tenofovir (which is the acyclic nucleotide analogue) for evaluation of anti- HIV activity. During the nonclinical antiviral activity studies and early PK studies the applicant found that tenofovir had low bioavailability and therefore developed and selected an orally available prodrug of tenofovir, tenofovir disoproxil fumarate for clinical development.



Tenofovir Tenofovir DF

Figure 1. Structure of tenofovir and its orally bioavailable salt form the tenofovir disoproxyl fumarate.

The structures of tenofovir and tenofovir DF are presented in Fig 1. Tenofovir, an acyclic nucleoside phsophonate analogue was used in the nonclinical antiviral activity studies. The clinical candidate, however, is tenofovir disoproxil fumarate (a prodrug of tenofovir)

which is a fumaric acid salt of *bis*-isopropoxycarbonyloxymethyl ester derivative of tenofovir. In vivo tenofovir-DF is converted into tenofovir, a nucleoside phsophonate analogue of adenosine 5'-monophosphate. The conversion of tenofovir DF to tenofovir requires two initial ester hydrolysis steps. Then tenofovir undergoes anabolic conversion to the active metabolite the tenofovir diphosphate that inhibits HIV-RT. The cellular enzymes responsible for tenofovir metabolism are adenylate kinase and nucleotide diphosphate kinase, which are ubiquitous in cells.

In vitro antiviral activity of tenofovir: In studies on the determination of the in vitro anti-HIV activity, two forms of the drug, tenofovir and tenofovir disoproxyl fumarate were used. Tenofovir being a nucleoside phosphonate (nucleotide analogue) contains two negative charges on the phosphonyl group compared to the classical nucleoside analogues, which lack the two negative charges. The negative charges on the tenofovir prevent its oral bioavailability and transport into the cells across the cell membrane. It is believed that tenofovir is taken into cells by endocytosis, a relatively inefficient process. Neutralization of the negative charges by esterification improves both the bioavailability as well as cellular uptake in vitro. To evaluate the differences in the antiviral activity of these two forms of tenofovir the applicant investigated the antiviral activity of these compounds in CD4 + T-cells.

Table 4. Anti-HIV activity (IC_{50}) and cytotoxicity (CC_{50}) of tenofovir and tenofovir-DF

Drug	Cells/virus						
	MT-2/HIV-1 IIIb			PBMC/HIV-1 IIIb			MM/HIV-1 Bal
	IC_{50} (μM)	CC_{50} (μM)	SI	IC_{50} (μM)	CC_{50}(μM)	SI	IC_{50} (μM)
Tenofovir	0.63	1250	1984	0.18	1200	6666	0.04
Tenofovir-DF	0.007	22	3142	0.005	29	5800	ND

MM =Monocyte-macrophages; SI = Selectivity index (ration of CC_{50} to IC_{50}); ND= not determined.

The anti-HIV activity of nucleoside/nucleotide analogues depends on many factors including the host cell type, the virus type and the assay used to determine the viral

replication. Therefore, the applicant determined the antiviral activity of these compounds in a variety of host cells including established human lymphocytes cell lines (MT-2 cells), primary lymphocytes (PBMC) and monocyte-macrophages. The HIV types used to infect the different cell types include laboratory and clinical isolates. In parallel experiments the applicant also determined the cytotoxicity of the drugs on the host cells to determine the selectivity index of tenofovir and tenofovir-DF.

Data presented in Table 4 and in other studies [(2)] show that the 50% inhibitory concentration (IC_{50}) of tenofovir ranged from 0.04 to 8.5 μM. Tenofovir-DF expressed increased activity against both laboratory adopted and primary clinical isolates of HIV strains in different culture systems. The inhibition of HIV in stimulated PBMC by tenofovir-DF was >25-fold better than the unmodified parental drug tenofovir.

The selectivity index data i.e., the ratio of 50% cytotoxic concentration (CC_{50}) to the 50% inhibitory concentration (IC_{50}) of HIV-RT show that the selectivity index for tenofovir-DF was ~6000, but for tenofovir it was less (~2000). The results suggest that the protective group on the phosphonate was not toxic in these cells.

Antiviral effects of tenofovir in combination with other anti-HIV compounds: Use of regimens containing combination of several anti-HIV agents is increasingly considered the most effective use of these agents for treatment of HIV infection. The use of drug combinations is considered likely to improve efficacy and to reduce the potential for selection of resistant variants. To select appropriate drug combinations for potential clinical use the applicant tested the combination of tenofovir with each of the antiretroviral agents approved to date.

In drug combination in vitro studies, tenofovir paired individually with 14 other antiretroviral agents was tested for additive, antagonistic, or synergistic antiviral activity

against HIV-1 in MT-2 cells. In these combination studies, MT-2 cells were infected with wild-type HIV-1 IIIb in the presence of graded concentrations of tenofovir in combination with graded concentrations of each of the drugs separately. After 5-days incubation the extent of HIV-1 infection was measured by determining the HIV-1 cytopathic effect. The data were analyzed with MacSynergy™ II software.

Data presented in Table 5 indicates that in vitro tenofovir showed synergistic activity in combination with the non-nucleoside analogue RT inhibitors: delavirdine, efavirenz, nevirapine; nucleoside analogue didanosine, zidovudine and protease inhibitors nelfinavir and amprenavir. Tenofovir showed additive effects with nucleoside RT inhibitors: stavudine, abacavir, lamivudine and zalcitabine, with the protease inhibitors: indinavir, saquinavir and ritonavir. It was stated that there was no significant antiviral antagonism with any of the tested compounds. The clinical significance of the in vitro combination effects is unknown.

Table 5. Antiviral effects of tenofovir in combinations other anti-HIV compounds

Drug Combination	Volume ($\mu M^2\%$)[1]		Combined Effect
	Synergy	Antagonism	
Tenofovir + Nucleoside Reverse Transcriptase Inhibitors (NRTIs)			
Tenofovir + Stavudine	4.1 ± 2.8	2.8 ± 1.3	Additive
Tenofovir + Abacavir	12.0 ± 12.1	20.3 ± 10.8	Additive
Tenofovir + Lamivudine	18.2 ± 9.6	3.9 ± 3.4	Additive
Tenofovir + Zalcitabine	22.0 ± 7.9	8.1 ± 8.1	Additive
Tenofovir + Didanosine	36.6 ± 2.0	22.0 ± 3.5	Minor Synergy
Tenofovir + Zidovudine	105.5 ± 12.9	0.5 ± 0.5	Strong Synergy
Tenofovir + Non Nucleoside Reverse Transcriptase Inhibitors (NNRTIs)			
Tenofovir + Delavirdine	138.3 ± 97	0 ± 0	Strong Synergy
Tenofovir + Nevirapine	167.8 ± 29.9	0 ± 0	Strong Synergy
Tenofovir + Efavirenz	210.5 ± 76.6	10.6 ± 0.8	Strong Synergy
Tenofovir + Protease Inhibitors			
Tenofovir + Indinavir	12.0 ± 2.9	8.7 ± 5.8	Additive
Tenofovir + Saquinavir	17.5 ± 1.9	3.6 ± 3.6	Additive
Tenofovir + Ritonavir	20.3 ± 5.3	12.6 ± 8.7	Additive
Tenofovir + Nelfinavir	49.9 ± 11.5	7.9 ± 7.9	Moderate Synergy
Tenofovir + Amprenavir	108.9 ± 95	12.5 ± 10.9	Strong Synergy

1=Volume of synergy and antagonism were computed by the MacSynergy™ II program using a 95% confidence interval and are defined by the program as follows: values < 25 $\mu M^2\%$ indicate insignificant synergy (additive); values ≥ 25 and < 50 $\mu M^2\%$ indicate minor synergy/antagonism; values ≥ 50 and < 100 $\mu M^2\%$ indicate moderate synergy/antagonism; and values ≥ 100 $\mu M^2\%$ indicate strong synergy/antagonism

Effect of tenofovir on simian immunodeficiency virus (SIV) infection: SIV infection of rhesus macaques is considered similar to HIV infection in humans and has been used to study the pathogenesis of HIV disease and to test potential antiretroviral therapies. In a study of juvenile rhesus monkeys chronically infected with SIV, tenofovir at 30 or 75

mg/kg was administered subcutaneously once daily for 4 weeks and the virology results were compared with the mock-treated control monkeys.

At week 4 in the 30 or 75 mg/kg tenofovir treatment groups, plasma SIV RNA levels were reduced 100to 1000-fold from baseline (as determined by the Chiron bDNA assay) and the CD4 cell count had risen a mean of 158 to 314 cells/mm^3 respectively. Results from the SIV-infected monkey model support that tenofovir exerts antiviral activity in vivo by reducing the viral load and increasing the CD4 cell count.

NON-CLINICAL RESISTANCE STUDIES

In vitro selection of HIV-1 variants resistant to tenofovir: Upon administration of any of the clinically available nucleoside analogue inhibitors, non-nucleoside analogue inhibitors of HIV-1 RT and protease inhibitors, HIV-infected individuals develop resistance to these drugs. In vitro experiments have been generally predictive of the potential for emergence of resistance. The rate of emergence of resistance appears to differ from drug to drug. To explore the potential for the emergence of resistance to tenofovir, the applicant attempted to select for tenofovir-resistant variants in vitro by serial passage of the HIV on permissive cells in the presence of increasing concentrations of tenofovir.

When MT-2 cells infected with HIV-1IIIb were grown in increasing concentrations of tenofovir, by 8 culture cycles variants with approximately 4-fold decreased susceptibility to tenofovir emerged (baseline IC_{50} =0.4 μM and passage 8 virus IC_{50} =1.5 μM). Genotypic analysis of these variants (4 out of the 15 clones analyzed) showed K65R[†] mutation in the viral RT. Recombinant HIV-1 constructs containing the K65R mutation

[†] Please see Appendix-2 for key to amino acid codes

in the HXB2D conferred 3-4 fold reduced susceptibility to tenofovir in vitro confirming that this mutation on HIV-RT confers resistance to tenofovir.

Table 6. Antiviral susceptibilities of molecular clones of HIV-1 expressing nucleoside-associated resistance mutations in RT

RT Mutation	IC_{50} Fold increase above wild-type (HXB2D or IIIb)						
	Tenofovir	ZDV	D4T	ddI	3TC	ddC	Abacavir
D67N + K70R	0.7	5.7	ND	ND	ND	ND	1.7
D67N+K70R+K219Q	1.8	23	ND	ND	3.1	ND	1.2
T69D	2	1.7	4	ND	19	4.3	ND
K70E	1.3	0.6	1.1	1	3.8	0.7	ND
L74V	1	2	1.1	2.8	2.4	3.9	3.7
Q151M[1]	0.8	39	69	42	1.9	7.3	ND
M184V	0.2–0.9	0.7	1.5	2.8	> 50	1.8	4.9
T215Y	1.8	6.9	1.6	2.2	1.1	1.3	2.2
T215Y + M184V	0.5	0.7	1.1	2	> 50	1.7	7
K65R	3.4	0.6	2.1	4	25	14	3.1
K65R + M184V	1.1	1	1	5.3	> 50	7.3	6.9

1=Site-directed recombinant also includes A62V, V75I, F77L, and F116Y RT mutations.
ND = not determined

Table 6 shows the antiviral susceptibility of tenofovir against selected recombinant HIV-1 mutant constructs. The data indicate that tenofovir retains activity against some of the recombinant molecular clones expressing some of the mutations that confer resistance to ddI, ddC, 3-TC and AZT. Based on the comparison of resistance mutations, tenofovir falls in the cross-resistance grouping with abacavir, ddI, ddC and 3TC although there are significant differences in the degree of decrease in susceptibilities. Because of the potential cross-resistance the combination use or sequential use of these may not be beneficial. It is to be noted that the recombinant constructs derived in these studies may not reflect the activities of resistant clinical HIV isolates as the latter may harbor a variety of other additional mutations.

It is to be noted that data derived from recombinant constructs while very useful also have limitations in addressing the issues of resistance and cross-resistance. The recombinant constructs contain only a portion of the HIV RT gene that recombines into a large backbone of a wild type HIV. The portion of the viral RT that was not included into the recombinant construct could have several mutations that would not be represented in the recombinant virus. Additionally, the resistance mutations can interact in synergistic and/or antagonistic manner and some of these potentially missed mutations and their interaction effects will not be represented in the recombinant viruses. Moreover, the recombinants tend to be clonal, enriching in those virus populations that have growth advantage in the host cell type used.

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Table 7. Tenofovir susceptibility of recombinant HIV-1 constructs derived from clinical isolates expressing selected nucleoside analogue resistance mutations

Resistance Group	N	Mean Fold Change in Susceptibility from Wild-Type [1] *(range)*					
		Tenofovir	**ZDV**	**3TC**	**ddI**	**d4T**	**Abacavir**
M184V	10	0.7	0.9	> 50	1.0	1.4	1.3
ZDV-HI	10	3.7	47	4.3	1.6	2.5	2.6
ZDV-HI + M184V	10	2.4	15	> 50	1.8	1.7	4.6
Q151M	5	1.8	43	2.1	13	20	11
Q151M + M184V	5	1.6	46	> 50	19	11	16
T69S Ins	5	23	101	28	4.1	9.3	20
T69S Ins + M184V	10	6.0	31	> 50	1.8	4.2	8.1
L74V	5	0.7	1.3	1.4	0.8	0.9	1.4
L74V + M184V	5	0.5	0.6	> 39	3.0	1.0	2.9
L74V + Y115F + M184V	5	0.6	0.6	> 39	1.5	0.5	7.3
K65R[2]	4	3.4	17	20	4.7	8.4	7.8
K65R[2] + M184V	4	1.5	20	> 50	12	8.7	13

1 Antivirogram assay results by [redacted] Fold changes of between 4 and 10-fold indicate reduced susceptibility, above 10-fold indicate possible resistance (both shown shaded). The wild-type IC_{50} values for tenofovir ranged from [redacted] in these analyses.

2 Virus from two patients in each K65R group also expressed the Q151M multinucleoside resistance complex.

The applicant determined the in vitro susceptibility of tenofovir against nucleoside-resistant recombinant HIV-1 constructs from clinical isolates of antiretroviral experienced patients. Seventy-eight patient isolates selectively expressing lamivudine (M184V), high-level zidovudine (T215Y + others ± M184V), multinucleoside (Q151M

complex and T69S insertions ± M184V), didanosine (L74V ± M184V), abacavir (L74V + Y115F + M184V) or K65R (± M184V) resistance mutations have been analyzed by [redacted] using the Antivirogram™ phenotypic resistance assay. As shown in Table 7 tenofovir showed a mean IC_{50} value of 0.9 µM against a panel of ten wild-type clinical isolates. Phenotypic classification is based upon IC_{50} changes relative to the wild-type reference where "sensitive" is < 4-fold, "intermediate" is 4 to 10-fold, and "resistant" is >10-fold. These classifications are for comparative purposes only and do not represent clinical cut-off for tenofovir or any other antiretroviral drug.

Clinical HIV isolates expressing M184V alone showed a 0.7 fold increase in their susceptibility to tenofovir. High-level zidovudine-resistant HIV (ZDV-HI; mean 47-fold ZDV resistant; mean 3.4 ZDV mutations including T215Y/F in all cases) remained susceptible to tenofovir demonstrating a mean 3.7-fold reduced susceptibility, with only three samples having an intermediate phenotype. Furthermore, the combination of M184V and high-level ZDV mutations exhibited a mean 2.4-fold change from wild-type (mean 3.2 ZDV mutations). HIV strains expressing the multinucleoside-resistant T69S double amino acid insertion mutations (T69S Ins) were resistant to tenofovir (23-fold). Intermediate susceptibility to tenofovir (6-fold) was observed when these insertions were combined with M184V. The frequency of the T69S insertion multinucleoside-resistant virus was less than 1% in a sample of over 12,000 patients' samples. The multinucleoside-resistant HIV with Q151M showed susceptibility to tenofovir (1.6 to 1.8-fold) regardless of M184V. HIV expressing the didanosine-associated L74V mutation, with or without M184V, were slightly hypersusceptible to tenofovir (0.5 to 0.7-fold). HIV expressing a common pattern of resistance mutations associated with abacavir (L74V + Y115F + M184V) were also hypersusceptible to tenofovir (0.6-fold) while showing reduced susceptibility to abacavir (7.3-fold). Finally, HIV expressing K65R showed a mean 3.4-fold reduced susceptibility to tenofovir but only 1.5-fold reduced

susceptibility in the presence of M184V, results which are nearly identical to those obtained with the site-directed recombinant viruses.

Antivirogram is a research-based methodology designed to derive chimeric constructs of HIV-1 composed of partial RT sequences and the entire viral protease sequence. The RT and protease RNA sequences amplified by RT-PCR and the amplicons are transfected into CD4T cells together with laboratory wild type HIV DNA from which RT and PR sequences are removed. After homologous recombination new viruses are produced from these cells. The recombinant virus population derived from the clinical specimen was used in the analysis. As indicated earlier in this review the recombinant constructs have limitations in evaluating for resistance and cross-resistance because these chimeras contain only a selected small DNA segments recombined on to a large backbone of wild type HIV strain and thus not a true representative of the clinical isolate

In vitro antiviral activity of tenofovir against HIV-1 expressing NNRTI resistant mutations: Non-nucleoside RT inhibitors (NNRTIs) bind to a structurally distinct portion of RT and cross-resistance to nucleoside and/or nucleotide RT inhibitors is generally not expected. Nevertheless, RT amino acid changes associated with NNRTIs may induce structural changes in RT potentially altering the antiviral susceptibility of any RT inhibitor. In published studies, HIV-1 molecular clones expressing the primary NNRTI-associated resistance mutations Y181C and K103N demonstrated increased susceptibility to tenofovir of 4 to 8-fold. Tenofovir and NNRTI susceptibilities of ten HIV-1 clinical isolates expressing these mutations in the context of additional NNRTI-associated mutations is shown in Table 8. For the clinical isolates expressing Y181C or K103N, the mean tenofovir IC_{50} values were 0.82 μM and 1.01 μM, respectively, in comparison to 0.96 μM for wild-type clinical isolates. Thus, mutations associated with high-level NNRTI resistances remain susceptible to tenofovir in vitro. This study once again is

subjected to the limitations stated earlier on comparing resistance and cross-resistance profiles with recombinant constructs

Table 8. Tenofovir susceptibility of HIV-1 clinical isolates and molecular clones expressing high-level NNRTI resistance mutations

Virus Source	Primary NNRTI Resistance Mutation	Mean IC_{50} (μM)			
		Tenofovir	Nevirapine	Efavirenz	Delavirdine
Clinical Isolates (n = 8)	Wild-Type	0.96	0.019	0.0007	0.008
Clinical Isolates (n = 5)	Y181C	0.82	> 1.21	0.0027	> 0.725
Clinical Isolates (n = 5)	K103N	1.01	> 0.89	0.095	0.484

Emergence of tenofovir resistance in rhesus monkeys infected with simian immunodeficiency virus (SIV): To study the therapeutic effect of tenofovir and the development of SIV resistance to tenofovir during treatment [3] eight new-born rhesus macaques were inoculated orally within three days after birth with 1.0 ml (10^5 $TCID_{50}$) of an uncloned SIV-mac 251 virus stock. Four of these eight SIV-inoculated animals were used as untreated controls while the other four animals were treated with tenofovir (30mg/kg subcutaneously, once daily) beginning at 3 weeks of age.

The four untreated SIV-infected newborn macaques developed persistently high viremia. Tenofovir treatment of the other four SIV-infected macaques, resulted in a persistent reduction of viremia in three of the four animals. In the animals with reduced viremia, there was a 100 to 1,000-fold reduction in PBMC-associated SIV titer within three weeks of treatment and undetectable SIV titers from plasma.

SIV with a 5-fold reduced susceptibility to tenofovir emerged in all four tenofovir-treated SIV-infected monkeys after 5-15 weeks of tenofovir therapy. The development of a K65R mutation in the RT was consistently observed and played a central role in the resistance to tenofovir. Thus this study demonstrates the in vivo development of K65R

mutation under the selection pressure of tenofovir treatment. The data also suggests that the K65R may arise in HIV infected patients receiving tenofovir therapy.

Table 9. Emergence of drug resistance in tenofovir-treated SIV-infected rhesus macaques.

Animal #	Mutations in RT sequence at indicated time after SIVmac infection								
	3 wk	6 wk	8 wk	12-14 wk	18 wk	6 mo	7 mo	8 mo	9 mo
29003	None	NA	None	None	K65R I118V	K65R I118V	K65R N69S I118V	NA	K65R N69S I118V
29008	None	NA	None	K65R	K65R I118V	K65R N69S I118V	K65R N69S I118V	K65R N69S I118V	K65R N69S I118V
29045	None	NA	None	K65R I118V	NA	K65R N69S I118V	K65R N69S I118V	K65R N69S I118V	K65R N69S I118V
29055	None	None	K65 R R82 K S211 N	K65R N69S R82K S211N	NA	K65R N69T R82K A158 S S211 N	K65R N69T R82K A158 S S211 N	K65R N69T R82K A158 S S211 N	K65R N69T R82K A158 S S211 N

NA= not available None = wild type virus and *= ½ approximately equal mixture of wild type and mutant amino acids.

The data in Table 9 shows that in addition to the K65R mutation, SIV isolates from the 4 tenofovir treated monkeys also showed progressive accumulation of other mutations in the viral RT. However, development of these additional mutations did not result in further increased resistance to tenofovir. Thus a possible role for these additional mutations may be to compensate for impaired replicative capacity induced by the K65R mutation, rather than to contribute directly to the drug-resistant phenotype.

Resistant virus from three of these animals had K65R, N69S, and I118V mutations in the RT. The fourth animal had persistently high viremia and died at 20 months; virus from this animal had K65R, N69T, R82K, A158S, and S211N mutations in the RT. To directly assess whether tenofovir resistance alters viral virulence and/or the therapeutic efficacy of

tenofovir treatment, two virus stocks resistant to tenofovir were made (SIVmac385: K65R, N69S, I118V; SIVmac055: K65R, N69T, R82K, A158S, S211N) [3]. Two groups of six newborn macaques were inoculated with either SIVmac385 or SIVmac055. Three animals of each group were started on tenofovir treatment at three weeks of age. The six untreated animals developed persistently high viremia and had rapid immunosuppression; all died within four months. In contrast, the six infants inoculated with virus resistant to tenofovir but treated with tenofovir also had high viremia but had a delayed disease progression. The three SIVmac055-infected animals treated with tenofovir developed fatal disease between five to nine months of age, while the three SIVmac385-infected animals treated with tenofovir survived ≥21 months. This result suggests that drug induced genetic changes in SIV mac can bring about changes in biological and pathological properties. The genotype and phenotype resistant to tenofovir in most animals, even those that did not get tenofovir treatment were stable. Specifically, K65R was not lost and phenotypic expression of altered susceptibility was maintained. Thus, although SIV-resistant to tenofovir is fully virulent, tenofovir treatment appears to have therapeutic benefits even in the presence of virus resistant to tenofovir.

Tenofovir resistance in clinical studies

In conjunction with the clinical trials of tenofovir and tenofovir DF the applicant conducted detailed virology studies and substudies in the treatment of HIV-infected patients. According to the applicant the objectives of these virology studies were to:

1. Determine whether RT resistance mutations develop during tenofovir DF therapy

2. Determine whether any RT mutations that develop correlate with reduced susceptibility of the mutant HIV to tenofovir in vitro or with increasing HIV viral load in vivo.

3. Determine whether the baseline HIV RT genotype in antiretroviral experienced patients affects treatment response to tenofovir DF.

Results from resistance analyses of four clinical trials (GS-96-701, GS-97-901, GS-98-902 and GS-99-907) are summarized below. In addition, there are individual virology study reports for trials GS-98-902 and GS-99-907.

Study 701

Study 701 was a Phase I monotherapy trial in which an intravenous infusion formulation of tenofovir was used. Patients were randomized into two dose groups, 1 mg/kg or 3 mg/kg, of tenofovir I.V. as a single dose for 7 consecutive days. HIV genotypic analyses were performed from baseline and day 14 plasma samples from all patients enrolled. None of the patients in the 1 mg/kg dose group entered the clinical trial with baseline resistance mutations and none developed any during the treatment period (Table 10).

Table 10. Genotypic Analyses of Plasma Samples from Study 701 Patients (3 mg/kg dose cohort)

Patient #	Baseline nucleoside resistance mutations	RT mutations at day 14	Average baseline HIV RNA (copies/ml)	Log HIV RNA change from baseline at day 14
1021	M41L D67E K70SGA (double insertion of G and A) T215Y	M41L D67E K70SGA (double insertion of G and A) T215Y		0.37
1023	M184V	M184V		-0.89
1024	None	None		-1.21
1025	None	None		-1.09
1026	M41L M184V T215Y	M41L T215Y		-1.06
1028	None	None		-1.28
1029	None	None		-0.54
1030	None	None		-1.24

In the 3 mg/kg dose group three patients randomized into the study showed nucleoside-associated RT mutations at baseline. Results from sequencing RT gene (nucleotides 1-900) are presented in Table 10. Patient 1021 had HIV with a double insertion mutation at

codon 70 (K70SGA), patient 1023 had a mutation M184V, and patient 1026 had mutations M41L, M184V, and T215Y. Patient 1021who did not show a response to therapy entered the trial with the double insertion mutation in the viral RT. Insertion mutation at codon position 70 on the HIV RT was known to confer multinucleoside resistance and thus this patient was expected to show reduced susceptibility to tenofovir. The other two patients with baseline mutations showed reduction in viral RNA. However, patient 1026 with amino acid valine at position 184 mutated to methionine. This result suggests that short-term exposure to tenofovir DF can bring about mutations in HIV and thus can change HIV genetic pools.

Study 901

Study 901 was a Phase I/II, randomized, double-blind, placebo-controlled study of the pharmacokinetics and anti-HIV-1 activity of tenofovir DF administered orally once daily for 28 consecutive days to HIV-1 infected patients with CD4 cell counts ≥ 200 cells/mm^3 and plasma HIV-1 RNA levels ≥ 10,000 copies/ml. In all of the quantification assays for HIV RNA an approved Roche Amplicor® HIV-1 Monitor™ was used. Fifty-nine HIV-1-infected patients were enrolled in the study. Twelve patients received DF at a dose of 75-mg daily, eight at a dose of 150-mg daily, eight at a dose of 300 mg daily, and ten at a dose of 600 mg daily. In addition eight patients received a dose of 75 mg daily plus hydroxyurea 500 mg daily; eleven received matching placebo and two received matching placebo plus hydroxyurea 500 mg daily. In this study, 66% of patients had prior antiretroviral experience.

Table 11. Genotypic analysis of RT of tenofovir DF-treated patients in study 901 with baseline nucleoside-associated RT mutations

Tenofovir DF	Patient number	Prior nucleoside therapy	Nucleoside-associated RT mutations[1]	
			Day 0	Day 35
75 mg	89	AZT/3TC/d4T/ ddI	M41L, T215Y	M41L, T215Y
	90	AZT/3TC	M184V	M184V
75 mg + HU	26	AZT/3TC	M41L/M, T215S	M41L/M, T215S
	30	AZT/3TC/ddI/d dC/d4T	M41L/M, K70R/K, L210W/L, T215Y/S/N, K219E/K	M41L/M, K70R/K, L210W/L, T215Y/S/N, K219E/K
150 mg	2	AZT/3TC	**M184V**[2]	None
	6	AZT/3TC/d4T/	**M184V/M**	None
	7	AZT/3TC/d4T	M184V	M184V
	8	AZT/3TC	**M184V/M**	M184V
	10	AZT/3TC/d4T	M184V	M184V
600 mg	105	AZT/3TC/d4T/ ddC	M41L/M, **M184V/M**, T215Y	M41L, T215Y
	112	AZT/3TC/d4T/ 3TC/ABC/	M41L/M, **T69A/T**, **M184V/M**, T215Y/T	M41L/M, T215Y/T

1=Some of the nucleoside-associated RT codons are M41, K65, D67, T69, K70, L74, V75, Q151, M184, L210, T215, and K219. 2= bold and underlined text shows the site at which mutations occurred after treatment with tenofovir.

Baseline and Day 35 HIV-1 RT sequences (amino acids 1-300) were analyzed from plasma HIV-1 RNA for all patients treated with tenofovir. Eleven of the patients had HIV-1 expressing nucleoside-associated RT mutations at baseline. The applicant stated that none of the patients developed detectable sequence changes in RT during the four-week dosing period. The data in Table 11 shows that mutation M184V (methionine is the amino acid at position 184 in the wild type HIV) was identified in the baseline plasma HIV-1 from eight patients treated with tenofovir DF (75 mg, 1 patient; 150 mg, 5 patients; 600 mg, 2 patients). By Day 35, there were mutations in the viral RT (bold and underlined in table 11) indicating that tenofovir treatment brings about mutations (in 4 of the 8 patient genotypes in this group in 28 days). Five patients expressed nucleoside

analogue-associated mutations at baseline, either as mixtures or full mutants (75 mg, 1 patient; 75 mg + HU, 2 patients; 600 mg, 2 patients). In one patient there was a mutation at amino acid position 69 (threonine at position 69 in the wild type HIV). The combined results from clinical study 701 and 901 suggest that treatment of HIV-infected subjects with Tenofovir/VIREAD™ for ≤28 days can result in genotypic changes in the HIV-1 RT. The relationship of these mutations to VIREAD™ susceptibility remains to be determined.

Study 902

Phenotypic and genotypic data on HIV isolates in clinical studies 902 and 907 included in this review is primarily the summary version of the applicant's submission with minor comments by the microbiology reviewer. For detailed comments please see the clinical review.

Study 902 was a randomized, double-blind, placebo-controlled, multicenter study of the safety and efficacy of tenofovir DF administered orally to HIV-1-infected patients with plasma HIV-1 RNA levels ≥ 400 copies/ml and ≤ 100,000 copies/ml. Patients on stable antiretroviral therapy (ART) containing no more than four active agents for ≥ 8 weeks at study entry were randomly assigned in a 2:2:2:1 ratio to add either tenofovir DF at one of three doses (75 mg, 150 mg, or 300 mg once daily) or placebo to their existing regimen in a double-blinded manner. Table 12 shows the baseline characteristics of patients enrolled into study 902 and 907. The patients in 902 were stratified by site according to HIV-1 RNA level, CD4 cell count, and number of antiretroviral drugs prior to study entry. At 24 weeks post-randomization, patients initially assigned to placebo treatment were crossed over to tenofovir DF 300 mg once daily in a blinded fashion for the remainder of the 48-week study.

A total of 189 patients were enrolled in this trial, 186 of whom received at least one dose of study medication and represent the protocol-defined intent-to-treat (ITT) population (n = 28, 53, 51 and 54 for the placebo, 75 mg, 150 mg and 300 mg randomization groups, respectively). The co-primary efficacy endpoints were the time-weighted change in $\log_{10}$ HIV-1 RNA levels from baseline average at week 4 post-randomization ($DAVG_4$) and week 24 post-randomization ($DAVG_{24}$). There were statistically significant changes in HIV RNA for all doses of tenofovir DF as compared to placebo for both efficacy endpoints that were durable through week 48.

Virology sub-study of study 902: The virology sub-study included all patients who enrolled in the trial (n = 189) and the final analyses consisted of the ITT population (n = 186). Both the HIV-1 RT and protease genes from banked plasma samples from all patients were genotypically analyzed at baseline, week 24, week 48, or upon early termination. A commercial company, [redacted] performed genotyping of the plasma HIV. In this procedure the genotyping included the amino terminal RT amino acids 1-400 (out of the full-length of the 560 amino acids of the RT) and all of the 99 amino acids of HIV protease.

Phenotypic analyses of tenofovir susceptibility were performed at baseline, week 48, or upon early termination for all patients who were assigned to 300-mg tenofovir DF therapy in the ITT population. Additional phenotypic analyses were performed for patients developing nucleoside-associated RT mutations, including all patients who developed the K65R mutation in RT from any dosing group. The method used for phenotypic analysis of the plasma HIV was a proprietary recombinant virus phenotypic assay. A commercial laboratory, [redacted] performed the assays. The basic principle of the method [5] involves the construction of chimeric HIV strains composed of the HIV RT and PR genetic elements. These genetic elements are amplified from the plasma viral RNA and subsequently recombined inside CD4+ T-cells

with an wild type HIV DNA construct from which the RT/PR genes were deleted. Measurement of drug susceptibility of the wild type and recombinant virus indicates the change in the drug susceptibility.

Baseline Characteristics and Baseline HIV Genotypes: Data in Table 12 shows that the overall mean CD4 count at baseline was 374 cells/mm^3 and the mean plasma HIV-1 RNA viral load at baseline was 4,571 copies/ml. The overall mean duration of prior antiretroviral therapy was 4 years and 7 months. The applicant stated that there were no significant differences between the four treatment groups in baseline viral load, CD4 count, or duration of prior ART.

Table 12: Baseline Characteristics of studies 902 and 907(intent-to-treat)

Characteristic		Study 902 (N=186)	Study 907 (N=550)
Baseline HIV RNA*	Mean	4,571	2291
Baseline HIV RNA	<5000 copies/ml ≥5000 copies/ml	51% 49%	78% 22%
Baseline CD+4 cells/mm^3	Mean	374	427
Baseline CD+4 cells/mm^3	<200 cells/mm^3 ≥ 200cells/mm^3	22% 78%	13% 87%
Prior ART experience	Mean	4 yr.7 mo.	5 yr.5 mo.
HIV status (CDG classification)	Asymptomatic Symptomatic AIDS	38% 15% 47%	50% 23% 27%
Prior ART use	< 4 drugs ≥ 4 drugs	21% 79%	20% 80%

*= HIV RNA copies/ml, ART= Antiretroviral treatment

At baseline, all patients were taking one or more nucleoside analogs with lamivudine and stavudine most commonly used (66% and 60% of patients, respectively). Additionally, 69% of patients were taking a least one protease inhibitor (PI) and 29% of patients were taking an NNRTI. Nelfinavir was the most commonly used PI (38%) and nevirapine (21%) was the most commonly used NNRTI. There were no significant differences among the treatment groups with regard to baseline ART usage.

Baseline HIV genotypic data were obtained from 184 of 186 patients in the ITT population. Plasma HIV RNA from two patients, both in the placebo arm, failed to generate a sufficient PCR product for genotypic analysis. Consistent with the extensive treatment experience of patients in this trial, baseline genotypic analysis revealed that 94% of analyzed patients had plasma HIV expressing one or more primary nucleoside-associated resistance mutations in RT, 57% expressed primary PI-associated resistance mutations and 32% expressed primary NNRTI-associated resistance mutations (Table 13). Most patients (74%) had HIV with typical ZDV analog-associated resistance mutations at RT codons M41L, D67N, K70R, L210W, T215Y/F, or K219Q/E/N (mean of 2.8 mutations); 66% had HIV with the lamivudine/abacavir-associated M184V/I mutations; and 47% had both of these types of resistance mutations. The prevalence of each of these baseline resistance mutations was similar across the four treatment arms in the study.

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Table 13. Baseline genotypes of selected subgroups of HIV from patients in study 902

RT and Protease Resistance Mutations at Baseline	Percent of Patients (n)				
	Placebo (n = 26)	**75 mg TDF (n = 53)**	**150 mg TDF (n = 51)**	**300 mg TDF (n = 54)**	**Total (n = 184)**
Nucleoside-Associated :	92% (24)	94% (50)	94% (48)	94% (51)	94% (173)
ZDV-R (M41L, D67N, K70R, L210W, T215Y/F, or K219Q)	77% (20)	70% (37)	76% (39)	74% (40)	74% (136)
M184V/I	62% (16)	72% (38)	71% (36)	59% (32)	66% (122)
T215Y/F	54% (14)	47% (25)	57% (29)	48% (26)	51% (94)
M184V/I + ZDV-R	46% (12)	47% (25)	53% (27)	43% (23)	47% (87)
T69D/N	19% (5)	17% (9)	12% (6)	9% (5)	14% (25)
L74V/I	27% (7)	11% (6)	8% (4)	6% (3)	11% (20)
A62V		4% (2)	4% (2)	2% (1)	3% (5)
V75T			2% (1)	2% (1)	1% (2)
K65R				2% (1)	0.5 % (1)
T69S Insertions, Q151M					0% (0)
NNRTI-Associated mutations: (K103N or Y181C in RT)	31% (8)	26% (14)	37% (19)	31% (17)	32% (58)
PI-Associated mutations: (Any substitution at codons 30, 48, 50, 82, 84, or 90 in PR)	69% (18)	51% (27)	53% (27)	61% (33)	57% (105)

- = Mutations M41L, A62V, K65R, D67N, T69D/N, K70R, L74V/I, V75T, F77L, Y115F, F116Y, Q151M, M184V, L210W, T215Y/F or K219Q of RT.

HIV RNA response to tenofovir DF therapy by baseline HIV genotype: Despite the presence of extensive RT resistance mutations at baseline, patients adding tenofovir DF 300 mg to their existing regimen demonstrated a statistically significant decline in their plasma HIV RNA by week 24 (-0.58 $\log_{10}$ $DAVG_{24}$, $p < 0.001$). This decline in plasma HIV RNA was durable through week 48 (-0.62 $\log_{10}$ $DAVG_{48}$). The HIV RNA responses among patients with HIV expressing specific types of resistance mutations at baseline are shown in Table 14 in an intent-to-treat analysis and in Table 15 in an as-treated. The

decrease in plasma HIV RNA among patients taking 300 mg of tenofovir DF was similar among patients expressing or not expressing ZDV- or other nucleoside associated (M184V) resistance mutations in their HIV. Patients with HIV expressing the M184V mutation in the absence of nucleoside-associated mutations had the largest decline in HIV RNA among all genotypic groups (-0.91 $\log_{10}$ $DAVG_{24}$ for 300-mg tenofovir DF). These responses were durable through 48 weeks of therapy. Patients with HIV expressing the high-level ZDV resistance mutation T215Y or F (51% of patients), NNRTI-associated, or PI-associated resistance mutations also responded durably to 300 mg of tenofovir DF therapy.

Table 14. HIV RNA responses by selected baseline resistance mutations in study 902 (n = 184, virology intent-to-treat[1])

Baseline Mutation Group	Mean $DAVG_{24}$[2] (n)				p-Value[3]	Mean $DAVG_{48}$[2,4]
	Placebo	75 mg TDF	150 mg TDF	300 mg TDF		300 mg TDF (n)
All Patients	+0.02 (28)[5]	-0.26 (53)	-0.34 (51)	-0.58 (54)	< 0.001	-0.62 (54)
No M184V	+0.28 (10)	-0.32 (15)	-0.30 (15)	-0.48 (22)	0.001	-0.57 (22)
M184V	-0.20 (16)	-0.23 (38)	-0.36 (36)	-0.65 (32)	0.025	-0.64 (32)
M184V / No ZDV-R[6]	+0.07 (4)	-0.31 (13)	-0.55 (9)	-0.91 (9)	0.025	-0.82 (9)
No ZDV-R[6]	+0.19 (6)	-0.30 (16)	-0.64 (12)	-0.61 (14)	0.019	-0.55 (14)
ZDV-R[6]	-0.08 (20)	-0.24 (37)	-0.25 (39)	-0.57 (40)	0.003	-0.64 (40)
ZDV-R[6] / No M184V	+0.24 (8)	-0.32 (12)	-0.14 (12)	-0.60 (17)	0.002	-0.72 (17)
ZDV-R[6] + M184V	-0.29 (12)	-0.20 (25)	-0.30 (27)	-0.55 (23)	0.217	-0.58 (23)
T215Y/F	-0.03 (14)	-0.16 (25)	-0.28 (29)	-0.47 (26)	0.046	-0.61 (26)
T215Y/F / No M184V	+0.29 (6)	-0.21 (9)	-0.26 (9)	-0.54 (11)	0.018	-0.72 (11)
T215Y/F + M184V	-0.27 (8)	-0.13 (16)	-0.28 (20)	-0.41 (15)	0.723	-0.53 (15)
T69D/N	-0.53 (5)	-0.15 (9)	-0.40 (6)	-0.74 (5)	0.676	-0.80 (5)
L74V/I	+0.09 (7)	-0.36 (6)	-0.35 (4)	+0.09 (3)	1.000	-0.11 (3)
NNRTI-R[7]	+0.23 (8)	-0.24 (14)	-0.24 (19)	-0.52 (17)	0.004	-0.63 (17)
Protease Inhibitor-R[8]	-0.03 (18)	-0.29 (27)	-0.29 (27)	-0.61 (33)	0.001	-0.66 (33)

1 Excluding 2 patients without baseline genotypic data.

2 Average HIV RNA changes from baseline through week 24 ($DAVG_{24}$) or week 48 ($DAVG_{48}$) in $\log_{10}$ copies/mL.
3 Wilcoxon rank sum test comparing 300 mg TDF to placebo in the same mutation group.
4 During weeks 24 through 48 placebo patients received 300 mg TDF precluding placebo comparisons.
5 Includes two patients without baseline genotypic data.
6 Zidovudine resistance mutations considered for this analysis are M41L, D67N, K70R, L210W, T215Y/F or K219Q in RT.
7 NNRTI resistance mutations considered in this analysis are K103N or Y181C in RT.
8 PI resistance mutations considered in this analysis are any amino acid substitution at codons 30, 48, 50, 82, 84, or 90 in protease.

Table 15. HIV RNA responses by selected baseline resistance mutations in study 902 (n = 184, virology as-treated[1])

Baseline Mutation Group	Mean $DAVG_{24}$[2] (n)				p-Value[3]	Mean $DAVG_{48}$[2,4]
	Placebo	75 mg TDF	150 mg TDF	300 mg TDF		300 mg TDF (n)
All Patients	+0.16 (28)[5]	-0.16 (52)	-0.32 (47)	-0.52 (53)	< 0.001	-0.49 (53)
No M184V	+0.33 (10)	-0.20 (14)	-0.24 (13)	-0.35 (22)	< 0.001	-0.36 (22)
M184V	+0.08 (16)	-0.14 (38)	-0.34 (34)	-0.64 (31)	< 0.001	-0.59 (31)
M184V / No ZDV-R[6]	+0.06 (4)	-0.15 (13)	-0.58 (8)	-0.86 (8)	0.034	-0.82 (8)
No ZDV-R[6]	+0.19 (6)	-0.19 (16)	-0.62 (10)	-0.51 (13)	0.032	-0.48 (13)
ZDV-R[6]	+0.17 (20)	-0.15 (36)	-0.23 (37)	-0.52 (40)	< 0.001	-0.50 (40)
ZDV-R[6] / No M184V	+0.30 (8)	-0.16 (11)	-0.15 (11)	-0.46 (17)	0.001	-0.49 (17)
ZDV-R[6] + M184V	+0.08 (12)	-0.14 (25)	-0.27 (26)	-0.56 (23)	0.001	-0.51 (23)
T215Y/F	+0.19 (14)	-0.11 (25)	-0.24 (27)	-0.44 (26)	0.002	-0.44 (26)
T215Y/F / No M184V	+0.30 (6)	-0.19 (9)	-0.23 (8)	-0.36 (11)	0.024	-0.39 (11)
T215Y/F + M184V	+0.12 (8)	-0.07 (16)	-0.25 (19)	-0.50 (15)	0.036	-0.48 (15)
T69D/N	-0.03 (5)	-0.05 (9)	-0.40 (6)	-0.65 (5)	0.210	-0.68 (5)
L74V/I	+0.14 (7)	-0.07 (5)	-0.30 (4)	+0.09 (3)	0.820	+0.05 (3)
NNRTI-R[7]	+0.25 (8)	-0.08 (13)	-0.32 (18)	-0.46 (17)	0.005	-0.46 (17)
Protease Inhibitor-R[8]	+0.21 (18)	-0.25 (27)	-0.28 (25)	-0.52 (32)	< 0.001	-0.49 (32)

1 Excluding HIV RNA data after permanent discontinuation of TDF or addition of new antiretroviral therapy.
2 Average HIV RNA changes from baseline through week 24 ($DAVG_{24}$) or week 48 ($DAVG_{48}$) in $\log_{10}$ copies/mL.
3 Wilcoxon rank sum test comparing 300 mg TDF to placebo in the same mutation group.
4 During weeks 24 through 48 placebo patients received 300 mg TDF precluding placebo comparisons.

5 Includes two patients without baseline genotypic data.
6 M41L, D67N, K70R, L210W, T215Y/F or K219Q in RT.
7 K103N or Y181C in RT.
8 Any amino acid substitution at codons 30, 48, 50, 82, 84, or 90 in protease.

The effect of the M184V mutation: Increased tenofovir susceptibility among viruses that express the M184V mutation has been observed *in vitro*. Therefore, an analysis was performed to determine whether patients whose HIV expresses the M184V mutation at baseline respond better than those that have wild-type HIV at RT position 184. In these analyses, a "net treatment effect" was protocol-defined as the difference between the $DAVG_{24}$ response among tenofovir DF-treated patients and placebo-treated patients in the same genotypic group. Although patients with HIV expressing the M184V mutation showed stronger responses to tenofovir DF than patients without M184V with $DAVG_{24}$ values of -0.65 vs. -0.48 log_{10} (Table 14) and -0.64 vs. -0.35 log_{10} (Table 15), respectively, subtracting the responses of the placebo group negated this effect. The in vitro observed hypersusceptibility with a single M184V has not translated in vivo. This result is consistent with the presence of multiple HIV mutations at base line, their dynamism and interactions (which can be synergistic, antagonistic or no effect) which cumulatively modulate the antiviral effects.

Development of RT mutations: Post-baseline genotypic data (week 24- week 48- or early termination) were obtained from 159 of 186 patients who received study medication (110 patients at week 48), with the remaining patients having insufficient HIV RNA to genotype (n = 27). Due to the detection limits of PCR amplification, plasma samples with less than 50 copies/ml of HIV RNA were not evaluated. The last available plasma sample while on treatment was analyzed for any patient who prematurely discontinued study treatment.

Development of nucleoside-associated RT mutations by treatment arm: Any patient with a post-baseline plasma sample showing a mutation resulting in an amino acid substitution

at any of the 16 amino acid residues in RT associated with nucleoside resistance (Resistance Collaborative Group definition; residues 41, 62, 65, 67, 69, 70, 74, 75, 77, 115, 116, 151, 184, 210, 215 and 219 of RT) was considered to have developed a nucleoside-associated RT mutation. Of the 159 genotypically evaluable patients, 79 patients developed one or more of the mutations during the 48-week study; 46 out of the 79 patients (58%) developed them during the 24-week placebo-controlled phase.

Table 16 shows the distribution of patients developing nucleoside-associated RT mutations across the placebo and three treatment arms of the study. During the placebo-controlled phase, 14% of patients in the placebo arm developed a nucleoside-associated RT mutation versus 21%, 37%, and 22% of patients in the 75 mg, 150 mg, and 300 mg tenofovir DF treatment arms, respectively. From logistic regression analyses using the Wald Chi-squared test and from Fisher's exact test comparisons, there were no statistically significant differences in the development of nucleoside-associated RT mutations between placebo and each of the treatment arms. Similarly, in patients developing RT mutations through 48 weeks there was no dose-response across the treatment arms with 34%, 57%, and 39% of patients developing a new nucleoside-associated RT mutation in the three dose groups originally randomized to tenofovir DF therapy. These comparisons suggest that background antiretroviral therapy and not tenofovir DF was responsible for the development of these mutations. Moreover, patients in this study developing nucleoside-associated RT mutations in the 300 mg tenofovir DF treatment group showed continued viral load suppression in HIV RNA at both week 24 and week 48 ($DAVG_{24}$ = -0.59 log_{10} and $DAVG_{48}$ = -0.59 log_{10}, (n = 21) similar to the -0.62 log_{10} decrease in $DAVG_{48}$ observed for all patients treated with 300 mg tenofovir DF (n = 54) or the -0.63 log_{10} $DAVG_{48}$ decrease observed for patients not developing a nucleoside-associated RT mutation (n = 33).

Table 16. Development of selected nucleoside-associated RT mutations by treatment arm in study 902

	Treatment arm				
	Placebo	**75 mg TDF**	**150 mg TDF**	**300 mg TDF**	**P-Value[1]**
% of Patients Developing RT Mutations by Week 24 (number of patients)	14% (4/28)	21% (11/53)	37% (19/51)	22% (12/54)	0.34
% of Patients Developing RT Mutations by Week 48 (number of patients)	NA	34% (18/53)	57% (29/51)	39% (21/54)	0.62

1 Logistic regression analysis and Wald Chi-squared test comparing placebo to tenofovir DF (week 24) or across tenofovir DF doses (week 48).
2 At week 24, patients in the placebo arm began receiving 300-mg TDF treatment.

Development of ZDV-associated RT mutations: The specific amino acid substitutions observed among the patients developing nucleoside-associated RT mutations also suggest that background therapy was responsible for the development of these mutations (Table 17). The majority of the patients (63 of 79) developed typical ZDV-associated mutations while taking either zidovudine, stavudine, abacavir, or lamivudine concomitantly and the majority of these patients (48 of 63) were adding additional ZDV-associated mutations onto a background of pre-existing ZDV resistance mutations. The capacity of stavudine and abacavir to also select for "zidovudine-associated" mutations *in vivo* has been reported.

Table 17. Development of selected antiretroviral-associated HIV mutations by week 48 (intent-to-treat, n = 186) in study 902

RT and Protease Resistance Mutations Developing	Percent of Patients (n)					
	Placebo[1] (n = 28)		75 mg TDF (n = 53)	150 mg TDF (n = 51)	300 mg TDF (n = 54)	Total (n = 168)
	Up to Week	Week 24 to 48				
Nucleoside-Associated: (concomitant ART)	14% (4)	25% (7)	34% (18)	57% (29)	39% (21)	42% (79)
M41L, D67N/G, K70R, L210W/S, T215Y/F/I or K219E/Q/N (d4T, ZDV, ABC or 3TC)	11% (3)	21% (6)	25% (13)	45% (23)	33% (18)	34% (63)[2]
M184V (3TC)		4% (1)	2% (1)	6% (3)	2% (1)	3% (6)
T69D/N (ZDV, ABC or d4T)			6% (3)	6% (3)		3% (6)
L74V/I (ddI, ABC or 3TC)			4% (2)	4% (2)	2% (1)	3% (5)
K65R (ddI or ABC)		4% (1)		2% (1)	4% (2)	2% (4)
A62V (ZDV or d4T)		4% (1)	4% (2)		2% (1)	2% (4)
V75L/A (ddI or d4T)	4% (1)		2% (1)		2% (1)	2% (3)
Y115F (ABC)			4% (2)			1% (2)
F77L (ZDV)					2% (1)	0.5 % (1)
Q151M (ABC and d4T)			2% (1)			0.5 % (1)
Primary NNRTI-Associated: (any change at residues 103 or 181 in RT)		7% (2)	8% (4)	8% (4)	9% (5)	8% (15)[3]
Primary PI-Associated: (any change at residues 30, 32, 48, 82, 84, or 90 in protease)	11% (3)	4% (1)	6% (3)	12% (6)	6% (3)	9% (16)[4]

1 Patients on placebo up-to week 24, 300 mg TDF between weeks 24 and 48
2 48 of these patients also had ZDV-associated mutations at codons 41, 67, 70, 210, 215, or 219 at baseline.
3 5 of these patients also had primary NNRTI-associated resistance mutations at baseline.
4 11 of these patients also had primary PI-associated resistance mutations at baseline.

Development of K65R RT mutations: Four patients (2%) developed the K65R mutation, a RT mutation associated with zalcitabine, didanosine and abacavir use *in vivo*. This mutation has also been shown in HIV cell cultures exposed to tenofovir and in SIV-infected rhesus monkeys treated with tenofovir. All four patients were taking either didanosine (n = 3) or abacavir (n = 1) concomitantly with tenofovir DF (150 mg or 300 mg). Phenotypic analysis of HIV from all four patients was performed and compared to

the patient's baseline HIV susceptibility to tenofovir. Recombinant HIV from the analyzed patients demonstrated a 2.8 to 3.9-fold reduction in tenofovir susceptibility after the acquisition of the K65R mutation, consistent with results from site-directed recombinant viruses expressing only the K65R mutation. In tenofovir treated patients who developed K65R mutation there was no decrease in the viral RNA, consistent with the conclusion that the K65R mutation in HIV RT confers decreased susceptibility to tenofovir .

Development of other nucleoside-associated RT mutations: Twenty-two patients developed one or more RT mutations at the other nucleoside-associated resistance codons 62, 69, 74, 75, 77, 115, 151 or 184. The emergence of mutations at these codons could be correlated with the use of concomitant nucleoside analogs previously shown to select for these mutations (didanosine, stavudine, abacavir and lamivudine). The concomitant antiretroviral therapies that patients were taking are listed in Table 17. At each of these RT residues a new mutation developed in less than 3% of patients. A single patient developed a mutation associated with multinucleoside drug resistance (Q151M) at week 48 while taking tenofovir DF as well as stavudine and abacavir concomitantly. The capacity of stavudine to potentially select for the Q151M multinucleoside resistance complex has been described. Interestingly, this patient has maintained viral load suppression through 60 weeks of tenofovir DF therapy (-0.79 $\log_{10}$ at week 48; -0.61 $\log_{10}$ at week 60), despite the development of the Q151M mutation.

Development of PI and NNRTI-associated mutations: Development of primary resistance mutations to NNRTIs and PIs was infrequent in this trial (8% and 9%, respectively, Table 17). Of the 15 patients who developed a new primary NNRTI-associated resistance mutation during the study period, five already had a primary NNRTI-associated mutation at baseline. Of the 16 patients who developed a primary PI-associated mutation, 11 already had primary PI-associated mutations at baseline. The most common protease mutation that developed in this group was L90M (4% of patients). In this study, there

were no apparent differences in the development of NNRTI- or PI-associated mutations among the treatment groups.

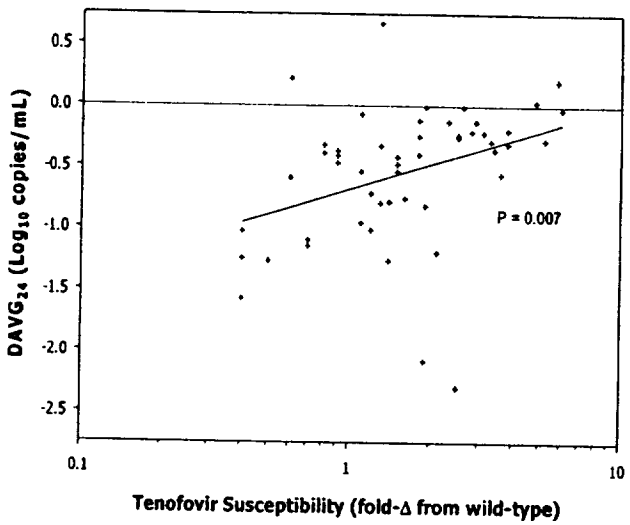
Development of potential novel resistance mutations: An analysis of all other RT residues not previously associated with nucleoside or NNRTI resistance was also performed. There were no RT amino acid substitutions that developed in three or more patients that were not either established polymorphic residues of RT or present in greater than 2% of baseline samples. Thus, from this protocol-specified definition, there was no evidence for the development of "novel" RT mutations that might be associated with resistance to tenofovir DF therapy.

It is important to note that the genotypic analyses reported in these studies have several caveats that limit the interpretation of the applicant's results. For example, in the genotypic analyses, mutations were scored only on a portion of the HIV RT, i.e the amino terminal 1-300 amino acids out of the full length 560 amino acid HIV RT. The carboxy terminal amino acids of the RT contain another important activity (RNase H) that is essential for HIV infection and spread. There may very well be mutations in the unsequenced 300-560 amino acid portion of the RT. The number, type or the contribution of mutations (in this portion of the RT) to alterations in drug susceptibility could not be evaluated by this genotypic analysis. The sequencing method used for identifying mutations selects only for those virus populations that represents 20 or greater percent of the plasma virus pool. The contribution of the minor populations not represented in this sequencing could have significant effects. The patient populations enrolled into the study to begin with (prior to treatment with VIREAD™) have multiple pre-existing mutations representing up to 10% of the sequenced portion of the viral RT. In view of the dynamic nature of these mutations and the mutational interactions including their antagonistic and synergistic effects, selecting a few mutations and attributing the selected mutations to changes in drug susceptibility could lead to misleading interpretations

Baseline Phenotypic Analyses: Baseline phenotypic analyses were attempted for all patients treated with 300 mg tenofovir DF at study entry (n = 54); successful phenotypic results were generated for 53 of these patients. Among these 53 patients with baseline phenotypic results, the mean baseline susceptibility was 1.9 fold above wild-type control for tenofovir (range 0.4 - 6.0) versus > 13.8-fold above wild type for ZDV (range 0.3 - > 150) and > 24.1-fold above wild-type control for lamivudine (range 0.2 - > 54.5). There were a total of four patients who had HIV with > 4-fold reduced susceptibility to tenofovir, including the single patient with the K65R, whose HIV demonstrated 5.2-fold reduced susceptibility to tenofovir. No patients had HIV with > 10-fold reduced susceptibility to tenofovir at baseline.

Baseline phenotype and response to 300 mg tenofovir DF therapy: Linear regression analyses were performed to examine the relationship between the baseline susceptibility to tenofovir and the patient's HIV RNA response to therapy with tenofovir DF during the first 24 weeks of therapy. A positive correlation between the baseline tenofovir susceptibility and $DAVG_{24}$ was observed. This relationship was significant for the as-treated population ($p = 0.007$) and approached significance for intent-to-treat population ($p = 0.053$). Figure 2 graphically depicts this relationship with the as-treated population in a scatter plot. Regression analyses were also performed adjusting for baseline viral load with similar results. These results suggest a pharmacodynamic interaction between the susceptibility of a patient's HIV to tenofovir and their anti-HIV response.

Figure 2. Effect of Baseline Tenofovir Susceptibility on Anti-HIV Response to 300mg mg Tenofovir DF in Study 902 ($DAVG_{24}$, As-Treated)



The HIV RNA response among various strata of baseline susceptibility to tenofovir is shown in Table 18. Patients with baseline tenofovir susceptibility within three-fold of wild-type all responded with $\geq$ 0.5 $\log_{10}$ decreases in HIV RNA which were durable through week 48. In the intent-to-treat analyses, patients with 3- to 4-fold reduced susceptibility to tenofovir responded to 300 mg tenofovir DF therapy (-0.55 $\log_{10}$ copies/ml $DAVG_{24}$ ITT, -0.32 $\log_{10}$ copies/ml $DAVG_{24}$ AT). There were four patients with > 4-fold reduced susceptibility to tenofovir at baseline and, as a group, these patients did not appear to respond to 300 mg tenofovir DF therapy.

Table 18. Response to 300 mg tenofovir DF therapy by baseline tenofovir susceptibility in study 902

Baseline Tenofovir Susceptibility (fold change from wild-	Intent-to-Treat			As-Treated		
	N	$DAVG_{?}$	$DAVG_{48}$[1]	N	DAVG	DAVG
≤ 1.0	14	-0.71	-0.61	14	-0.69	-0.64
> 1.0 and ≤ 2.0	21	-0.63	-0.68	20	-0.59	-0.55
> 2.0 and ≤ 3.0	8	-0.57	-0.56	8	-0.56	-0.53
> 3.0 and ≤ 4.0	6	-0.55	-0.55	6	-0.32	-0.35
> 4.0	4	-0.17	-0.72	4	-0.02	-0.02
All Patients Analyzed	53	-0.60	-0.63	52	-0.54	-0.51

1 Mean $DAVG_{xx}$ for all patients in group (log_{10} copies/ml).

The HIV RNA responses to 300-mg tenofovir DF therapy among patients within various strata of ZDV resistance are shown in Table 19. Patients with < 4-fold ZDV resistance at baseline had HIV RNA responses of -0.68 to -0.73 log_{10} $DAVG_{24}$ that were durable through week 48. Patients with 4 to 10-fold ZDV resistance or > 10-fold resistance to ZDV also responded to 300 mg TDF therapy with ≥ 0.41 log_{10} decreases in HIV RNA ($DAVG_{24}$, intent-to-treat) (Table 19). Although these responses appear diminished when compared to strata with less ZDV resistance, the HIV RNA results suggest the continued activity of tenofovir DF in patients with high-level ZDV resistance.

Table 19. Response to 300 mg tenofovir DF therapy by baseline zidovudine susceptibility in study 902

Baseline ZDV Susceptibility(fold change from wild-type)	Intent-to-Treat			As-Treated		
	N	$DAVG_{24}$[1]	$DAVG_{48}$[1]	N	$DAVG_{24}$[1]	$DAVG_{48}$[1]
≤ 1.0	11	-0.68	-0.77	11	-0.56	-0.55
> 1.0 and ≤ 4.0	21	-0.73	-0.65	20	-0.68	-0.63
> 4.0 and ≤ 10.0	9	-0.41	-0.49	9	-0.48	-0.47
> 10.0	12	-0.43	-0.58	12	-0.31	-0.28
All Patients Analyzed	53	-0.60	-0.63	52	-0.54	-0.51

1 Mean $DAVG_{xx}$ for all patients in group (lo g_{10} copies/ml).

Post-baseline phenotypic analysis: Week 48 phenotypic analyses were attempted for all patients originally treated with 300-mg tenofovir DF. The last available plasma sample was substituted for patients who discontinued treatment prior to week 48. Of the 54 patients in the 300-mg treatment group, a post-baseline phenotype was obtained from 30 patients who also had matching baseline phenotypic results.

Changes in tenofovir susceptibility during treatment: Table 20 shows the distribution of patients developing changes in tenofovir susceptibility over 48 weeks grouped according to the changes from baseline susceptibility. Of the 30 patients with both baseline and post-baseline phenotypic results, identical numbers of patients showed a decrease or no change in tenofovir susceptibility (n = 15), as an increase in tenofovir susceptibility (n = 15), when compared to their baseline. The mean post-baseline fold change in tenofovir susceptibility among all 30 patients was 1.5 fold (Table 20). Six patients had changes in tenofovir susceptibility of greater than 2.5-fold, which corresponds to the threshold of inter-assay variation for the phenotyping assay, with a range of 2.7 to 4.3-fold. For two of these patients, the 48-week tenofovir susceptibility was still within 2-fold of wild-type since hypersusceptibility was observed at baseline. This group of patients also includes the two patients who developed the K65R mutation in this dosing group (each showing a 3-fold decrease in tenofovir susceptibility). For the remaining patients, increasing numbers of zidovudine-associated mutations while taking concomitantly ZDV or stavudine may explain the susceptibility changes.

Table 20. Summary of tenofovir susceptibility changes by week 48 in patients treated with 300 mg tenofovir DF (n = 30) in study 902

Tenofovir Susceptibility Group (fold-change from baseline)	Number of Patients	Patients Developing New NRTI Mutation (%)	Patients with Evidence of Viral Load Rebound[2] (%)
≤ 1.0	15	7 (47%)	5 (33%)
> 1.0 and ≤ 2.5	9	5 (55%)	3 (33%)
> 2.5	6[1]	4[1] (67%)	2 (33%)
All Patients Analyzed	30	16 (53%)	10 (33%)

1. Includes 2 patients who developed the K65R mutation in this dose group; neither patient had evidence of viral load rebound.
2. Viral load rebound defined as a response of ≥ 0.5 $\log_{10}$ decrease in HIV RNA from baseline within the first 12 weeks followed by a confirmed increase in HIV RNA of ≥ 0.5 $\log_{10}$ from nadir by week 48.

Correlation with RT mutations and viral load rebound: Of the 30 patients analyzed, 16 patients (53%) developed new nucleoside-associated mutations during the treatment period. However, with the exception of the development of the K65R mutation, the development of RT mutations did not appear to correlate with changes in tenofovir susceptibility as similar proportions of patients developed mutations regardless of tenofovir susceptibility change (Table 20). Moreover, patients who showed evidence of viral load rebound (≥ 0.5 $\log_{10}$ response followed by confirmed ≥ 0.5 $\log_{10}$ increase in HIV RNA) were equally distributed amongst the tenofovir susceptibility groups (Table 20). The patient who developed the K65R mutation showed evidence of viral load rebound, although they showed a mean decrease in tenofovir susceptibility of 3-fold (Table 21). Overall, these results suggest that changes in tenofovir susceptibility during tenofovir DF treatment are generally low-level and are not associated with viral load rebound.

Table 21. Phenotypic analysis of 300 mg tenofovir DF patients after 48 weeks of treatment in study 902

Patients Developing New NRTI Mutation	N (% ebound[1])	Mean Fold Change in Susceptibility from Baseline					
		Tenofovir	ZDV	d4T	ddI	3TC	ABC
None by Week 48	14 (36%)	1.3	1.7	1.4	1.6	1.3	1.2
Yes by Week 48	14 (36%)	1.5	3.5	1.5	1.4	2.4	1.6
K65R	2 (0%)	3.0	1.4	2.4	1.4	10.7	4.5
All Patients Analyzed	30 (33%)	1.5	2.5	1.5	1.5	2.4	1.6

1 Viral load rebound defined as a response of ≥ 0.5 $\log_{10}$ decrease in HIV RNA from baseline within the first 12 weeks followed by a confirmed increase in HIV RNA of ≥ 0.5 $\log_{10}$ from nadir by week 48.

Changes in susceptibility to other nucleoside analogs: Table 21 shows the mean change in susceptibility for tenofovir, as well as for zidovudine, stavudine, didanosine, lamivudine, and abacavir, for the 30 patients analyzed. When these patients are stratified according to the results of the week 48 genotypic analysis, patients who did not develop nucleoside-associated RT mutations by week 48 showed less than 2-fold changes in susceptibility for all analyzed drugs. Patients whose HIV developed nucleoside-associated RT mutations by week 48 other than K65R showed evidence of decreased susceptibility to ZDV and mildly decreased susceptibility to lamivudine as well. These results are consistent with the identity of the specific mutations that developed in these patients, ZDV-associated RT mutations that also have mild cross-resistance to lamivudine. For the two patients who developed K65R mutations in this phenotypic analysis, decreased susceptibility for lamivudine and abacavir was also observed.

Phenotypic analysis of specific types of NRTI-associated mutations: During the course of the treatment-blinded genotypic analyses, a number of patients developed RT mutations that were specifically investigated for their potential phenotypic effects on tenofovir susceptibility. A total of 18 patients were included in this analysis, including all four patients who developed the K65R mutation, 10 patients who developed ZDV-associated

mutations, two patients who developed T69D/N mutations and two patients who developed an L74V mutation. These phenotypic results are summarized in Table 22. For the patients developing the K65R mutation, a mean reduction in tenofovir susceptibility of 3.2-fold was observed. Patients developing ZDV-associated mutations, while taking either ZDV or stavudine concomitantly, showed a notable decrease in ZDV susceptibility and a minor decrease in abacavir susceptibility, but no significant change in tenofovir susceptibility. The final two groups of patients, representing development of the T69D/N and L74V mutations showed slightly increased susceptibility to tenofovir and ZDV, and mild decreases in susceptibility to abacavir and lamivudine (L74V group only). These results are consistent with the observations from earlier preclinical analyses, which showed the activity of tenofovir against the T69D and the didanosine-associated L74V mutations.

Table 22. Phenotypic analysis of patients developing specific types of NRTI mutations in study 902

Type of Mutation Developing	N	Mean Fold Change in Susceptibility from Baseline					
		Tenofovir	ZDV	d4T	ddI	3TC	ABC
ZDV	10	1.6	6.3	1.9	1.2	1.1	2.7
K65R	4	3.2	1.1	1.7	2.2	19.1	3.2
T69N/D	2	0.8	0.8	1.2	1.0	1.0	3.1
L74V	2	0.6	0.4	1.3	1.8	3.7	6.6

Study 907

Study 907 was a Phase III randomized, double blind, placebo-controlled, multicenter study of the safety and efficacy of tenofovir DF administered orally to HIV-1-infected patients with plasma HIV-1 RNA levels ≥ 400 copies/ml and ≤ 10,000 copies/ml. Patients on stable antiretroviral therapy containing no more than four active agents for ≥ 8 weeks at study entry were randomly assigned in a 2:1 ratio to add either tenofovir DF 300 mg once daily or placebo to their existing regimen in a double-blinded manner.

Patients were stratified by site according to HIV-1 RNA level, CD4 cell count, and number of antiretroviral drugs prior to study entry (see Table 12 for baseline characteristics).

A total of 552 patients were enrolled in this trial, 550 of whom received at least one dose of study medication and represent the protocol-defined intent-to-treat (ITT) population (n = 182 and 368, respectively, for the placebo and tenofovir DF randomization groups). The primary efficacy endpoint was the time-weighted change in $\log_{10}$ HIV-1 RNA from baseline average at week 24 post-randomization ($DAVG_{24}$). There were statistically significant reductions in HIV RNA for patients treated with tenofovir DF (-0.61 $\log_{10}$ copies/ml) as compared to placebo (-0.03 $\log_{10}$ copies/ml, $p < 0.0001$) for this primary endpoint.

A virology substudy of Study 907 was conducted from a cohort of patients randomly assigned throughout the study into the virology substudy. These analyses were prospectively defined. The virology substudy randomization was balanced for all strata that were utilized for the treatment assignment randomization and, additionally, maintained the 2:1 balance of tenofovir DF versus placebo-treated patients. According to the randomization plan, approximately 50% of enrolled patients were included in the genotypic analyses substudy (n = 274) and 50% of these patients were included in the phenotypic analyses substudy (n = 137). Patients without baseline genotypic data were excluded from the analysis creating a virology ITT population of 253 patients. Both the HIV-1 RT and protease genes from banked plasma samples from the patients in the genotyping substudy were genotypically analyzed at baseline, week 24, or upon early termination. Phenotypic analyses of susceptibility to tenofovir and all approved nucleoside analogs were performed at baseline, week 24, or upon early termination for all patients in the phenotyping substudy.

Baseline characteristics and baseline HIV genotypes: The overall mean CD4 cell count at baseline was 427 cells/mm^3, and the mean plasma HIV-1 RNA viral load at baseline was 2291 copies/ml (n = 550). The overall mean duration of prior antiretroviral therapy was 5 years and 5 months. There were no significant differences between the two treatment groups in baseline viral load and CD4 count. Moreover, patients assigned into the virology substudy had a similar overall mean baseline viral load 2239 copies/ml (n = 274) and no significant difference between the treatment groups. Overall, patients in this trial were heavily antiretroviral experienced with an overall mean duration of prior antiretroviral therapy of 65 months. Consistent with the heavy ART experience, base line genotyping showed that the enrolled patients had approximately 10-44 mutation in the 400 amino acid portion of the HIV RT sequence

At baseline, 99% of patients were taking one or more nucleoside analogs with lamivudine and stavudine most commonly used (69% and 59% of patients, respectively). Additionally, 55% of patients were taking at least one protease inhibitor (PI) and 41% of patients were taking an NNRTI. Nelfinavir was the most commonly used PI (29%); nevirapine (20%) and efavirenz (18%) were the most commonly used NNRTIs. There were no significant differences among the treatment groups with regard to baseline ART usage and there were no significant differences in baseline ART usage among patients assigned into the virology substudy (n = 274) in comparison to all patients enrolled in the trial (n = 550).

Baseline HIV genotypic data were obtained from 253 of the 274 patients in the virology genotyping substudy; plasma HIV from 21 patients (14 tenofovir DF; 7 placebo) failed to yield a sufficient PCR product for genotypic analysis. Consistent with the extensive treatment experience of patients in this trial, baseline genotypic analysis revealed that 94% of analyzed patients had plasma HIV expressing one or more primary nucleoside-associated resistance mutations in RT (58% expressed primary PI-associated resistance

mutations, and 48% expressed primary NNRTI-associated resistance mutations (Table 23). Most patients (69%) had HIV with typical ZDV-associated resistance mutations at RT codons M41L, D67N, K70R, L210W, T215Y/F, or K219Q/E/N (mean of 2.8 mutations); 68% had HIV with the lamivudine/abacavir-associated M184V/I mutations; and 45% had both of these types of resistance mutations. The prevalence of each of these baseline resistance mutations is similar across the two treatment arms in the study.

Table 23. Baseline genotypes of selected subgroups of HIV from patients in study 907 (n = 253, virology intent-to-treat)

RT and Protease Resistance Mutations at Baseline	Percent of Patients (n)		
	Placebo (n = 84)	Tenofovir DF (n = 169)	Total (n = 253)
Nucleoside-Associated [1]:	94% (79)	94% (159)	94% (238)
ZDV-R (M41L, D67N, K70R, L210W, T215Y/F, or K219Q/E/N)	73% (61)	67% (114)	69% (175)
M184V/I	64% (54)	70% (118)	68% (172)
T215Y/F	46% (39)	47% (80)	47% (119)
M184V/I + ZDV-R	45% (38)	44% (75)	45% (113)
T69D/N	17% (14)	12% (20)	13% (34)
L74V/I	11% (9)	9% (15)	9% (24)
A62V	1% (1)	3% (15)	2% (6)
V75T/I	1% (1)	2% (4)	2% (5)
K65R		3% (5)	2% (5)
Q151M	2% (2)	1% (2)	2% (4)
T69S Insertions	0% (0)	1% (2)	1% (2)
NNRTI-Associated mutations [2]:	52% (44)	46% (77)	48% (121)
PI-Associated mutations [3]:	62% (52)	57% (96)	58% (148)

1 Mutations M41L, A62V, K65R, D67N, T69D/N, K70R, L74V/I, V75T/I, F77L, Y115F, F116Y, Q151M, M184V, L210W, T215Y/F or K219Q/E/N in RT.

2 NNRTI resistance mutations are K103N, V106A, V108I, Y181C/I, Y188C/L/H, G190A/S/E or P236L in RT.

3 Protease inhibitor resistance mutations are D30N, V32I, G48V, I50V, V82A/F/T/S, I84V or L90M in protease

HIV RNA response to tenofovir DF therapy by baseline HIV genotype: Despite the presence of extensive RT resistance mutations at baseline, patients in the virology genotyping substudy who added tenofovir DF to their existing regimen demonstrated a statistically significant mean decline in their plasma HIV RNA by week 24 (-0.59 $\log_{10}$ $DAVG_{24}$, $p < 0.0001$). This decline was similar to the decline observed among tenofovir DF treated patients in the overall study (-0.61 $\log_{10}$ $DAVG_{24}$, $p < 0.0001$). The HIV RNA responses among patients with HIV expressing specific types of resistance mutations at baseline are shown in Table 24 in an intent-to-treat analysis. In both intent-to-treat and as-treated analyses, treatment with tenofovir DF resulted in statistically significant decreases in plasma HIV RNA among patients expressing ZDV-associated or lamivudine (M184V) mutations in their HIV.

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Table 24. HIV RNA responses by baseline resistance mutations in study 907 (n = 253, virology intent-to-treat)

Baseline Mutation Group	Mean $DAVG_{24}$[1] (n)		Net Treatment Effect [2]	P-Value[3]
	Placebo	Tenofovir DF		
All Patients	-0.03 (84)	-0.59 (168)	-0.56	< 0.0001
No M184V	+0.02 (30)	-0.40 (51)	-0.42	0.0006
M184V	-0.05 (54)	-0.68 (117)	-0.63	< 0.0001
M184V / No ZDV-R[4]	-0.16 (16)	-0.97 (42)	-0.81	< 0.0001
No ZDV-R[4]	-0.18 (23)	-0.85 (54)	-0.67	< 0.0001
ZDV-R[4]	+0.03 (61)	-0.47 (114)	-0.50	< 0.0001
ZDV-R[4] / No M184V	+0.09 (23)	-0.39 (39)	-0.48	0.0002
ZDV-R[4] + M184V	-0.01 (38)	-0.51 (75)	-0.50	< 0.0001
T215Y/F	+0.05 (39)	-0.32 (80)	-0.37	< 0.0001
T215Y/F / No M184V	+0.08 (18)	-0.31 (33)	-0.39	0.002
T215Y/F + M184V	+0.01 (21)	-0.32 (47)	-0.33	0.0018
T69D/N	+0.08 (14)	-0.42 (20)	-0.50	0.002
L74V/I	+0.13 (9)	-0.22 (15)	-0.35	0.027
K65R	0	+0.12 (5)	+0.12	NA[7]
Q151M	+0.05 (2)	+0.38 (2)	+0.33	0.698
T69S Insertions	0	+0.29 (2)	+0.29	NA[7]
NNRTI-R[5]	+0.02 (44)	-0.49 (77)	-0.51	< 0.0001
Protease Inhibitor-R[6]	-0.00 (52)	-0.55 (96)	-0.55	< 0.0001

1 Average HIV RNA changes from baseline through week 24 ($DAVG_{24}$) in log_{10} copies/mL.
2 Difference between $DAVG_{24}$ values of tenofovir DF- versus placebo-treated patients.
3 Wilcoxon rank sum test comparing tenofovir DF to placebo in the same mutation group.
4 Zidovudine resistance mutations are M41L, D67N, K70R, L210W, T215Y/F or K219Q/E/N in RT.
5 NNRTI resistance mutations are K103N, V106A, V108I, Y181C/I, Y188C/L/H, G190A/S/E or P236L in RT.
6 Protease inhibitor resistance mutations are D30N, V32I, G48V, I50V, V82A/F/T/S, I84V or L90M in protease.
7 Not applicable (no comparator patients in placebo arm).

Patients with HIV expressing the M184V mutation in the absence of ZDV-associated mutations had the largest decline in HIV RNA among all genotypic groups (-0.97 log_{10}

$DAVG_{24}$). Patients with HIV expressing the high-level ZDV resistance mutation T215Y or F (47% of patients), NNRTI-associated, or PI-associated resistance mutations also responded significantly to tenofovir DF therapy. Additionally, patients expressing the less common nucleoside-associated RT mutations T69N/D, associated with zalcitabine and other nucleoside therapies, or L74V/I, associated with didanosine or abacavir therapy, also responded significantly to tenofovir DF therapy.

Treatment of HIV infection by abacavir, didanosine and zalcitabine selects the K65R mutation resulting in the reduced susceptibility to these drugs. Both cell culture experiments and SIV-infected Rhesus monkey studies, showed that the K65R mutation is selected by tenofovir treatment. At baseline, five patients had HIV expressing the K65R RT mutation. These five patients were all randomly assigned to tenofovir DF therapy and did not respond to tenofovir DF therapy (+0.12 $\log_{10}$ mean $DAVG_{24}$). This result indicates that patients with HIV expressing the K65R mutation do not respond to tenofovir treatment. Fewer patients assigned to take tenofovir DF had HIV expressing mutations at the multinucleoside drug resistance site Q151M (n = 2) or the multinucleoside resistance insertion mutation after codon T69 (n = 2). Neither of these groups responded to tenofovir DF therapy with mean $DAVG_{24}$ values of +0.38 $\log_{10}$ and +0.29 $\log_{10}$, respectively.

Development of mutations: Post-baseline genotypic data (week 24 or early termination) were obtained from 171 of 274 patients in the genotypic analyses substudy, with the remaining patients having insufficient HIV RNA to genotype (n = 102) or no post-baseline plasma sample (n = 1). Proportionally fewer patients in the tenofovir DF treatment arm (54%) than in the placebo arm (80%) had week 24 genotypic results due to the greater number of tenofovir DF treated patients having insufficient HIV RNA for analysis.

Forty-seven patients developed one or more RT mutations at known nucleoside-associated resistance sites during the first 24 weeks (Table 25). Slightly fewer patients in the tenofovir DF treatment arm than in the placebo arm developed nucleoside-associated RT mutations (15% vs. 22%, respectively, p = 0.17, Fisher's Exact Test). Development of NNRTI- associated mutations were less common, but also occurred less frequently in the tenofovir DF arm (5% vs. 9%, p = 0.17). There were significantly fewer patients developing PI-associated mutations in the tenofovir DF arm than in the placebo arm (2% vs. 8%, respectively, p = 0.02, Fisher's Exact Test). Thus, it appears that tenofovir DF therapy was contributing to the suppression of nucleoside, NNRTI- and PI-associated mutation development, consistent with significant decreases in viral load observed among tenofovir DF-treated patients.

Comparison of data on the emergence of RT mutations in study 902 and 907 show different results. In study 902 there were more RT mutations in the tenofovir DF treatment arm than the placebo arm (Table 16) and in study 907 there were more mutations in the placebo arm than tenofovir DF treatment arm Table 25). The applicant has not offered an explanation for the opposing results between the two studies.

Table 25. Development of selected antiretroviral-associated HIV mutations by week 24 in study 907 (genotyping substudy, n = 274)

RT and Protease Resistance Mutations Developing	Concomitant Nucleoside ART	Percent of Patients (n)		
		Placebo (n = 91)	Tenofovir DF (n = 183)	Total (n = 274)
Nucleoside-Associated:		22% (20)	15% (27)	17% (47)
Any ZDV-Associated [1]*:*		13% (12)	10% (19)	11% (31)[2]
M41L	d4T, ZDV, ddI, ABC, 3TC	3% (3)	4% (8)	4% (11)
K70R/Q/N	d4T, ZDV, ddI, 3TC	3% (3)	3% (6)	3% (9)
D67N	d4T, ZDV, ddI, 3TC	1% (1)	4% (7)	3% (8)
T215Y/F/I	d4T, ZDV, ddI, ABC, 3TC	5% (5)	2% (3)	3% (8)
L74V/I	d4T, ddI, ABC, 3TC	5% (5)	0.5% (1)	2% (6)
K65R	d4T, ZDV, ddI, ABC, 3TC		3% (5)	2% (5)
K219E/Q/R	d4T, ZDV, ddI, ABC, 3TC	2% (2)	1% (2)	1% (4)
L210W/S	d4T, ddI	1% (1)	1% (2)	1% (3)
M184V	ZDV, ABC, 3TC	2% (2)		1% (2)
T69N/I	d4T, ddI	1% (1)	0.5% (1)	1% (2)
V75I/A	d4T, ddI, ABC	1% (1)	0.5% (1)	1% (2)
A62V	ZDV, 3TC	1% (1)		0.4% (1)
Y115F	d4T, ABC, 3TC		0.5% (1)	0.4% (1)
Q151M	d4T, ABC, 3TC		0.5% (1)	0.4% (1)
Primary NNRTI-Associated [3]*:*		9% (8)	5% (9)	6% (17)[4]
Primary PI-Associated [5]*:*	-	8% (7)	2% (3)	4% (10)[6]

1 Zidovudine resistance mutations are M41L, D67N, K70R, L210W, T215Y/F or K219Q/E/N in RT.
2 22 of these patients also had ZDV-associated mutations at codons 41, 67, 70, 210, 215, or 219 at baseline (10 placebo, 12 tenofovir DF).
3 NNRTI resistance mutations are K103N, V106A, V108I, Y181C/I, Y188C/L/H, G190A/S/E, or P236L in RT.
4 11 of these patients also had primary NNRTI-associated resistance mutations at baseline (6 placebo, 5 tenofovir DF).
5 Protease inhibitor resistance mutations are D30N, V32I, G48V, I50V, V82A/F/T/S, I84V, or L90M in protease.
6 7 of these patients also had primary PI-associated resistance mutations at baseline (6 placebo, 1 tenofovir DF).

Development of nucleoside-associated RT mutations: The majority of the patients (31 of 47) who developed nucleoside-associated mutations developed typical ZDV-associated mutations while taking zidovudine, stavudine, abacavir, or didanosine concomitantly.

There were no significant differences in the development of any of the ZDV-associated RT mutations between patients in the placebo and tenofovir DF arms of the study. Development of the D67N mutation appeared to occur more frequently in the tenofovir DF arm, but this was also not statistically significant ($p = 0.28$, Fisher's Exact Test). Among the seven patients who developed a D67N mutation, there was continued viral load suppression (-0.94 $\log_{10}$ $DAVG_{24}$). Overall, the concomitant use of antiretroviral agents known to select for ZDV-associated mutations and their similar distribution between the treatment arms suggests that the concomitant antiretroviral agents were primarily responsible for their development.

Patients who developed nucleoside-associated RT mutations in the tenofovir DF treatment group showed continued viral load suppression in HIV RNA at week 24 (-0.51 $\log_{10}$ $DAVG_{24}$, $n = 27$) similar to the -0.60 $\log_{10}$ decrease in $DAVG_{24}$ observed for all patients treated with tenofovir DF in the virology substudy. Moreover, using the secondary endpoint of absolute change in HIV RNA from baseline, tenofovir DF treated patients who developed nucleoside-associated RT mutations during the first 24 weeks still showed a statistically significant mean HIV RNA decrease of -0.41 $\log_{10}$ at week 24. This suggests continued anti-HIV activity despite the development of these mutations.

Development of K65R RT mutations: Five patients (2% of all patients, 3% of tenofovir DF treated patients) developed the K65R mutation, an RT mutation associated with zalcitabine, didanosine and abacavir in vivo, and also selected by tenofovir in vitro. All five patients were in the tenofovir DF treatment arm. Two of these patients were taking either didanosine or abacavir concomitantly and three were taking lamivudine concomitantly along with tenofovir DF. Among these five patients, there was a notable

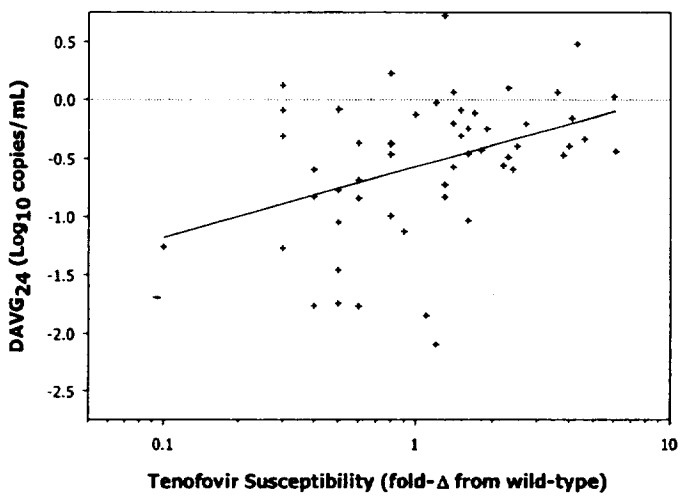
variation in their response to tenofovir DF therapy, with a mean $DAVG_{24}$ of -0.29 log_{10} copies/ml (range of -1.10 to +0.72). Overall, few patients developed the K65R mutation and there was no consistent pattern of HIV RNA increases observed coincident with its development that would reflect treatment failure.

Baseline phenotypic analyses: Baseline phenotypic analyses were attempted for all patients randomly assigned into the virology phenotyping substudy (n = 137) with the [redacted]Antivirogram™ assay. Successful phenotypic results were generated for 85 of these patients (56 tenofovir DF, 29 placebo). Among these 85 patients, the mean number of ZDV-associated resistance mutations was 2.1 and the mean number of NRTI-associated resistance mutations was 3.2. Overall, the mean baseline susceptibility was 1.7-fold above wild-type control for tenofovir versus 7.6-fold above wild type for ZDV and > 31.8-fold above wild type for lamivudine. There were a total of nine patients who had HIV with > 4-fold reduced susceptibility to tenofovir. None of these nine patients had the K65R mutation at baseline, but had multiple nucleoside-associated mutations (mean = 4.8). No patient had HIV with > 10-fold reduced susceptibility to tenofovir at baseline as compared to wild-type HIV.

Baseline phenotype and response to tenofovir DF therapy: Linear regression analyses were performed to examine the relationship between the baseline susceptibility to tenofovir and the patient's HIV RNA response to therapy with tenofovir DF during the first 24 weeks. Using a first order linear model and log_{10} transformed tenofovir susceptibility data, a positive correlation between the baseline tenofovir susceptibility and $DAVG_{24}$ was observed. This relationship was significant for both the intent-to-treat ($p = 0.0051$) and as-treated population ($p = 0.0028$). Figure 3 graphically depicts this relationship with the intent-to-treat population in a scatter plot. Regression analyses were

also performed adjusting for baseline viral load with similar results. In these multivariate analyses, patients with HIV that is more susceptible to tenofovir and those who had higher baseline viral loads had greater decreases in $DAVG_{24}$. The increased response observed in patients who had higher baseline viral loads may be associated with the potential for a larger observed decrease in HIV RNA before the lower limit of quantification of the assay is reached. The results with baseline tenofovir susceptibility suggest a pharmacodynamic interaction between drug susceptibility in vitro and anti-HIV responses in vivo.

Figure 3. Effect of Baseline Tenofovir Susceptibility on Anti-HIV Response to Tenofovir DF in Study 907 ($DAVG_{24}$, Intent-to-Treat)



The HIV RNA response among various strata of baseline susceptibility to tenofovir is shown in Table 26. In intent-to-treat analyses, patients with baseline tenofovir susceptibility within 3-fold of wild-type responded with -0.42 to -0.72 log_{10} decreases in HIV RNA through week 24. There were few patients within the other phenotypic strata,

but there appeared to be a reduction in response consistent with the linear regression modeling. There were only five patients in the tenofovir DF arm with > 4-fold reduced susceptibility to tenofovir at baseline and, as a group, these patients did not appear to respond to tenofovir DF therapy.

Table 26. Response to tenofovir DF therapy by baseline tenofovir susceptibility in study 907

Baseline Tenofovir Susceptibility (fold change from wild-type)	Tenofovir DF		Placebo	
	Mean $DAVG_{24}$[1] (n)		Mean $DAVG_{24}$[1] (n)	
	Intent-to-Treat	As-Treated	Intent-to-Treat	As-Treated
≤ 1.0	-0.72 (25)	-0.74 (25)	-0.05 (13)	-0.04 (13)
> 1.0 and ≤ 2.0	-0.50 (17)	-0.50 (17)	+0.03 (10)	+0.03 (10)
> 2.0 and ≤ 3.0	-0.42 (6)	-0.36 (6)	+0.41 (2)	+0.41 (2)
> 3.0 and ≤ 4.0	-0.27 (3)	-0.27 (3)		
> 4.0	-0.08 (5)	-0.08 (5)	-0.22 (4)	-0.22 (4)
All Patients Analyzed	-0.54 (56)	-0.54 (56)	-0.02 (29)	-0.01 (29)

1 Mean $DAVG_{24}$ for all patients in group (log_{10} copies/mL).

The HIV RNA responses to tenofovir DF therapy among patients within various strata of ZDV resistance are shown in Table 27. In intent-to-treat analyses, patients with < 4-fold ZDV resistance at baseline had HIV RNA responses of -0.72 to -0.93 log_{10} $DAVG_{24}$. Patients with 4 to 10-fold ZDV resistance also responded to tenofovir DF therapy with -0.39 log_{10} decreases in HIV RNA ($DAVG_{24}$). Patients with > 10-fold ZDV resistance appeared to respond more poorly to tenofovir DF therapy (-0.17 log_{10} mean $DAVG_{24}$). Overall, the HIV RNA results suggest the continued activity of tenofovir DF in patients with up to 10-fold ZDV resistance and diminished responses with > 10-fold ZDV resistance.

Table 27. Response to tenofovir DF therapy by baseline zidovudine susceptibility in study 907

Baseline ZDV susceptibility (fold change from wild-type)	Tenofovir DF		Placebo	
	Mean $DAVG_{24}$[1] (n)		Mean $DAVG_{24}$[1] (n)	
	Intent-to-Treat	As-Treated	Intent-to-Treat	As-Treated
≤ 1.0	-0.93 (10)	-0.93 (10)	0.06 (8)	0.07 (8)
> 1.0 and ≤ 4.0	-0.72 (18)	-0.73 (18)	-0.26 (8)	-0.26 (8)
> 4.0 and ≤ 10.0	-0.39 (14)	-0.37 (14)	0.13 (6)	0.13 (6)
> 10.0	-0.17[2] (13)	-0.17[2] (13)	0.05 (7)	0.05 (7)
All Patients Analyzed[3]	-0.54 (55)	-0.54 (55)	-0.02 (29)	-0.01 (29)

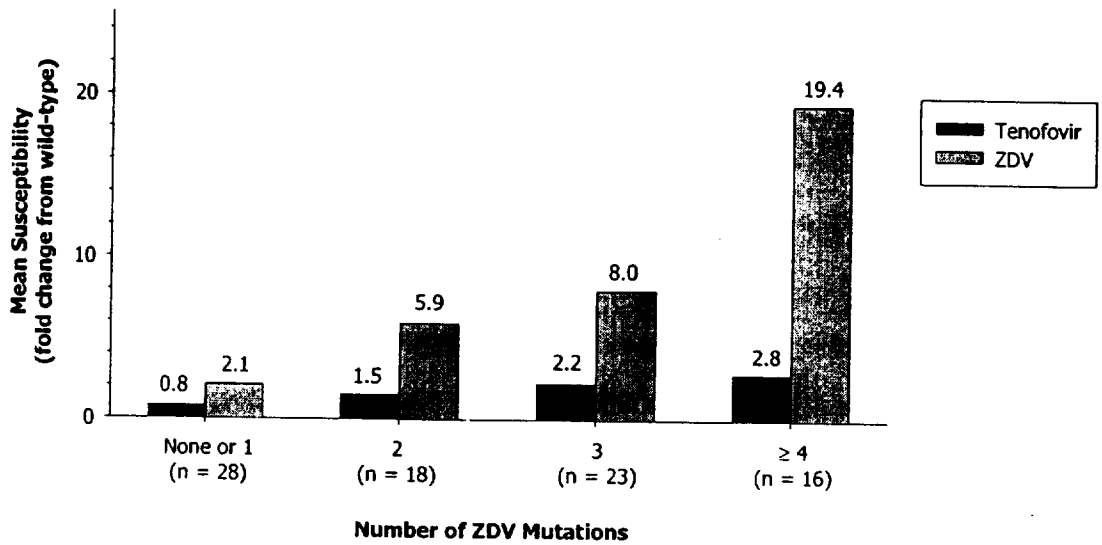
1 Mean $DAVG_{24}$ for all patients in group ($\log_{10}$ copies/mL).
2 Includes two patients with multinucleoside resistant HIV (Q151M and T69S Ins) with DAVG24 values of +0.72 and +0.48, respectively. Excluding these patients the mean DAVG24 for patients with > 10-fold ZDV resistance was -0.31 $\log_{10}$ for both ITT and AT analyses (n=11).
3 One patient (RNR, Pat. ID 407-3070) with baseline tenofovir susceptibility results did not have baseline ZDV susceptibility results.

Relationship between baseline phenotype and genotype

Effect of the M184V Mutation: Previous phenotypic analyses have indicated that the M184V mutation increases the susceptibility of HIV to tenofovir. Among the 85 patients with baseline phenotypic data, an approximate 2-fold increase in tenofovir susceptibility was associated with the M184V mutation among all analyzed patients or among those patients with baseline ZDV resistance mutations. However, this increase in tenofovir susceptibility did not result in a tenofovir DF-specific enhancement of the anti-HIV response. Figure 4 shows the effects of increasing number of ZDV mutations on susceptibility to ZDV and tenofovir among the 85 patients with baseline phenotypic data. In patients with HIV with ≥ 4 ZDV mutations, a mean of 19.4-fold ZDV resistance was observed. Although there is a decrease in susceptibility to tenofovir with greater numbers of ZDV mutations, HIV with ≥ 4 ZDV mutations showed only a mean 2.8-fold reduced susceptibility to tenofovir. This group of 16 patients had $DAVG_{24}$ responses to tenofovir

DF therapy of -0.30 $\log_{10}$ copies/ml (n = 12) versus +0.11 $\log_{10}$ copies/ml (n = 4) for the placebo-treated patients in intent-to-treat analyses.

Figure 4. Baseline comparison of susceptibility to tenofovir and ZDV to number of ZDV resistance mutations



Genotypic correlation of >4 fold reduced tenofovir susceptibility at baseline: Nine of 85 patients demonstrated greater than 4-fold reduced susceptibility to tenofovir at baseline in these analyses. These patients as a group did not appear to respond to tenofovir DF therapy. One patient expressed a T69S insertion mutation that is the likely basis for the 4.3-fold reduced susceptibility to tenofovir. For the remaining patients, a variety of patterns of pre-existing nucleoside-associated mutations were observed all in the presence of the T215Y/F mutation, with a mean of 4.8 nucleoside-associated mutations and a mean of 3.8 ZDV-associated mutations. For comparison, among the 85 patients with a baseline phenotype, the mean number of nucleoside-associated and ZDV-associated mutations was 3.2 and 2.1, respectively. Overall, there were few patients with > 4-fold reduced

susceptibility to tenofovir at baseline and there was no consistent genotypic pattern from which to clearly determine the genotypic basis for the reduced susceptibility.

Post-baseline phenotypic analyses: Week 24 phenotypic analyses were done for all patients randomly assigned into the virology phenotyping substudy (n = 137). Of these 137 patients, a post-baseline phenotype was obtained from 75 patients and 59 of these patients also had matching baseline phenotypic results, permitting a direct comparison of changes in drug susceptibilities over the treatment period (35 tenofovir DF, 24 placebo). As a result of the sensitivity limitation of phenotypic assays to analyze HIV at low copy numbers, fewer patients in the tenofovir DF arm than in the placebo arm had post-baseline phenotypic results reflecting the lower post-treatment HIV RNA levels in patients receiving tenofovir DF.

Changes in tenofovir susceptibility during treatment: Of the 35 tenofovir DF-treated patients with both baseline and post-baseline phenotypic results, the mean post-baseline fold change in tenofovir susceptibility was 2.2-fold (Table 28), slightly greater than the 1.5-fold mean change observed for the 24 placebo-treated patients. The majority of patients analyzed in both arms showed no change or a change of less than 2.5-fold in tenofovir susceptibility from baseline, the threshold of inter-assay variation for the Antivirogram™ assay. Twelve tenofovir DF-treated patients had changes in tenofovir susceptibility of greater than 2.5-fold, with a range of 2.9 to 8.8-fold (6). For five of the 12 patients, the week 24 tenofovir susceptibility was still within 2.5-fold of wild-type since hypersusceptibility was observed at baseline. One patient who developed the K65R mutation showed a 4.3-fold decrease in tenofovir susceptibility. For the remaining seven patients, there was no consistent pattern of genotypic change and, in fact, only one of these seven patients had developed any new NRTI-associated mutation (D67N and K70R). For the two patients with the greatest change in susceptibility (5.8-fold and 8.8-fold), no changes at any RT residue were detected, although the patients had HIV with numerous nucleoside-associated RT mutations at baseline. Overall, there were few

patients with changes in tenofovir susceptibility beyond normal assay variation of 2.5-fold and genotypic analysis of these patients did not suggest the presence of uncharacterized resistance mutations to tenofovir

Table 28. Comparison of baseline and week 24 phenotypic analyses in study 907 (n = 59)

Patients Developing New NRTI Mutation	Mean Fold Change in Susceptibility from Baseline						
	N	Tenofovir	ZDV	d4T	ddI	3TC	ABC
Tenofovir DF Patients	35	2.2	3.6	2.2	1.7	1.4	1.7
None by Week 24	27	2.1	3.2	2.2	1.6	1.3	1.7
Yes by Week 24	6	2.5	5.5	2.4	2.2	1.6	2.0
K65R	2	3.0	2.5	2.2	1.2	2.2	0.5
Placebo Patients	24	1.5	2.6	2.5	1.3	1.8	1.5
None by Week 24	19	1.6	2.4	2.3	1.1	1.3	1.4
Yes by Week 24	5	1.2	3.3	3.2	2.1	3.7	2.2
All Patients Analyzed	59	1.9	3.2	2.3	1.5	1.6	1.6

Correlation with viral load rebound: There was an infrequent development of viral load rebound, as defined by a ≥ 0.5 $\log_{10}$ response followed by confirmed ≥ 0.5 $\log_{10}$ increase in HIV RNA, among the tenofovir DF-treated patients with complete baseline and post-baseline phenotypic analyses (n = 7, 20%). Four of the 12 patients who had > 2.5-fold changes in tenofovir susceptibility had evidence of viral load rebound. Three of the patients had no genotypic changes in HIV RT and the fourth patient had developed both a D67N and K70R mutation. Phenotypic data was obtained from 2 of the 5 patients who developed the K65R mutation (Table 28). They showed a mean decrease in tenofovir susceptibility of 3-fold but no viral load rebound. Overall, these results suggest that reductions in tenofovir susceptibility during tenofovir DF treatment are generally not

associated with viral load rebound and, if they are, there are generally no genetic changes associated with the rebound.

Changes in susceptibility to other nucleoside analogs: Both treatment arms showed similar and low-level mean changes in susceptibility to tenofovir and the other nucleosides during the 24 week period (Table 28). The greatest changes were observed for zidovudine susceptibility and these were most marked in patients who had also developed nucleoside-associated mutations during this period. For the two patients who developed the K65R mutation in this phenotypic analysis, changes in the susceptibility to other nucleoside analogs were within 2.5-fold of the baseline susceptibility results for these nucleoside analogs. Reductions in susceptibility to lamivudine and abacavir might be expected in these patients, however both patients had > 30 and > 6.8-fold reduced susceptibility to these drugs at baseline, respectively, due to pre-existing mutations. Thus, in comparison with placebo, treatment with tenofovir DF is not potentiating the development of resistance to other nucleoside analogs and changes in tenofovir susceptibility are minimal.

CONCLUSIONS

The in vitro antiviral activity studies show that tenofovir, an inhibitor of HIV-RT, blocks HIV replication in a variety of host cell /virus infection test systems. These in vitro nonclinical studies provide support for clinical studies. In the cell /virus infection test systems, the applicant stated that 50% inhibitory concentration of tenofovir was in the range of 1.0 to 6.0 μM. PK studies at the treatment selected dose of 300-mg once daily demonstrated that the C_{max} was 326 ng/ml, which corresponds to approximately 1.0μM of tenofovir in the plasma. Comparison of the in vitro IC_{50} to that available in plasma suggests that the plasma concentration of tenofovir may be subtherapeutic at the 300-mg once daily dose.

HIV variants with reduced susceptibility to tenofovir have been selected in cell culture in vitro. Similarly in the in vivo studies, SIV variants with reduced susceptibility to tenofovir have been found in four out of the four rhesus monkeys infected with SIV. Both of the in vitro and in vivo generated resistant viruses showed mutations primarily at K65R of the viral RT and in some cases at other additional sites. Previous studies showed that zalcitabine, didanosine and abacavir also select for the K65R mutation suggesting potential cross-resistance among these drugs. Only about 3% of the patients in the placebo controlled clinical trials developed the K65R mutation. Tenofovir treatment showed no decrease of the viral RNA in patients with this mutation, suggesting that K65R is a tenofovir selected resistant mutation and that there may be cross-resistance among didanosine, abacavir and tenofovir resistant variants of HIV.

In several of the nonclinical and clinical reports, the applicant attempted to correlate genotypic changes with phenotypic susceptibility, by use of recombinant constructs of HIV instead of the 'pure' HIV isolates themselves. This procedure evaluates local/regional effects of the inserted sequence into the hybrid unlike the global effects manifested by the 'pure' HIV isolates. Results derived from such recombinant constructs may not be extrapolatable to clinical settings. As an example, the hypersensitivity effect of M184V mutation to tenofovir observed in the recombinant constructs (Tables 6 and 7) could not be translated in clinical studies (Tables 14 and 15). This result also emphasizes the importance of mutational interaction effects in the manifestation of antiviral activity of antiretroviral agents.

In the clinical trials conducted, addition of tenofovir-DF to heavily ART experienced (mean of ~5years) patients lead to a significant decrease in the viral RNA through 24 to 48 weeks. There appears to be a low incidence of genotypic and phenotypic resistance to tenofovir during 24 to 48 weeks of tenofovir-DF therapy. In these clinical trials the HIV-

infected subjects were relatively healthy in that their immune system was well preserved with mean CD4 cell count of approximately 400, with a low viral load of approximately 3500 copies/ml. The combination of higher CD4 cells and lower viral load and thus a low existing viral variation and competent immune system due to the higher CD4 cell count provide conditions for minimizing the potential for the emergence of resistance and maximizing the potential for antiviral activity. The antiviral benefit of tenofovir-DF and its ability to bring about mutations in the viral genomes in patients with higher viral RNA and lower CD4 cells remains to be evaluated in future clinical trials.

In preliminary clinical studies, treatment of HIV-infected subjects (study 701 and 901) with tenofovir or tenofovir-DF for ≤28 days, mutations in the viral RT have emerged indicating that tenofovir treatment brings about mutations and thus changes the genetic pools of HIV. The clinical significance of these mutations is unknown, as of yet no follow up on the patients with regard to their viral load and CD4 cell count is available.

The enormous plasticity of HIV due to its inherent genetic variation and rapid replication is reflected in the large number of mutations found in viral sequences from antiretroviral agent challenged isolates. By the early part of the year 2001, there were 107 mutations in the 1-400 amino terminal amino acid sequences of the full length 560 amino acid HIV RT, and 56 mutations in the full length 99 amino acid HIV PR[7]. In the ART experienced patients enrolled in studies 902 and 907 the base line HIV isolates had mutations in up to 44 amino acid positions of the 400 amino acid portion of the HIV RT. In consideration of the large number of existing (background) mutations, the dynamic nature of the mutations, and their recognized antagonistic and synergistic interactions, it is problematic to isolate and attribute the contribution of single, pairs or small groups of mutations to response or lack of response to antiretroviral agents.

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RECOMMENDATIONS

The microbiology section of the draft package insert as currently written is acceptable. With respect to microbiology this NDA is approved.

Phase 4 considerations: The following four microbiology phase 4 commitments were submitted to the applicant:

1. Conduct genotypic and phenotypic analyses of clinical isolates from all adult and pediatric patients in studies 903 and 928 who experience loss of virologic response.
2. Evaluate the virologic response of VIREAD in patients with baseline reduced susceptibility to didanosine and abacavir. Isolates with mutations conferring resistance to didanosine or abacavir should be evaluated in order to discern meaningful differences in virologic response.
3. Characterize the role of the K65R mutation in conferring resistance to VIREAD and cross-resistance between VIREAD and other nucleoside reverse transcriptase inhibitors, specifically didanosine, abacavir and zalcitabine.
4. Investigate whether the M184V increases virologic response, if present alone or in combination with other NRTI mutations. Isolates should be evaluated in order to discern meaningful differences in virologic responses.

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Mechanism of Action: Tenofovir disoproxil fumarate is an acyclic nucleoside phosphonate diester analog of adenosine monophosphate. Tenofovir disoproxil fumarate requires initial diester hydrolysis for conversion to tenofovir and subsequent phosphorylations by cellular enzymes to form tenofovir diphosphate. Tenofovir diphosphate inhibits the activity of HIV reverse transcriptase by competing with the natural substrate deoxyadenosine 5'-triphosphate and, after incorporation into DNA, by DNA chain termination. Tenofovir diphosphate is a weak inhibitor of mammalian DNA polymerases α, β, and mitochondrial DNA polymerase γ.

Antiviral Activity In Vitro: The in vitro antiviral activity of tenofovir against laboratory and clinical isolates of HIV was assessed in lymphoblastoid cell lines, primary monocyte/ macrophage cells and peripheral blood lymphocytes. The IC_{50} (50% inhibitory concentrations) for tenofovir was in the range of 0.04 μM to 8.5 μM. In drug combination studies of tenofovir with nucleoside and non-nucleoside analog inhibitors of HIV reverse transcriptase, and protease inhibitors, additive to synergistic effects were observed. Most of these drug combinations have not been studied in humans.

In Vitro Resistance: HIV isolates with reduced susceptibility to tenofovir have been selected in vitro. These viruses expressed a K65R mutation in reverse transcriptase and showed a 3-4 fold reduction in susceptibility to tenofovir.

Cross-resistance: Cross-resistance among certain reverse transcriptase inhibitors has been recognized. The in vitro activity of tenofovir against HIV-1 strains with zidovudine-associated reverse transcriptase mutations (M41L, D67N, K70R, L210W, T215Y/F or K219Q/E/N) was evaluated. Zidovudine-associated mutations may also confer reductions in susceptibility to other NRTIs and these mutations have been reported to emerge during combination therapy with stavudine and didanosine. In 20 samples that had multiple zidovudine-associated mutations (mean 3), a mean 3.1-fold increase of the IC_{50} of tenofovir was observed (range 0.8 to 8.4). The K65R mutation is selected both in vitro and in some HIV-infected subjects treated with didanosine, zalcitabine, or abacavir;

therefore, some cross-resistance may occur in patients who develop this mutation following treatment with these drugs. Multinucleoside resistant HIV-1 with a T69S double insertion mutation in the reverse transcriptase showed reduced susceptibility to tenofovir.

Genotypic and Phenotypic Analyses of VIREAD in Patients with Previous Antiretroviral Therapy (Studies 902 and 907): See Description of Clinical Studies

In Vivo Resistance:

Post baseline genotyping in Studies 902 and 907 showed that seven of 237 VIREAD-treated patients' HIV (3%) developed the K65R mutation, a mutation selected by VIREAD and other NRTIs in vitro. Among VIREAD-treated patients whose HIV developed NRTI-associated mutations, there was continued HIV RNA suppression through 24 weeks. The rate and extent of tenofovir-associated resistance mutations has not been characterized in antiretroviral naïve patients initiating VIREAD treatment.

Phenotypic analyses of HIV isolates after 48 weeks (Study 902, n=30) or 24 weeks (Study 907, n=35) of VIREAD therapy showed no significant changes in VIREAD susceptibility unless the K65R mutation had developed.

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CONCURRENCES

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_____ Date:_____

HFD-530/TL Micro/F Marsik

cc:

HFD-530/Original NDA

HFD-530/Division File

HFD-530/RPM/M Holloman

HFD-530/Micro/N Battula

APPENDIX-1

GLOSSARY OF ABBREVIATIONS

ABC	Abacavir
AIDS	acquired immunodeficiency syndrome
AK	adenylate kinase
ART	Antiretroviral therapy
AUC	area under the time-concentration curve
BDNA	branched DNA
CC_{50}	Concentration required for 50% cytotoxicity
CD4	antigenic marker on helper/inducer T cells
C_{max}	maximum serum concentration
D4T	Stavudine
DATP	Deoxyadenosine triphosphate
DNA	Deoxyribonucleic acid
DNTP	Deoxynucleoside triphosphate
DTTP	thymidine triphosphate
HAART	highly active antiretroviral therapy
HBV	hepatitis B virus
HBV DNA	hepatitis B virus deoxyribonucleic acid
HBeAg	hepatitis B envelope antigen
HBeAb	hepatitis B envelope antibody

HBsAg	hepatitis B surface antigen
HIV, HIV-1, HIV-2	human immunodeficiency virus, type 1, and type 2
HIV-RT	HIV reverse transcriptase
HPLC	high-pressure (performance) liquid chromatography
IC_{50}	concentration that inhibits 50%
IV	Intravenous
K_I	kinetic inhibition constant
K_m	Michaelis-Menton constant
Mg	milligram(s)
mg/kg	milligrams per kilogram
NADPH	nicotinamide adenine dinucleotide phosphate
ND	not determined
NRTI	nucleoside reverse transcriptase inhibitor
NNRTI	non-nucleoside reverse transcriptase inhibitor
NVP	Nevirapine
PBMC	peripheral blood mononuclear cell
PCR	polymerase chain reaction
PHA	Phytohemagglutinin
PI	Protease inhibitor
PK	Pharmacokinetic
PMEGpp	2-phosphonylmethoxyethylguanosine diphosphate
PMPA	9-[*(R)*-2(phosphonomethoxy)propyl]adenine

PMPAp	Tenofovir monophosphate
PMPApp	Tenofovir diphosphate
Po	Orally
RNA	Ribonucleic acid
RT	reverse transcriptase
RTI	reverse transcriptase inhibitor
Sc	Subcutaneously
SIVmac251	simian immunodeficiency virus macaque strain 251
3TC	Lamivudine
3TCTP	lamivudine triphosphate
TDF	Tenofovir disoproxil fumarate
TK	Thymidine kinase
TP	Triphosphate
ZDV	Zidovudine

APPENDIX-2

KEY TO AMINO ACID CODES

A	Ala	alanine
C	Cys	cysteine
D	Asp	aspartic acid
E	Glu	glutamic acid
F	Phe	phenylalanine
G	Gly	glycine
H	His	histidine
I	Ile	isoleucine
K	Lys	lysine
L	Len	leucine
M	Met	methionine
N	Asn	asparagine
P	Pro	proline
Q	Gln	glutamine
R	Arg	arginine
S	Ser	serine
T	Thr	threonine

V	Val	valine
W	Trp	tryptophan
Y	Tyr	tyrosine