CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

207561Orig1s000

MICROBIOLOGY / VIROLOGY REVIEW(S)

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)

AMENDED VIROLOGY REVIEW

NDA: 207561 **SDN:** 001 **DATE REVIEWED:** 06/29/15

Clinical Virology Reviewer: Lisa K. Naeger, Ph.D.

NDA#: 207,561 **Supporting Document #:** 000

Reviewer's Name(s): Lisa K. Naeger, Ph.D.

Applicant Name and Address:

Gilead Sciences, Inc.
333 Lakeside Drive
Foster City, CA 94404

Initial Submission Dates:

Correspondence Date: November 5, 2014
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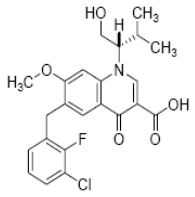
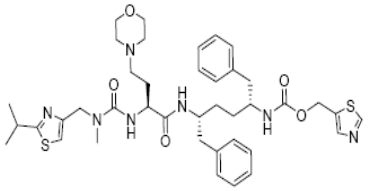
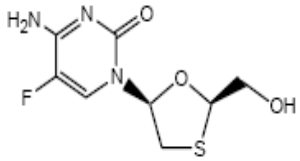
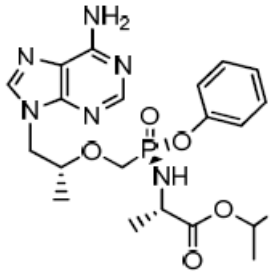
Amendments:

SDN	CDER Stamp Date	Assigned Date
003	11/25/14	01/28/15
005	01/26/15	01/28/15
011	03/13/15	03/16/15
020	05/29/15	06/01/15

Product Name(s): GENVOYA tablet is a fixed-dose combination product containing four active pharmaceutical ingredients, elvitegravir (EVG), cobicistat (COBI), emtricitabine (FTC), and tenofovir alafenamide fumarate (TAF).
Proprietary: GENVOYA
Non-Proprietary/USAN: EVG/COBI/FTC/TAF tablet
Code Name/Number: single-tablet regimen of EVG 150 mg, COBI 150 mg, FTC 200 mg, and TAF (GS-7340) 10 mg

Individual Component	**EVG**	**COBI**
Structure		
Chemical Name	6-(3-chloro-2-fluorobenzyl)-1-[(2S)-1-hydroxy-3-methylbutan-2-yl]-7-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid	1,3-thiazol-5-ylmethyl[(2R,5R)-5-{[(2S)-2-[(methyl{[2-(propan-2-yl)-1,3-thiazol-4-yl]methyl}carbamoyl)amino]-4-(morpholin-4-yl)butanoyl]amino}-1,6-diphenylhexan-2-yl]carbamate
Molecular Formula	$C_{23}H_{23}ClFNO_5$	$C_{40}H_{53}N_7O_5S_2$
Molecular	447.88	776.02

Weight		
Drug Class	INSTI	Pharmacoenhancer (No anti-HIV-1 activity in cell culture)
Supporting Document	IND 72,177; NDA 203093; NDA 203100	IND 102,283; NDA 203094
Individual Component	**FTC**	**TAF**
Structure		
Chemical Names	5-fluoro-1-(2R,5S)-[2-(hydroxymethyl) -1,3-oxathiolan-5-yl]cytosine	L-Alanine,N-[(S)-[[(1R)-2-(6-amino-9H-purin-9-yl)-1-methylethoxy] methyl]phenoxyphosphinyl]-,1-methylethyl ester,(2E)-2-butenedioate (1:1)
Molecular Formula	$C_8H_{10}FN_3O_3S$	$C_{25}H_{33}O_9N_6P$
Molecular Weight	247.24	592.54 Da
Drug Class	NRTI	NRTI
Supporting Document	IND (b) (4); IND 53971; NDA 21500;	IND 63737; IND 111007

Indication(s): Treatment of HIV-1
Dosage Form(s): Tablet (EVG 150 mg, COBI 150 mg, FTC 200 mg, and TAF, GS-7340 10 mg)
Route(s) of Administration: Oral
Recommended Dosage: One tablet taken once daily with food
Dispensed: Rx _X_ OTC ___ (Discipline relevant)
Abbreviations: ABC, abacavir; ADV, adefovir; AIDS, acquired immunodeficiency syndrome; APV, amprenavir; ARV, antiretroviral; ATR, Atripla; ATV, atazanavir; ATV/r, ritonavir-boosted atazanavir; AZT, zidovudine; bp, base pair; CC50, 50% cytotoxic concentration; CI, combination index; COBI, cobicistat; ddI, didanosine; DRV, darunavir; d4T, stavudine; EC50, effective concentration inhibiting viral replication by 50%; EFV, efavirenz; ELISA, enzyme-linked immunosorbent assay; ETR, etravirine; ETV, entecavir; EVG, elvitegravir; FTC, emtricitabine; HAART, highly active antiretroviral therapy; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV,

human immunodeficiency virus; IN, HIV-1 integrase; INSTI, HIV-1 integrase strand transfer inhibitor; LAM, lamivudine; LPV, lopinavir; MDR, multidrug-resistant; mtDNA, mitochondrial DNA; MVC, maraviroc; NDA, new drug application; NNRTI, HIV-1 non-nucleoside reverse transcriptase inhibitor; NRTI, HIV-1 nucleoside/nucleotide reverse transcriptase inhibitor; PBMC, peripheral blood mononuclear cell; PCR, polymerase chain reaction; HIV-1 protease inhibitor; PI/r, PK, pharmacokinetics; PR, HIV-1 protease; QD, once daily; RAL, raltegravir; RBV, ribavirin; RPV, rilpivirine; RT, HIV-1 reverse transcriptase;; RTV, ritonavir; SD, standard deviation; SQV, saquinavir; TAF, tenofovir alafenamide fumarate; TAM, thymidine analogue mutations; TDF, tenofovir disoproxil fumarate; TFV, tenofovir (active moiety of the diester prodrug TDF); TPV, tipranavir; T-20, enfuvirtide; VF, virologic failure; VR, virologic rebound;

TABLE OF CONTENTS

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)

AMENDED VIROLOGY REVIEW

NDA: 207561 **SDN:** 001 **DATE REVIEWED:** 06/29/15

Clinical Virology Reviewer: Lisa K. Naeger, Ph.D.

EXECUTIVE SUMMARY

This NDA for a fixed dose combination of elvitegravir, cobicistat, emtricitabine and tenofovir alafenamide fumarate is approvable from a virology perspective for the treatment of HIV-1 infection in adults and pediatric patients 12 years of age and older who have no antiretroviral treatment history or to replace the current antiretroviral regimen in those who are virologically-suppressed (HIV-1 RNA <50 copies/mL) on a stable antiretroviral regimen for at least 6 months with no history of treatment failure. Elvitegravir (NDA203093) and cobicistat (NDA 203094) were approved for the treatment of HIV-1 infection in September 2014 and emtricitiabine (NDA 21500) was approved for the treatment of HIV-1 infection in July 2003. The fixed dose combination of elvitegravir, cobicistat ,emtricitabine and tenofovir disoproxil fumarate (Stribild) (NDA 203100) was approved in August 2012. (b) (4)

Tenofovir alafenamide fumarate (TAF) is a prodrug that is metabolized intracellularly to the active metabolite, tenofovir diphosphate (TFV-DP). TAF has EC_{50} values ranging from 0.14 to 12.0 nM, with a mean of 3.5 nM, against primary HIV-1 isolates. TAF has the same cytotoxicity profile as tenofovir disoproxil fumarate (TDF) and tenofovir. In addition, TAF and TDF have a similar resistance profile in cell culture and in clinical trials.

In treatment-naïve studies (Study 104 and 111) comparing the efficacy of elvitegravir, cobicistat, emtricitabine and tenofovir alafenamide fumarate (E/C/F/TAF) to elvitegravir, cobicistat, emtricitabine and tenofovir disoproxil fumarate (STRIBILD®; STB), there were a similar number of virologic failures in the E/C/F/TAF and STB arms with a similar resistance pattern. At Week 48, the development of one or more primary elvitegravir, emtricitabine, or tenofovir alafenamide fumarate substitutions associated with resistance was observed in 7 of 14 subjects with evaluable genotypic data from paired baseline and E/C/F/TAF treatment-failure isolates compared with 6 of 17 treatment-failure isolates from subjects in the STB treatment group. Of the 7 subjects with resistance development in the E/C/F/TAF group, the substitutions that emerged were M184V/I (N = 7) and K65R (N = 1) in reverse transcriptase and T66T/A/I/V (N = 2), E92Q (N = 2), E138K (N = 1), Q148Q/R (N = 1) and N155H (N = 1) in integrase. Of the 6 subjects with resistance development in the STB group, the substitutions that emerged were M184V/I (N = 5) and K65R (N = 1) in reverse transcriptase and E92E/Q (N = 2), E138K (n = 3) and Q148R (N = 2) in integrase. In both treatment groups, most subjects who developed substitutions associated with resistance to elvitegravir also developed emtricitabine resistance-associated substitutions.

In a clinical study of virologically-suppressed subjects (Study 109, N = 799) who switched from a regimen containing emtricitabine/tenofovir disoproxil fumarate and a third agent to E/C/F/TAF), one subject had emergent emtricitabine resistance, with the emergence of M184M/I, out of 4 virologic failure subjects.

1.1. RECOMMENDATION AND CONCLUSION ON APPROVABILITY:

This NDA for a fixed dose combination of elvitegravir, cobicistat, emtricitabine and tenofovir alafenamide fumarate is approvable from a virology perspective for the treatment of HIV-1 infection in adults and pediatric patients 12 years of age and older who have no antiretroviral treatment history or to replace the current antiretroviral regimen in those who are virologically-suppressed (HIV-1 RNA <50 copies/mL) on a stable antiretroviral regimen for at least 6 months with no history of treatment failure.

1.2. RECOMMENDATION ON PHASE 4 (POST-MARKETING) COMMITMENTS, AGREEMENTS, AND/OR RISK MANAGEMENT STEPS, IF APPROVABLE:

There are no phase 4 recommendations for this application.

2. SUMMARY OF OND VIROLOGY ASSESSMENTS

2.1 Nonclinical Virology

TAF is a prodrug that is metabolized intracellularly to the active metabolite, tenofovir diphosphate (TFV-DP). TFV-DP is an inhibitor of HIV-1 RT that competes with deoxyadenosine triphosphate (dATP) for incorporation into nascent DNA and terminates the elongation of the viral DNA chain during the process of retroviral reverse transcription and blocks HIV replication. EC_{50} values for TAF ranged from 0.14 to 12.0 nM, with a mean of 3.5 nM, against 26 primary HIV-1 isolates. TAF exhibits anti-HIV activity in lymphoid T-cells, primary human PBMCs, and macrophages with EC_{50} values ranging from 3 to 14 nM. The activity of TAF against HIV-1 in cell culture is 100- to 600-fold greater than TFV and 4- to 6-fold greater than TDF. In addition, TAF has activity against HIV-2 with a mean EC_{50} value of 1.8 nM and is an inhibitor of HBV replication, exhibiting cell culture activity comparable to that of TDF with an EC_{50} value of 18 nM. TAF exhibited minimal antiviral activity against adenovirus, dengue type 2, influenza A, parainfluenza 3, RSV, coxsackie B virus, rhinovirus, HSV-1, HSV-2, HCMV, VZV, vaccinia virus, or HCV.

In MT-2 cells, TAF shows low cytotoxicity with a selectivity index of >10,000. CC_{50} values for TAF ranged from 23.2 μM in MT-4 cells to >53.0 μM in MT-2 T-cells and >44.4 μM in HepG2 cells. A variety of cell culture studies have been conducted to evaluate the potential of TFV and TAF to exert mitochondrial toxicity. Results from cell growth; extracellular production of lactic acid; relative cellular content of mtDNA and mtDNA-encoded cytochrome coxidase II (COX II); and intracellular lipid accumulation studies indicate that TFV has limited capability to inhibit human DNA polymerases or to mediate cytotoxicity or mitochondrial damage. In addition, no antagonistic antiviral interaction was found between TAF and the clinically relevant classes of antiretroviral drugs.

Cell culture resistance selection experiments with TAF selected for the K65R substitution. Phenotypic analyses showed 6.5-fold reduced TAF susceptibility of K65R selected viruses. A K70E substitution as a mixture with wild-type was observed along with a K65R substitution as a

mixture with wild-type in the second experiment with TAF. The K70E substitution has been observed previously in clinical studies of TDF and is associated with slight decreases in tenofovir susceptibility in phenotypic assays. In addition, TAF showed a 2.1- and 5.4-fold reduced susceptibility in cell culture that was associated with the presence of D67N, K70R, M184V, and K219E, and multi-drug resistance Q151M mutation complex (A62V, V75I, F116Y, Q151M) + K65R + TAMs (M41L, K65R, D67N, L210W, and T215Y), respectively.

2.2 Clinical Virology

In the phase 3 active controlled studies, Studies 104 and 111, which assessed the efficacy of the E/C/F/TAF FDC compared with Stribild® (STB) in HIV-infected, ART-naive adults, there were 14 virologic failures in the E/C/F/TAF arm and 17 virologic failures in the STB arm in the FDA-defined virologic failure resistance subset. Of these virologic failures, 7 subjects (50%) had emergent resistance substitutions in the E/C/F/TAF arm and 6 subjects (35%) had emergent resistance substitutions in the STB arm. In the E/C/F/TAF arm, all 7 of the virologic failures with emergent substitutions had the M184V substitution and one subject had the K65R substitution; 5 of these 7 subjects had emergent INSTI resistance substitutions. In the STB arm, 5 of the 6 virologic failures with emergent substitutions had emergent M184V substitutions and one had the K65R substitution; 4 of the 6 subjects had emergent INSTI resistance substitutions. The number and type of emergent NRTI and INSTI resistance substitutions is similar in both arms. TAF did not reduce the frequency of virologic failure or number of emergent resistance substitutions compared to TDF. In addition, the resistance pathways of TAF are similar to TDF.

In Study 109, where virologically suppressed subjects switched from a FTC/TDF regimen to a E/C/F/TAF regimen, 4 subjects in the E/C/F/TAF arm were virologic failures and 1 subject was a virologic failure in the comparison FTC/TDF+ 3rd agent arm. One subject in the E/C/FTAF arm demonstrated genotypic and phenotypic resistance to FTC (M184M/I; FTC FC = 3.8) at Week 8. The other 4 subjects did not have detectable resistance to FTC or TAF.

3. ADMINISTRATIVE

3.1. Reviewer's Signatures

Lisa K. Naeger
Lisa K. Naeger, Ph.D.
Sr. Clinical Virology Reviewer

3.2. Concurrence

HFD-530/MicroTL/J. O'Rear, Ph.D.

4 OND VIROLOGY REVIEW

4.1 Important Milestones in Development

STRIBILD (STB), a fixed-dose combination of elvitegravir (EVG, 150 mg), an HIV-1 integrase strand transfer inhibitor (INSTI), and cobicistat (COBI, 150 mg), a pharmacoenhancer, combined with two FDA-approved nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), emtricitabine (200 mg; Emtriva®) and tenofovir disoproxil fumarate (300 mg; Viread®) was approved on August 27, 2012 for the treatment of HIV-1 infection in antiretroviral treatment-naïve adult patients. This application for a FDC for GENVOYA is a fixed dose combination of EVG, COBI, FTC and the new component, tenofovir alafenamide fumarate (TAF). TAF is a prodrug, which is metabolized to the active metabolite, tenofovir diphosphate – the same active metabolite of TDF.

4.2 Methodology

Criteria for Resistance Testing

Genotyping of the protease (PR)/RT genes and the integrase (IN) gene was performed at screening for all subjects enrolled in Studies GS-US-292-0104, GS-US-292-0111, GS-US-292-0106, and GS-US-292-0112 Cohort 2. For all subjects in Study GS-US-292-0109, historical PR/RT genotypes were used because genotyping was not performed at screening since subjects entered the study with suppressed HIV-1 RNA <50 copies/mL.

Resistance testing was performed for any subject meeting the criteria of the resistance analysis population, which included any subject who received at least 1 dose of study drug, maintained their study drug regimen (or within 72 hours after interruption or discontinuation of study drugs), and met one of the following virologic failure (VF) criteria:

- Suboptimal virologic response (SVR): HIV-1 RNA <1 $\log_{10}$ reduction from baseline and ≥50 copies/mL HIV-1 RNA at the Week 8 visit, confirmed at a scheduled or unscheduled visit at least 2 weeks following Week 8
- Virologic rebound (VR): At any visit, after achieving <50 copies/mL HIV-1 RNA, a rebound in HIV-1 RNA above 50 copies/mL, which was subsequently confirmed at the following scheduled or unscheduled visit;

OR

At any visit, a >1 $\log_{10}$ increase in HIV-1 RNA from the nadir, which was subsequently confirmed at the following scheduled or unscheduled visit

- Viremic at Final Timepoint: Any subject with HIV-1 RNA ≥400 copies/mL at the study endpoint or study discontinuation who did not meet any of the criteria above also had PR/RT and IN genotyping and phenotyping performed.

Ad hoc resistance analyses could be conducted at the request of the medical monitor or the treating physician. A subject was included in the resistance analysis population if the HIV-1 RNA level at failure was ≥400 copies/mL. If a subject remained on study drug and was later suppressed HIV-1 RNA to <50 copies/mL, this subject was not included in the final resistance analysis population. However, these subjects could still have resistance data available which are included.

Resistance testing included genotyping and phenotyping of PR/RT and IN at the VF time point. (b) (4) was the designated reference laboratory for all resistance analyses at screening and virologic failure. Resistance testing was only conducted when HIV-1 RNA was ≥400 copies/mL, which is close to the validated limits of detection of the Monogram Biosciences assays (500 copies/mL). For subjects with confirmed virologic failure, the plasma sample corresponding to the confirmed virologic failure time point (second time point) was analyzed. If needed, the first plasma sample could also have been used for the resistance analysis.

Genotypes/Phenotypes

HIV-1 genotyping of the PR/RT and IN genes were conducted at screening to assess for preexisting resistance as part of enrollment criteria for most studies. As defined in the protocol, subjects were to have genotypic sensitivity to EVG, FTC, and tenofovir. The GenoSure MG® or GenoSure PRIme® assays, which determine HIV-1 PR/RT or PR/RT/IN genotype, respectively, were performed for all subjects at screening in Studies GS-US-292-0104, GS-US-292-0111, GS-US-292-0106, and GS-US-292-0112. The GenoSure MG assay covers the entire PR gene and amino acids 1-400 of RT. The GenoSure PRIme assay covers the entire PR gene, amino acids 1-400 of RT, and the entire IN gene. In cases where subjects had assay failure for both GenoSure MG and GenoSure PRIme, an alternative commercially available assay was used to obtain genotype information. For subjects enrolled in Studies GS-US-292-0104 and GS-US-292-0111 harboring subtype AE virus that failed several IN genotyping assays, IN genotyping was conducted at Gilead using RT polymerase chain reaction (PCR) and standard Sanger population sequencing.

The PhenoSense® GT assay, GenoSure® IN assay, and PhenoSense® IN assay (Monogram Biosciences, South San Francisco, CA) were used to determine subjects' genotypes and phenotypes for PR/RT and IN, respectively, at the time of confirmed virologic failure. For subjects with virologic failure who were missing screening genotype information, baseline plasma samples were evaluated for genotypic and phenotypic resistance. The PhenoSense GT assay tests for genotypic and phenotypic resistance to all currently approved antiretroviral drugs in the NRTI, NNRTI, and PI classes. The GenoSure IN assay tests for IN genotype, while the PhenoSense IN assay tests for IN phenotype. These data were made available to study investigators in real time for cases of suboptimal virologic response and virologic rebound.

Baseline PR, RT, and IN sequences were analyzed for the presence of previously identified resistance-associated substitutions to tenofovir (delivered as TDF or TAF), FTC, and EVG for all studies (Table 1). Detection of resistance substitutions to any study drug excluded treatment-naïve subjects from enrollment.

Post-baseline sequences were compared with subject-specific baseline sequences to determine if resistance-associated substitutions had developed in PR, RT, and/or IN during treatment. A substitution was considered to have emerged if it was detected post-baseline but not at baseline. If a substitution was detected as a mixture at baseline and then resolved to the consensus amino acid post-baseline, it was not considered to have emerged.

Table 1. Previously Identified Drug Resistance Substitutions by Antiretroviral Class

Resistance Mutations[a]		
Drug Class	**Codon Mutations**	
Nucleoside and Nucleotide Reverse Transcriptase Inhibitors (NRTIs)	M41L, E44D, A62V, K65R, D67N, T69 insertion, T69D/N, K70E/R, L74V/I, V75I, F77L, Y115F, F116Y, V118I, Q151M, M184V/I, L210W, T215Y/F, K219E/Q/N/R	
Thymidine Analogue Mutations (TAMs)	M41L, D67N, K70R, L210W, T215Y/F[b], K219Q/N/E/R	
Nucleoside-Associated Mutations (NAMs)	TAMs plus E44D[c], K65R, T69D/N[c], K70E, L74V/I, Y115F, V118I[c], M184V/I	
Multi-NRTI Resistance Mutations	Q151M Complex: A62V, V75I, F77L, F116Y, Q151M	
Multi-NRTI Resistance Mutations	T69 Insertion Complex: T69S-SS, -SA, -SG, or others	
Nonnucleoside Reverse Transcriptase Inhibitors (NNRTIs)	V90I, A98G, L100I, K101E/H/P, K103N/S, V106M/A/I, V108I, E138A/G/K/Q/R, V179D/F/L/T, Y181C/I/V, Y188C/H/L, G190A/E/Q/S, H221Y, P225H, F227C, M230L/I	
Protease Inhibitors (PIs)	**Primary**	**Secondary**
	D30N, V32I, L33F, M46I/L, I47V/A, G48V, I50V/L, I54M/L, Q58E, T74P, L76V, V82A/F/L/S/T, I84V, N88S, L90M	L10I/F/R/V/C, V11I, I13V, G16E, K20I/M/R/T/V, L24I, L33I/V, E34Q, E35G, M36I/L/V, K43T, F53L/Y, I54A/S/T/V, D60E, I62V, L63P, I64L/M/V, H69K, A71V/T/I/L, G73A/C/S/T, V77I, V82I, N83D, I85V, N88D, L89V, I93L/M
Entry Inhibitors	—	G36D/S, I37V, V38A/E/M, Q39R, Q40H, N42T, N43D
Integrase Strand Transfer Inhibitors (INSTIs)[d]	**Primary**	**Secondary**
	T66I/A/K, E92Q/G, T97A, Y143R/H/C, S147G, Q148H/K/R, N155H/S	M50I, H51Y, L68V/I, V72A/N/T, L74M, Q95K/R, G118R, S119P/R/T, F121C/Y, A128T, E138K/A, G140A/C/S, P145S, Q146R/I/K/L/P, V151L/A, S153A/F/Y, E157K/Q, G163K/R, E170A, R263K

a Adapted from the current International Antiviral Society-USA (IAS-USA) Guidelines lists with some modifications.
b Reversion mutations at RT codon T215, including T215A/C/D/E/G/H/I/L/N/S/V have not been definitively shown to be associated with reduced response to either FTC or TDF.
c E44D, T69D/N, and V118I mutations can be natural polymorphisms in RT and have not been shown to be associated with reduced response to either FTC or TDF.
d Primary and secondary IN mutations observed in clinical studies of INSTIs.

4.3 Prior FDA Reviews

The original NDA-203100 for STRIBILD® was reviewed by clinical virologists, Sung Rhee, Ph.D. and Takashi Komatsu, Ph.D. STRIBILD® tablets contain a fixed-dose combination of elvitegravir (EVG, 150 mg), an HIV-1 integrase strand transfer inhibitor (INSTI), and cobicistat (COBI, 150 mg), a pharmacoenhancer, combined with two FDA-approved nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), emtricitabine (200 mg; Emtriva®) and tenofovir disoproxil fumarate (300 mg; Viread®). The proposed indication for the STRIBILD® tablet is for once daily use as a complete regimen for the treatment of HIV-1 infection in adult patients, aged 18 years and over, who are antiretroviral treatment-naïve and have no known substitutions associated with resistance to the individual components of the regimen. It was approved on August 27, 2012 for the treatment of HIV-1 infection in antiretroviral treatment-naïve adult patients, based on Week-48 data from 2 Phase 3 studies and Week-60 data from a supportive Phase 2 study.

4.4 State of Antivirals Used for the Indication Sought

Since highly-active antiviretroviral therapy (HAART) regimens have been introduced, the number of AIDS cases has decreased dramatically; however, HAART does not clear HIV-1 from subjects and even though the number of serum HIV-1 RNA copies is reduced to undetectable levels, HIV-1 re-emerges quickly after discontinuation of HAART. Therefore, with the currently available regimens, it is likely that HIV-infected subjects will require antiretroviral therapy throughout their lives.

There are currently greater than 25 FDA-approved anti-HIV-1 drugs including including integrase strand transfer inhibitors (INSTIs) (dolutegravir, elvitegravir and raltegravir), NNRTIs (delavirdine, efavirenz, etravirine, nevirapine, rilpivirine), NRTIs (abacavir, didanosine, emtricitabine, lamivudine, stavudine, tenofovir, zalcitabine, zidovudine), PIs (atazanavir, darunavir, fosamprenavir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir, tipranivir), the fusion inhibitor enfuvirtide, and the CCR5 coreceptor antagonist maraviroc. NRTIs mimic nucleosides and target HIV-1 RT by competing with natural deoxynucleoside triphosphates for binding to RT and by incorporating into newly synthesized viral DNA resulting in chain-termination. NNRTIs inhibit HIV-1 RT by binding near the catalytic site of RT and acting as noncompetitive inhibitors. Integrase catalyzes the integration of linear viral DNA into host cell DNA forming the provirus. INSTIs bind to the integrase active site and block the strand transfer step of retroviral DNA integration. PIs work at the late stage of viral replication to prevent virus production from infected cells. They block the HIV-1 protease enzyme, which is necessary for the production of mature virions, resulting in defective particles which are unable to infect new cells. Maraviroc inhibits the interaction between the viral envelope glycoprotein gp120 and the human CCR5 receptor membrane protein and inhibits entry of the virus into the cell. Enfuvirtide is a gp41 fusion inhibitor preventing the joining of the viral and cellular membranes necessary for virus entry.

Additionally, there are multiple fixed dose combinations (FDC) of anti-HIV-1 drugs. One FDA-approved FDC, STRIBILD®, contains elvitegravir, emtricitabine, tenofovir disoproxil and cobicistat. Cobicistat, or COBI, was designed as a specific inhibitor of CYP3A, the body's major drug-metabolizing enzyme, for use as a PK enhancer (booster) to increase the systemic levels of coadministered agents metabolized by this enzyme system. Enzyme inactivation studies have demonstrated that COBI is an efficient inactivator of human hepatic microsomal CYP3A activity, with enzyme kinetic parameters (K_i and k_{inact}) comparable to those of ritonavir. CYP3A-mediated oxidative metabolism is the major biotransformation pathway for COBI, as it is for ritonavir; however, unlike ritonavir, COBI is a more specific CYP enzyme inhibitor. It is a weak inhibitor of CYP2D6 and does not inhibit CYP1A2, CYP2C9, or CYP2C19. In addition, COBI displays low liability for induction through activation of xenobiotic receptors, including the aryl hydrocarbon receptor, pregnane X receptor, and the constitutive androstane receptor, in human hepatocytes. In contrast, ritonavir, a known potent pregnane X receptor activator, produces significant induction of phase I enzymes, including CYP3A, as well as phase II uridine 5'-diphospho-glucuronosyltransferase enzymes and drug transporters, including P-gp, that lead to clinically significant drug–drug interactions. Other favorable characteristics of COBI are the absence of HIV-1 protease inhibition and anti-HIV activity in general (half-maximal effective concentration value of >30 μM) and reduced perturbation of the normal adipocyte functions of lipid accumulation and/or response to insulin.

This NDA application is for a fixed dose combination of elvitegravir, cobicistat, emtricitabine and tenofovir alafenamide fumarate. The 3 antiviral components (elvitegravir, emtricitabine and tenofovir alafenamide fumarate) of the new regimen target independently two essential viral enzymes, integrase (IN) and reverse transcriptase (RT), that are required during the early stages of the HIV-1 life cycle. The new component of this FDC is tenofovir alafenamide fumarate, which is a prodrug that is metabolized to the active moiety, tenofovir diphosphate – the same active moiety as the approved NRTI, tenofovir disoproxil fumate (TDF).

5 NONCLINICAL VIROLOGY

Previous nonclinical reports for EVG and COBI have been reviewed in NDA-203100. The following nonclinical review is for TAF.

MECHANISM OF ACTION

Following its release intracellularly from the TAF prodrug bycathepsin A, tenofovir (TFV) is metabolized intracellularly to the active metabolite, tenofovir diphosphate (TFV-DP). TFV-DP inhibits HIV-1, HIV-2 and HBV polymerases, and is an inhibitor of HIV-1 RT that competes with deoxyadenosine triphosphate (dATP) for incorporation into nascent DNA and terminates the elongation of the viral DNA chain during the process of retroviral reverse transcription, thereby effectively blocking the replication of HIV. The kinetic inhibition (K_i) constant for TFV-DP against HIV-1 reverse transcriptase (ribonucleic acid [RNA]-directed DNA synthesis) is 0.02 μM, more than 200-fold lower than its K_i for human DNA polymerase α and more than 3000-fold lower than its K_i values for human DNA polymerases β and γ. Unlike TDF, TAF is relatively stable in human plasma ($t_{½}$ ~90 minutes), but rapidly converts to TFV inside cells. Assessment of the intracellular metabolism of TAF in various types of immune cells including cluster determinant 4 $(CD4)^+$ T-cells, lymphocytes, and monocytes showed efficient conversion of the prodrug to the active metabolite TFV-DP.

ANTIVIRAL ACTIVITY IN CELL CULTURE

TAF was tested in cell culture in activated peripheral blood mononuclear cells (PBMCs) against HIV isolates by (b) (4) The HIV isolates were wild-type clinical isolates, representing HIV-1 isolates from group M (subtypes A to G), groups N and O, as well as HIV-2. Overall, for the 26 primary HIV-1 isolates tested, TAF EC_{50} values ranged from 0.14 to 12.0 nM, with a mean of 3.5 nM (Table 2; Report PC-120-2004 derived from data on Pages 6-8). For the 3 HIV-2 isolates, the mean EC_{50} value was 1.8 nM. TAF antiviral activity was also evaluated against 7 HIV-1 primary isolates with resistance substitutions across multiple drug classes. Five of the 7 isolates were single class resistant mutants, including one NNRTI resistant mutant (NNRTI-R), 2 PI resistant mutants (PI-R), and 2 INSTI resistant mutants (INSTI-R). The 2 remaining isolates had either NRTI resistance (NRTI-R) plus NNRTI-R, or NRTI-R plus PI-R. TAF demonstrated antiviral activity against the NNRTI-R, the PI-R and the INSTI-R resistant mutants. For the 2 viruses that contained NRTI resistance substitutions, TAF showed a 2.1- and 5.4-fold reduced susceptibility that was associated with the presence of D67N, K70R, M184V, and K219E in the first isolate (5705-72), and multi-drug resistance Q151M mutation complex (A62V, V75I, F116Y, Q151M) + K65R + TAMs (M41L, K65R, D67N, L210W, and T215Y) in the second isolate (MDR 769). Overall, TAF showed antiviral activity against all HIV-1 groups/subtypes evaluated, HIV-2, and drug resistant isolates from other classes.

Table 2. Antiviral Activity of TAF in PMBCs

Virus Isolate	EC_{50} (nM)	TC_{50} (nM)	Therapeutic Index
92UG029 Subtype A	8.75	> 500	> 57.1
92UG037 Subtype A	0.69	> 500	> 727
92RW016 Subtype A	0.71	> 500	> 700
93BR021 Subtype B	9.69	> 500	> 51.6
JR-CSF Subtype B	1.05	> 500	> 477
90US873 Subtype B	3.87	> 500	> 129
92BR025 Subtype C	4.16	> 500	> 120
98BR004 Subtype C	1.75	> 500	> 120
93IN101 Subtype C	1.50	> 500	> 333
92UG001 Subtype D	3.79	> 500	> 132
92UG046 Subtype D	0.99	> 500	> 503
92UG024 Subtype D	6.27	> 500	> 79.8
93TH073 Subtype E	1.18	> 500	> 423
CMU06 Subtype E	1.36	> 500	> 368
CMU08 Subtype E	5.88	> 500	> 85.0
93BR019 Subtype F	0.73	> 500	> 682
92BR024 Subtype F	5.97	> 500	> 83.8
93BR020 Subtype F	2.22	> 500	> 225
93BR029 Subtype F	0.14	> 500	> 3,679
G3 Subtype G	5.34	> 500	> 93.7
RU570 Subtype G	12.0	> 500	> 41.7
JV1083 Subtype G	9.85	> 500	> 50.8

BCF02 Group O	1.30	> 500	> 384
BCF03 Group O	3.01	> 500	> 166
BCF07 Group O	0.10	> 500	> 4,873
YBF30 Group N	1.98	> 500	> 252
CDC 310319 HIV-2	2.63	> 500	> 190
CDC 310342 HIV-2	1.96	> 500	> 255
CBL-20 HIV-2	0.91	> 500	> 552
MDR 769 NRTI-R (M41L, K65R,D67N, V75I, Q151M, T215Y) NNRTI R (Y181I) PI-R (M46L,I54V, V82A, I84V, L90M)	18.6	> 500	> 26.8
A-17 NNRTI-R (K103N, Y181C)	5.80	> 500	> 86.2
5705-72 NRTI-R (D67N, K70R, M184V, K219E) NNRTI-R (K103N)	7.09	> 500	> 70.6
1064-52 PI-R (I54V,V82F, L90M)	1.78	> 500	> 281
52-52 PI-R(M46I,I54V, V82T)	1.24	> 500	> 402
8070_1 INI-R (G140S, Y143H, Q148H)	0.67	> 500	> 750
4736_4 INI-R (E92Q, N155H)	0.34	> 500	> 1,293

TAF exhibited antiviral activity against all virus isolates tested in this assay system (range of EC_{50} values = 0.10 nM to 18.6 nM). No cytotoxicity was observed with this compound at the concentrations evaluated (TC_{50} >500 nM). TAF exhibits anti-HIV activity in lymphoid T-cells, primary human PBMCs, and macrophages with EC_{50} values ranging from 3 to 14 nM. The activity of TAF against HIV-1 in cell culture is 100- to 600-fold greater than TFV and 4- to 6-fold greater than TDF. In MT-2 cells, TAF shows low cytotoxicity with a selectivity index (SI) of

>10,000). In addition, TAF is an inhibitor of HBV replication, exhibiting cell culture activity comparable to that of TDF with an EC_{50} value of 18 nM.

A critical step in the intracellular metabolic activation of TAF is mediated by the lysosomal protease cathepsin A (CatA). Intracellular CatA activity together with intracellular metabolism and antiretroviral activity of TAF in $CD4^+$ T cells (CD4s) and monocyte-derived macrophages (MDMs) obtained from a demographically diverse group of donors was assessed. The levels of CatA and intracellular TAF metabolites differed minimally in CD4s and MDMs among 13 tested donors (Table 3; Report PC-120-2017, Page 10). The mean ± SD rate of TFV-alanine and TFV formation (products of the CatA-mediated hydrolysis of TAF) was comparable in cellular extracts prepared from quiescent and activated CD4s (2.7 ± 0.9 and 3.0 ± 0.6 pmol/min•µg, respectively) with not more than a 3-fold difference across individual donors. Overall, CatA activity in MDMs was approximately 2-fold greater than that observed in CD4s, averaging 7.1 ± 3.3 pmol/min•µg, with a range of 3.1 to 13.9 pmol/min•µg among the individual donors.

Table 3. Cathepsin A Activity in Quiescent and Activated Primary Human CD4s and MDMs

Donor	Gender	Ethnicity	TAF Conversion Rate (pmol/min•µg protein)[a]		
			Resting CD4s	Activated CD4s	MDMs
1	F	Caucasian	1.0	2.8	8.2
2	M	Caucasian	2.4	2.9	5.3
3	M	African Descent	4.3	4.2	5.9
4	F	Non-Hispanic White	3.5	3.8	6.7
5	F	Mixed Descent	4.2	3.7	6.1
6	F	Mixed Descent	2.6	2.7	5.3
7	M	Caucasian	2.4	2.9	13.1
8	F	African Descent	2.5	2.9	13.9
9	M	Caucasian	1.7	2.6	10.1
10	F	Hispanic	3.2	2.1	3.1
11	M	Caucasian	2.6	2.9	5.1
12	M	Non-Hispanic White	2.4	2.5	4.4
13	F	Hispanic	2.5	2.9	5.5
Mean ± SD			**2.7 ± 0.9**	**3.0 ± 0.6**	**7.1 ± 3.3**

a The rate of TAF (30 µM) hydrolysis was determined by HPLC analysis of TAF, TFV-Ala, and TFV upon incubation with CD4 and MDM extracts for 10, 30, and 120 minutes. The activity was normalized to the total amount of protein in each cell extract. The rate of CatA-specific activity was calculated over the 3 time points and expressed as picomoles of metabolites produced per minute per microgram protein.

The intracellular accumulation of TAF metabolites in isolated populations of activated $CD4^+$ T cells and MDMs was measured from a subset of 8 donors. Intracellular TFV and TFV-DP were readily detected, as was the intermediate TFV-MP at lower concentrations. Among the 8 donors analyzed, the total mean ± SD intracellular TAF metabolite levels were 20.8 ± 4.9 µM (range

14.5 to 24.5 µM) and 23.5 ± 2.8 µM (range 15.1 to 24.2 µM) in CD4s and MDMs, respectively. Accumulation of parent TFV occurred uniformly within all analyzed samples, indicative of CatA-mediated TAF intracellular activation being consistent across the individual tested donors. Moreover, the intracellular conversion of TFV to TFV-DP also occurred within a narrow 3-fold range both in CD4s and MDMs, indicating that the phosphorylation of TFV occurs uniformly in both target cell types. Taken together, these data demonstrate that the intracellular accumulation and conversion of TAF to TFV-DP occurred consistently in both CD4 and MDM cells across all tested donors.

TAF antiviral activity was assessed in the 13 donors. The mean EC_{50} value of TAF in all tested donors was 11.0 nM (range 6.6 to 19.9 nM) and 9.7 nM (range 2.5 to 15.7 nM) in CD4s and MDMs, respectively. The relative range of TAF antiviral activity across all tested donors was comparable to that of other HIV-1 RT inhibitors. Across all tested donors, TAF had >600-fold and >80-fold lower EC_{50} values than TFV in CD4s and MDMs, respectively, a result consistent with previous findings in T-cell lines. These results indicate consistent intracellular metabolism and antiretroviral activity of TAF in relevant target cells of HIV-1 infection across demographically diverse donors.

Activity of TAF against Other Animal Viruses

TAF antiviral activity was evaluated against a panel of 18 human viruses and compared with TFV by (b) (4) The viruses tested included one or several isolates of the following: adenovirus, dengue type 2, influenza A, parainfluenza 3, RSV, coxsackie B virus, rhinovirus, HSV-1, HSV-2, HCMV, VZV, vaccinia virus, HCV, HIV-1 and SIV. With the exception of the known antiviral activity against HIV-1 and SIV, TAF and TFV exhibited minimal antiviral activity against most of the viruses evaluated in this study with EC_{50} values >1,000 nM and >1,000 µM, respectively (Table 4; Report PC-120-2003, compiled from Tables 3-17 on pages 16-20). Neither TFV nor TAF exhibited cytotoxicity up to the high-test concentrations of 1,000 µM or 1,000 nM, respectively, used for these evaluations.

Table 4. Activity of TAF and TFV against Human Viruses

Virus	TAF	TFV
Adenovirus Type 50	>1,000 nM	>1,000 µM
Dengue Virus Type 2 Strain New Guinea C	>1,000 nM	>1,000 µM
Influenza A Strain A/PR/8/34	>1,000 nM	>1,000 µM
Parainfluenza 3 Strain C 243	843 nM	>1,000 µM
RSV Strain Long	>1,000 nM	>1,000 µM
Coxsackie B3	>1,000 nM	>1,000 µM
Rhinovirus Strain 1B	>1,000 nM	>1,000 µM
HSV-1 Strain HF	>1,000 nM	>1,000 µM
HSV-2 Strain KW	>1,000 nM	29 µM
HCMV Strain AD169	>1,000 nM	>1,000 µM
VZV Strain Ellen	>1,000 nM	>1,000 µM
Vaccinia Strain Western	>1,000 nM	>1,000 µM

Reserve		
HCV RNA Replicons Strain Con1	>1,000 nM	>1,000 μM
Two SIV Strains	1.21 nM 0.51 nM	0.73 μM 0.35 μM
Two HIV-1 Strains	2.04 nM 5.89 nM	1.05 μM 2.30 μM

CYTOTOXICITY

The cytotoxicity of TAF was evaluated in 2 T-lymphoblastoid cell lines (MT-2 and MT-4) and one hepatic cell line (HepG2) following 5 days of continuous compound exposure. CC_{50} values for TAF ranged from 23.2 μM in MT-4 cells to >53.0 μM in MT-2 T-cells and >44.4 μM in HepG2 cells (Table 5; Report PC-120-2007, page 11). The CC_{50} values for the prodrug TDF were comparable to TAF, and ranged from 22.9 μM (MT-4) to 37.1 μM (MT-2) in T-cells and >44.4 μM in HepG2 cells based on the highest concentration tested. TFV, the parent drug for both TAF and TDF, exhibited higher CC_{50} values which ranged from 6,264 μM (MT-4) to 7,605 μM (MT-2) in T-cells and >44.4 μM in HepG2 cells.

Table 5. Cellular Cytotoxicity Evaluation of TAF and other HIV Inhibitors in Multiple Cell Lines after Five Days of Drug Exposure

			Cytotoxicity CC_{50}, μM (MSD)[a]		
			Hepatic	T-Cell	
Compound	Class	Abbrev.	HepG2	MT-2	MT-4
GS-007340	NtRTI	TAF	>44.4 (1)	>53.0 (1)	23.2 (1.13)
GS-004331	NtRTI	TDF	>44.4 (1)	37.1 (1.02)	22.9 (1.04)
GS-001278	NtRTI	TFV	>44.4 (1)	7605 (1.06)	6264 (1.13)

TAF had EC_{50} values of 14.7 nM and 11.6 nM in MT-2 and MT-4 cells, respectively. Based on these values, therapeutic indices of TAF ranged from >3,607 in MT-2 to 1,997 in MT-4 cells. By comparison, TDF exhibited a therapeutic index of 604 in MT-2 cells and 1,167 in MT-4 cells, and TFV exhibited a therapeutic index of 2,265 in MT-2 cells and 438 in MT-4 cells.

In addition, the cytotoxicity of TAF was investigated in resting and dividing human peripheral blood mononuclear cells (PBMCs) over a 5-day continuous drug incubation from up to 10 different donors. The mean CC_{50} values of TAF were 6.8 μM in dividing PBMCs (range 3.4 - 9.3 μM) and 25.1 μM in resting PBMCs (range 6.8 - 43.4 μM), leading to a therapeutic index of >1,900 in dividing PBMCs when compared to the EC_{50} value of 3.5 nM.

Mitochondrial Toxicity

A variety of clinical symptoms observed in HIV-1 patients treated with prolonged NRTI therapy appear to be linked to mitochondrial toxicity. Several representatives of this class of HIV-1 drugs inhibit mitochondrial DNA polymerase γ, by direct binding and competition with the natural deoxyribonucleotide substrate, incorporation into DNA, leading to DNA chain termination.

A variety of cell culture studies have been conducted to evaluate the potential of TFV and TAF to exert mitochondrial toxicity. Results from these studies suggest that TFV have limited capability to inhibit human DNA polymerases or to mediate cytotoxicity or mitochondrial damage. Cell culture combination studies have also been conducted in HepG2 cells to further evaluate the potential mitochondrial toxicity of TFV. HepG2 cells were exposed for 5 days to FTC and TFV (as well as other nucleosides), either alone or in combination. Assay endpoints included cell growth; extracellular production of lactic acid; relative cellular content of mtDNA and mtDNA-encoded cytochrome c oxidase II (COX II); and intracellular lipid accumulation. Tenofovir and FTC alone or in combination with each other or other nucleosides generally had no time- or concentration-dependent effects on cytotoxicity (cell counts) or mitochondrial parameters in HepG2 liver cells. The dual combination of high-dose FTC+ZDV, with or without TFV, appeared to have greater cytotoxicity than the agents alone, but showed no increase in mitochondrial effects.

Tenofovir alafenamide fumarate did not cause a specific depletion of mtDNA in HepG2 cells at concentrations as high as 1.0 µM, a level exceeding the maximum clinical systemic exposure of the 25 mg dose of TAF by more than 2-fold (C_{max} = 0.48 µM; Study GS-US-120-0104). Thus, TAF should have a low potential for inhibiting mtDNA synthesis and inducing NRTI-related mitochondrial toxicities at the anticipated human exposure.

No effect of TFV was seen on the synthesis of mtDNA or lactic acid production in HepG2 human liver cells or in normal human skeletal muscle cells (SkMCs). The results of these studies indicate a low potential for TFV to interfere with mitochondrial functions. These studies confirmed that the potential of FTC and TFV to interfere with mitochondrial functions is low, whether administered alone or in combination with other NRTIs. Further, because administration of TAF results in lower exposure to TFV compared to TDF, the potential for mitochondrial toxicity is also low with the E/C/F/TAF FDC. No additional nonclinical studies were done with the combination of EVG, COBI, FTC, and TAF.

COMBINATION ACTIVITY IN CELL CULTURE

The anti-HIV activity of TAF was examined in combination with representatives from 4 major classes of antiretrovirals in cell culture using HIV-1_{IIIB} infected MT-2 cells. TAF was tested in various combinations with nucleo(t)side reverse transcriptase inhibitors (TFV, and FTC), non-nucleoside reverse transcriptase inhibitors (NNRTI, EFV and NVP), integrase strand transfer inhibitors (INSTI; EVG, RAL, DTG), and protease inhibitors (PI, ATV and DRV). Combinations of ddI + RBV, d4T + RBV, and TAF with itself were used as controls for synergy, antagonism, and additivity, respectively. The combination of TAF with TFV resulted in an additive effect, as expected, as both deliver TFV-DP to the cells. When combined with any of the NRTIs or NNRTIs, TAF exhibited moderate to high synergistic effects, with synergy score values ranging from 41 to 131 (Table 6; Report PC-120-2002, page 9). The combination of TAF with INSTIs resulted in the highest level of synergy (271, 205, and 179 for EVG, RAL, and DTG, respectively). When TAF was combined with PIs, it resulted in moderate synergy, with synergy scores of 96 and 56 for ATV and DRV, respectively. No antagonistic antiviral interaction was found between TAF and the clinically relevant classes of antiretroviral drugs.

Table 6. TAF Anti-HIV-1 Activity in Combination with Selected Antiretrovirals

Drug combination[a]	Class	Net Effect	Synergy Score[b]	Antagonism Score[b]
TAF + TFV	NRTI	Additive	24	-14
TAF + FTC	NRTI	Strong synergy	131	-9
TAF + EFV	NNRTI	Strong synergy	100	-7
TAF + NVP	NNRTI	Minor synergy	41	-14
TAF + EVG	INSTI	Strong synergy	271	-9
TAF + RAL	INSTI	Strong synergy	205	-10
TAF + DTG	INSTI	Strong synergy	179	-10
TAF + ATV	PI	Moderate synergy	96	-10
TAF + DRV	PI	Moderate synergy	56	-12
TAF + COBI	PK enhancer	Additive	17	-22
TAF + TAF	Control	Additive	20	-17
ddI + RBV	Control	Strong synergy	302	-20
d4T + RBV	Control	Strong antagonism	20	-340

a TAF, tenofovir alafenamide; TFV, tenofovir; FTC, emtricitabine; EFV, efavirenz; NVP, nevirapine; EVG, elvitegravir; RAL, raltegravir; DTG, dolutegravir; ATV, atazanavir; DRV, darunavir; COBI, cobicistat; RBV, ribavirin; d4T, stavudine; ddI, didanosine

b Data shown represent the mean from >3 independent experiments performed in triplicate.

Additive to synergistic effects were observed in cell culture combination interaction studies of TFV, the active metabolite of TAF, with NRTIs (abacavir, FTC, lamivudine, stavudine, zalcitabine, zidovudine [ZDV]), nonnucleoside reverse transcriptase inhibitors (NNRTIs) (delavirdine, efavirenz [EFV], nevirapine), PIs (amprenavir, indinavir, nelfinavir, RTV, saquinavir), and the IN inhibitor EVG. No antagonistic interactions were observed for any of these 2-drug combinations in a T lymphoblastoid cell line.

Cathepsin A (CatA) plays an essential role in the intracellular activation of TAF. Since certain viral protease inhibitors such as telaprevir were shown to be inhibitors of CatA, the potential for drug-drug interactions between TAF and viral protease inhibitors was investigated. The HIV protease inhibitors atazanavir, darunavir, lopinavir and ritonavir, as well as boosting agent cobicistat, did not inhibit CatA-mediated hydrolysis of TAF up to a concentration of 50 μM in an enzymatic assay. Similarly, HCV protease inhibitors TMC-435, BI-201355, MK-5172, GS-9256 and GS-9451 showed little-to-no inhibition of CatA, with IC_{50} values ranging from 25 μM to >50 μM.

On the other hand, both boceprevir and telaprevir, two inhibitors of the HCV protease, were identified as inhibitors of CatA-mediated hydrolysis of TAF, with IC_{50} values of 0.3 μM and 0.2 μM, respectively. The effect of these compounds on the antiviral activity of TAF in CD4+ T lymphocytes was measured (Table 7; Report PC-120-2001, page 11). Telaprevir and boceprevir, when tested at their respective C_{max} concentrations of 5.2 μM and 3.3 μM, reduced the activity of TAF by 23-fold and 3-fold, respectively. In contrast, the CYP3A inhibitor cobicistat

and other non-covalent acting HIV and HCV PIs, including darunavir, showed no pharmacological antagonism with TAF. These data indicate co-administering TAF with the irreversible HCV protease inhibitors boceprevir and telaprevir in HIV/HCV co-infected individuals has the potential to adversely affect the intracellular activation and antiviral efficacy of TAF in vivo. The other tested HCV and HIV protease inhibitors, as well as the boosting agent cobicistat, exhibited low potential for interfering with the intracellular conversion of TAF to parent tenofovir.

Table 7. Effect of PIs on the Antiviral Activity of TAF in Primary Human CD4+ T Lymphocytes

Inhibitor Class	Compound	Fold EC_{50} increase in the presence of tested PI[a]	
		TAF[b]	TDF[b]
HIV PI	Darunavir	0.88 ± 0.24	1.39 ± 0.77
	Atazanavir	1.08 ± 0.29	1.47 ± 0.75
HCV PI	**Telaprevir**	**23.93 ± 12.88**	1.64 ± 0.84
	Boceprevir	**2.91 ± 0.40**	1.32 ± 0.74
	TMC-435	1.07 ± 0.19	1.67 ± 1.13
CYP3A inhibitor	Cobicistat	0.94 ± 0.20	1.80 ± 1.17
Factor Xa inhibitor	Apixaban	1.68 ± 0.24	1.07 ± 0.11
	Rivaroxaban	1.23 ± 0.80	1.17 ± 0.40
Thrombin inhibitor	Argatroban	1.55 ± 1.15	1.27 ± 0.11
	Dabigatran	1.39 ± 0.27	1.20 ± 0.69
DPP4 inhibitor	Sitagliptin	1.49 ± 1.44	0.97 ± 0.21

a Fold change in TAF and TDF EC_{50} values (mean ± standard deviation) obtained in CD4+ T cells from 4 donors performed in triplicate, expressed as an EC_{50} ratio (with test compound/without test compound).
b EC_{50} (TAF) = 5.6 ± 1.2 nM; EC_{50} (TDF) = 3.1 ± 1.0 nM, determined from at least 4 donors assayed in triplicate.

RESISTANCE DEVELOPMENT IN CELL CULTURE

Two resistance selection experiments with TAF and TFV were conducted in parallel. TFV is used in cell culture instead of TDF in these experiments due to the limited stability of TDF in culture media. The first experiment started at concentrations of TAF and TFV below the EC_{50} value for each drug and the second experiment started at concentrations of TAF and TFV corresponding to twice the EC_{50} value for each drug. The duration of the experiments was >115 days and >147 days for the first and second experiment, respectively. In all 4 selections, the outcome of the experiments was nearly identical for both drugs with the development of the K65R substitution in reverse transcriptase, which was substitution accompanied by an S68N substitution in 3 of 4 cases, either as a full substitution or as a mixture. The development of the S68N substitution alongside K65R has been observed previously in TDF/TFV studies both clinically and in cell culture where it may play a role in restoring reduced replication capacity induced by the K65R substitution. The L214F polymorphism was observed in the first experiment for both drugs and was also observed in the no drug control. Therefore, this polymorphism may be present at low levels in the starting virus quasispecies. A K70E substitution as a mixture with wild-type was observed along with a K65R substitution as a mixture with wild-type in the second experiment with TAF, but was not detected at subsequent

time points. This K70E substitution has been observed previously in clinical studies of TDF and is associated with minor decreases in TFV susceptibility in phenotypic assays. TAF and TFV concentrations at which the K65R substitution first appeared ranged from 4X to 15X the EC_{50} value. The K65R substitution developed from approximately 45 - 90 days in both TAF and TFV selections. Attempts to increase the drug concentrations beyond 16X EC_{50} value of each drug (to 24X EC_{50} or 336 nM for TAF, and 24X EC_{50} or 84 µM for TFV) over >5 weeks in the second experiment did not yield additional resistance nor viable virus.

Phenotypic analyses were conducted with the two final mutant viruses obtained in the second selection experiment. The fold changes from wild-type control observed for the viruses selected either by TAF or TFV were similar, with TAF activity reduced 6.5-fold for both selected viruses and TFV activity reduced 5.5- and 5.1-fold for the TAF and TFV selected viruses, respectively (Table 8; Report PC-120-2011, page 12). Reduced FTC susceptibility was also observed at similar levels between the 2 selected viruses (8.5- and 6.7-fold from wild-type), while susceptibility to the control drugs EFV (NNRTI) and EVG (INSTI) was near wild-type levels. These data provide evidence that TAF and TFV have the same resistance profile in selection experiments, resulting in a mutant virus with the K65R substitution with similar phenotypic changes.

Table 8. Genotype and Phenotype of Selected Resistance Viruses

Selected Viruses				EC_{50} Fold Change (FC) from wild-type control (HIV-1_{IIIB})[a]				
Drug	Concentration (FC over EC_{50})	Time Point	Genotype	TAF	TFV	FTC	EFV	EVG
TAF	224 nM (16X)	day 148	K65R	6.5*	5.5*	8.5*	1.4	1.7
TFV	56 µM (16X)	day 154	K65R S68S/N/R/K	6.5*	5.1*	6.7*	1.5	1.4

a EC_{50} against HIV-1_{IIIB} in MT-2 standard assay was 10 nM, 2.9 µM, 1.2 nM, 0.77 µM, and 1.5 nM for TAF, TFV, EVG, FTC, and EFV respectively. TAF: tenofovir alafenamide; TFV: tenofovir; FTC: emtricitabine; EFV: efavirenz; EVG: elvitegravir. Fold changes of the average EC_{50} were obtained from 5 independent experiments.
(*) t-test *p*-value <0.05 as compared to wild-type control.

Resistance selection experiments using HIV-1 isolates with pre-existing TDF-resistance (K65R, 3 thymidine analog substitutions, and Q151M complex) were carried out with TAF, TFV, and raltegravir (RAL), to investigate the potential for additional resistance development in the presence of TAF/TFV. The 3 NRTI-resistant clonal HIV-1 isolates HIV-$1_{LAI\text{-}K65R}$, HIV-$1_{LAI\text{-}3TAM}$, and HIV-$1_{LAI\text{-}Q151M}$ have phenotypic resistance to TAF and TFV measured at 3-fold, 3-fold, and 13-fold above wild-type, respectively, and all 3 isolates have near wild-type susceptibility to the RAL. The selection experiments were initiated using drug concentrations corresponding to 2-times the respective EC_{50} values for TAF, TFV, and RAL.
With the mutant isolate HIV-$1_{LAI\text{-}K65R}$, the maximum viable TAF or TFV concentration supporting virus growth was 3X the starting concentration for TAF and 2X the starting concentration for TFV (18- and 12-fold increase based on the wild-type EC_{50} values, respectively). The selected viruses acquired the S68N substitution. Phenotypic analyses of the 3 resulting mutant viruses showed 5.9- to 6.8-fold decreased susceptibility to TAF, and 5.9- to 6.3-fold reduced susceptibility to TFV (Table 9; Report PC-120-2012, page 17). These phenotypic fold changes for viruses with K65R plus S68N are consistent with the fold changes observed in the wild-type selections.

Table 9. Drug Susceptibilities of Selected Resistant Viruses Starting from TDF-Resistant HIV-1

Virus Name	Selecting Drug	Drug Concentration (FC from WT EC_{50})[a]	Genotype (reverse transcriptase, RT; integrase, IN)[b]	EC_{50}s and Fold Change (FC) from Wild Type Control ($HIV\text{-}1_{LAI}$)[c]				
				TAF	TFV	RAL	AZT	EFV
$HIV\text{-}1_{LAI}$			RT: WT	0.014	2.6	0.003	0.17	0.002
WT-A3	TAF	120 nM (8X)	RT: **K65R**	3.5*	3.7*	1.0	0.7	0.6
WT-F3	TFV	28 µM (8X)	RT: **K65R, S68N**	5.2*	5.4*	1.6	1.2	0.6
WT-R6	RAL	640 nM (64X)	RT: WT IN: **E92Q, V151I**	0.9	1.0	13.4*	1.0	0.7
WT-ND	None	NA	RT: WT	1.0	1.1	1.9	0.7	1.0
$HIV\text{-}1_{LAI\text{-}K65R}$			RT: K65R	4.4*	4.9*	1.4	0.9	0.9
K65R-A2	TAF	180 nM (12X)	RT: K65R, **S68N**	5.9*	6.3*	1.9	2.8*	1.3
K65R-A2.5	TAF	270 nM (18X)	RT: K65R, **S68N**	6.8*	6.3*	1.5	2.3*	1.0
K65R-F2	TFV	42 µM (12X)	RT: K65R, **S68N**	6.1*	5.9*	1.2	2.2	1.4
K65R-R6	RAL	640 nM (64X)	RT: **WT** (loss of K65R) IN: **E138K, Q148R, V151I**	0.8	1.0	>200*	0.9	0.7
K65R-ND	None	NA	RT: **WT** (loss of K65R)	1.0	1.1	2.2	0.9	1.1
$HIV\text{-}1_{LAI\text{-}3TAM}$			RT: M41L, Y181C, G190A, L210W, T215Y ("3TAM") IN: WT	3.8*	3.7*	1.1	>90*	>54*
3TAM-A2	TAF	180 nM (12X)	RT: "3TAM" + **L429I**	8.5*	6.1*	2.2*	>90*	>54*
3TAM-A2.5	TAF	270 nM (18X)	RT: "3TAM" + **L429I**	8.6*	6.1*	1.9	>90*	>54*
3TAM-F2	TFV	42 µM (12X)	RT: "3TAM" + **L429I**	9*	6.1*	1.7	>90*	>54*
3TAM-F2.5	TFV	63 µM (18X)	RT: "3TAM" + **L429I**	ND	ND	ND	ND	ND
3TAM-R6	RAL	640 nM (64X)	RT: "3TAM" IN: **Q148R, D232N**	4.8*	4.9*	>204*	>90*	>54*
3TAM-ND	None	NA	RT: "3TAM"	3.5*	4.2*	2.1*	>57*	>54*
$HIV\text{-}1_{LAI\text{-}Q151M}$			RT: A62V, K65R, S68G, V75I, F77L, F116Y, Q151M ("Q151M") IN: WT	18.6*	19*	1.7	>90*	3.3*
Q151M-A1.5	TAF	585 nM (39X)	RT: "Q151M" + **T69I**	26.1*	22.1*	1.5	>90*	3.4*
Q151M-F1.5	TFV	137 µM (39X)	RT: "Q151M" + **T69I**	32.5*	34.5*	2.2*	>90*	2.7*
Q151M-R6	RAL	640 nM (64X)	RT: " Q151M" IN: **L74M, Q148R, S230R**	19.3*	15.7*	>264*	>90*	3.0*
Q151M-ND	None	NA	RT: " Q151M"	15.3*	15.2*	2.0	>90*	2.4*

a Concentrations based on previously defined EC_{50}s against $HIV\text{-}1_{LAI}$ in MT-2 assay of 0.015, 3.5, and 0.010 µM for TAF, TFV, and RAL, respectively.
b Boldface font indicates changes from starting material. WT IN sequences not shown.
c Fold changes (FC) calculated from EC_{50}s for HIV-1LAI shown in first row (shaded). TAF: tenofovir alafenamide; TFV: tenofovir; RAL: raltegravir; AZT: zidovudine; EFV: efavirenz. Data averaged from at least 4 independent experiments.
(*) P-value <0.01 (student t-test comparing mutant EC_{50}s with wild-type EC_{50}s)
NA: not applicable; WT: wild-type; ND: not determined.

The maximum viable TAF or TFV concentration supporting virus growth when starting with the mutant isolate $HIV\text{-}1_{LAI\text{-}3TAM}$ (M41L, L210W, T215Y) was 3X the starting concentration for either drug (18-fold increase based on the wild-type EC_{50} value). The selected viruses acquired the RT substitution L429I, which is located in the RT connection domain. This substitution has not been previously seen or characterized, as it lies beyond the range of standard genotypic assays (amino acid residues 1-400). Phenotypic characterization was obtained for only 3 of the 4 viruses selected with TAF or TFV, due to very weak infectivity of one virus. A 2- to 3-fold

increase for both TAF and TFV was observed for all 3 viruses with the added L429I substitution compared to HIV-$1_{LAI-3TAM}$ (Table x).

For the HIV-$1_{LAI-Q151M}$ isolate (A62V, K65R, S68G, V75I, F77L, F116Y, and Q151M), the maximum TAF or TFV concentration supporting virus growth was 1.5X the starting concentration for either drug (39-fold increase based on the wild-type EC_{50} value) and the viruses obtained acquired the RT substitution T69I. Phenotypic susceptibilities to TAF and TFV for the 2 selected viruses were very similar for the 2 drugs, ranging from 22.1- to 34.5-fold above wild-type, within 2-fold of the value for the starting material that was determined concurrently (Table 9). The sponsor states that this indicates a very limited impact on resistance for the emerging T69I substitution in that isolate.

Viral growth was sustained at much higher concentration with the control compound RAL (32X starting concentration), where the virus acquired the INSTI-resistance substitutions E138K and Q148R with >200-fold decrease in RAL susceptibility when starting from the HIV-$1_{LAI-K65R}$ (Table 9). The virus acquired the INSTI-resistance substitution Q148R with >204-fold decrease in RAL susceptibility when starting from HIV-$1_{LAI-3TAM}$, and acquired the INSTI-resistance substitutions L74M and Q148R with >264-fold decrease in RAL susceptibility when starting from HIV-$1_{LAI-Q151M.}$

Extended resistance selection of these viruses with TAF or TFV did not lead to the accumulation of additional known resistance-associated substitutions, or phenotypic fold-change increases above 2.5-fold, after 6 months in culture. Viral survival in the presence of drug could not be sustained above a 2-3X drug increase, indicating a lack of alternative resistance pathways for the viruses. In contrast, resistance selections with RAL resulted in mutant viruses with high-level of phenotypic resistance to RAL. Interestingly, the K65R mutant reverted to wild-type in the absence of TAF or TFV selection pressure after 6 months in culture, confirming its fitness defect in the absence of drug.

Viral breakthrough experiments were conducted with TAF and TFV in MT-2 cells using TDF-resistant viruses in order to compare the antiviral effect of TAF and TFV at concentrations adjusted to reflect the ≥5X higher concentration of TFV-DP observed in vivo when dosed with TAF vs. TDF. TAF inhibited viral breakthrough for the duration of the experiment (28 days) for most viruses (8 of 10) with the exception of viruses with 5 TAMs (Table 10; Report PC120-2013, consolidated from Tables on pages 7 and 10). In contrast, viral breakthrough was only inhibited for 1 of 10 viruses in the presence of TFV with breakthrough ranging from 4-18 days.

Table 10. Time to Viral Breakthrough of TFV-Resistant Viruses

Isolate	Mutant Category	RT Sequence	TAF EC_{50} FC from WT*	TFV EC_{50} FC from WT*	Time to Viral BT TAF	Time to Viral BT TFV
PD9	3TAMs	D67N K70R M184V K219Q	4.3	3.7	>28 days	>28 days
PD6	3TAMs	M41L L210W T215Y	3.9	3.1	>28 days	13 days

PD20	3TAMs	M41L L210W T215Y	10.3	7.2	>28 days	4 days
K65R	K65R	K65R	3.3	3.3	>28 days	18 days
PD11	K65R	K65R M184V	3.3	3.1	>28 days	4 days
PD15	Q151M/K65R	M41L A62V K65R T69I K70T L74V V75I Y115F F116Y Q151M M184V	5.9	4.1	>28 days	8 days
PD25	4TAMs	D67N K70R T215F K219Q	6.1	5.1	>28 days	8 days
PD30	T69ins	D67E T69SSG	10.1	10.1	>28 days	4 days
PD31	5TAMs	M41L D67N T69D L210W T215Y K219R	25.5	21.9	8 days	4 days
PD34	5TAMs	M41L D67N L210W T215Y K219R	14.8	14.7	8 days	4 days

*MT-2 Assay

CROSS-RESISTANCE

The antiviral phenotypic susceptibility of TAF and TFV was analyzed against a panel of 24 patient-derived HIV-1 recombinant isolates in the Monogram Biosciences PhenoSense assay (Table 11; Report PC-120-2014, page 8). The mutants in the panel were chosen to represent a wide array of NRTI resistance substitutions and were expected to display a wide spectrum of TFV susceptibilities. In the PhenoSense assay, clinical susceptibility cutoffs for TDF have been established at 1.4. The panel of recombinant mutants showed TFV fold changes from wild-type ranging from 0.41 to 20, with 11 isolates showing sensitivity to TFV and 13 isolates showing resistance to TFV. Isolates resistant to TFV had either 6 TAMs, Q151M complex + K65R, or double insertions at T69 + other NRTI substitutions and/or TAMs. The highest level of resistance to TFV was observed for isolates with T69 double insertions. Susceptibility to TAF for this panel of mutants was similar to TFV, ranging from 0.34 to 23 (fold of the wild-type EC_{50} value). There was a strong correlation between the fold change for TFV and TAF with R^2 = 0.97, indicating that TAF and TFV have a similar resistance profile against NRTI resistant mutants in the PhenoSense assay.

In addition, 21 patient-derived and 4 site-directed HIV-1 recombinant clones harboring NRTI-resistance substitutions with reduced susceptibility to TFV were generated using the pXXLAI recombinant clone and tested for their susceptibility in a multi-cycle MT-2 assay. The samples used for this study were either resistant or had reduced sensitivity to TDF as determined by the Monogram PhenoSense assay, with TFV fold change ranging from 1.6 to 16 and substitution patterns such as K65R, 3 TAMs, 4 TAMs, 5 TAMs, T69 insertions, and Q151M complex. In addition to the patient-derived recombinants, 4 site-directed mutants with either K65R, K70E, M184V, or K65R/Q151M complex (A62V, K65R, S68G, V75I, F77L, F116Y, Q151M) were analyzed phenotypically.

Table 11. Phenotypic Susceptibilities of 24 Recombinant HIV-1 Isolates with NRTI Mutations against TAF and TFV in the Monogram PhenoSense Assay

Virus ID	EC_{50} FC[a]		Mutation Category	NRTI mutations
	TAF	TFV		
13	0.34	0.41	NRTI	L74V
16	0.40	0.47	NRTI + M184V	L74V Y115F M184V*
5	0.50	0.48	M184V	M184V
14	0.43	0.50	NRTI	L74V
22	0.56	0.53	Q151M + M184V	Q151M M184V*
15	0.50	0.59	NRTI + M184V	L74V Y115F M184V*
6	0.67	0.65	M184V	M184V*
21	0.82	0.82	Q151M + M184V	A62V V75V/I F116Y Q151M M184V*
20	0.91	0.93	Q151M	F116Y Q151M*
11	0.78	0.98	K65R + M184V	A62A/V K65R M184V*
12	1.09	1.18	K65R + M184V	K65R M184V*
9	1.68	1.48	K65R	K65R*
10	1.91	1.71	K65R	K65R*
17	1.62	1.81	3 TAMs	M41L L74V L210W T215Y
3	2.11	2.27	6 TAMs + M184V	M41L D67N K70R M184V L210W T215Y K219E
19	3.43	2.82	Q151M Complex	A62V V75I F77L Y115F F116Y Q151M*
1	3.62	3.48	6 TAMs	M41L D67N K70R L210W T215F K219Q*
18	8.80	3.80	5 TAMs	M41L D67N T69D L74V L210W T215Y K219R*
2	4.77	4.01	6 TAMs	M41L D67N K70R L210W T215Y K219E*
4	9.16	6.11	6 TAMs + M184V	M41L D67N K70R M184V L210W T215Y K219E*
24	9.07	9.60	Q151M Complex + K65R	A62V K65R K70K/R V75I F77L F116Y Q151M*
8	20.0	18.0	T69 Insertion	M41L T69ins L74V L210W T215Y*
23	22.0	19.0	T69 Insertion	M41L A62V T69ins L210W T215Y*
7	23.0	20.0	T69 Insertion	A62V T69ins V75I*

a Susceptibilities are expressed as fold changes (FC) in EC_{50} from wild-type control. Wild-type EC_{50} for TAF and TFV was 10 nM and 0.6 µM, respectively.
(*) Indicates the additional presence of NNRTI resistance mutations (not shown).

Phenotypic analyses using these clones in the HIV-1 multi-cycle MT-2 assay showed that susceptibility of patient-derived isolates to TAF ranged from 2.2- to 25.5-fold change from wild-type control. Most samples had very high levels of AZT and FTC resistance due to the presence of TAMs and M184V, respectively. The highest level of TAF phenotypic resistance was observed in patient-derived isolates harboring 5 TAMs including T215Y and L210W in the absence of the M184V (Table 12; Report PC-120-2015, page 12). The lowest level of TAF phenotypic resistance was observed with a sample harboring K65R. Notably, samples within same mutation categories showed some fold change variability inherent to the genetic diversity found in patient samples, similar to results observed in the single-cycle PhenoSense assay. Among the site-directed mutants, the highest fold change was observed for the mutant carrying the Q151M substitution complex plus K65R (13.2-fold), followed by K65R alone (3.3-fold), and K70E (2.2-fold). The mutant with M184V alone showed wild-type susceptibility to TAF.

Phenotypic susceptibility to TAF and TFV were highly correlated (R^2 = 0.9671) in this multi-cycle MT-2 assay. In summary, TAF and TFV showed very similar fold changes from wild-type against these recombinant HIV-1 isolates, consistent with the activity of each compound being driven by the same active entity in these assays, TFV-DP, and both compounds having a similar resistance profile.

Table 12. Phenotypic Susceptibilities of Patient-Derived and Site-Directed Recombinant Mutant Clones into the MT-2 Assay

		Fold Change from Wild-Type Control[b] (MT-2 assay)					
Virus ID	Mutation Category[a]	TAF	TFV	AZT[c]	FTC[c]	DRV	EVG
2	K65R/M184V	5.0 *	3.9 *	1.1	>>	1.1	1.1
6	3 TAMs	3.0 *	3.8 *	>>	2.0 *	0.7	0.6
7	K65R/M184V	8.9 *	6.2 *	4.6 *	>>	1.2	1.8 *
8	4 TAMs	6.3 *	4.7 *	>>	>>	1.5	2.0
9	3 TAMs	4.3 *	3.7 *	25.5 *	>>	1.2	1.9 *
10	4 TAMs	2.3 *	2.6 *	>>	>>	0.7	0.9
11	K65R/M184V	3.3 *	3.1 *	1.8	>>	0.8	0.7
12	K65R	2.2 *	2.3 *	0.4	6.0 *	n/a	0.7
14	5 TAMs	4.5 *	4.1 *	>>	>>	1	0.6
15	Q151M/K65R	5.9 *	4.1 *	>>	>>	1.1	0.9
16	3 TAMs	3.6 *	3.3 *	>>	>>	0.8	0.9
20	3 TAMs	10.3 *	7.2 *	>>	5.0 *	1	1.0
21	5 TAMs	6.9 *	6.5 *	>>	>>	1.1	1.1
24	4 TAMs	6.4 *	5.2 *	>>	15.2 *	0.7	0.7
25	4 TAMs	6.1 *	5.1 *	>>	3.9 *	1.1	1.2
30	T69 insertion	10.1 *	10.1 *	>>	9.0 *	1.2	1.6
31	5 TAMs	25.5 *	21.9 *	>>	28.4 *	1.0	0.9
32	3 TAMs	3.4 *	3.8 *	>>	2.8 *	1.2	1.3
33	T69 insertion	14.3 *	10.9 *	>>	>>	0.5	0.9
34	5 TAMs	14.8 *	14.7 *	>>	14.5 *	0.9	1.3
37	5 TAMs	22.9 *	19.6 *	>>	5.4 *	1.7	1.3
K65R	K65R	3.3 *	3.3 *	0.6	6.5 *	0.9	0.8
K70E	K70E	2.2 *	2.1 *	0.3	2.5 *	1.0	1.0
M184V	M184V	1.1	0.9	0.5	>>	0.9	1.0
Q151M Complex	Q151M/K65R	13.2 *	13.9 *	>>	>>	1.0	1.1

a Thymidine analog-associated mutations (TAMs): M41L, D67N, K70R, L210W, T215F/Y, and K219Q/E/N/R.
b EC_{50} values for wild-type control ($HIV\text{-}1_{XXLAI}$) were 14 nM, 3.4 µM, 0.3 µM, 1.1 µM, 7 nM, and 2 nM, for TAF, TFV, AZT, FTC, DRV, and EVG, respectively, in the MT-2 assay.
c ">>" denotes that at least one value was >55, or >48 fold above wild-type for FTC, and AZT, respectively.
Data were averaged from at least 3 independent experiments.
(*) indicates p-value <0.01 as compared to wild-type control (Student's T-test).

6 CLINICAL STUDIES

GS-US-292-0104
Study GS-US-292-0104 is an ongoing Phase 3, randomized, double-blinded, multicenter, active-controlled study to assess the efficacy and safety of the E/C/F/TAF FDC compared with the STB in HIV-infected, ART-naive adults. Subjects were randomized in a 1:1 ratio to 1 of the following 2 treatment groups: E/C/F/TAF (150/150/200/10 mg) + placebo-to-match STB (n = 420) and STB (150/150/200/300 mg) + placebo-to-match E/C/F/TAF (n = 420). Randomization was stratified by HIV-1 RNA level (≤100,000 copies/mL, >100,000 copies/mL to ≤400,000 copies/mL, or >400,000 copies/mL), $CD4^+$ cell count (<50 cells/μL, 50 to 199 cells/μL, or ≥200 cells/μL), and region (United States [US] or ex-US) at screening. The primary efficacy endpoint was the percentage of subjects with HIV-1 RNA <50 copies/mL at Week 48 using the FDA-defined snapshot algorithm. Virologic outcomes at Week 48 were similar between the 2 treatment groups for the primary endpoint analysis based on the full analysis set. Virologic success rates were >90% in both groups (E/C/F/TAF 93.1%; STB 92.4%) and E/C/F/TAF was determined to be non-inferior to STB.

GS-US-292-0111
Study GS-US-292-0111 is an ongoing Phase 3, randomized, double-blinded, multicenter, active-controlled study to assess the efficacy and safety of the E/C/F/TAF FDC compared with the STB FDC in HIV-infected, ART-naive adults. Subjects were randomized in a 1:1 ratio to 1 of the following 2 treatment groups: E/C/F/TAF (150/150/200/10 mg) + placebo-to-match STB once daily (n = 420) and STB (150/150/200/300 mg) + placebo-to-match E/C/F/TAF once daily (n = 420). Randomization was stratified by HIV-1 RNA level (≤100,000 copies/mL, >100,000 copies/mL to ≤400,000 copies/mL, or >400,000 copies/mL), CD4 count (<50 cells/μL, 50 to 199 cells/μL, or ≥200 cells/μL), and region (US or ex-US) at screening. The primary efficacy endpoint was the percentage of subjects with HIV-1 <50 copies/mL at Week 48 using the FDA-defined snapshot algorithm. Virologic outcomes at Week 48 were similar between the 2 treatment groups for the primary endpoint analysis based on the full analysis set. Virologic success rates were 91.6% in E/C/F/TAF 91.6% group and 88.5% in the STB group and E/C/F/TAF was determined to be non-inferior to STB.

GS-US-292-0106
Study GS-US-292-0106 is an ongoing Phase 2/3, open-label, multicenter, 2-part, single group study of the PK, safety, tolerability, and antiviral activity of the E/C/F/TAF FDC in HIV-infected, ART-naive adolescents. Adolescent (12 to < 18 years of age) subjects of either sex were enrolled to receive E/C/F/TAF once daily with food. In Part A, 24 subjects were enrolled to evaluate the steady-state PK and to confirm the dose of the E/C/F/TAF. Subjects participated in an intensive PK evaluation at Week 4 and continued to receive E/C/F/TAF through Week 48. In Part B, 24 subjects were enrolled to evaluate the safety, tolerability, and antiviral activity of E/C/F/TAF through Week 48. At the Week 24 interim analysis, 48 subjects were enrolled into the study (24 in Part A and 24 in Part B) and received at least 1 dose of study drug. Twenty-three of the 48 enrolled subjects were enrolled in the study by 11 February 2014 and completed their Week 24 study visit; these subjects were included in the Week 24 full analysis set. At the Week 24 interim analysis, 91.3% of subjects (21 of 23) at Week 24 had virologic success (FDA snapshot algorithm, HIV-1 RNA <50 copies/mL)

GS-US-292-0109
Study GS-US-292-0109 is an ongoing, Phase 3, randomized, open-label, multicenter, active-controlled study to evaluate the efficacy and safety of E/C/F/TAF in virologically suppressed subjects who switch from a FTC/TDF regimen. All subjects are HIV-infected adults with virologic suppression on STB, efavirenz (EFV)/FTC/TDF, cobicistat-boosted atazanavir (ATV)/COBI+FTC/TDF, or ritonavir-boosted ATV (ATV/r)+FTC/TDF. Subjects were randomized in a 2:1 ratio to 1 of the following 2 treatment groups: switch to E/C/F/TAF (150/150/200/10 mg) (n = 1,000) or maintain preexisting regimen FTC/TDF+3rd Agent regimen (EVG/COBI/FTC/TDF [150/150/200/300 mg]; EFV/FTC/TDF [600/200/300 mg]; ATV/r [300/100 mg] + FTC/TDF [200/300 mg]; or ATV/COBI [300/150 mg] + FTC/TDF [200/300 mg]) (n = 500)
Randomization was stratified by prior treatment regimen group at screening. Overall, 1,436 subjects (E/C/F/TAF = 959 subjects; FTC/TDF+3rd Agent = 477 subjects) were randomized and received at least 1 dose of study drug. The Week 48 full analysis set included any subject randomized by 31-October-2013 who received at least 1 dose of study drug; in total, 1,196 subjects were included in the Week 48 full analysis set (E/C/F/TAF = 799; FTC/TDF+3rd Agent = 397). The primary efficacy endpoint was the percentage of subjects with HIV-1 RNA <50 copies/mL at Week 48 using the FDA-defined snapshot algorithm. Virologic success rates at Week 48 were >90% in both groups (E/C/F/TAF 95.6%; FTC/TDF+3rd Agent 92.9%), with E/C/F/TAF determined to be non-inferior to FTC/TDF+3rd Agent.

7 CLINICAL VIROLOGY

EMERGENCE OF RESISTANCE
In the studies from the original STIBILD® NDA, resistance development to TRUVADA components of the regimen occurred more frequently in the E/C/F/T-treatment failures, 54.6% versus 13.3% and 0% of the ATR and ATV/r +TVD treatment failures with evaluable genotypic data, respectively.

Studies 104 and 111
In Studies 104 and 111, there were 17 virologic failures in the EVG/COBI/FTC/TAF (E/C/F/TAF) arm and 21 virologic failures in the STB arm. The FDA resistance analysis includes subjects who had confirmed viral load >400 copies/mL at discontinuation, final timepoint or after suppression to <50 copies/mL (i.e., rebound). In addition, the FDA resistance analysis includes subjects who may not have met these criteria, but had evidence of resistance emergence in the data provided by the sponsor. Therefore, 3 subjects in the E/C/F/TAF arm (0104-0115-4389, 0111-0255-5554, 0111-2348-5568) and 4 subjects in the STB arm (0104-2855-4619, 0104-3612-4362, 0111-1236-5061, 0111-2675-5138) were removed from the FDA virologic failure subset because they did not have confirmed >400 copies/mL. Thus, the FDA virologic failure subset had 14 virologic failures in the E/C/F/TAF arm compared to 17 virologic failures in the STB arm (Table 13).

Table 13. Emergent Substitutions in Virologic Failures from Studies 104 and 111

PID	ARM	Timepoint with Resistance Data	Viral Load	RT Emergent Substitutions	INSTI Emergent Substitutions	Phenotype Resistance
0104-0115-4389	E/C/F/TAF	WK16	606 resuppressed	NO DATA	NO DATA	NO DATA
0104-0255-4091	E/C/F/TAF	WK48	986	I178I/M	NO DATA	
0104-1965-4201	E/C/F/TAF	WK48 DC	1,260	**M41M/K K65R M184V**	G70G/R **N155H D232D/N**	FTC-R >63 EVG-R 60 TDF 1.01
0104-2704-4329	E/C/F/TAF	WK48	8,340	**M184V**	**E92Q**	FTC-R >70 EVG-R 56
0104-2734-4394	E/C/F/TAF	WK16 DC	62600	**M184M/V**	A129A/S	FTC-2X
0111-0255-5554	E/C/F/TAF	WK2 DC	6480	NO DATA	NO DATA	NO DATA
0111-0729-5139	E/C/F/TAF	WK24 DC	6250	P170P/L V179V/I **M184V**	L68V **E92Q**	FTC-R >84 NO INSTI DATA
0111-0994-5313	E/C/F/TAF	WK48	2750	I195I/M	A21A/T I182I/F	
0111-1790-5185	E/C/F/TAF	WK16 DC	63000	**M184V**	**T66T/I/A/V E138E/K Q148Q/R** D232N	FTC-R >81 EVG-R 11
0111-2348-5568	E/C/F/TAF	WK16	597 resuppressed	NO DATA	NO DATA	NO DATA
0111-2348-5661	E/C/F/TAF	WK36	759	D177E Q207E	NO DATA	
0111-2480-5585	E/C/F/TAF	WK24 DC	500	K64K/R	NONE	

0111-2704-5057	E/C/F/TAF	WK12	6,230	D121H/Y **M184M/I/V** Q207Q/H	A265A/V	FTC-R >68
0111-2873-5544	E/C/F/TAF	WK36	47,800 REBOUND		S17N	
0111-4735-5238	E/C/F/TAF	WK36	412 <400 at WK48 and 60	W71P T139T/A E204E/K	NO DATA	
0111-5083-5289	E/C/F/TAF	WK16 DC	3,850 resuppressed	**M184V** H208Y E248K	**T66A** V72I	FTC-R >89 EVG-R 12
0111-7710-5649	E/C/F/TAF	WK48	322,000	T200T/A Q207Q/R		NONE
0104-0031-4253	STB	WK16 DC	1,130	V60I **K65R M184V**	**E92Q**	NO RT DATA EVG-R 21
0104-0783-4492	STB	WK16	1,340 resuppressed	NONE	S24N	EVG 2.0
0104-1541-4150	STB	WK36 DC	364,000	NONE	D6N, E35K, E69K, R107K **E138K**,E152K **M154I, E157K**	EVG-NO DATA
0104-1609-4268	STB	WK24 DC	1,100 resuppressed	E36E/Q E44E/D K49K/Q **M184M/V**	NONE	FTC 1.5
0104-2855-4619	STB	WK36	592	NONE	NO DATA	NO INSTI DATA
0104-3612-4362	STB	WK12 DC	1,020	R78R/K D86D/N E224E/K	NONE	NONE
0104-4735-4448	STB	WK12 DC	53,400	A98G/R **M184V**	G70G/E V72I P90P/S **E92E/Q** H114H/Y **E138E/K Q148R**	FTC-R >71 EVG-R >156

					G190G/E	
0111-0031-5129	STB	WK24	2,820	V179V/I V245V/A	NO DATA	NO INSTI DATA
0111-0044-5800	STB	WK48	871	G196E		NO INSTI DATA
0111-0255-5134	STB	WK8 DC	603,000	NO DATA	NO DATA	NO DATA
0111-0729-5836	STB	WK36	576	NONE	NONE	NONE
0111-0986-5527	STB	WK48	60,400	NONE	NO INSTI DATA	NO INSTI DATA
0111-0994-5655	STB	WK48	1,280	NONE	S17N L101L/I K127K/R L172L/F	NONE
0111-1236-5061	STB	WK36	20,500	NONE	G59G/E T112I	EVG 2.2
0111-1480-5728	STB	WK24	1,010	NONE	L45I	EVG 1.7
0111-1534-5156	STB	WK48	73	**M184V** T200I		FTC-R >64 EVG 1.9
0111-2106-5415	STB	WK48	11,700	NONE	V249I/V	
0111-2348-5569	STB	WK36	803	NO DATA	NO DATA	NO DATA
0111-2348-5662	STB	WK36	1,410	NO DATA	NO DATA	NO DATA
0111-2675-5138	STB	WK2 DC	603	NO DATA	NO DATA	NO DATA

0111-2704-5132	STB	WK36	44,800	**K65R M184V**	S17N **E138K Q148R**	FTC-R >82 TDF 1.3 EVG-R >95

Bolded subjects PIDs had emergent RT or IN substitutions.
Italicized subject PIDs were removed from denominator because they resuppressed and had no resistance data.
The clinical phenotypic cutoffs were as follows: TFV = 1.4 to 4, FTC = 3.5, and EVG = 2.5.

Of the final FDA-defined virologic failure set, 7 subjects (50%) had emergent resistance substitutions in the E/C/F/TAF arm and 6 subjects (35%) had emergent resistance substitutions in the STB arm (Table 14). In the E/C/F/TAF arm, all 7 of the virologic failures with emergent substitutions had the M184V substitution and one subject had the K65R substitution. Three subjects had emergent Q207E/H/R in reverse transcriptase. Five of the 7 subjects had emergent INSTI resistance substitutions. In the STB arm, 5 of the 6 subjects with emergent substitutions had emergent M184V substitutions and two had the K65R substitution. Four the 6 subjects had emergent INSTI resistance substitutions. Additionally, 1 subject in each arm developed a substitution in RT at T200 (T200T/A in the GENVOYA arm and T200I in the STB arm). The number and type of emergent NRTI and INSTI resistance substitutions is similar in both arms. TAF did not reduce the frequency of virologic failure or number of emergent resistance substitutions compared to TDF. In addition, the resistance pathways of TAF are similar to TDF.

Table 14. Summary of Emergent Substitutions in Virologic Failure Subjects of Study 104 and 111

	E/C/F/TAF (n=14)	**STB (n=17)**
# Subjects with Emerging Substitutions	7 (50%)	6 (35%)
NRTI	7 (100%)	5 (83%)
M184V	7 (100%)	5 (100%)
K65R	1 (14%)	2 (40%)
INSTI	5 (71%)	4 (67%)
T66A	2 (40%)	
E92Q	2 (40%)	2 (50%)
E138K	1 (20%)	3 (75%)
Q148R	1 (20%)	2 (50%)
M154I		1 (25%)
N155H	1 (20%)	
E157K		1 (25%)

Study 109

HIV-1 genotyping was not conducted at screening since all subjects who entered Study GS-US-292-0109 had HIV RNA <50 copies/mL at screening. Historical genotyping results for the PR/RT genes was available for all subjects as they were previously enrolled in Gilead Studies GS-US-236-0102, GS-US-236-0103, GS-US-216-0114, GS-US-264-0110 (limited to subjects on a EFV-based regimen), GS-US-236-0104, or GS-US-216-0105, all of which enrolled ART-naïve subjects. Consistent with the enrollment criteria for these studies, all enrolled subjects demonstrated full sensitivity to FTC and tenofovir (delivered as TDF or TAF) based on the proprietary algorithm from (b) (4) NRTI-associated resistance substitutions were observed in 9% of subjects, NNRTI-associated resistance substitutions were observed in 14.5% of subjects, and primary PI-associated resistance substitutions were observed in 2.6% of subjects. The distribution of baseline resistance-associated substitutions was comparable between treatment groups.

Of the 1,196 subjects in Study GS-US-292-0109 at Week 48, 5 (0.4%) met the virologic failure criteria and were included in the sponsor's resistance analysis set with 4 of 799 subjects (0.5%) in the E/C/F/TAF group and 1 of 397 subjects (0.3%) in the FTC/TDF+3rd Agent regimen group analyzed. Four subjects (Subjects 2838-6691, 2840-6507, 5083-6551 and 0959-6571) achieved HIV-1 RNA resuppression to <50 copies/mL with further treatment and were not included in the sponsor's final resistance analysis population. However, all these virologic failures subjects were included FDA resistance analysis because they had confirmed >400 copies/mL and resistance data. Subject 2838-6691 demonstrated genotypic and phenotypic resistance to FTC (M184M/I; FTC FC = 3.8) at Week 8 (Table 15) and the HIV-1 RNA level resuppressed to <50 copies/mL by early study drug discontinuation when the subject switched to a new drug regimen. The remaining 2 subjects (Subjects 2840-6507 and 5083-6551) did not have resistance to emtricitabine or TAF detected, and no IN resistance data was available. Subject 2824-6999 in the E/C/F/TAF group that was included in the sponsor's final resistance analysis set had no detectable resistance to any study drug.

Table 15. Emergent Substitutions in Virologic Failures from Study 109

PID	ARM	Timepoint with Resistance Data	Viral Load	RT Emergent Substitutions	INSTI Emergent Substitutions	Phenotype Resistance
0109-2824-6999	E/C/F/TAF	WK24	4970	NONE	S17N S39C	NONE
0109-2838-6691	E/C/F/TAF	WK8 resuppressed	662	**M184M/I**	I84M	**FTC-R 3.8**
0109-2840-6507	E/C/F/TAF	WK12 resuppressed	438	NONE	NO DATA	NO INSTI DATA
0109-5083-	E/C/F/TAF	WK4 resuppressed	617	NONE	NO DATA	NO INSTI

6551						DATA
0109-0959-6571	STB	WK12 resuppressed	4760	NO DATA	NO DATA	NO DATA

Study 106

Of the 23 subjects in the Week 24 full analysis set for Study GS-US-292-0106, 1 subject of 23 (4.3%) had virologic rebound that was unconfirmed. Subject 8275-2008 was suppressed (HIV-1 RNA <50 copies/mL) at Weeks 12 and 16 and had unconfirmed virologic rebound to HIV-1 RNA of 1,010 copies/mL at Week 24. This subject resuppressed HIV-1 RNA to <50 copies/mL at the next study visit upon continued treatment with E/C/F/TAF. Therefore, this subject's virus was not analyzed for resistance development.

8 CONCLUSION

TAF has the same cytotoxicity profile as TDF and tenofovir. TAF and TDF have a similar resistance profile in cell culture and in clinical trials. In treatment-naïve studies, there were a similar number of virologic failures in the E/C/F/TAF and STB arms with a similar resistance pattern. At Week 48, the development of one or more primary elvitegravir, emtricitabine, or tenofovir alafenamide fumarate substitutions associated with resistance was observed in 7 of 14 subjects with evaluable genotypic data from paired baseline and GENVOYA treatment-failure isolates (7 of 978 subjects [0.7%]) compared with 6 of 17 treatment-failure isolates from subjects in the STRIBILD treatment group (7 of 925 subjects [0.8%]). Of the 7 subjects with resistance development in the GENVOYA group, the substitutions that emerged were M184V/I (N = 7) and K65R (N = 1) in reverse transcriptase and T66T/A/I/V (N = 2), E92Q (N = 2), E138K (N = 1), Q148Q/R (N = 1) and N155H (N = 1) in integrase. Of the 6 subjects with resistance development in the STRIBILD group, the substitutions that emerged were M184V/I (N = 5) and K65R (N = 2) in reverse transcriptase and E92E/Q (N = 2), E138K (n = 3) and Q148R (N = 2) in integrase. In both treatment groups, most subjects who developed substitutions associated with resistance to elvitegravir also developed emtricitabine resistance-associated substitutions.

In a clinical study of virologically-suppressed subjects (Study 109, N = 799) who switched from a regimen containing emtricitabine/tenofovir disoproxil fumarate and a third agent to E/C/F/TAF), 4 subjects were virologic failures of which one had emergent emtricitabine resistance with the emergence of M184M/I.

APPLICANT PROPOSED PACKAGE INSERT

12.4 Microbiology

Mechanism of Action

Elvitegravir: Elvitegravir inhibits the strand transfer activity of HIV-1 integrase (integrase strand transfer inhibitor; INSTI), an HIV-1 encoded enzyme that is required for viral replication. Inhibition of integrase prevents the integration of HIV-1 DNA into host genomic DNA, blocking the formation of the HIV-1 provirus and propagation of the viral infection. Elvitegravir does not inhibit human topoisomerases I or II.

Cobicistat: Cobicistat is a selective, mechanism-based inhibitor of cytochromes P450 of the CYP3A subfamily. Inhibition of CYP3A-mediated metabolism by cobicistat enhances the systemic exposure of CYP3A substrates, such as elvitegravir, where bioavailability is limited and half-life is shortened by CYP3A-dependent metabolism.

Emtricitabine: Emtricitabine, a synthetic nucleoside analog of cytidine, is phosphorylated by cellular enzymes to form emtricitabine 5'-triphosphate. Emtricitabine 5'-triphosphate inhibits the activity of the HIV-1 RT by competing with the natural substrate deoxycytidine 5'-triphosphate and by being incorporated into nascent viral DNA which results in chain termination. Emtricitabine 5′-triphosphate is a weak inhibitor of mammalian DNA polymerases α, β, Ɛ, and mitochondrial DNA polymerase γ.

Tenofovir Alafenamide: Tenofovir alafenamide is a phosphonoamidate prodrug of tenofovir (2'-deoxyadenosine monophosphate analogue). (b) (4) through hydrolysis by cathepsin A, (b) (4) tenofovir is subsequently phosphorylated (b) (4) active metabolite tenofovir diphosphate. Tenofovir diphosphate inhibits HIV replication through incorporation into viral DNA by the HIV reverse transcriptase, which results in DNA chain-termination.

Tenofovir has activity that is specific to human immunodeficiency virus (b) (4) and hepatitis B virus. (b) (4) have shown that both emtricitabine and tenofovir can be fully phosphorylated when combined in cells. Tenofovir diphosphate is a weak inhibitor of mammalian DNA polymerases that include mitochondrial DNA polymerase γ and there is no evidence of toxicity to mitochondria *in vitro*.

Antiviral Activity

Elvitegravir, Cobicistat, Emtricitabine, and Tenofovir Alafenamide: (b) (4) elvitegravir, emtricitabine, and tenofovir alafenamide (b) (4) .

Elvitegravir: The antiviral activity of elvitegravir against laboratory and clinical isolates of HIV-1 was assessed in T lymphoblastoid cell lines, monocyte/macrophage cells, and primary peripheral blood lymphocytes. The 50% effective concentrations (EC_{50}) ranged from 0.02 to 1.7

nM. Elvitegravir displayed antiviral activity in cell culture against HIV-1 clades A, B, C, D, E, F, G, and O (EC_{50} values ranged from 0.1 to 1.3 nM) and activity against HIV-2 (EC_{50} value of 0.53 nM). Elvitegravir did not show inhibition of replication of HBV or HCV in cell culture.

Cobicistat: Cobicistat has no detectable antiviral activity in cell culture against HIV-1, HBV, or HCV and does not antagonize the antiviral activity of elvitegravir, emtricitabine, or tenofovir.

Emtricitabine: The antiviral activity of emtricitabine against laboratory and clinical isolates of HIV-1 was assessed in T lymphoblastoid cell lines, the MAGI-CCR5 cell line, and primary peripheral blood mononuclear cells. The EC_{50} values for emtricitabine were in the range of 0.0013–0.64 micromolar. Emtricitabine displayed antiviral activity in cell culture against HIV-1 clades A, B, C, D, E, F, and G (EC_{50} values ranged from 0.007–0.075 micromolar) and showed strain specific activity against HIV-2 (EC_{50} values ranged from 0.007–1.5 micromolar).

Tenofovir Alafenamide: The antiviral activity of tenofovir alafenamide against laboratory and clinical isolates of HIV-1 subtype B was assessed in lymphoblastoid cell lines, PBMCs, primary monocyte/macrophage cells and CD4-T lymphocytes. The EC_{50} values for tenofovir alafenamide were in the range of 2.0 to 14.7 nM.

Tenofovir alafenamide displayed antiviral activity in cell culture against all HIV-1 groups (M, N, O), including sub-types A, B, C, D, E, F, and G (EC_{50} values ranged from 0.10 to 12.0 nM) and strain specific activity against HIV-2 (EC_{50} values ranged from 0.91 to 2.63 nM).

In a study of tenofovir alafenamide with a broad panel of representatives from the major classes of approved anti-HIV agents (NRTIs, NNRTIs, INSTIs, and PIs), additive to synergistic effects were observed. No antagonism was observed for these combinations.

Resistance

In Cell Culture

Elvitegravir: HIV-1 isolates with reduced susceptibility to elvitegravir have been selected in cell culture. Reduced susceptibility to elvitegravir was associated with the primary integrase substitutions T66A/I, E92G/Q, S147G, and Q148R. Additional integrase substitutions observed in cell culture selection included D10E, S17N, H51Y, F121Y, S153F/Y, E157Q, D232N, R263K, and V281M.

Emtricitabine: HIV-1 isolates with reduced susceptibility to emtricitabine have been selected in cell culture. Reduced susceptibility to emtricitabine was associated with M184V/I substitutions in HIV-1 RT.

Tenofovir Alafenamide: HIV-1 isolates with reduced susceptibility to tenofovir alafenamide have been selected in cell culture. HIV-1 isolates selected by tenofovir alafenamide expressed a K65R substitution in HIV-1 RT; in addition, a K70E substitution in HIV-1 RT has been (b) (4) observed. HIV-1 isolates with the K65R substitution have (b) (4) reduced susceptibility to abacavir, emtricitabine, tenofovir, and lamivudine. (b) (4)

In Clinical Trials

In Treatment-Naïve Subjects: In a pooled analysis of antiretroviral-naive subjects receiving [TRADENAME] in Studies 104 and 111, (b) (4) genotyping was performed on plasma HIV-1 isolates from all subjects with HIV-1 RNA > 400 copies/mL at confirmed virologic failure, at Week 48, or at time of early study drug discontinuation. As of Week 48, the development of (b) (4) elvitegravir, emtricitabine, or tenofovir alafenamide (b) (4) was observed in 7 of 14 subjects with evaluable (b) (4) data from paired baseline and [TRADENAME] treatment-failure isolates (7 of (b) (4) subjects [(b) (4)%]) compared with (b) (4) treatment-failure isolates from subjects in the STRIBILD treatment group (b) (4) subjects [(b) (4)%]). Of the 7 subjects with resistance development in the [TRADENAME] group, the substitutions that emerged were M184V/I (N = 7) and K65R (N = 1) in reverse transcriptase and T66T/A/I/V (N = 2), E92Q (N = 2), Q148Q/R (N = 1) and N155H (N = 1) in integrase. Of the (b) (4) subjects with resistance development in the STRIBILD group, the substitutions that emerged were M184V/I (N = (b) (4) and K65R (N = 2) in reverse transcriptase and E92E/Q (N = (b) (4), and Q148R (N = 2) in integrase. (b) (4) subjects (b) (4) who developed substitutions associated with resistance to elvitegravir developed substitutions (b) (4)

(b) (4)

In Virologically Suppressed Subjects: (b) (4) emergent resistance to [TRADENAME] (b) (4) in a clinical study of virologically-suppressed subjects who switched from a regimen containing emtricitabine/tenofovir disoproxil fumarate and a third agent (Study 109, N = 799).

Cross Resistance

(b) (4) No cross-resistance has been demonstrated for elvitegravir-resistant HIV-1 isolates and emtricitabine or tenofovir, or for emtricitabine- or tenofovir-resistant isolates and elvitegravir.

Elvitegravir: Cross-resistance has been observed among INSTIs. Elvitegravir-resistant viruses showed varying degrees of cross-resistance in cell culture to raltegravir depending on the type and number of substitutions in HIV-1 integrase. Of the primary elvitegravir resistance-associated substitutions tested (T66A/I/K, E92G/Q, T97A, S147G, Q148H/K/R, and N155H), all but three (T66I, E92G, and S147G) conferred greater than 1.5-fold reduced susceptibility to raltegravir (above the biological cutoff for raltegravir) when introduced individually into a wild-type virus by site-directed mutagenesis. Of the primary raltegravir resistance-associated substitutions (Y143C/H/R, Q148H/K/R, and N155H), all but Y143C/H conferred greater than 2.5-fold reductions in susceptibility to elvitegravir (above the biological cutoff for elvitegravir). Viruses expressing elvitegravir or raltegravir resistance mutations maintain susceptibility to dolutegravir.

Emtricitabine: Cross-resistance has been observed among NRTIs. Emtricitabine-resistant isolates harboring an M184V/I substitution in HIV-1 RT were cross-resistant to lamivudine. HIV-

1 isolates containing the K65R RT substitution, selected in vivo by abacavir, didanosine, and tenofovir, demonstrated reduced susceptibility to inhibition by emtricitabine.

Tenofovir Alafenamide: The K65R and K70E mutations result in reduced susceptibility to abacavir, didanosine, lamivudine, emtricitabine, and tenofovir (b) (4)

(b) (4)

FDA NEGOTIATED PACKAGE INSERT

12.4 Microbiology

Mechanism of Action

Elvitegravir: Elvitegravir inhibits the strand transfer activity of HIV-1 integrase (integrase strand transfer inhibitor; INSTI), an HIV-1 encoded enzyme that is required for viral replication. Inhibition of integrase prevents the integration of HIV-1 DNA into host genomic DNA, blocking the formation of the HIV-1 provirus and propagation of the viral infection. Elvitegravir does not inhibit human topoisomerases I or II.

Cobicistat: Cobicistat is a selective, mechanism-based inhibitor of cytochromes P450 of the CYP3A subfamily. Inhibition of CYP3A-mediated metabolism by cobicistat enhances the systemic exposure of CYP3A substrates, such as elvitegravir, where bioavailability is limited and half-life is shortened by CYP3A-dependent metabolism.

Emtricitabine: Emtricitabine, a synthetic nucleoside analog of cytidine, is phosphorylated by cellular enzymes to form emtricitabine 5'-triphosphate. Emtricitabine 5'-triphosphate inhibits the activity of the HIV-1 reverse transcriptase by competing with the natural substrate deoxycytidine 5'-triphosphate and by being incorporated into nascent viral DNA which results in chain termination. Emtricitabine 5′-triphosphate is a weak inhibitor of mammalian DNA polymerases α, β, Ɛ, and mitochondrial DNA polymerase γ.

Tenofovir Alafenamide Fumarate: Tenofovir alafenamide is a phosphonoamidate prodrug of tenofovir (2'-deoxyadenosine monophosphate analog). (b) (4) is intracellularly converted to tenofovir through hydrolysis by cathepsin A. Tenofovir is subsequently phosphorylated by cellular kinases to the active metabolite tenofovir diphosphate. Tenofovir diphosphate inhibits HIV-1 replication through incorporation into viral DNA by the HIV reverse transcriptase, which results in DNA chain-termination.

Tenofovir has activity that is specific to human immunodeficiency virus (HIV-1) and hepatitis B virus. Cell culture studies have shown that both emtricitabine and tenofovir can be fully

phosphorylated when combined in cells. Tenofovir diphosphate is a weak inhibitor of mammalian DNA polymerases that include mitochondrial DNA polymerase γ and there is no evidence of toxicity to mitochondria in cell culture.

Antiviral Activity in Cell Culture

Elvitegravir, Cobicistat, Emtricitabine, and Tenofovir Alafenamide Fumarate: The combination of elvitegravir, emtricitabine, and tenofovir alafenamide fumarate was not antagonistic in cell culture combination antiviral activity assays and was not affected by the addition of cobicistat. In addition, elvitegravir, cobicistat, emtricitabine and tenofovir alafenamide fumarate were not antagonistic with a panel of representatives from the major classes of approved anti-HIV-1 agents (INSTIs, NNRTIs, NRTIs, and PIs).

Elvitegravir: The antiviral activity of elvitegravir against laboratory and clinical isolates of HIV-1 was assessed in T lymphoblastoid cell lines, monocyte/macrophage cells, and primary peripheral blood lymphocytes. The 50% effective concentrations (EC_{50}) ranged from 0.02 to 1.7 nM. Elvitegravir displayed antiviral activity in cell culture against HIV-1 clades A, B, C, D, E, F, G, and O (EC_{50} values ranged from 0.1 to 1.3 nM) and activity against HIV-2 (EC_{50} value of 0.53 nM). Elvitegravir did not show inhibition of replication of HBV or HCV in cell culture.

Cobicistat: Cobicistat has no detectable antiviral activity in cell culture against HIV-1, HBV, or HCV and does not antagonize the antiviral activity of elvitegravir, emtricitabine, or tenofovir.

Emtricitabine: The antiviral activity of emtricitabine against laboratory and clinical isolates of HIV-1 was assessed in T lymphoblastoid cell lines, the MAGI-CCR5 cell line, and primary peripheral blood mononuclear cells. The EC_{50} values for emtricitabine were in the range of 0.0013–0.64 micromolar. Emtricitabine displayed antiviral activity in cell culture against HIV-1 clades A, B, C, D, E, F, and G (EC_{50} values ranged from 0.007–0.075 micromolar) and showed strain specific activity against HIV-2 (EC_{50} values ranged from 0.007–1.5 micromolar).

Tenofovir Alafenamide Fumarate: The antiviral activity of tenofovir alafenamide fumarate against laboratory and clinical isolates of HIV-1 subtype B was assessed in lymphoblastoid cell lines, PBMCs, primary monocyte/macrophage cells and CD4-T lymphocytes. The EC_{50} values for tenofovir alafenamide fumarate ranged from (b) (4) to 14.7 nM.

Tenofovir alafenamide fumarate displayed antiviral activity in cell culture against all HIV-1 groups (M, N, O), including sub-types A, B, C, D, E, F, and G (EC_{50} values ranged from 0.10 to 12.0 nM) and strain specific activity against HIV-2 (EC_{50} values ranged from 0.91 to 2.63 nM).

Resistance

In Cell Culture

Elvitegravir: HIV-1 isolates with reduced susceptibility to elvitegravir have been selected in cell culture. Reduced susceptibility to elvitegravir was associated with the primary integrase substitutions T66A/I, E92G/Q, S147G, and Q148R. Additional integrase substitutions observed in cell culture selection included D10E, S17N, H51Y, F121Y, S153F/Y, E157Q, D232N, R263K, and V281M.

Emtricitabine: HIV-1 isolates with reduced susceptibility to emtricitabine have been selected in cell culture. Reduced susceptibility to emtricitabine was associated with M184V or I substitutions in HIV-1 RT.

Tenofovir Alafenamide Fumarate: HIV-1 isolates with reduced susceptibility to tenofovir alafenamide fumarate have been selected in cell culture. HIV-1 isolates selected by tenofovir alafenamide fumarate expressed a K65R substitution in HIV-1 RT, sometimes in the presence of S68N or L429I substitutions; in addition, a K70E substitution in HIV-1 RT has been observed. HIV-1 isolates with the K65R substitution have reduced susceptibility to abacavir, emtricitabine, tenofovir, and lamivudine.

In Clinical Trials

In Treatment-Naïve Subjects: In a pooled analysis of antiretroviral-naive subjects receiving [TRADENAME] in Studies 104 and 111, genotyping was performed on plasma HIV-1 isolates from all subjects with HIV-1 RNA > 400 copies/mL at confirmed virologic failure, at Week 48, or at time of early study drug discontinuation. As of Week 48, the development of genotypic resistance to elvitegravir, emtricitabine, or tenofovir alafenamide fumarate was observed in 7 of 14 subjects with evaluable resistance data from paired baseline and [TRADENAME] treatment-failure isolates (7 of 866 subjects [0.8%]) compared with 6 of 17 in the STRIBILD treatment group (6 of 867 subjects [0.7%]). Of the 7 subjects with resistance development in the [TRADENAME] group, the resistance-associated substitutions that emerged were M184V/I (N = 7) and K65R (N = 1) in reverse transcriptase and T66T/A/I/V (N = 2), E92Q (N = 2), E138K (n=1), Q148Q/R (N = 1) and N155H (N = 1) in integrase. Of the 6 subjects with resistance development in the STRIBILD group, the resistance-associated substitutions that emerged were M184V/I (N = 5) and K65R (N = 2) in reverse transcriptase and E92E/Q (N = 2), E138K (n=3) and Q148R (N = 2) in integrase. In both treatment groups, most subjects who developed substitutions associated with resistance to elvitegravir also developed emtricitabine resistance-associated substitutions. The genotypic resistance results were confirmed by phenotypic analyses.

In Virologically Suppressed Subjects : One subject was identified with emergent resistance to [TRADENAME] (M184M/I) out of 4 virologic failure subjects in a clinical study of virologically-suppressed subjects who switched from a regimen containing emtricitabine/tenofovir disoproxil fumarate and a third agent to [TRADENAME] (Study 109, N = 799).

Cross-Resistance

No cross-resistance has been demonstrated for elvitegravir-resistant HIV-1 isolates and emtricitabine or tenofovir, or for emtricitabine- or tenofovir-resistant isolates and elvitegravir.

Elvitegravir: Cross-resistance has been observed among INSTIs. Elvitegravir-resistant viruses showed varying degrees of cross-resistance in cell culture to raltegravir depending on the type and number of amino acid substitutions in HIV-1 integrase. Of the primary elvitegravir resistance-associated substitutions tested (T66A/I/K, E92G/Q, T97A, S147G, Q148H/K/R, and N155H), all but three (T66I, E92G, and S147G) conferred greater than 1.5-fold reduced susceptibility to raltegravir (above the biological cutoff for raltegravir) when introduced individually into a wild-type virus by site-directed mutagenesis. Of the primary raltegravir resistance-associated substitutions (Y143C/H/R, Q148H/K/R, and N155H), all but Y143C/H

conferred greater than 2.5-fold reductions in susceptibility to elvitegravir (above the biological cutoff for elvitegravir). Some viruses expressing elvitegravir or raltegravir resistance mutations maintain susceptibility to dolutegravir.

Emtricitabine: Cross-resistance has been observed among NRTIs. Emtricitabine-resistant isolates harboring an M184V/I substitution in HIV-1 RT were cross-resistant to lamivudine. HIV-1 isolates containing the K65R RT substitution, selected in vivo by abacavir, didanosine, and tenofovir, demonstrated reduced susceptibility to inhibition by emtricitabine.

Tenofovir Alafenamide Fumarate: Tenofovir resistance substitutions, K65R and K70E, result in reduced susceptibility to abacavir, didanosine, emtricitabine, lamivudine, and tenofovir.

HIV-1 with multiple TAMs (M41L, D67N, K70R, L210W, T215F/Y, K219Q/E/N/R), or multinucleoside resistant HIV-1 with a T69S double insertion mutation or with a Q151M mutation complex including K65R showed reduced susceptibility to tenofovir alafenamide fumarate in cell culture.

APPENDIX

03/31/15 Correspondence

In Phase 3 Studies 104 and 111, there were 17 virologic failures (VF) in the E/C/F/TAF arm and 21 virologic failures in the STB arm. The FDA VF subset included subjects who had confirmed viral load >400 copies/mL at discontinuation, final timepoint or after suppression to <50 copies/mL (i.e., rebound) (Subject IDs in Table A below). In addition, the FDA subset includes subjects that may not have met these criteria, but had evidence of resistance emergence in the data provided by the sponsor. Thus, 3 subjects in the E/C/F/TAF arm (0104-0115-4389, 0111-0255-5554, 0111-2348-5568) and 4 subjects in the STB arm (0104-2855-4619, 0104-3612-4362, 0111-1236-5061, 0111-2675-5138) were removed from the VF analysis because they did not have confirmed >400 copies/mL and resuppressed. Of the final FDA subset of virologic failures (E/C/F/TAF = 14; STB = 17), 7 subjects had emergent resistance substitutions in the E/C/F/TAF arm and 6 subjects had emergent resistance substitutions in the STB arm (Bolded).

Table A. Emergent Substitutions in Virologic Failures from Studies 104 and 111

PID	ARM	Timepoint with Resistance Data	Viral Load	RT Emergent Substitutions	INSTI Emergent Substitutions	Phenotype Resistance
0104-0255-4091	E/C/F/TAF	WK48	986	I178I/M	NO DATA	
0104-1965-4201	E/C/F/TAF	WK48 DC	1260	**M41M/K K65R M184V**	G70G/R **N155H D232D/N**	FTC-R >63 EVG-R 60 TDF 1.01
0104-2704-4329	E/C/F/TAF	WK48	8340	**M184V**	**E92Q**	FTC-R >70 EVG-R 56
0104-2734-4394	E/C/F/TAF	WK16 DC	62600	**M184M/V**	A129A/S	FTC-2X
0111-0729-5139	E/C/F/TAF	WK24 DC	6250	P170P/L V179V/I **M184V**	L68V **E92Q**	FTC-R >84 NO INSTI DATA
0111-0994-5313	E/C/F/TAF	WK48	2750	I195I/M	A21A/T I182I/F	
0111-1790-5185	E/C/F/TAF	WK16 DC	63000	**M184V**	**T66T/I/A/V E138E/K Q148Q/R** D232N	FTC-R >81 EVG-R 11
0111-2348-5661	E/C/F/TAF	WK36	759	D177E Q207E	NO DATA	

0111-2480-5585	E/C/F/TAF	WK24 DC	500	K64K/R	NONE	
0111-2704-5057	E/C/F/TAF	WK12	6230	D121H/Y **M184M/I/V** Q207Q/H	A265A/V	FTC-R >68
0111-2873-5544	E/C/F/TAF	WK36	47800 REBOUND		S17N	
0111-4735-5238	E/C/F/TAF	WK36	412 <400 at WK48 and 60	W71P T139T/A E204E/K	NO DATA	
0111-5083-5289	E/C/F/TAF	WK16 DC	3850 resuppressed	**M184V** H208Y E248K	**T66A** V72I	FTC-R >89 EVG-R 12
0111-7710-5649	E/C/F/TAF	WK48	322000	T200T/A Q207Q/R		NONE
0104-0031-4253	STB	WK16 DC	1130	V60I **K65R M184V**	**E92Q**	NO RT DATA EVG-R 21
0104-0783-4492	STB	WK16	1340 resuppressed	NONE	S24N	EVG 2.0
0104-1541-4150	STB	WK36 DC	364000	NONE	D6N, E35K, E69K, R107K **E138K**,E152K **M154I, E157K**	EVG-NO DATA
0104-1609-4268	STB	WK24 DC	1100 resuppressed	E36E/Q E44E/D K49K/Q **M184M/V**	NONE	FTC 1.5
0104-4735-4448	STB	WK12 DC	53400	A98G/R **M184V**	G70G/E V72I P90P/S **E92E/Q** H114H/Y **E138E/K Q148R** G190G/E	FTC-R >71 EVG-R >156
0111-0031-5129	STB	WK24	2820	V179V/I V245V/A	NO DATA	NO INSTI DATA

0111-0044-5800	STB	WK48	871	G196E		NO INSTI DATA
0111-0255-5134	STB	WK8 DC	603000	NO DATA	NO DATA	NO DATA
0111-0729-5836	STB	WK36	576	NONE	NONE	NONE
0111-0986-5527	STB	WK48	60400	NONE	NO INSTI DATA	NO INSTI DATA
0111-0994-5655	STB	WK48	1280	NONE	S17N L101L/I K127K/R L172L/F	NONE
0111-1480-5728	STB	WK24	1010	NONE	L45I	EVG 1.7
0111-1534-5156	STB	WK48	73	**M184V** T200I		FTC-R >64 EVG 1.9
0111-2106-5415	STB	WK48	11700	NONE	V249I/V	
0111-2348-5569	STB	WK36	803	NO DATA	NO DATA	NO DATA
0111-2348-5662	STB	WK36	1410	NO DATA	NO DATA	NO DATA
0111-2704-5132	STB	WK36	44800	**K65R M184V**	S17N **E138K Q148R**	FTC-R >82 TDF 1.3 EVG-R >95

Bolded subjects PIDs had emergent RT on IN substitutions.
Italicized subject PIDs were removed from denominator because they resuppressed and had no resistance data.

In Study 109, 4 subjects (Subjects 2838-6691, 2840-6507, 5083-6551 and 0959-6571) achieved HIV-1 RNA resuppression to <50 copies/mL with further treatment and were not included in the sponsor's final resistance analysis population. However, all these virologic failures subjects were included FDA resistance analysis because they have confirmed >400 copies/mL and resistance data (Table B). Subject 2838-6691 demonstrated genotypic phenotypic resistance to

FTC (M184M/I; FTC FC = 3.8) at Week 8 and the HIV-1 RNA level resuppressed to <50 copies/mL by early study drug discontinuation when the subject switched to a new drug regimen. The remaining 2 subjects (Subjects 2840-6507 and 5083-6551) did not have resistance to emtricitabine or TAF detected, and no IN resistance data was available. Subject 2824-6999 in the E/C/F/TAF group that was included in the sponsor's final resistance analysis set had no detectable resistance to any study drug.

Table B. Emergent Substitutions in Virologic Failures from Study 109

PID	ARM	Timepoint with Resistance Data	Viral Load	RT Emergent Substitutions	INSTI Emergent Substitutions	Phenotype Resistance
0109-2824-6999	E/C/F/TAF	WK24	4970	NONE	S17N S39C	NONE
0109-2838-6691	E/C/F/TAF	WK8 resuppressed	662	**M184M/I**	I84M	**FTC-R 3.8**
0109-2840-6507	E/C/F/TAF	WK12 resuppressed	438	NONE	NO DATA	NO INSTI DATA
0109-5083-6551	E/C/F/TAF	WK4 resuppressed	617	NONE	NO DATA	NO INSTI DATA
0109-0959-6571	STB	WK12 resuppressed	4760	NO DATA	NO DATA	NO DATA

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/s/

LISA K NAEGER
10/26/2015

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)

AMENDED VIROLOGY REVIEW

NDA: 207561 **SDN:** 001 **DATE REVIEWED:** 06/29/15
Clinical Virology Reviewer: Lisa K. Naeger, Ph.D.

NDA#: 207,561 **Supporting Document #:** 000
Reviewer's Name(s): Lisa K. Naeger, Ph.D.

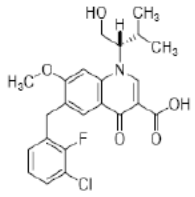
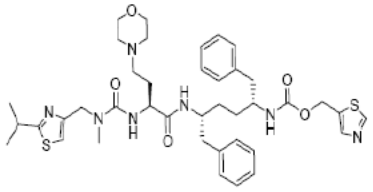
Applicant Name and Address:
Gilead Sciences, Inc.
333 Lakeside Drive
Foster City, CA 94404

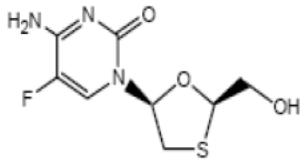
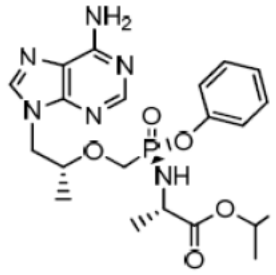
Initial Submission Dates:
Correspondence Date: November 5, 2014
CDER Receipt Date: November 5, 2014
Assigned Date: November 5, 2014
Review Complete Date: June 29, 2015
PDUFA Date: November 5, 2015

Amendments:

SDN	CDER Stamp Date	Assigned Date
003	11/25/14	01/28/15
005	01/26/15	01/28/15
011	03/13/15	03/16/15
020	05/29/15	06/01/15

Product Name(s): GENVOYA tablet is a fixed-dose combination product containing four active pharmaceutical ingredients, elvitegravir (EVG), cobicistat (COBI), emtricitabine (FTC), and tenofovir alafenamide fumarate (TAF).
Proprietary: GENVOYA
Non-Proprietary/USAN: EVG/COBI/FTC/TAF tablet
Code Name/Number: single-tablet regimen of EVG 150 mg, COBI 150 mg, FTC 200 mg, and TAF (GS-7340) 10 mg

Individual Component	EVG	COBI
Structure		
Chemical Name	6-(3-chloro-2-fluorobenzyl)-1-[(2S)-1-hydroxy-3-methylbutan-2-yl]-7-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid	1,3-thiazol-5-ylmethyl[(2R,5R)-5-{[(2S)-2-[(methyl{[2-(propan-2-yl)-1,3-thiazol-4-yl]methyl}carbamoyl)amino]-4-(morpholin-4-yl)butanoyl]amino}-1,6-diphenylhexan-2-yl]carbamate
Molecular Formula	$C_{23}H_{23}ClFNO_5$	$C_{40}H_{53}N_7O_5S_2$
Molecular	447.88	776.02

Weight		
Drug Class	INSTI	Pharmacoenhancer (No anti-HIV-1 activity in cell culture)
Supporting Document	IND 72,177; NDA 203093; NDA 203100	IND 102,283; NDA 203094
Individual Component	**FTC**	**TAF**
Structure		
Chemical Names	5-fluoro-1-(2R,5S)-[2-(hydroxymethyl) -1,3-oxathiolan-5-yl]cytosine	L-Alanine,N-[(S)-[[(1R)-2-(6-amino-9H-purin-9-yl)-1-methylethoxy] methyl]phenoxyphosphinyl]-,1-methylethyl ester,(2E)-2-butenedioate (1:1)
Molecular Formula	$C_8H_{10}FN_3O_3S$	$C_{25}H_{33}O_9N_6P$
Molecular Weight	247.24	592.54 Da
Drug Class	NRTI	NRTI
Supporting Document	IND (b) (4); IND 53971; NDA 21500;	IND 63737; IND 111007

Indication(s): Treatment of HIV-1
Dosage Form(s): Tablet (EVG 150 mg, COBI 150 mg, FTC 200 mg, and TAF, GS-7340 10 mg)
Route(s) of Administration: Oral
Recommended Dosage: One tablet taken once daily with food
Dispensed: Rx _X_ OTC ___ (Discipline relevant)
Abbreviations: ABC, abacavir; ADV, adefovir; AIDS, acquired immunodeficiency syndrome; APV, amprenavir; ARV, antiretroviral; ATR, Atripla; ATV, atazanavir; ATV/r, ritonavir-boosted atazanavir; AZT, zidovudine; bp, base pair; CC50, 50% cytotoxic concentration; CI, combination index; COBI, cobicistat; ddI, didanosine; DRV, darunavir; d4T, stavudine; EC50, effective concentration inhibiting viral replication by 50%; EFV, efavirenz; ELISA, enzyme-linked immunosorbent assay; ETR, etravirine; ETV, entecavir; EVG, elvitegravir; FTC, emtricitabine; HAART, highly active antiretroviral therapy; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV,

human immunodeficiency virus; IN, HIV-1 integrase; INSTI, HIV-1 integrase strand transfer inhibitor; LAM, lamivudine; LPV, lopinavir; MDR, multidrug-resistant; mtDNA, mitochondrial DNA; MVC, maraviroc; NDA, new drug application; NNRTI, HIV-1 non-nucleoside reverse transcriptase inhibitor; NRTI, HIV-1 nucleoside/nucleotide reverse transcriptase inhibitor; PBMC, peripheral blood mononuclear cell; PCR, polymerase chain reaction; HIV-1 protease inhibitor; PI/r, PK, pharmacokinetics; PR, HIV-1 protease; QD, once daily; RAL, raltegravir; RBV, ribavirin; RPV, rilpivirine; RT, HIV-1 reverse transcriptase;; RTV, ritonavir; SD, standard deviation; SQV, saquinavir; TAF, tenofovir alafenamide fumarate; TAM, thymidine analogue mutations; TDF, tenofovir disoproxil fumarate; TFV, tenofovir (active moiety of the diester prodrug TDF); TPV, tipranavir; T-20, enfuvirtide; VF, virologic failure; VR, virologic rebound;

TABLE OF CONTENTS

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)

AMENDED VIROLOGY REVIEW

NDA: 207561 **SDN:** 001 **DATE REVIEWED:** 06/29/15

Clinical Virology Reviewer: Lisa K. Naeger, Ph.D.

EXECUTIVE SUMMARY

This NDA for a fixed dose combination of elvitegravir, cobicistat, emtricitabine and tenofovir alafenamide fumarate is approvable from a virology perspective for the treatment of HIV-1 infection in adults and pediatric patients 12 years of age and older who have no antiretroviral treatment history or to replace the current antiretroviral regimen in those who are virologically-suppressed (HIV-1 RNA <50 copies/mL) on a stable antiretroviral regimen for at least 6 months with no history of treatment failure. Elvitegravir (NDA203093) and cobicistat (NDA 203094) were approved for the treatment of HIV-1 infection in September 2014 and emtricitiabine (NDA 21500) was approved for the treatment of HIV-1 infection in July 2003. The fixed dose combination of elvitegravir, cobicistat ,emtricitabine and tenofovir disoproxil fumarate (Stribild) (NDA 203100) was approved in August 2012. (b) (4) .

Tenofovir alafenamide fumarate (TAF) is a prodrug that is metabolized intracellularly to the active metabolite, tenofovir diphosphate (TFV-DP). TAF has EC_{50} values ranging from 0.14 to 12.0 nM, with a mean of 3.5 nM, against primary HIV-1 isolates. TAF has the same cytotoxicity profile as tenofovir disoproxil fumarate (TDF) and tenofovir. In addition, TAF and TDF have a similar resistance profile in cell culture and in clinical trials.

In treatment-naïve studies (Study 104 and 111) comparing the efficacy of elvitegravir, cobicistat, emtricitabine and tenofovir alafenamide fumarate (E/C/F/TAF) to elvitegravir, cobicistat, emtricitabine and tenofovir disoproxil fumarate (STRIBILD®; STB), there were a similar number of virologic failures in the E/C/F/TAF and STB arms with a similar resistance pattern. At Week 48, the development of one or more primary elvitegravir, emtricitabine, or tenofovir alafenamide fumarate substitutions associated with resistance was observed in 7 of 14 subjects with evaluable genotypic data from paired baseline and E/C/F/TAF treatment-failure isolates compared with 6 of 17 treatment-failure isolates from subjects in the STB treatment group. Of the 7 subjects with resistance development in the E/C/F/TAF group, the substitutions that emerged were M184V/I (N = 7) and K65R (N = 1) in reverse transcriptase and T66T/A/I/V (N = 2), E92Q (N = 2), E138K (N = 1), Q148Q/R (N = 1) and N155H (N = 1) in integrase. Of the 6 subjects with resistance development in the STB group, the substitutions that emerged were M184V/I (N = 5) and K65R (N = 1) in reverse transcriptase and E92E/Q (N = 2), E138K (n = 3) and Q148R (N = 2) in integrase. In both treatment groups, most subjects who developed substitutions associated with resistance to elvitegravir also developed emtricitabine resistance-associated substitutions.

In a clinical study of virologically-suppressed subjects (Study 109, N = 799) who switched from a regimen containing emtricitabine/tenofovir disoproxil fumarate and a third agent to E/C/F/TAF), one subject had emergent emtricitabine resistance, with the emergence of M184M/I, out of 4 virologic failure subjects.

1.1. RECOMMENDATION AND CONCLUSION ON APPROVABILITY:

This NDA for a fixed dose combination of elvitegravir, cobicistat, emtricitabine and tenofovir alafenamide fumarate is approvable from a virology perspective for the treatment of HIV-1 infection in adults and pediatric patients 12 years of age and older who have no antiretroviral treatment history or to replace the current antiretroviral regimen in those who are virologically-suppressed (HIV-1 RNA <50 copies/mL) on a stable antiretroviral regimen for at least 6 months with no history of treatment failure.

1.2. RECOMMENDATION ON PHASE 4 (POST-MARKETING) COMMITMENTS, AGREEMENTS, AND/OR RISK MANAGEMENT STEPS, IF APPROVABLE:

There are no phase 4 recommendations for this application.

2. SUMMARY OF OND VIROLOGY ASSESSMENTS

2.1 Nonclinical Virology

TAF is a prodrug that is metabolized intracellularly to the active metabolite, tenofovir diphosphate (TFV-DP). TFV-DP is an inhibitor of HIV-1 RT that competes with deoxyadenosine triphosphate (dATP) for incorporation into nascent DNA and terminates the elongation of the viral DNA chain during the process of retroviral reverse transcription and blocks HIV replication. EC_{50} values for TAF ranged from 0.14 to 12.0 nM, with a mean of 3.5 nM, against 26 primary HIV-1 isolates. TAF exhibits anti-HIV activity in lymphoid T-cells, primary human PBMCs, and macrophages with EC_{50} values ranging from 3 to 14 nM. The activity of TAF against HIV-1 in cell culture is 100- to 600-fold greater than TFV and 4- to 6-fold greater than TDF. In addition, TAF has activity against HIV-2 with a mean EC_{50} value of 1.8 nM and is an inhibitor of HBV replication, exhibiting cell culture activity comparable to that of TDF with an EC_{50} value of 18 nM. TAF exhibited minimal antiviral activity against adenovirus, dengue type 2, influenza A, parainfluenza 3, RSV, coxsackie B virus, rhinovirus, HSV-1, HSV-2, HCMV, VZV, vaccinia virus, or HCV.

In MT-2 cells, TAF shows low cytotoxicity with a selectivity index of >10,000. CC_{50} values for TAF ranged from 23.2 μM in MT-4 cells to >53.0 μM in MT-2 T-cells and >44.4 μM in HepG2 cells. A variety of cell culture studies have been conducted to evaluate the potential of TFV and TAF to exert mitochondrial toxicity. Results from cell growth; extracellular production of lactic acid; relative cellular content of mtDNA and mtDNA-encoded cytochrome coxidase II (COX II); and intracellular lipid accumulation studies indicate that TFV has limited capability to inhibit human DNA polymerases or to mediate cytotoxicity or mitochondrial damage. In addition, no antagonistic antiviral interaction was found between TAF and the clinically relevant classes of antiretroviral drugs.

Cell culture resistance selection experiments with TAF selected for the K65R substitution. Phenotypic analyses showed 6.5-fold reduced TAF susceptibility of K65R selected viruses. A K70E substitution as a mixture with wild-type was observed along with a K65R substitution as a

mixture with wild-type in the second experiment with TAF. The K70E substitution has been observed previously in clinical studies of TDF and is associated with slight decreases in tenofovir susceptibility in phenotypic assays. In addition, TAF showed a 2.1- and 5.4-fold reduced susceptibility in cell culture that was associated with the presence of D67N, K70R, M184V, and K219E, and multi-drug resistance Q151M mutation complex (A62V, V75I, F116Y, Q151M) + K65R + TAMs (M41L, K65R, D67N, L210W, and T215Y), respectively.

2.2 Clinical Virology

In the phase 3 active controlled studies, Studies 104 and 111, which assessed the efficacy of the E/C/F/TAF FDC compared with Stribild® (STB) in HIV-infected, ART-naive adults, there were 14 virologic failures in the E/C/F/TAF arm and 17 virologic failures in the STB arm in the FDA-defined virologic failure resistance subset. Of these virologic failures, 7 subjects (50%) had emergent resistance substitutions in the E/C/F/TAF arm and 6 subjects (35%) had emergent resistance substitutions in the STB arm. In the E/C/F/TAF arm, all 7 of the virologic failures with emergent substitutions had the M184V substitution and one subject had the K65R substitution; 5 of these 7 subjects had emergent INSTI resistance substitutions. In the STB arm, 5 of the 6 virologic failures with emergent substitutions had emergent M184V substitutions and one had the K65R substitution; 4 of the 6 subjects had emergent INSTI resistance substitutions. The number and type of emergent NRTI and INSTI resistance substitutions is similar in both arms. TAF did not reduce the frequency of virologic failure or number of emergent resistance substitutions compared to TDF. In addition, the resistance pathways of TAF are similar to TDF.

In Study 109, where virologically suppressed subjects switched from a FTC/TDF regimen to a E/C/F/TAF regimen, 4 subjects in the E/C/F/TAF arm were virologic failures and 1 subject was a virologic failure in the comparison FTC/TDF+ 3rd agent arm. One subject in the E/C/FTAF arm demonstrated genotypic and phenotypic resistance to FTC (M184M/I; FTC FC = 3.8) at Week 8. The other 4 subjects did not have detectable resistance to FTC or TAF.

3. ADMINISTRATIVE

3.1. Reviewer's Signatures

Lisa K. Naeger
Lisa K. Naeger, Ph.D.
Sr. Clinical Virology Reviewer

3.2. Concurrence

HFD-530/MicroTL/J. O'Rear, Ph.D.

4 OND VIROLOGY REVIEW

4.1 Important Milestones in Development

STRIBILD (STB), a fixed-dose combination of elvitegravir (EVG, 150 mg), an HIV-1 integrase strand transfer inhibitor (INSTI), and cobicistat (COBI, 150 mg), a pharmacoenhancer, combined with two FDA-approved nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), emtricitabine (200 mg; Emtriva®) and tenofovir disoproxil fumarate (300 mg; Viread®) was approved on August 27, 2012 for the treatment of HIV-1 infection in antiretroviral treatment-naïve adult patients. This application for a FDC for GENVOYA is a fixed dose combination of EVG, COBI, FTC and the new component, tenofovir alafenamide fumarate (TAF). TAF is a prodrug, which is metabolized to the active metabolite, tenofovir diphosphate – the same active metabolite of TDF.

4.2 Methodology

Criteria for Resistance Testing

Genotyping of the protease (PR)/RT genes and the integrase (IN) gene was performed at screening for all subjects enrolled in Studies GS-US-292-0104, GS-US-292-0111, GS-US-292-0106, and GS-US-292-0112 Cohort 2. For all subjects in Study GS-US-292-0109, historical PR/RT genotypes were used because genotyping was not performed at screening since subjects entered the study with suppressed HIV-1 RNA <50 copies/mL.

Resistance testing was performed for any subject meeting the criteria of the resistance analysis population, which included any subject who received at least 1 dose of study drug, maintained their study drug regimen (or within 72 hours after interruption or discontinuation of study drugs), and met one of the following virologic failure (VF) criteria:

- Suboptimal virologic response (SVR): HIV-1 RNA <1 $\log_{10}$ reduction from baseline and ≥50 copies/mL HIV-1 RNA at the Week 8 visit, confirmed at a scheduled or unscheduled visit at least 2 weeks following Week 8
- Virologic rebound (VR): At any visit, after achieving <50 copies/mL HIV-1 RNA, a rebound in HIV-1 RNA above 50 copies/mL, which was subsequently confirmed at the following scheduled or unscheduled visit;

OR

At any visit, a >1 $\log_{10}$ increase in HIV-1 RNA from the nadir, which was subsequently confirmed at the following scheduled or unscheduled visit

- Viremic at Final Timepoint: Any subject with HIV-1 RNA ≥400 copies/mL at the study endpoint or study discontinuation who did not meet any of the criteria above also had PR/RT and IN genotyping and phenotyping performed.

Ad hoc resistance analyses could be conducted at the request of the medical monitor or the treating physician. A subject was included in the resistance analysis population if the HIV-1 RNA level at failure was ≥400 copies/mL. If a subject remained on study drug and was later suppressed HIV-1 RNA to <50 copies/mL, this subject was not included in the final resistance analysis population. However, these subjects could still have resistance data available which are included.

Resistance testing included genotyping and phenotyping of PR/RT and IN at the VF time point. [redacted] (b) (4) was the designated reference laboratory for all resistance analyses at screening and virologic failure. Resistance testing was only conducted when HIV-1 RNA was ≥400 copies/mL, which is close to the validated limits of detection of the Monogram Biosciences assays (500 copies/mL). For subjects with confirmed virologic failure, the plasma sample corresponding to the confirmed virologic failure time point (second time point) was analyzed. If needed, the first plasma sample could also have been used for the resistance analysis.

Genotypes/Phenotypes

HIV-1 genotyping of the PR/RT and IN genes were conducted at screening to assess for preexisting resistance as part of enrollment criteria for most studies. As defined in the protocol, subjects were to have genotypic sensitivity to EVG, FTC, and tenofovir. The GenoSure MG® or GenoSure PRIme® assays, which determine HIV-1 PR/RT or PR/RT/IN genotype, respectively, were performed for all subjects at screening in Studies GS-US-292-0104, GS-US-292-0111, GS-US-292-0106, and GS-US-292-0112. The GenoSure MG assay covers the entire PR gene and amino acids 1-400 of RT. The GenoSure PRIme assay covers the entire PR gene, amino acids 1-400 of RT, and the entire IN gene. In cases where subjects had assay failure for both GenoSure MG and GenoSure PRIme, an alternative commercially available assay was used to obtain genotype information. For subjects enrolled in Studies GS-US-292-0104 and GS-US-292-0111 harboring subtype AE virus that failed several IN genotyping assays, IN genotyping was conducted at Gilead using RT polymerase chain reaction (PCR) and standard Sanger population sequencing.

The PhenoSense® GT assay, GenoSure® IN assay, and PhenoSense® IN assay (Monogram Biosciences, South San Francisco, CA) were used to determine subjects' genotypes and phenotypes for PR/RT and IN, respectively, at the time of confirmed virologic failure. For subjects with virologic failure who were missing screening genotype information, baseline plasma samples were evaluated for genotypic and phenotypic resistance. The PhenoSense GT assay tests for genotypic and phenotypic resistance to all currently approved antiretroviral drugs in the NRTI, NNRTI, and PI classes. The GenoSure IN assay tests for IN genotype, while the PhenoSense IN assay tests for IN phenotype. These data were made available to study investigators in real time for cases of suboptimal virologic response and virologic rebound.

Baseline PR, RT, and IN sequences were analyzed for the presence of previously identified resistance-associated substitutions to tenofovir (delivered as TDF or TAF), FTC, and EVG for all studies (Table 1). Detection of resistance substitutions to any study drug excluded treatment-naïve subjects from enrollment.

Post-baseline sequences were compared with subject-specific baseline sequences to determine if resistance-associated substitutions had developed in PR, RT, and/or IN during treatment. A substitution was considered to have emerged if it was detected post-baseline but not at baseline. If a substitution was detected as a mixture at baseline and then resolved to the consensus amino acid post-baseline, it was not considered to have emerged.

Table 1. Previously Identified Drug Resistance Substitutions by Antiretroviral Class

Resistance Mutations[a]		
Drug Class	**Codon Mutations**	
Nucleoside and Nucleotide Reverse Transcriptase Inhibitors (NRTIs)	M41L, E44D, A62V, K65R, D67N, T69 insertion, T69D/N, K70E/R, L74V/I, V75I, F77L, Y115F, F116Y, V118I, Q151M, M184V/I, L210W, T215Y/F, K219E/Q/N/R	
Thymidine Analogue Mutations (TAMs)	M41L, D67N, K70R, L210W, T215Y/F[b], K219Q/N/E/R	
Nucleoside-Associated Mutations (NAMs)	TAMs plus E44D[c], K65R, T69D/N[c], K70E, L74V/I, Y115F, V118I[c], M184V/I	
Multi-NRTI Resistance Mutations	Q151M Complex: A62V, V75I, F77L, F116Y, Q151M	
Multi-NRTI Resistance Mutations	T69 Insertion Complex: T69S-SS, -SA, -SG, or others	
Nonnucleoside Reverse Transcriptase Inhibitors (NNRTIs)	V90I, A98G, L100I, K101E/H/P, K103N/S, V106M/A/I, V108I, E138A/G/K/Q/R, V179D/F/L/T, Y181C/I/V, Y188C/H/L, G190A/E/Q/S, H221Y, P225H, F227C, M230L/I	
Protease Inhibitors (PIs)	**Primary**	**Secondary**
	D30N, V32I, L33F, M46I/L, I47V/A, G48V, I50V/L, I54M/L, Q58E, T74P, L76V, V82A/F/L/S/T, I84V, N88S, L90M	L10I/F/R/V/C, V11I, I13V, G16E, K20I/M/R/T/V, L24I, L33I/V, E34Q, E35G, M36I/L/V, K43T, F53L/Y, I54A/S/T/V, D60E, I62V, L63P, I64L/M/V, H69K, A71V/T/I/L, G73A/C/S/T, V77I, V82I, N83D, I85V, N88D, L89V, I93L/M
Entry Inhibitors	—	G36D/S, I37V, V38A/E/M, Q39R, Q40H, N42T, N43D
Integrase Strand Transfer Inhibitors (INSTIs)[d]	**Primary**	**Secondary**
	T66I/A/K, E92Q/G, T97A, Y143R/H/C, S147G, Q148H/K/R, N155H/S	M50I, H51Y, L68V/I, V72A/N/T, L74M, Q95K/R, G118R, S119P/R/T, F121C/Y, A128T, E138K/A, G140A/C/S, P145S, Q146R/I/K/L/P, V151L/A, S153A/F/Y, E157K/Q, G163K/R, E170A, R263K

a Adapted from the current International Antiviral Society-USA (IAS-USA) Guidelines lists with some modifications.
b Reversion mutations at RT codon T215, including T215A/C/D/E/G/H/I/L/N/S/V have not been definitively shown to be associated with reduced response to either FTC or TDF.
c E44D, T69D/N, and V118I mutations can be natural polymorphisms in RT and have not been shown to be associated with reduced response to either FTC or TDF.
d Primary and secondary IN mutations observed in clinical studies of INSTIs.

4.3 Prior FDA Reviews

The original NDA-203100 for STRIBILD® was reviewed by clinical virologists, Sung Rhee, Ph.D. and Takashi Komatsu, Ph.D. STRIBILD® tablets contain a fixed-dose combination of elvitegravir (EVG, 150 mg), an HIV-1 integrase strand transfer inhibitor (INSTI), and cobicistat (COBI, 150 mg), a pharmacoenhancer, combined with two FDA-approved nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), emtricitabine (200 mg; Emtriva®) and tenofovir disoproxil fumarate (300 mg; Viread®). The proposed indication for the STRIBILD® tablet is for once daily use as a complete regimen for the treatment of HIV-1 infection in adult patients, aged 18 years and over, who are antiretroviral treatment-naïve and have no known substitutions associated with resistance to the individual components of the regimen. It was approved on August 27, 2012 for the treatment of HIV-1 infection in antiretroviral treatment-naïve adult patients, based on Week-48 data from 2 Phase 3 studies and Week-60 data from a supportive Phase 2 study.

4.4 State of Antivirals Used for the Indication Sought

Since highly-active antiviretroviral therapy (HAART) regimens have been introduced, the number of AIDS cases has decreased dramatically; however, HAART does not clear HIV-1 from subjects and even though the number of serum HIV-1 RNA copies is reduced to undetectable levels, HIV-1 re-emerges quickly after discontinuation of HAART. Therefore, with the currently available regimens, it is likely that HIV-infected subjects will require antiretroviral therapy throughout their lives.

There are currently greater than 25 FDA-approved anti-HIV-1 drugs including including integrase strand transfer inhibitors (INSTIs) (dolutegravir, elvitegravir and raltegravir), NNRTIs (delavirdine, efavirenz, etravirine, nevirapine, rilpivirine), NRTIs (abacavir, didanosine, emtricitabine, lamivudine, stavudine, tenofovir, zalcitabine, zidovudine), PIs (atazanavir, darunavir, fosamprenavir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir, tipranivir), the fusion inhibitor enfuvirtide, and the CCR5 coreceptor antagonist maraviroc. NRTIs mimic nucleosides and target HIV-1 RT by competing with natural deoxynucleoside triphosphates for binding to RT and by incorporating into newly synthesized viral DNA resulting in chain-termination. NNRTIs inhibit HIV-1 RT by binding near the catalytic site of RT and acting as noncompetitive inhibitors. Integrase catalyzes the integration of linear viral DNA into host cell DNA forming the provirus. INSTIs bind to the integrase active site and block the strand transfer step of retroviral DNA integration. PIs work at the late stage of viral replication to prevent virus production from infected cells. They block the HIV-1 protease enzyme, which is necessary for the production of mature virions, resulting in defective particles which are unable to infect new cells. Maraviroc inhibits the interaction between the viral envelope glycoprotein gp120 and the human CCR5 receptor membrane protein and inhibits entry of the virus into the cell. Enfuvirtide is a gp41 fusion inhibitor preventing the joining of the viral and cellular membranes necessary for virus entry.

Additionally, there are multiple fixed dose combinations (FDC) of anti-HIV-1 drugs. One FDA-approved FDC, STRIBILD®, contains elvitegravir, emtricitabine, tenofovir disoproxil and cobicistat. Cobicistat, or COBI, was designed as a specific inhibitor of CYP3A, the body's major drug-metabolizing enzyme, for use as a PK enhancer (booster) to increase the systemic levels of coadministered agents metabolized by this enzyme system. Enzyme inactivation studies have demonstrated that COBI is an efficient inactivator of human hepatic microsomal CYP3A activity, with enzyme kinetic parameters (K_i and k_{inact}) comparable to those of ritonavir. CYP3A-mediated oxidative metabolism is the major biotransformation pathway for COBI, as it is for ritonavir; however, unlike ritonavir, COBI is a more specific CYP enzyme inhibitor. It is a weak inhibitor of CYP2D6 and does not inhibit CYP1A2, CYP2C9, or CYP2C19. In addition, COBI displays low liability for induction through activation of xenobiotic receptors, including the aryl hydrocarbon receptor, pregnane X receptor, and the constitutive androstane receptor, in human hepatocytes. In contrast, ritonavir, a known potent pregnane X receptor activator, produces significant induction of phase I enzymes, including CYP3A, as well as phase II uridine 5'-diphospho-glucuronosyltransferase enzymes and drug transporters, including P-gp, that lead to clinically significant drug–drug interactions. Other favorable characteristics of COBI are the absence of HIV-1 protease inhibition and anti-HIV activity in general (half-maximal effective concentration value of >30 μM) and reduced perturbation of the normal adipocyte functions of lipid accumulation and/or response to insulin.

This NDA application is for a fixed dose combination of elvitegravir, cobicistat, emtricitabine and tenofovir alafenamide fumarate. The 3 antiviral components (elvitegravir, emtricitabine and tenofovir alafenamide fumarate) of the new regimen target independently two essential viral enzymes, integrase (IN) and reverse transcriptase (RT), that are required during the early stages of the HIV-1 life cycle. The new component of this FDC is tenofovir alafenamide fumarate, which is a prodrug that is metabolized to the active moiety, tenofovir diphosphate – the same active moiety as the approved NRTI, tenofovir disoproxil fumate (TDF).

5 NONCLINICAL VIROLOGY

Previous nonclinical reports for EVG and COBI have been reviewed in NDA-203100. The following nonclinical review is for TAF.

MECHANISM OF ACTION

Following its release intracellularly from the TAF prodrug bycathepsin A, tenofovir (TFV) is metabolized intracellularly to the active metabolite, tenofovir diphosphate (TFV-DP). TFV-DP inhibits HIV-1, HIV-2 and HBV polymerases, and is an inhibitor of HIV-1 RT that competes with deoxyadenosine triphosphate (dATP) for incorporation into nascent DNA and terminates the elongation of the viral DNA chain during the process of retroviral reverse transcription, thereby effectively blocking the replication of HIV. The kinetic inhibition (K_i) constant for TFV-DP against HIV-1 reverse transcriptase (ribonucleic acid [RNA]-directed DNA synthesis) is 0.02 μM, more than 200-fold lower than its K_i for human DNA polymerase α and more than 3000-fold lower than its K_i values for human DNA polymerases β and γ. Unlike TDF, TAF is relatively stable in human plasma ($t_{½}$ ~90 minutes), but rapidly converts to TFV inside cells. Assessment of the intracellular metabolism of TAF in various types of immune cells including cluster determinant 4 $(CD4)^+$ T-cells, lymphocytes, and monocytes showed efficient conversion of the prodrug to the active metabolite TFV-DP.

ANTIVIRAL ACTIVITY IN CELL CULTURE

TAF was tested in cell culture in activated peripheral blood mononuclear cells (PBMCs) against HIV isolates by (b) (4) The HIV isolates were wild-type clinical isolates, representing HIV-1 isolates from group M (subtypes A to G), groups N and O, as well as HIV-2. Overall, for the 26 primary HIV-1 isolates tested, TAF EC_{50} values ranged from 0.14 to 12.0 nM, with a mean of 3.5 nM (Table 2; Report PC-120-2004 derived from data on Pages 6-8). For the 3 HIV-2 isolates, the mean EC_{50} value was 1.8 nM. TAF antiviral activity was also evaluated against 7 HIV-1 primary isolates with resistance substitutions across multiple drug classes. Five of the 7 isolates were single class resistant mutants, including one NNRTI resistant mutant (NNRTI-R), 2 PI resistant mutants (PI-R), and 2 INSTI resistant mutants (INSTI-R). The 2 remaining isolates had either NRTI resistance (NRTI-R) plus NNRTI-R, or NRTI-R plus PI-R. TAF demonstrated antiviral activity against the NNRTI-R, the PI-R and the INSTI-R resistant mutants. For the 2 viruses that contained NRTI resistance substitutions, TAF showed a 2.1- and 5.4-fold reduced susceptibility that was associated with the presence of D67N, K70R, M184V, and K219E in the first isolate (5705-72), and multi-drug resistance Q151M mutation complex (A62V, V75I, F116Y, Q151M) + K65R + TAMs (M41L, K65R, D67N, L210W, and T215Y) in the second isolate (MDR 769). Overall, TAF showed antiviral activity against all HIV-1 groups/subtypes evaluated, HIV-2, and drug resistant isolates from other classes.

Table 2. Antiviral Activity of TAF in PMBCs

Virus Isolate	EC_{50} (nM)	TC_{50} (nM)	Therapeutic Index
92UG029 Subtype A	8.75	> 500	> 57.1
92UG037 Subtype A	0.69	> 500	> 727
92RW016 Subtype A	0.71	> 500	> 700
93BR021 Subtype B	9.69	> 500	> 51.6
JR-CSF Subtype B	1.05	> 500	> 477
90US873 Subtype B	3.87	> 500	> 129
92BR025 Subtype C	4.16	> 500	> 120
98BR004 Subtype C	1.75	> 500	> 120
93IN101 Subtype C	1.50	> 500	> 333
92UG001 Subtype D	3.79	> 500	> 132
92UG046 Subtype D	0.99	> 500	> 503
92UG024 Subtype D	6.27	> 500	> 79.8
93TH073 Subtype E	1.18	> 500	> 423
CMU06 Subtype E	1.36	> 500	> 368
CMU08 Subtype E	5.88	> 500	> 85.0
93BR019 Subtype F	0.73	> 500	> 682
92BR024 Subtype F	5.97	> 500	> 83.8
93BR020 Subtype F	2.22	> 500	> 225
93BR029 Subtype F	0.14	> 500	> 3,679
G3 Subtype G	5.34	> 500	> 93.7
RU570 Subtype G	12.0	> 500	> 41.7
JV1083 Subtype G	9.85	> 500	> 50.8

BCF02 Group O	1.30	> 500	> 384
BCF03 Group O	3.01	> 500	> 166
BCF07 Group O	0.10	> 500	> 4,873
YBF30 Group N	1.98	> 500	> 252
CDC 310319 HIV-2	2.63	> 500	> 190
CDC 310342 HIV-2	1.96	> 500	> 255
CBL-20 HIV-2	0.91	> 500	> 552
MDR 769 NRTI-R (M41L, K65R,D67N, V75I, Q151M, T215Y) NNRTI-R (Y181I) PI-R (M46L,I54V, V82A, I84V, L90M)	18.6	> 500	> 26.8
A-17 NNRTI-R (K103N, Y181C)	5.80	> 500	> 86.2
5705-72 NRTI-R (D67N, K70R, M184V, K219E) NNRTI-R (K103N)	7.09	> 500	> 70.6
1064-52 PI-R (I54V,V82F, L90M)	1.78	> 500	> 281
52-52 PI-R(M46I,I54V, V82T)	1.24	> 500	> 402
8070_1 INI-R (G140S, Y143H, Q148H)	0.67	> 500	> 750
4736_4 INI-R (E92Q, N155H)	0.34	> 500	> 1,293

TAF exhibited antiviral activity against all virus isolates tested in this assay system (range of EC_{50} values = 0.10 nM to 18.6 nM). No cytotoxicity was observed with this compound at the concentrations evaluated (TC_{50} >500 nM). TAF exhibits anti-HIV activity in lymphoid T-cells, primary human PBMCs, and macrophages with EC_{50} values ranging from 3 to 14 nM. The activity of TAF against HIV-1 in cell culture is 100- to 600-fold greater than TFV and 4- to 6-fold greater than TDF. In MT-2 cells, TAF shows low cytotoxicity with a selectivity index (SI) of

>10,000). In addition, TAF is an inhibitor of HBV replication, exhibiting cell culture activity comparable to that of TDF with an EC_{50} value of 18 nM.

A critical step in the intracellular metabolic activation of TAF is mediated by the lysosomal protease cathepsin A (CatA). Intracellular CatA activity together with intracellular metabolism and antiretroviral activity of TAF in $CD4^+$ T cells (CD4s) and monocyte-derived macrophages (MDMs) obtained from a demographically diverse group of donors was assessed. The levels of CatA and intracellular TAF metabolites differed minimally in CD4s and MDMs among 13 tested donors (Table 3; Report PC-120-2017, Page 10). The mean ± SD rate of TFV-alanine and TFV formation (products of the CatA-mediated hydrolysis of TAF) was comparable in cellular extracts prepared from quiescent and activated CD4s (2.7 ± 0.9 and 3.0 ± 0.6 pmol/min•µg, respectively) with not more than a 3-fold difference across individual donors. Overall, CatA activity in MDMs was approximately 2-fold greater than that observed in CD4s, averaging 7.1 ± 3.3 pmol/min•µg, with a range of 3.1 to 13.9 pmol/min•µg among the individual donors.

Table 3. Cathepsin A Activity in Quiescent and Activated Primary Human CD4s and MDMs

Donor	Gender	Ethnicity	TAF Conversion Rate (pmol/min•µg protein)[a]		
			Resting CD4s	Activated CD4s	MDMs
1	F	Caucasian	1.0	2.8	8.2
2	M	Caucasian	2.4	2.9	5.3
3	M	African Descent	4.3	4.2	5.9
4	F	Non-Hispanic White	3.5	3.8	6.7
5	F	Mixed Descent	4.2	3.7	6.1
6	F	Mixed Descent	2.6	2.7	5.3
7	M	Caucasian	2.4	2.9	13.1
8	F	African Descent	2.5	2.9	13.9
9	M	Caucasian	1.7	2.6	10.1
10	F	Hispanic	3.2	2.1	3.1
11	M	Caucasian	2.6	2.9	5.1
12	M	Non-Hispanic White	2.4	2.5	4.4
13	F	Hispanic	2.5	2.9	5.5
Mean ± SD			**2.7 ± 0.9**	**3.0 ± 0.6**	**7.1 ± 3.3**

a The rate of TAF (30 µM) hydrolysis was determined by HPLC analysis of TAF, TFV-Ala, and TFV upon incubation with CD4 and MDM extracts for 10, 30, and 120 minutes. The activity was normalized to the total amount of protein in each cell extract. The rate of CatA-specific activity was calculated over the 3 time points and expressed as picomoles of metabolites produced per minute per microgram protein.

The intracellular accumulation of TAF metabolites in isolated populations of activated $CD4^+$ T cells and MDMs was measured from a subset of 8 donors. Intracellular TFV and TFV-DP were readily detected, as was the intermediate TFV-MP at lower concentrations. Among the 8 donors analyzed, the total mean ± SD intracellular TAF metabolite levels were 20.8 ± 4.9 µM (range

14.5 to 24.5 µM) and 23.5 ± 2.8 µM (range 15.1 to 24.2 µM) in CD4s and MDMs, respectively. Accumulation of parent TFV occurred uniformly within all analyzed samples, indicative of CatA-mediated TAF intracellular activation being consistent across the individual tested donors. Moreover, the intracellular conversion of TFV to TFV-DP also occurred within a narrow 3-fold range both in CD4s and MDMs, indicating that the phosphorylation of TFV occurs uniformly in both target cell types. Taken together, these data demonstrate that the intracellular accumulation and conversion of TAF to TFV-DP occurred consistently in both CD4 and MDM cells across all tested donors.

TAF antiviral activity was assessed in the 13 donors. The mean EC_{50} value of TAF in all tested donors was 11.0 nM (range 6.6 to 19.9 nM) and 9.7 nM (range 2.5 to 15.7 nM) in CD4s and MDMs, respectively. The relative range of TAF antiviral activity across all tested donors was comparable to that of other HIV-1 RT inhibitors. Across all tested donors, TAF had >600-fold and >80-fold lower EC_{50} values than TFV in CD4s and MDMs, respectively, a result consistent with previous findings in T-cell lines. These results indicate consistent intracellular metabolism and antiretroviral activity of TAF in relevant target cells of HIV-1 infection across demographically diverse donors.

Activity of TAF against Other Animal Viruses

TAF antiviral activity was evaluated against a panel of 18 human viruses and compared with TFV by (b) (4). The viruses tested included one or several isolates of the following: adenovirus, dengue type 2, influenza A, parainfluenza 3, RSV, coxsackie B virus, rhinovirus, HSV-1, HSV-2, HCMV, VZV, vaccinia virus, HCV, HIV-1 and SIV. With the exception of the known antiviral activity against HIV-1 and SIV, TAF and TFV exhibited minimal antiviral activity against most of the viruses evaluated in this study with EC_{50} values >1,000 nM and >1,000 µM, respectively (Table 4; Report PC-120-2003, compiled from Tables 3-17 on pages 16-20). Neither TFV nor TAF exhibited cytotoxicity up to the high-test concentrations of 1,000 µM or 1,000 nM, respectively, used for these evaluations.

Table 4. Activity of TAF and TFV against Human Viruses

Virus	TAF	TFV
Adenovirus Type 50	>1,000 nM	>1,000 µM
Dengue Virus Type 2 Strain New Guinea C	>1,000 nM	>1,000 µM
Influenza A Strain A/PR/8/34	>1,000 nM	>1,000 µM
Parainfluenza 3 Strain C 243	843 nM	>1,000 µM
RSV Strain Long	>1,000 nM	>1,000 µM
Coxsackie B3	>1,000 nM	>1,000 µM
Rhinovirus Strain 1B	>1,000 nM	>1,000 µM
HSV-1 Strain HF	>1,000 nM	>1,000 µM
HSV-2 Strain KW	>1,000 nM	29 µM
HCMV Strain AD169	>1,000 nM	>1,000 µM
VZV Strain Ellen	>1,000 nM	>1,000 µM
Vaccinia Strain Western	>1,000 nM	>1,000 µM

Reserve		
HCV RNA Replicons Strain Con1	>1,000 nM	>1,000 μM
Two SIV Strains	1.21 nM 0.51 nM	0.73 μM 0.35 μM
Two HIV-1 Strains	2.04 nM 5.89 nM	1.05 μM 2.30 μM

CYTOTOXICITY

The cytotoxicity of TAF was evaluated in 2 T-lymphoblastoid cell lines (MT-2 and MT-4) and one hepatic cell line (HepG2) following 5 days of continuous compound exposure. CC_{50} values for TAF ranged from 23.2 μM in MT-4 cells to >53.0 μM in MT-2 T-cells and >44.4 μM in HepG2 cells (Table 5; Report PC-120-2007, page 11). The CC_{50} values for the prodrug TDF were comparable to TAF, and ranged from 22.9 μM (MT-4) to 37.1 μM (MT-2) in T-cells and >44.4 μM in HepG2 cells based on the highest concentration tested. TFV, the parent drug for both TAF and TDF, exhibited higher CC_{50} values which ranged from 6,264 μM (MT-4) to 7,605 μM (MT-2) in T-cells and >44.4 μM in HepG2 cells.

Table 5. Cellular Cytotoxicity Evaluation of TAF and other HIV Inhibitors in Multiple Cell Lines after Five Days of Drug Exposure

			Cytotoxicity CC_{50}, μM (MSD)[a]		
			Hepatic	T-Cell	
Compound	Class	Abbrev.	HepG2	MT-2	MT-4
GS-007340	NtRTI	TAF	>44.4 (1)	>53.0 (1)	23.2 (1.13)
GS-004331	NtRTI	TDF	>44.4 (1)	37.1 (1.02)	22.9 (1.04)
GS-001278	NtRTI	TFV	>44.4 (1)	7605 (1.06)	6264 (1.13)

TAF had EC_{50} values of 14.7 nM and 11.6 nM in MT-2 and MT-4 cells, respectively. Based on these values, therapeutic indices of TAF ranged from >3,607 in MT-2 to 1,997 in MT-4 cells. By comparison, TDF exhibited a therapeutic index of 604 in MT-2 cells and 1,167 in MT-4 cells, and TFV exhibited a therapeutic index of 2,265 in MT-2 cells and 438 in MT-4 cells.

In addition, the cytotoxicity of TAF was investigated in resting and dividing human peripheral blood mononuclear cells (PBMCs) over a 5-day continuous drug incubation from up to 10 different donors. The mean CC_{50} values of TAF were 6.8 μM in dividing PBMCs (range 3.4 - 9.3 μM) and 25.1 μM in resting PBMCs (range 6.8 - 43.4 μM), leading to a therapeutic index of >1,900 in dividing PBMCs when compared to the EC_{50} value of 3.5 nM.

Mitochondrial Toxicity

A variety of clinical symptoms observed in HIV-1 patients treated with prolonged NRTI therapy appear to be linked to mitochondrial toxicity. Several representatives of this class of HIV-1 drugs inhibit mitochondrial DNA polymerase γ, by direct binding and competition with the natural deoxyribonucleotide substrate, incorporation into DNA, leading to DNA chain termination.

A variety of cell culture studies have been conducted to evaluate the potential of TFV and TAF to exert mitochondrial toxicity. Results from these studies suggest that TFV have limited capability to inhibit human DNA polymerases or to mediate cytotoxicity or mitochondrial damage. Cell culture combination studies have also been conducted in HepG2 cells to further evaluate the potential mitochondrial toxicity of TFV. HepG2 cells were exposed for 5 days to FTC and TFV (as well as other nucleosides), either alone or in combination. Assay endpoints included cell growth; extracellular production of lactic acid; relative cellular content of mtDNA and mtDNA-encoded cytochrome c oxidase II (COX II); and intracellular lipid accumulation. Tenofovir and FTC alone or in combination with each other or other nucleosides generally had no time- or concentration-dependent effects on cytotoxicity (cell counts) or mitochondrial parameters in HepG2 liver cells. The dual combination of high-dose FTC+ZDV, with or without TFV, appeared to have greater cytotoxicity than the agents alone, but showed no increase in mitochondrial effects.

Tenofovir alafenamide fumarate did not cause a specific depletion of mtDNA in HepG2 cells at concentrations as high as 1.0 µM, a level exceeding the maximum clinical systemic exposure of the 25 mg dose of TAF by more than 2-fold (C_{max} = 0.48 µM; Study GS-US-120-0104). Thus, TAF should have a low potential for inhibiting mtDNA synthesis and inducing NRTI-related mitochondrial toxicities at the anticipated human exposure.

No effect of TFV was seen on the synthesis of mtDNA or lactic acid production in HepG2 human liver cells or in normal human skeletal muscle cells (SkMCs). The results of these studies indicate a low potential for TFV to interfere with mitochondrial functions. These studies confirmed that the potential of FTC and TFV to interfere with mitochondrial functions is low, whether administered alone or in combination with other NRTIs. Further, because administration of TAF results in lower exposure to TFV compared to TDF, the potential for mitochondrial toxicity is also low with the E/C/F/TAF FDC. No additional nonclinical studies were done with the combination of EVG, COBI, FTC, and TAF.

COMBINATION ACTIVITY IN CELL CULTURE

The anti-HIV activity of TAF was examined in combination with representatives from 4 major classes of antiretrovirals in cell culture using HIV-1_{IIIB} infected MT-2 cells. TAF was tested in various combinations with nucleo(t)side reverse transcriptase inhibitors (TFV, and FTC), non-nucleoside reverse transcriptase inhibitors (NNRTI, EFV and NVP), integrase strand transfer inhibitors (INSTI; EVG, RAL, DTG), and protease inhibitors (PI, ATV and DRV). Combinations of ddI + RBV, d4T + RBV, and TAF with itself were used as controls for synergy, antagonism, and additivity, respectively. The combination of TAF with TFV resulted in an additive effect, as expected, as both deliver TFV-DP to the cells. When combined with any of the NRTIs or NNRTIs, TAF exhibited moderate to high synergistic effects, with synergy score values ranging from 41 to 131 (Table 6; Report PC-120-2002, page 9). The combination of TAF with INSTIs resulted in the highest level of synergy (271, 205, and 179 for EVG, RAL, and DTG, respectively). When TAF was combined with PIs, it resulted in moderate synergy, with synergy scores of 96 and 56 for ATV and DRV, respectively. No antagonistic antiviral interaction was found between TAF and the clinically relevant classes of antiretroviral drugs.

Table 6. TAF Anti-HIV-1 Activity in Combination with Selected Antiretrovirals

Drug combination[a]	Class	Net Effect	Synergy Score[b]	Antagonism Score[b]
TAF + TFV	NRTI	Additive	24	-14
TAF + FTC	NRTI	Strong synergy	131	-9
TAF + EFV	NNRTI	Strong synergy	100	-7
TAF + NVP	NNRTI	Minor synergy	41	-14
TAF + EVG	INSTI	Strong synergy	271	-9
TAF + RAL	INSTI	Strong synergy	205	-10
TAF + DTG	INSTI	Strong synergy	179	-10
TAF + ATV	PI	Moderate synergy	96	-10
TAF + DRV	PI	Moderate synergy	56	-12
TAF + COBI	PK enhancer	Additive	17	-22
TAF + TAF	Control	Additive	20	-17
ddI + RBV	Control	Strong synergy	302	-20
d4T + RBV	Control	Strong antagonism	20	-340

a TAF, tenofovir alafenamide; TFV, tenofovir; FTC, emtricitabine; EFV, efavirenz; NVP, nevirapine; EVG, elvitegravir; RAL, raltegravir; DTG, dolutegravir; ATV, atazanavir; DRV, darunavir; COBI, cobicistat; RBV, ribavirin; d4T, stavudine; ddI, didanosine

b Data shown represent the mean from >3 independent experiments performed in triplicate.

Additive to synergistic effects were observed in cell culture combination interaction studies of TFV, the active metabolite of TAF, with NRTIs (abacavir, FTC, lamivudine, stavudine, zalcitabine, zidovudine [ZDV]), nonnucleoside reverse transcriptase inhibitors (NNRTIs) (delavirdine, efavirenz [EFV], nevirapine), PIs (amprenavir, indinavir, nelfinavir, RTV, saquinavir), and the IN inhibitor EVG. No antagonistic interactions were observed for any of these 2-drug combinations in a T lymphoblastoid cell line.

Cathepsin A (CatA) plays an essential role in the intracellular activation of TAF. Since certain viral protease inhibitors such as telaprevir were shown to be inhibitors of CatA, the potential for drug-drug interactions between TAF and viral protease inhibitors was investigated. The HIV protease inhibitors atazanavir, darunavir, lopinavir and ritonavir, as well as boosting agent cobicistat, did not inhibit CatA-mediated hydrolysis of TAF up to a concentration of 50 μM in an enzymatic assay. Similarly, HCV protease inhibitors TMC-435, BI-201355, MK-5172, GS-9256 and GS-9451 showed little-to-no inhibition of CatA, with IC_{50} values ranging from 25 μM to >50 μM.

On the other hand, both boceprevir and telaprevir, two inhibitors of the HCV protease, were identified as inhibitors of CatA-mediated hydrolysis of TAF, with IC_{50} values of 0.3 μM and 0.2 μM, respectively. The effect of these compounds on the antiviral activity of TAF in CD4+ T lymphocytes was measured (Table 7; Report PC-120-2001, page 11). Telaprevir and boceprevir, when tested at their respective C_{max} concentrations of 5.2 μM and 3.3 μM, reduced the activity of TAF by 23-fold and 3-fold, respectively. In contrast, the CYP3A inhibitor cobicistat

and other non-covalent acting HIV and HCV PIs, including darunavir, showed no pharmacological antagonism with TAF. These data indicate co-administering TAF with the irreversible HCV protease inhibitors boceprevir and telaprevir in HIV/HCV co-infected individuals has the potential to adversely affect the intracellular activation and antiviral efficacy of TAF in vivo. The other tested HCV and HIV protease inhibitors, as well as the boosting agent cobicistat, exhibited low potential for interfering with the intracellular conversion of TAF to parent tenofovir.

Table 7. Effect of PIs on the Antiviral Activity of TAF in Primary Human CD4+ T Lymphocytes

Inhibitor Class	Compound	Fold EC_{50} increase in the presence of tested PI[a]	
		TAF[b]	TDF[b]
HIV PI	Darunavir	0.88 ± 0.24	1.39 ± 0.77
	Atazanavir	1.08 ± 0.29	1.47 ± 0.75
HCV PI	**Telaprevir**	**23.93 ± 12.88**	1.64 ± 0.84
	Boceprevir	**2.91 ± 0.40**	1.32 ± 0.74
	TMC-435	1.07 ± 0.19	1.67 ± 1.13
CYP3A inhibitor	Cobicistat	0.94 ± 0.20	1.80 ± 1.17
Factor Xa inhibitor	Apixaban	1.68 ± 0.24	1.07 ± 0.11
	Rivaroxaban	1.23 ± 0.80	1.17 ± 0.40
Thrombin inhibitor	Argatroban	1.55 ± 1.15	1.27 ± 0.11
	Dabigatran	1.39 ± 0.27	1.20 ± 0.69
DPP4 inhibitor	Sitagliptin	1.49 ± 1.44	0.97 ± 0.21

a Fold change in TAF and TDF EC_{50} values (mean ± standard deviation) obtained in CD4+ T cells from 4 donors performed in triplicate, expressed as an EC_{50} ratio (with test compound/without test compound).
b EC_{50} (TAF) = 5.6 ± 1.2 nM; EC_{50} (TDF) = 3.1 ± 1.0 nM, determined from at least 4 donors assayed in triplicate.

RESISTANCE DEVELOPMENT IN CELL CULTURE

Two resistance selection experiments with TAF and TFV were conducted in parallel. TFV is used in cell culture instead of TDF in these experiments due to the limited stability of TDF in culture media. The first experiment started at concentrations of TAF and TFV below the EC_{50} value for each drug and the second experiment started at concentrations of TAF and TFV corresponding to twice the EC_{50} value for each drug. The duration of the experiments was >115 days and >147 days for the first and second experiment, respectively. In all 4 selections, the outcome of the experiments was nearly identical for both drugs with the development of the K65R substitution in reverse transcriptase, which was substitution accompanied by an S68N substitution in 3 of 4 cases, either as a full substitution or as a mixture. The development of the S68N substitution alongside K65R has been observed previously in TDF/TFV studies both clinically and in cell culture where it may play a role in restoring reduced replication capacity induced by the K65R substitution. The L214F polymorphism was observed in the first experiment for both drugs and was also observed in the no drug control. Therefore, this polymorphism may be present at low levels in the starting virus quasispecies. A K70E substitution as a mixture with wild-type was observed along with a K65R substitution as a mixture with wild-type in the second experiment with TAF, but was not detected at subsequent

time points. This K70E substitution has been observed previously in clinical studies of TDF and is associated with minor decreases in TFV susceptibility in phenotypic assays. TAF and TFV concentrations at which the K65R substitution first appeared ranged from 4X to 15X the EC_{50} value. The K65R substitution developed from approximately 45 - 90 days in both TAF and TFV selections. Attempts to increase the drug concentrations beyond 16X EC_{50} value of each drug (to 24X EC_{50} or 336 nM for TAF, and 24X EC_{50} or 84 µM for TFV) over >5 weeks in the second experiment did not yield additional resistance nor viable virus.

Phenotypic analyses were conducted with the two final mutant viruses obtained in the second selection experiment. The fold changes from wild-type control observed for the viruses selected either by TAF or TFV were similar, with TAF activity reduced 6.5-fold for both selected viruses and TFV activity reduced 5.5- and 5.1-fold for the TAF and TFV selected viruses, respectively (Table 8; Report PC-120-2011, page 12). Reduced FTC susceptibility was also observed at similar levels between the 2 selected viruses (8.5- and 6.7-fold from wild-type), while susceptibility to the control drugs EFV (NNRTI) and EVG (INSTI) was near wild-type levels. These data provide evidence that TAF and TFV have the same resistance profile in selection experiments, resulting in a mutant virus with the K65R substitution with similar phenotypic changes.

Table 8. Genotype and Phenotype of Selected Resistance Viruses

Selected Viruses				EC_{50} Fold Change (FC) from wild-type control (HIV-1_{IIIB})[a]				
Drug	Concentration (FC over EC_{50})	Time Point	Genotype	TAF	TFV	FTC	EFV	EVG
TAF	224 nM (16X)	day 148	K65R	6.5*	5.5*	8.5*	1.4	1.7
TFV	56 µM (16X)	day 154	K65R S68S/N/R/K	6.5*	5.1*	6.7*	1.5	1.4

a EC_{50} against HIV-1_{IIIB} in MT-2 standard assay was 10 nM, 2.9 µM, 1.2 nM, 0.77 µM, and 1.5 nM for TAF, TFV, EVG, FTC, and EFV respectively. TAF: tenofovir alafenamide; TFV: tenofovir; FTC: emtricitabine; EFV: efavirenz; EVG: elvitegravir. Fold changes of the average EC_{50} were obtained from 5 independent experiments.
(*) t-test *p*-value <0.05 as compared to wild-type control.

Resistance selection experiments using HIV-1 isolates with pre-existing TDF-resistance (K65R, 3 thymidine analog substitutions, and Q151M complex) were carried out with TAF, TFV, and raltegravir (RAL), to investigate the potential for additional resistance development in the presence of TAF/TFV. The 3 NRTI-resistant clonal HIV-1 isolates HIV-$1_{LAI\text{-}K65R}$, HIV-$1_{LAI\text{-}3TAM}$, and HIV-$1_{LAI\text{-}Q151M}$ have phenotypic resistance to TAF and TFV measured at 3-fold, 3-fold, and 13-fold above wild-type, respectively, and all 3 isolates have near wild-type susceptibility to the RAL. The selection experiments were initiated using drug concentrations corresponding to 2-times the respective EC_{50} values for TAF, TFV, and RAL.
With the mutant isolate HIV-$1_{LAI\text{-}K65R}$, the maximum viable TAF or TFV concentration supporting virus growth was 3X the starting concentration for TAF and 2X the starting concentration for TFV (18- and 12-fold increase based on the wild-type EC_{50} values, respectively). The selected viruses acquired the S68N substitution. Phenotypic analyses of the 3 resulting mutant viruses showed 5.9- to 6.8-fold decreased susceptibility to TAF, and 5.9- to 6.3-fold reduced susceptibility to TFV (Table 9; Report PC-120-2012, page 17). These phenotypic fold changes for viruses with K65R plus S68N are consistent with the fold changes observed in the wild-type selections.

Table 9. Drug Susceptibilities of Selected Resistant Viruses Starting from TDF-Resistant HIV-1

Virus Name	Selecting Drug	Drug Concentration (FC from WT EC_{50})[a]	Genotype (reverse transcriptase, RT; integrase, IN)[b]	EC_{50}s and Fold Change (FC) from Wild Type Control ($HIV\text{-}1_{LAI}$)[c]				
				TAF	TFV	RAL	AZT	EFV
$HIV\text{-}1_{LAI}$			RT: WT	0.014	2.6	0.003	0.17	0.002
WT-A3	TAF	120 nM (8X)	RT: **K65R**	3.5*	3.7*	1.0	0.7	0.6
WT-F3	TFV	28 µM (8X)	RT: **K65R, S68N**	5.2*	5.4*	1.6	1.2	0.6
WT-R6	RAL	640 nM (64X)	RT: WT IN: **E92Q, V151I**	0.9	1.0	13.4*	1.0	0.7
WT-ND	None	NA	RT: WT	1.0	1.1	1.9	0.7	1.0
$HIV\text{-}1_{LAI\text{-}K65R}$			RT: K65R	4.4*	4.9*	1.4	0.9	0.9
K65R-A2	TAF	180 nM (12X)	RT: K65R, **S68N**	5.9*	6.3*	1.9	2.8*	1.3
K65R-A2.5	TAF	270 nM (18X)	RT: K65R, **S68N**	6.8*	6.3*	1.5	2.3*	1.0
K65R-F2	TFV	42 µM (12X)	RT: K65R, **S68N**	6.1*	5.9*	1.2	2.2	1.4
K65R-R6	RAL	640 nM (64X)	RT: **WT** (loss of K65R) IN: **E138K, Q148R, V151I**	0.8	1.0	>200*	0.9	0.7
K65R-ND	None	NA	RT: **WT** (loss of K65R)	1.0	1.1	2.2	0.9	1.1
$HIV\text{-}1_{LAI\text{-}3TAM}$			RT: M41L, Y181C, G190A, L210W, T215Y ("3TAM") IN: WT	3.8*	3.7*	1.1	>90*	>54*
3TAM-A2	TAF	180 nM (12X)	RT: "3TAM" + **L429I**	8.5*	6.1*	2.2*	>90*	>54*
3TAM-A2.5	TAF	270 nM (18X)	RT: "3TAM" + **L429I**	8.6*	6.1*	1.9	>90*	>54*
3TAM-F2	TFV	42 µM (12X)	RT: "3TAM" + **L429I**	9*	6.1*	1.7	>90*	>54*
3TAM-F2.5	TFV	63 µM (18X)	RT: "3TAM" + **L429I**	ND	ND	ND	ND	ND
3TAM-R6	RAL	640 nM (64X)	RT: "3TAM" IN: **Q148R, D232N**	4.8*	4.9*	>204*	>90*	>54*
3TAM-ND	None	NA	RT: "3TAM"	3.5*	4.2*	2.1*	>57*	>54*
$HIV\text{-}1_{LAI\text{-}Q151M}$			RT: A62V, K65R, S68G, V75I, F77L, F116Y, Q151M ("Q151M") IN: WT	18.6*	19*	1.7	>90*	3.3*
Q151M-A1.5	TAF	585 nM (39X)	RT: "Q151M" + **T69I**	26.1*	22.1*	1.5	>90*	3.4*
Q151M-F1.5	TFV	137 µM (39X)	RT: "Q151M" + **T69I**	32.5*	34.5*	2.2*	>90*	2.7*
Q151M-R6	RAL	640 nM (64X)	RT: " Q151M" IN: **L74M, Q148R, S230R**	19.3*	15.7*	>264*	>90*	3.0*
Q151M-ND	None	NA	RT: " Q151M"	15.3*	15.2*	2.0	>90*	2.4*

a Concentrations based on previously defined EC_{50}s against $HIV\text{-}1_{LAI}$ in MT-2 assay of 0.015, 3.5, and 0.010 µM for TAF, TFV, and RAL, respectively.
b Boldface font indicates changes from starting material. WT IN sequences not shown.
c Fold changes (FC) calculated from EC_{50}s for HIV-1LAI shown in first row (shaded). TAF: tenofovir alafenamide; TFV: tenofovir; RAL: raltegravir; AZT: zidovudine; EFV: efavirenz. Data averaged from at least 4 independent experiments.
(*) P-value <0.01 (student t-test comparing mutant EC_{50}s with wild-type EC_{50}s)
NA: not applicable; WT: wild-type; ND: not determined.

The maximum viable TAF or TFV concentration supporting virus growth when starting with the mutant isolate $HIV\text{-}1_{LAI\text{-}3TAM}$ (M41L, L210W, T215Y) was 3X the starting concentration for either drug (18-fold increase based on the wild-type EC_{50} value). The selected viruses acquired the RT substitution L429I, which is located in the RT connection domain. This substitution has not been previously seen or characterized, as it lies beyond the range of standard genotypic assays (amino acid residues 1-400). Phenotypic characterization was obtained for only 3 of the 4 viruses selected with TAF or TFV, due to very weak infectivity of one virus. A 2- to 3-fold

increase for both TAF and TFV was observed for all 3 viruses with the added L429I substitution compared to HIV-$1_{LAI-3TAM}$ (Table x).

For the HIV-$1_{LAI-Q151M}$ isolate (A62V, K65R, S68G, V75I, F77L, F116Y, and Q151M), the maximum TAF or TFV concentration supporting virus growth was 1.5X the starting concentration for either drug (39-fold increase based on the wild-type EC_{50} value) and the viruses obtained acquired the RT substitution T69I. Phenotypic susceptibilities to TAF and TFV for the 2 selected viruses were very similar for the 2 drugs, ranging from 22.1- to 34.5-fold above wild-type, within 2-fold of the value for the starting material that was determined concurrently (Table 9). The sponsor states that this indicates a very limited impact on resistance for the emerging T69I substitution in that isolate.

Viral growth was sustained at much higher concentration with the control compound RAL (32X starting concentration), where the virus acquired the INSTI-resistance substitutions E138K and Q148R with >200-fold decrease in RAL susceptibility when starting from the HIV-$1_{LAI-K65R}$ (Table 9). The virus acquired the INSTI-resistance substitution Q148R with >204-fold decrease in RAL susceptibility when starting from HIV-$1_{LAI-3TAM}$, and acquired the INSTI-resistance substitutions L74M and Q148R with >264-fold decrease in RAL susceptibility when starting from HIV-$1_{LAI-Q151M.}$

Extended resistance selection of these viruses with TAF or TFV did not lead to the accumulation of additional known resistance-associated substitutions, or phenotypic fold-change increases above 2.5-fold, after 6 months in culture. Viral survival in the presence of drug could not be sustained above a 2-3X drug increase, indicating a lack of alternative resistance pathways for the viruses. In contrast, resistance selections with RAL resulted in mutant viruses with high-level of phenotypic resistance to RAL. Interestingly, the K65R mutant reverted to wild-type in the absence of TAF or TFV selection pressure after 6 months in culture, confirming its fitness defect in the absence of drug.

Viral breakthrough experiments were conducted with TAF and TFV in MT-2 cells using TDF-resistant viruses in order to compare the antiviral effect of TAF and TFV at concentrations adjusted to reflect the ≥5X higher concentration of TFV-DP observed in vivo when dosed with TAF vs. TDF. TAF inhibited viral breakthrough for the duration of the experiment (28 days) for most viruses (8 of 10) with the exception of viruses with 5 TAMs (Table 10; Report PC120-2013, consolidated from Tables on pages 7 and 10). In contrast, viral breakthrough was only inhibited for 1 of 10 viruses in the presence of TFV with breakthrough ranging from 4-18 days.

Table 10. Time to Viral Breakthrough of TFV-Resistant Viruses

Isolate	Mutant Category	RT Sequence	TAF EC_{50} FC from WT*	TFV EC_{50} FC from WT*	Time to Viral BT TAF	Time to Viral BT TFV
PD9	3TAMs	D67N K70R M184V K219Q	4.3	3.7	>28 days	>28 days
PD6	3TAMs	M41L L210W T215Y	3.9	3.1	>28 days	13 days

PD20	3TAMs	M41L L210W T215Y	10.3	7.2	>28 days	4 days
K65R	K65R	K65R	3.3	3.3	>28 days	18 days
PD11	K65R	K65R M184V	3.3	3.1	>28 days	4 days
PD15	Q151M/K65R	M41L A62V K65R T69I K70T L74V V75I Y115F F116Y Q151M M184V	5.9	4.1	>28 days	8 days
PD25	4TAMs	D67N K70R T215F K219Q	6.1	5.1	>28 days	8 days
PD30	T69ins	D67E T69SSG	10.1	10.1	>28 days	4 days
PD31	5TAMs	M41L D67N T69D L210W T215Y K219R	25.5	21.9	8 days	4 days
PD34	5TAMs	M41L D67N L210W T215Y K219R	14.8	14.7	8 days	4 days

*MT-2 Assay

CROSS-RESISTANCE

The antiviral phenotypic susceptibility of TAF and TFV was analyzed against a panel of 24 patient-derived HIV-1 recombinant isolates in the Monogram Biosciences PhenoSense assay (Table 11; Report PC-120-2014, page 8). The mutants in the panel were chosen to represent a wide array of NRTI resistance substitutions and were expected to display a wide spectrum of TFV susceptibilities. In the PhenoSense assay, clinical susceptibility cutoffs for TDF have been established at 1.4. The panel of recombinant mutants showed TFV fold changes from wild-type ranging from 0.41 to 20, with 11 isolates showing sensitivity to TFV and 13 isolates showing resistance to TFV. Isolates resistant to TFV had either 6 TAMs, Q151M complex + K65R, or double insertions at T69 + other NRTI substitutions and/or TAMs. The highest level of resistance to TFV was observed for isolates with T69 double insertions. Susceptibility to TAF for this panel of mutants was similar to TFV, ranging from 0.34 to 23 (fold of the wild-type EC_{50} value). There was a strong correlation between the fold change for TFV and TAF with R^2 = 0.97, indicating that TAF and TFV have a similar resistance profile against NRTI resistant mutants in the PhenoSense assay.

In addition, 21 patient-derived and 4 site-directed HIV-1 recombinant clones harboring NRTI-resistance substitutions with reduced susceptibility to TFV were generated using the pXXLAI recombinant clone and tested for their susceptibility in a multi-cycle MT-2 assay. The samples used for this study were either resistant or had reduced sensitivity to TDF as determined by the Monogram PhenoSense assay, with TFV fold change ranging from 1.6 to 16 and substitution patterns such as K65R, 3 TAMs, 4 TAMs, 5 TAMs, T69 insertions, and Q151M complex. In addition to the patient-derived recombinants, 4 site-directed mutants with either K65R, K70E, M184V, or K65R/Q151M complex (A62V, K65R, S68G, V75I, F77L, F116Y, Q151M) were analyzed phenotypically.

Table 11. Phenotypic Susceptibilities of 24 Recombinant HIV-1 Isolates with NRTI Mutations against TAF and TFV in the Monogram PhenoSense Assay

Virus ID	EC_{50} FC[a]		Mutation Category	NRTI mutations
	TAF	TFV		
13	0.34	0.41	NRTI	L74V
16	0.40	0.47	NRTI + M184V	L74V Y115F M184V*
5	0.50	0.48	M184V	M184V
14	0.43	0.50	NRTI	L74V
22	0.56	0.53	Q151M + M184V	Q151M M184V*
15	0.50	0.59	NRTI + M184V	L74V Y115F M184V*
6	0.67	0.65	M184V	M184V*
21	0.82	0.82	Q151M + M184V	A62V V75V/I F116Y Q151M M184V*
20	0.91	0.93	Q151M	F116Y Q151M*
11	0.78	0.98	K65R + M184V	A62A/V K65R M184V*
12	1.09	1.18	K65R + M184V	K65R M184V*
9	1.68	1.48	K65R	K65R*
10	1.91	1.71	K65R	K65R*
17	1.62	1.81	3 TAMs	M41L L74V L210W T215Y
3	2.11	2.27	6 TAMs + M184V	M41L D67N K70R M184V L210W T215Y K219E
19	3.43	2.82	Q151M Complex	A62V V75I F77L Y115F F116Y Q151M*
1	3.62	3.48	6 TAMs	M41L D67N K70R L210W T215F K219Q*
18	8.80	3.80	5 TAMs	M41L D67N T69D L74V L210W T215Y K219R*
2	4.77	4.01	6 TAMs	M41L D67N K70R L210W T215Y K219E*
4	9.16	6.11	6 TAMs + M184V	M41L D67N K70R M184V L210W T215Y K219E*
24	9.07	9.60	Q151M Complex + K65R	A62V K65R K70K/R V75I F77L F116Y Q151M*
8	20.0	18.0	T69 Insertion	M41L T69ins L74V L210W T215Y*
23	22.0	19.0	T69 Insertion	M41L A62V T69ins L210W T215Y*
7	23.0	20.0	T69 Insertion	A62V T69ins V75I*

a Susceptibilities are expressed as fold changes (FC) in EC_{50} from wild-type control. Wild-type EC_{50} for TAF and TFV was 10 nM and 0.6 µM, respectively.
(*) Indicates the additional presence of NNRTI resistance mutations (not shown).

Phenotypic analyses using these clones in the HIV-1 multi-cycle MT-2 assay showed that susceptibility of patient-derived isolates to TAF ranged from 2.2- to 25.5-fold change from wild-type control. Most samples had very high levels of AZT and FTC resistance due to the presence of TAMs and M184V, respectively. The highest level of TAF phenotypic resistance was observed in patient-derived isolates harboring 5 TAMs including T215Y and L210W in the absence of the M184V (Table 12; Report PC-120-2015, page 12). The lowest level of TAF phenotypic resistance was observed with a sample harboring K65R. Notably, samples within same mutation categories showed some fold change variability inherent to the genetic diversity found in patient samples, similar to results observed in the single-cycle PhenoSense assay. Among the site-directed mutants, the highest fold change was observed for the mutant carrying the Q151M substitution complex plus K65R (13.2-fold), followed by K65R alone (3.3-fold), and K70E (2.2-fold). The mutant with M184V alone showed wild-type susceptibility to TAF.

Phenotypic susceptibility to TAF and TFV were highly correlated (R^2 = 0.9671) in this multi-cycle MT-2 assay. In summary, TAF and TFV showed very similar fold changes from wild-type against these recombinant HIV-1 isolates, consistent with the activity of each compound being driven by the same active entity in these assays, TFV-DP, and both compounds having a similar resistance profile.

Table 12. Phenotypic Susceptibilities of Patient-Derived and Site-Directed Recombinant Mutant Clones into the MT-2 Assay

		Fold Change from Wild-Type Control[b] (MT-2 assay)					
Virus ID	Mutation Category[a]	TAF	TFV	AZT[c]	FTC[c]	DRV	EVG
2	K65R/M184V	5.0 *	3.9 *	1.1	>>	1.1	1.1
6	3 TAMs	3.0 *	3.8 *	>>	2.0 *	0.7	0.6
7	K65R/M184V	8.9 *	6.2 *	4.6 *	>>	1.2	1.8 *
8	4 TAMs	6.3 *	4.7 *	>>	>>	1.5	2.0
9	3 TAMs	4.3 *	3.7 *	25.5 *	>>	1.2	1.9 *
10	4 TAMs	2.3 *	2.6 *	>>	>>	0.7	0.9
11	K65R/M184V	3.3 *	3.1 *	1.8	>>	0.8	0.7
12	K65R	2.2 *	2.3 *	0.4	6.0 *	n/a	0.7
14	5 TAMs	4.5 *	4.1 *	>>	>>	1	0.6
15	Q151M/K65R	5.9 *	4.1 *	>>	>>	1.1	0.9
16	3 TAMs	3.6 *	3.3 *	>>	>>	0.8	0.9
20	3 TAMs	10.3 *	7.2 *	>>	5.0 *	1	1.0
21	5 TAMs	6.9 *	6.5 *	>>	>>	1.1	1.1
24	4 TAMs	6.4 *	5.2 *	>>	15.2 *	0.7	0.7
25	4 TAMs	6.1 *	5.1 *	>>	3.9 *	1.1	1.2
30	T69 insertion	10.1 *	10.1 *	>>	9.0 *	1.2	1.6
31	5 TAMs	25.5 *	21.9 *	>>	28.4 *	1.0	0.9
32	3 TAMs	3.4 *	3.8 *	>>	2.8 *	1.2	1.3
33	T69 insertion	14.3 *	10.9 *	>>	>>	0.5	0.9
34	5 TAMs	14.8 *	14.7 *	>>	14.5 *	0.9	1.3
37	5 TAMs	22.9 *	19.6 *	>>	5.4 *	1.7	1.3
K65R	K65R	3.3 *	3.3 *	0.6	6.5 *	0.9	0.8
K70E	K70E	2.2 *	2.1 *	0.3	2.5 *	1.0	1.0
M184V	M184V	1.1	0.9	0.5	>>	0.9	1.0
Q151M Complex	Q151M/K65R	13.2 *	13.9 *	>>	>>	1.0	1.1

a Thymidine analog-associated mutations (TAMs): M41L, D67N, K70R, L210W, T215F/Y, and K219Q/E/N/R.
b EC_{50} values for wild-type control ($HIV\text{-}1_{XXLAI}$) were 14 nM, 3.4 µM, 0.3 µM, 1.1 µM, 7 nM, and 2 nM, for TAF, TFV, AZT, FTC, DRV, and EVG, respectively, in the MT-2 assay.
c ">>" denotes that at least one value was >55, or >48 fold above wild-type for FTC, and AZT, respectively.
Data were averaged from at least 3 independent experiments.
(*) indicates p-value <0.01 as compared to wild-type control (Student's T-test).

6 CLINICAL STUDIES

GS-US-292-0104
Study GS-US-292-0104 is an ongoing Phase 3, randomized, double-blinded, multicenter, active-controlled study to assess the efficacy and safety of the E/C/F/TAF FDC compared with the STB in HIV-infected, ART-naive adults. Subjects were randomized in a 1:1 ratio to 1 of the following 2 treatment groups: E/C/F/TAF (150/150/200/10 mg) + placebo-to-match STB (n = 420) and STB (150/150/200/300 mg) + placebo-to-match E/C/F/TAF (n = 420). Randomization was stratified by HIV-1 RNA level (≤100,000 copies/mL, >100,000 copies/mL to ≤400,000 copies/mL, or >400,000 copies/mL), $CD4^+$ cell count (<50 cells/μL, 50 to 199 cells/μL, or ≥200 cells/μL), and region (United States [US] or ex-US) at screening. The primary efficacy endpoint was the percentage of subjects with HIV-1 RNA <50 copies/mL at Week 48 using the FDA-defined snapshot algorithm. Virologic outcomes at Week 48 were similar between the 2 treatment groups for the primary endpoint analysis based on the full analysis set. Virologic success rates were >90% in both groups (E/C/F/TAF 93.1%; STB 92.4%) and E/C/F/TAF was determined to be non-inferior to STB.

GS-US-292-0111
Study GS-US-292-0111 is an ongoing Phase 3, randomized, double-blinded, multicenter, active-controlled study to assess the efficacy and safety of the E/C/F/TAF FDC compared with the STB FDC in HIV-infected, ART-naive adults. Subjects were randomized in a 1:1 ratio to 1 of the following 2 treatment groups: E/C/F/TAF (150/150/200/10 mg) + placebo-to-match STB once daily (n = 420) and STB (150/150/200/300 mg) + placebo-to-match E/C/F/TAF once daily (n = 420). Randomization was stratified by HIV-1 RNA level (≤100,000 copies/mL, >100,000 copies/mL to ≤400,000 copies/mL, or >400,000 copies/mL), CD4 count (<50 cells/μL, 50 to 199 cells/μL, or ≥200 cells/μL), and region (US or ex-US) at screening. The primary efficacy endpoint was the percentage of subjects with HIV-1 <50 copies/mL at Week 48 using the FDA-defined snapshot algorithm. Virologic outcomes at Week 48 were similar between the 2 treatment groups for the primary endpoint analysis based on the full analysis set. Virologic success rates were 91.6% in E/C/F/TAF 91.6% group and 88.5% in the STB group and E/C/F/TAF was determined to be non-inferior to STB.

GS-US-292-0106
Study GS-US-292-0106 is an ongoing Phase 2/3, open-label, multicenter, 2-part, single group study of the PK, safety, tolerability, and antiviral activity of the E/C/F/TAF FDC in HIV-infected, ART-naive adolescents. Adolescent (12 to < 18 years of age) subjects of either sex were enrolled to receive E/C/F/TAF once daily with food. In Part A, 24 subjects were enrolled to evaluate the steady-state PK and to confirm the dose of the E/C/F/TAF. Subjects participated in an intensive PK evaluation at Week 4 and continued to receive E/C/F/TAF through Week 48. In Part B, 24 subjects were enrolled to evaluate the safety, tolerability, and antiviral activity of E/C/F/TAF through Week 48. At the Week 24 interim analysis, 48 subjects were enrolled into the study (24 in Part A and 24 in Part B) and received at least 1 dose of study drug. Twenty-three of the 48 enrolled subjects were enrolled in the study by 11 February 2014 and completed their Week 24 study visit; these subjects were included in the Week 24 full analysis set. At the Week 24 interim analysis, 91.3% of subjects (21 of 23) at Week 24 had virologic success (FDA snapshot algorithm, HIV-1 RNA <50 copies/mL)

GS-US-292-0109
Study GS-US-292-0109 is an ongoing, Phase 3, randomized, open-label, multicenter, active-controlled study to evaluate the efficacy and safety of E/C/F/TAF in virologically suppressed subjects who switch from a FTC/TDF regimen. All subjects are HIV-infected adults with virologic suppression on STB, efavirenz (EFV)/FTC/TDF, cobicistat-boosted atazanavir (ATV)/COBI+FTC/TDF, or ritonavir-boosted ATV (ATV/r)+FTC/TDF. Subjects were randomized in a 2:1 ratio to 1 of the following 2 treatment groups: switch to E/C/F/TAF (150/150/200/10 mg) (n = 1,000) or maintain preexisting regimen FTC/TDF+3rd Agent regimen (EVG/COBI/FTC/TDF [150/150/200/300 mg]; EFV/FTC/TDF [600/200/300 mg]; ATV/r [300/100 mg] + FTC/TDF [200/300 mg]; or ATV/COBI [300/150 mg] + FTC/TDF [200/300 mg]) (n = 500)
Randomization was stratified by prior treatment regimen group at screening. Overall, 1,436 subjects (E/C/F/TAF = 959 subjects; FTC/TDF+3rd Agent = 477 subjects) were randomized and received at least 1 dose of study drug. The Week 48 full analysis set included any subject randomized by 31-October-2013 who received at least 1 dose of study drug; in total, 1,196 subjects were included in the Week 48 full analysis set (E/C/F/TAF = 799; FTC/TDF+3rd Agent = 397). The primary efficacy endpoint was the percentage of subjects with HIV-1 RNA <50 copies/mL at Week 48 using the FDA-defined snapshot algorithm. Virologic success rates at Week 48 were >90% in both groups (E/C/F/TAF 95.6%; FTC/TDF+3rd Agent 92.9%), with E/C/F/TAF determined to be non-inferior to FTC/TDF+3rd Agent.

7 CLINICAL VIROLOGY

EMERGENCE OF RESISTANCE
In the studies from the original STIBILD® NDA, resistance development to TRUVADA components of the regimen occurred more frequently in the E/C/F/T-treatment failures, 54.6% versus 13.3% and 0% of the ATR and ATV/r +TVD treatment failures with evaluable genotypic data, respectively.

Studies 104 and 111
In Studies 104 and 111, there were 17 virologic failures in the EVG/COBI/FTC/TAF (E/C/F/TAF) arm and 21 virologic failures in the STB arm. The FDA resistance analysis includes subjects who had confirmed viral load >400 copies/mL at discontinuation, final timepoint or after suppression to <50 copies/mL (i.e., rebound). In addition, the FDA resistance analysis includes subjects who may not have met these criteria, but had evidence of resistance emergence in the data provided by the sponsor. Therefore, 3 subjects in the E/C/F/TAF arm (0104-0115-4389, 0111-0255-5554, 0111-2348-5568) and 4 subjects in the STB arm (0104-2855-4619, 0104-3612-4362, 0111-1236-5061, 0111-2675-5138) were removed from the FDA virologic failure subset because they did not have confirmed >400 copies/mL. Thus, the FDA virologic failure subset had 14 virologic failures in the E/C/F/TAF arm compared to 17 virologic failures in the STB arm (Table 13).

Table 13. Emergent Substitutions in Virologic Failures from Studies 104 and 111

PID	ARM	Timepoint with Resistance Data	Viral Load	RT Emergent Substitutions	INSTI Emergent Substitutions	Phenotype Resistance
0104-0115-4389	E/C/F/TAF	WK16	606 resuppressed	NO DATA	NO DATA	NO DATA
0104-0255-4091	E/C/F/TAF	WK48	986	I178I/M	NO DATA	
0104-1965-4201	E/C/F/TAF	WK48 DC	1,260	**M41M/K K65R M184V**	G70G/R **N155H D232D/N**	FTC-R >63 EVG-R 60 TDF 1.01
0104-2704-4329	E/C/F/TAF	WK48	8,340	**M184V**	**E92Q**	FTC-R >70 EVG-R 56
0104-2734-4394	E/C/F/TAF	WK16 DC	62600	**M184M/V**	A129A/S	FTC-2X
0111-0255-5554	E/C/F/TAF	WK2 DC	6480	NO DATA	NO DATA	NO DATA
0111-0729-5139	E/C/F/TAF	WK24 DC	6250	P170P/L V179V/I **M184V**	L68V **E92Q**	FTC-R >84 NO INSTI DATA
0111-0994-5313	E/C/F/TAF	WK48	2750	I195I/M	A21A/T I182I/F	
0111-1790-5185	E/C/F/TAF	WK16 DC	63000	**M184V**	**T66T/I/A/V E138E/K Q148Q/R** D232N	FTC-R >81 EVG-R 11
0111-2348-5568	E/C/F/TAF	WK16	597 resuppressed	NO DATA	NO DATA	NO DATA
0111-2348-5661	E/C/F/TAF	WK36	759	D177E Q207E	NO DATA	
0111-2480-5585	E/C/F/TAF	WK24 DC	500	K64K/R	NONE	

0111-2704-5057	E/C/F/TAF	WK12	6,230	D121H/Y **M184M/I/V** Q207Q/H	A265A/V	FTC-R >68
0111-2873-5544	E/C/F/TAF	WK36	47,800 REBOUND		S17N	
0111-4735-5238	E/C/F/TAF	WK36	412 <400 at WK48 and 60	W71P T139T/A E204E/K	NO DATA	
0111-5083-5289	E/C/F/TAF	WK16 DC	3,850 resuppressed	**M184V** H208Y E248K	**T66A** V72I	FTC-R >89 EVG-R 12
0111-7710-5649	E/C/F/TAF	WK48	322,000	T200T/A Q207Q/R		NONE
0104-0031-4253	STB	WK16 DC	1,130	V60I **K65R M184V**	**E92Q**	NO RT DATA EVG-R 21
0104-0783-4492	STB	WK16	1,340 resuppressed	NONE	S24N	EVG 2.0
0104-1541-4150	STB	WK36 DC	364,000	NONE	D6N, E35K, E69K, R107K **E138K**,E152K **M154I, E157K**	EVG-NO DATA
0104-1609-4268	STB	WK24 DC	1,100 resuppressed	E36E/Q E44E/D K49K/Q **M184M/V**	NONE	FTC 1.5
0104-2855-4619	STB	WK36	592	NONE	NO DATA	NO INSTI DATA
0104-3612-4362	STB	WK12 DC	1,020	R78R/K D86D/N E224E/K	NONE	NONE
0104-4735-4448	STB	WK12 DC	53,400	A98G/R **M184V**	G70G/E V72I P90P/S **E92E/Q** H114H/Y **E138E/K Q148R**	FTC-R >71 EVG-R >156

					G190G/E	
0111-0031-5129	STB	WK24	2,820	V179V/I V245V/A	NO DATA	NO INSTI DATA
0111-0044-5800	STB	WK48	871	G196E		NO INSTI DATA
0111-0255-5134	STB	WK8 DC	603,000	NO DATA	NO DATA	NO DATA
0111-0729-5836	STB	WK36	576	NONE	NONE	NONE
0111-0986-5527	STB	WK48	60,400	NONE	NO INSTI DATA	NO INSTI DATA
0111-0994-5655	STB	WK48	1,280	NONE	S17N L101L/I K127K/R L172L/F	NONE
0111-1236-5061	STB	WK36	20,500	NONE	G59G/E T112I	EVG 2.2
0111-1480-5728	STB	WK24	1,010	NONE	L45I	EVG 1.7
0111-1534-5156	STB	WK48	73	**M184V** T200I		FTC-R >64 EVG 1.9
0111-2106-5415	STB	WK48	11,700	NONE	V249I/V	
0111-2348-5569	STB	WK36	803	NO DATA	NO DATA	NO DATA
0111-2348-5662	STB	WK36	1,410	NO DATA	NO DATA	NO DATA
0111-2675-5138	STB	WK2 DC	603	NO DATA	NO DATA	NO DATA

0111-2704-5132	STB	WK36	44,800	**K65R M184V**	S17N **E138K Q148R**	FTC-R >82 TDF 1.3 EVG-R >95

Bolded subjects PIDs had emergent RT or IN substitutions.
Italicized subject PIDs were removed from denominator because they resuppressed and had no resistance data.
The clinical phenotypic cutoffs were as follows: TFV = 1.4 to 4, FTC = 3.5, and EVG = 2.5.

Of the final FDA-defined virologic failure set, 7 subjects (50%) had emergent resistance substitutions in the E/C/F/TAF arm and 6 subjects (35%) had emergent resistance substitutions in the STB arm (Table 14). In the E/C/F/TAF arm, all 7 of the virologic failures with emergent substitutions had the M184V substitution and one subject had the K65R substitution. Three subjects had emergent Q207E/H/R in reverse transcriptase. Five of the 7 subjects had emergent INSTI resistance substitutions. In the STB arm, 5 of the 6 subjects with emergent substitutions had emergent M184V substitutions and two had the K65R substitution. Four the 6 subjects had emergent INSTI resistance substitutions. Additionally, 1 subject in each arm developed a substitution in RT at T200 (T200T/A in the GENVOYA arm and T200I in the STB arm). The number and type of emergent NRTI and INSTI resistance substitutions is similar in both arms. TAF did not reduce the frequency of virologic failure or number of emergent resistance substitutions compared to TDF. In addition, the resistance pathways of TAF are similar to TDF.

Table 14. Summary of Emergent Substitutions in Virologic Failure Subjects of Study 104 and 111

	E/C/F/TAF (n=14)	**STB (n=17)**
# Subjects with Emerging Substitutions	7 (50%)	6 (35%)
NRTI	7 (100%)	5 (83%)
M184V	7 (100%)	5 (100%)
K65R	1 (14%)	2 (40%)
INSTI	5 (71%)	4 (67%)
T66A	2 (40%)	
E92Q	2 (40%)	2 (50%)
E138K	1 (20%)	3 (75%)
Q148R	1 (20%)	2 (50%)
M154I		1 (25%)
N155H	1 (20%)	
E157K		1 (25%)

Study 109

HIV-1 genotyping was not conducted at screening since all subjects who entered Study GS-US-292-0109 had HIV RNA <50 copies/mL at screening. Historical genotyping results for the PR/RT genes was available for all subjects as they were previously enrolled in Gilead Studies GS-US-236-0102, GS-US-236-0103, GS-US-216-0114, GS-US-264-0110 (limited to subjects on a EFV-based regimen), GS-US-236-0104, or GS-US-216-0105, all of which enrolled ART-naïve subjects. Consistent with the enrollment criteria for these studies, all enrolled subjects demonstrated full sensitivity to FTC and tenofovir (delivered as TDF or TAF) based on the proprietary algorithm from (b) (4) NRTI-associated resistance substitutions were observed in 9% of subjects, NNRTI-associated resistance substitutions were observed in 14.5% of subjects, and primary PI-associated resistance substitutions were observed in 2.6% of subjects. The distribution of baseline resistance-associated substitutions was comparable between treatment groups.

Of the 1,196 subjects in Study GS-US-292-0109 at Week 48, 5 (0.4%) met the virologic failure criteria and were included in the sponsor's resistance analysis set with 4 of 799 subjects (0.5%) in the E/C/F/TAF group and 1 of 397 subjects (0.3%) in the FTC/TDF+3rd Agent regimen group analyzed. Four subjects (Subjects 2838-6691, 2840-6507, 5083-6551 and 0959-6571) achieved HIV-1 RNA resuppression to <50 copies/mL with further treatment and were not included in the sponsor's final resistance analysis population. However, all these virologic failures subjects were included FDA resistance analysis because they had confirmed >400 copies/mL and resistance data. Subject 2838-6691 demonstrated genotypic and phenotypic resistance to FTC (M184M/I; FTC FC = 3.8) at Week 8 (Table 15) and the HIV-1 RNA level resuppressed to <50 copies/mL by early study drug discontinuation when the subject switched to a new drug regimen. The remaining 2 subjects (Subjects 2840-6507 and 5083-6551) did not have resistance to emtricitabine or TAF detected, and no IN resistance data was available. Subject 2824-6999 in the E/C/F/TAF group that was included in the sponsor's final resistance analysis set had no detectable resistance to any study drug.

Table 15. Emergent Substitutions in Virologic Failures from Study 109

PID	ARM	Timepoint with Resistance Data	Viral Load	RT Emergent Substitutions	INSTI Emergent Substitutions	Phenotype Resistance
0109-2824-6999	E/C/F/TAF	WK24	4970	NONE	S17N S39C	NONE
0109-2838-6691	E/C/F/TAF	WK8 resuppressed	662	**M184M/I**	I84M	**FTC-R 3.8**
0109-2840-6507	E/C/F/TAF	WK12 resuppressed	438	NONE	NO DATA	NO INSTI DATA
0109-5083-	E/C/F/TAF	WK4 resuppressed	617	NONE	NO DATA	NO INSTI

6551						DATA
0109-0959-6571	STB	WK12 resuppressed	4760	NO DATA	NO DATA	NO DATA

Study 106

Of the 23 subjects in the Week 24 full analysis set for Study GS-US-292-0106, 1 subject of 23 (4.3%) had virologic rebound that was unconfirmed. Subject 8275-2008 was suppressed (HIV-1 RNA <50 copies/mL) at Weeks 12 and 16 and had unconfirmed virologic rebound to HIV-1 RNA of 1,010 copies/mL at Week 24. This subject resuppressed HIV-1 RNA to <50 copies/mL at the next study visit upon continued treatment with E/C/F/TAF. Therefore, this subject's virus was not analyzed for resistance development.

8 CONCLUSION

TAF has the same cytotoxicity profile as TDF and tenofovir. TAF and TDF have a similar resistance profile in cell culture and in clinical trials. In treatment-naïve studies, there were a similar number of virologic failures in the E/C/F/TAF and STB arms with a similar resistance pattern. At Week 48, the development of one or more primary elvitegravir, emtricitabine, or tenofovir alafenamide fumarate substitutions associated with resistance was observed in 7 of 14 subjects with evaluable genotypic data from paired baseline and GENVOYA treatment-failure isolates (7 of 978 subjects [0.7%]) compared with 6 of 17 treatment-failure isolates from subjects in the STRIBILD treatment group (7 of 925 subjects [0.8%]). Of the 7 subjects with resistance development in the GENVOYA group, the substitutions that emerged were M184V/I (N = 7) and K65R (N = 1) in reverse transcriptase and T66T/A/I/V (N = 2), E92Q (N = 2), E138K (N = 1), Q148Q/R (N = 1) and N155H (N = 1) in integrase. Of the 6 subjects with resistance development in the STRIBILD group, the substitutions that emerged were M184V/I (N = 5) and K65R (N = 2) in reverse transcriptase and E92E/Q (N = 2), E138K (n = 3) and Q148R (N = 2) in integrase. In both treatment groups, most subjects who developed substitutions associated with resistance to elvitegravir also developed emtricitabine resistance-associated substitutions.

In a clinical study of virologically-suppressed subjects (Study 109, N = 799) who switched from a regimen containing emtricitabine/tenofovir disoproxil fumarate and a third agent to E/C/F/TAF), 4 subjects were virologic failures of which one had emergent emtricitabine resistance with the emergence of M184M/I.

APPLICANT PROPOSED PACKAGE INSERT

12.4 Microbiology

Mechanism of Action

Elvitegravir: Elvitegravir inhibits the strand transfer activity of HIV-1 integrase (integrase strand transfer inhibitor; INSTI), an HIV-1 encoded enzyme that is required for viral replication. Inhibition of integrase prevents the integration of HIV-1 DNA into host genomic DNA, blocking the formation of the HIV-1 provirus and propagation of the viral infection. Elvitegravir does not inhibit human topoisomerases I or II.

Cobicistat: Cobicistat is a selective, mechanism-based inhibitor of cytochromes P450 of the CYP3A subfamily. Inhibition of CYP3A-mediated metabolism by cobicistat enhances the systemic exposure of CYP3A substrates, such as elvitegravir, where bioavailability is limited and half-life is shortened by CYP3A-dependent metabolism.

Emtricitabine: Emtricitabine, a synthetic nucleoside analog of cytidine, is phosphorylated by cellular enzymes to form emtricitabine 5'-triphosphate. Emtricitabine 5'-triphosphate inhibits the activity of the HIV-1 RT by competing with the natural substrate deoxycytidine 5'-triphosphate and by being incorporated into nascent viral DNA which results in chain termination. Emtricitabine 5′-triphosphate is a weak inhibitor of mammalian DNA polymerases α, β, Ɛ, and mitochondrial DNA polymerase γ.

Tenofovir Alafenamide: Tenofovir alafenamide is a phosphonoamidate prodrug of tenofovir (2'-deoxyadenosine monophosphate analogue). (b) (4) through hydrolysis by cathepsin A, (b) (4) tenofovir is subsequently phosphorylated (b) (4) active metabolite tenofovir diphosphate. Tenofovir diphosphate inhibits HIV replication through incorporation into viral DNA by the HIV reverse transcriptase, which results in DNA chain-termination.

Tenofovir has activity that is specific to human immunodeficiency virus (b) (4) and hepatitis B virus. (b) (4) have shown that both emtricitabine and tenofovir can be fully phosphorylated when combined in cells. Tenofovir diphosphate is a weak inhibitor of mammalian DNA polymerases that include mitochondrial DNA polymerase γ and there is no evidence of toxicity to mitochondria *in vitro*.

Antiviral Activity

Elvitegravir, Cobicistat, Emtricitabine, and Tenofovir Alafenamide: (b) (4), elvitegravir, emtricitabine, and tenofovir alafenamide (b) (4) t.

Elvitegravir: The antiviral activity of elvitegravir against laboratory and clinical isolates of HIV-1 was assessed in T lymphoblastoid cell lines, monocyte/macrophage cells, and primary peripheral blood lymphocytes. The 50% effective concentrations (EC_{50}) ranged from 0.02 to 1.7

nM. Elvitegravir displayed antiviral activity in cell culture against HIV-1 clades A, B, C, D, E, F, G, and O (EC_{50} values ranged from 0.1 to 1.3 nM) and activity against HIV-2 (EC_{50} value of 0.53 nM). Elvitegravir did not show inhibition of replication of HBV or HCV in cell culture.

Cobicistat: Cobicistat has no detectable antiviral activity in cell culture against HIV-1, HBV, or HCV and does not antagonize the antiviral activity of elvitegravir, emtricitabine, or tenofovir.

Emtricitabine: The antiviral activity of emtricitabine against laboratory and clinical isolates of HIV-1 was assessed in T lymphoblastoid cell lines, the MAGI-CCR5 cell line, and primary peripheral blood mononuclear cells. The EC_{50} values for emtricitabine were in the range of 0.0013–0.64 micromolar. Emtricitabine displayed antiviral activity in cell culture against HIV-1 clades A, B, C, D, E, F, and G (EC_{50} values ranged from 0.007–0.075 micromolar) and showed strain specific activity against HIV-2 (EC_{50} values ranged from 0.007–1.5 micromolar).

Tenofovir Alafenamide: The antiviral activity of tenofovir alafenamide against laboratory and clinical isolates of HIV-1 subtype B was assessed in lymphoblastoid cell lines, PBMCs, primary monocyte/macrophage cells and CD4-T lymphocytes. The EC_{50} values for tenofovir alafenamide were in the range of 2.0 to 14.7 nM.

Tenofovir alafenamide displayed antiviral activity in cell culture against all HIV-1 groups (M, N, O), including sub-types A, B, C, D, E, F, and G (EC_{50} values ranged from 0.10 to 12.0 nM) and strain specific activity against HIV-2 (EC_{50} values ranged from 0.91 to 2.63 nM).

In a study of tenofovir alafenamide with a broad panel of representatives from the major classes of approved anti-HIV agents (NRTIs, NNRTIs, INSTIs, and PIs), additive to synergistic effects were observed. No antagonism was observed for these combinations.

Resistance

In Cell Culture

Elvitegravir: HIV-1 isolates with reduced susceptibility to elvitegravir have been selected in cell culture. Reduced susceptibility to elvitegravir was associated with the primary integrase substitutions T66A/I, E92G/Q, S147G, and Q148R. Additional integrase substitutions observed in cell culture selection included D10E, S17N, H51Y, F121Y, S153F/Y, E157Q, D232N, R263K, and V281M.

Emtricitabine: HIV-1 isolates with reduced susceptibility to emtricitabine have been selected in cell culture. Reduced susceptibility to emtricitabine was associated with M184V/I substitutions in HIV-1 RT.

Tenofovir Alafenamide: HIV-1 isolates with reduced susceptibility to tenofovir alafenamide have been selected in cell culture. HIV-1 isolates selected by tenofovir alafenamide expressed a K65R substitution in HIV-1 RT; in addition, a K70E substitution in HIV-1 RT has been (b) (4) observed. HIV-1 isolates with the K65R substitution have (b) (4) reduced susceptibility to abacavir, emtricitabine, tenofovir, and lamivudine. (b) (4)

In Clinical Trials

In Treatment-Naïve Subjects: In a pooled analysis of antiretroviral-naive subjects receiving [TRADENAME] in Studies 104 and 111, (b) (4) genotyping was performed on plasma HIV-1 isolates from all subjects with HIV-1 RNA > 400 copies/mL at confirmed virologic failure, at Week 48, or at time of early study drug discontinuation. As of Week 48, the development of (b) (4) elvitegravir, emtricitabine, or tenofovir alafenamide substitutions associated with resistance was observed in 7 of 14 subjects with evaluable (b) (4) data from paired baseline and [TRADENAME] treatment-failure isolates (7 of (b) (4) subjects [0 (b) (4)%]) compared with (b) (4) treatment-failure isolates from subjects in the STRIBILD treatment group (b) (4) subjects [0 (b) (4)%]). Of the 7 subjects with resistance development in the [TRADENAME] group, the substitutions that emerged were M184V/I (N = 7) and K65R (N = 1) in reverse transcriptase and T66T/A/I/V (N = 2), E92Q (N = 2), Q148Q/R (N = 1) and N155H (N = 1) in integrase. Of th (b) (4) subjects with resistance development in the STRIBILD group, the substitutions that emerged were M184V/I (N (b) (4) and K65R (N = 2) in reverse transcriptase and E92E/Q (N = (b) (4)), and Q148R (N = 2) in integrase. (b) (4) subjects (b) (4) (b) (4) who developed substitutions associated with resistance to elvitegravir developed substitutions (b) (4).

(b) (4)

In Virologically Suppressed Subjects: (b) (4) emergent resistance to [TRADENAME] (b) (4) in a clinical study of virologically-suppressed subjects who switched from a regimen containing emtricitabine/tenofovir disoproxil fumarate and a third agent (Study 109, N = 799).

Cross Resistance

(b) (4) No cross-resistance has been demonstrated for elvitegravir-resistant HIV-1 isolates and emtricitabine or tenofovir, or for emtricitabine- or tenofovir-resistant isolates and elvitegravir.

Elvitegravir: Cross-resistance has been observed among INSTIs. Elvitegravir-resistant viruses showed varying degrees of cross-resistance in cell culture to raltegravir depending on the type and number of substitutions in HIV-1 integrase. Of the primary elvitegravir resistance-associated substitutions tested (T66A/I/K, E92G/Q, T97A, S147G, Q148H/K/R, and N155H), all but three (T66I, E92G, and S147G) conferred greater than 1.5-fold reduced susceptibility to raltegravir (above the biological cutoff for raltegravir) when introduced individually into a wild-type virus by site-directed mutagenesis. Of the primary raltegravir resistance-associated substitutions (Y143C/H/R, Q148H/K/R, and N155H), all but Y143C/H conferred greater than 2.5-fold reductions in susceptibility to elvitegravir (above the biological cutoff for elvitegravir). Viruses expressing elvitegravir or raltegravir resistance mutations maintain susceptibility to dolutegravir.

Emtricitabine: Cross-resistance has been observed among NRTIs. Emtricitabine-resistant isolates harboring an M184V/I substitution in HIV-1 RT were cross-resistant to lamivudine. HIV-

1 isolates containing the K65R RT substitution, selected in vivo by abacavir, didanosine, and tenofovir, demonstrated reduced susceptibility to inhibition by emtricitabine.

Tenofovir Alafenamide: The K65R and K70E mutations result in reduced susceptibility to abacavir, didanosine, lamivudine, emtricitabine, and tenofovir, (b) (4)

(b) (4)

FDA NEGOTIATED PACKAGE INSERT

12.4 Microbiology

Mechanism of Action

Elvitegravir: Elvitegravir inhibits the strand transfer activity of HIV-1 integrase (integrase strand transfer inhibitor; INSTI), an HIV-1 encoded enzyme that is required for viral replication. Inhibition of integrase prevents the integration of HIV-1 DNA into host genomic DNA, blocking the formation of the HIV-1 provirus and propagation of the viral infection. Elvitegravir does not inhibit human topoisomerases I or II.

Cobicistat: Cobicistat is a selective, mechanism-based inhibitor of cytochromes P450 of the CYP3A subfamily. Inhibition of CYP3A-mediated metabolism by cobicistat enhances the systemic exposure of CYP3A substrates, such as elvitegravir, where bioavailability is limited and half-life is shortened by CYP3A-dependent metabolism.

Emtricitabine: Emtricitabine, a synthetic nucleoside analog of cytidine, is phosphorylated by cellular enzymes to form emtricitabine 5'-triphosphate. Emtricitabine 5'-triphosphate inhibits the activity of the HIV-1 reverse transcriptase by competing with the natural substrate deoxycytidine 5'-triphosphate and by being incorporated into nascent viral DNA which results in chain termination. Emtricitabine 5′-triphosphate is a weak inhibitor of mammalian DNA polymerases α, β, Ɛ, and mitochondrial DNA polymerase γ.

Tenofovir Alafenamide Fumarate: Tenofovir alafenamide is a phosphonoamidate prodrug of tenofovir (2'-deoxyadenosine monophosphate analog). (b) (4) is intracellularly converted to tenofovir through hydrolysis by cathepsin A. Tenofovir is subsequently phosphorylated by cellular kinases to the active metabolite tenofovir diphosphate. Tenofovir diphosphate inhibits HIV-1 replication through incorporation into viral DNA by the HIV reverse transcriptase, which results in DNA chain-termination.

Tenofovir has activity that is specific to human immunodeficiency virus (HIV-1) and hepatitis B virus. Cell culture studies have shown that both emtricitabine and tenofovir can be fully

phosphorylated when combined in cells. Tenofovir diphosphate is a weak inhibitor of mammalian DNA polymerases that include mitochondrial DNA polymerase γ and there is no evidence of toxicity to mitochondria *in vitro*.

Antiviral Activity in Cell Culture

Elvitegravir, Cobicistat, Emtricitabine, and Tenofovir Alafenamide Fumarate: The combination of elvitegravir, emtricitabine, and tenofovir alafenamide fumarate was not antagonistic in cell culture combination antiviral activity assays and was not affected by the addition of cobicistat. In addition, elvitegravir, cobicistat, emtricitabine and tenofovir alafenamide fumarate were not antagonistic with a panel of representatives from the major classes of approved anti-HIV-1 agents (INSTIs, NNRTIs, NRTIs, , and PIs).

Elvitegravir: The antiviral activity of elvitegravir against laboratory and clinical isolates of HIV-1 was assessed in T lymphoblastoid cell lines, monocyte/macrophage cells, and primary peripheral blood lymphocytes. The 50% effective concentrations (EC_{50}) ranged from 0.02 to 1.7 nM. Elvitegravir displayed antiviral activity in cell culture against HIV-1 clades A, B, C, D, E, F, G, and O (EC_{50} values ranged from 0.1 to 1.3 nM) and activity against HIV-2 (EC_{50} value of 0.53 nM). Elvitegravir did not show inhibition of replication of HBV or HCV in cell culture.

Cobicistat: Cobicistat has no detectable antiviral activity in cell culture against HIV-1, HBV, or HCV and does not antagonize the antiviral activity of elvitegravir, emtricitabine, or tenofovir.

Emtricitabine: The antiviral activity of emtricitabine against laboratory and clinical isolates of HIV-1 was assessed in T lymphoblastoid cell lines, the MAGI-CCR5 cell line, and primary peripheral blood mononuclear cells. The EC_{50} values for emtricitabine were in the range of 0.0013–0.64 micromolar. Emtricitabine displayed antiviral activity in cell culture against HIV-1 clades A, B, C, D, E, F, and G (EC_{50} values ranged from 0.007–0.075 micromolar) and showed strain specific activity against HIV-2 (EC_{50} values ranged from 0.007–1.5 micromolar).

Tenofovir Alafenamide Fumarate: The antiviral activity of tenofovir alafenamide fumarate against laboratory and clinical isolates of HIV-1 subtype B was assessed in lymphoblastoid cell lines, PBMCs, primary monocyte/macrophage cells and CD4-T lymphocytes. The EC_{50} values for tenofovir alafenamide fumarate ranged from (b) (4) to 14.7 nM.

Tenofovir alafenamide fumarate displayed antiviral activity in cell culture against all HIV-1 groups (M, N, O), including sub-types A, B, C, D, E, F, and G (EC_{50} values ranged from 0.10 to 12.0 nM) and strain specific activity against HIV-2 (EC_{50} values ranged from 0.91 to 2.63 nM).

Resistance

In Cell Culture

Elvitegravir: HIV-1 isolates with reduced susceptibility to elvitegravir have been selected in cell culture. Reduced susceptibility to elvitegravir was associated with the primary integrase substitutions T66A/I, E92G/Q, S147G, and Q148R. Additional integrase substitutions observed in cell culture selection included D10E, S17N, H51Y, F121Y, S153F/Y, E157Q, D232N, R263K, and V281M.

Emtricitabine: HIV-1 isolates with reduced susceptibility to emtricitabine have been selected in cell culture. Reduced susceptibility to emtricitabine was associated with M184V or I substitutions in HIV-1 RT.

Tenofovir Alafenamide Fumarate: HIV-1 isolates with reduced susceptibility to tenofovir alafenamide fumarate have been selected in cell culture. HIV-1 isolates selected by tenofovir alafenamide fumarate expressed a K65R substitution in HIV-1 RT, sometimes in the presence of S68N substitution; in addition, a K70E substitution in HIV-1 RT has been observed. HIV-1 isolates with the K65R substitution have reduced susceptibility to abacavir, emtricitabine, tenofovir, and lamivudine.

In Clinical Trials

In Treatment-Naïve Subjects: In a pooled analysis of antiretroviral-naive subjects receiving [TRADENAME] in Studies 104 and 111, genotyping was performed on plasma HIV-1 isolates from all subjects with HIV-1 RNA > 400 copies/mL at confirmed virologic failure, at Week 48, or at time of early study drug discontinuation. As of Week 48, the development of genotypic resistance to elvitegravir, emtricitabine, or tenofovir alafenamide fumarate was observed in 7 of 14 subjects with evaluable resistance data from paired baseline and [TRADENAME] treatment-failure isolates (7 of 866 subjects [0.78%]) compared with 6 of 17 in the STRIBILD treatment group (6 of 867 subjects [0.67%]). Of the 7 subjects with resistance development in the [TRADENAME] group, the resistance-associated substitutions that emerged were M184V/I (N = 7) and K65R (N = 1) in reverse transcriptase and T66T/A/I/V (N = 2), E92Q (N = 2), E138K (n=1), Q148Q/R (N = 1) and N155H (N = 1) in integrase. Of the 6 subjects with resistance development in the STRIBILD group, the resistance-associated substitutions that emerged were M184V/I (N = 5) and K65R (N = 2) in reverse transcriptase and E92E/Q (N = 2), E138K (n=3) and Q148R (N = 2) in integrase. In both treatment groups, most subjects who developed substitutions associated with resistance to elvitegravir also developed emtricitabine resistance-associated substitutions. The genotypic resistance results were confirmed by phenotypic analyses.

In Virologically Suppressed Subjects : One subject was identified with emergent resistance to [TRADENAME] (M184M/I) out of 4 virologic failure subjects in a clinical study of virologically-suppressed subjects who switched from a regimen containing emtricitabine/tenofovir disoproxil fumarate and a third agent to [TRADENAME] (Study 109, N = 799).

Cross-Resistance

No cross-resistance has been demonstrated for elvitegravir-resistant HIV-1 isolates and emtricitabine or tenofovir, or for emtricitabine- or tenofovir-resistant isolates and elvitegravir.

Elvitegravir: Cross-resistance has been observed among INSTIs. Elvitegravir-resistant viruses showed varying degrees of cross-resistance in cell culture to raltegravir depending on the type and number of amino acid substitutions in HIV-1 integrase. Of the primary elvitegravir resistance-associated substitutions tested (T66A/I/K, E92G/Q, T97A, S147G, Q148H/K/R, and N155H), all but three (T66I, E92G, and S147G) conferred greater than 1.5-fold reduced susceptibility to raltegravir (above the biological cutoff for raltegravir) when introduced individually into a wild-type virus by site-directed mutagenesis. Of the primary raltegravir resistance-associated substitutions (Y143C/H/R, Q148H/K/R, and N155H), all but Y143C/H

conferred greater than 2.5-fold reductions in susceptibility to elvitegravir (above the biological cutoff for elvitegravir). Viruses expressing elvitegravir or raltegravir resistance mutations maintain susceptibility to dolutegravir.

Emtricitabine: Cross-resistance has been observed among NRTIs. Emtricitabine-resistant isolates harboring an M184V/I substitution in HIV-1 RT were cross-resistant to lamivudine. HIV-1 isolates containing the K65R RT substitution, selected in vivo by abacavir, didanosine, and tenofovir, demonstrated reduced susceptibility to inhibition by emtricitabine.

Tenofovir Alafenamide Fumarate: Tenofovir resistance substitutions, K65R and K70E, result in reduced susceptibility to abacavir, didanosine, emtricitabine, lamivudine, and tenofovir.

HIV-1 with multiple TAMs (M41L, D67N, K70R, L210W, T215F/Y, K219Q/E/N/R), or multinucleoside resistant HIV-1 with a T69S double insertion mutation or with a Q151M mutation complex including K65R showed reduced susceptibility to tenofovir alafenamide fumarate in cell culture.

APPENDIX

03/31/15 Correspondence

In Phase 3 Studies 104 and 111, there were 17 virologic failures (VF) in the E/C/F/TAF arm and 21 virologic failures in the STB arm. The FDA VF subset included subjects who had confirmed viral load >400 copies/mL at discontinuation, final timepoint or after suppression to <50 copies/mL (i.e., rebound) (Subject IDs in Table A below). In addition, the FDA subset includes subjects that may not have met these criteria, but had evidence of resistance emergence in the data provided by the sponsor. Thus, 3 subjects in the E/C/F/TAF arm (0104-0115-4389, 0111-0255-5554, 0111-2348-5568) and 4 subjects in the STB arm (0104-2855-4619, 0104-3612-4362, 0111-1236-5061, 0111-2675-5138) were removed from the VF analysis because they did not have confirmed >400 copies/mL and resuppressed. Of the final FDA subset of virologic failures (E/C/F/TAF = 14; STB = 17), 7 subjects had emergent resistance substitutions in the E/C/F/TAF arm and 6 subjects had emergent resistance substitutions in the STB arm (Bolded).

Table A. Emergent Substitutions in Virologic Failures from Studies 104 and 111

PID	ARM	Timepoint with Resistance Data	Viral Load	RT Emergent Substitutions	INSTI Emergent Substitutions	Phenotype Resistance
0104-0255-4091	E/C/F/TAF	WK48	986	I178I/M	NO DATA	
0104-1965-4201	E/C/F/TAF	WK48 DC	1260	**M41M/K K65R M184V**	G70G/R **N155H D232D/N**	FTC-R >63 EVG-R 60 TDF 1.01
0104-2704-4329	E/C/F/TAF	WK48	8340	**M184V**	**E92Q**	FTC-R >70 EVG-R 56
0104-2734-4394	E/C/F/TAF	WK16 DC	62600	**M184M/V**	A129A/S	FTC-2X
0111-0729-5139	E/C/F/TAF	WK24 DC	6250	P170P/L V179V/I **M184V**	L68V **E92Q**	FTC-R >84 NO INSTI DATA
0111-0994-5313	E/C/F/TAF	WK48	2750	I195I/M	A21A/T I182I/F	
0111-1790-5185	E/C/F/TAF	WK16 DC	63000	**M184V**	**T66T/I/A/V E138E/K Q148Q/R** D232N	FTC-R >81 EVG-R 11
0111-2348-5661	E/C/F/TAF	WK36	759	D177E Q207E	NO DATA	

0111-2480-5585	E/C/F/TAF	WK24 DC	500	K64K/R	NONE	
0111-2704-5057	E/C/F/TAF	WK12	6230	D121H/Y **M184M/I/V** Q207Q/H	A265A/V	FTC-R >68
0111-2873-5544	E/C/F/TAF	WK36	47800 REBOUND		S17N	
0111-4735-5238	E/C/F/TAF	WK36	412 <400 at WK48 and 60	W71P T139T/A E204E/K	NO DATA	
0111-5083-5289	E/C/F/TAF	WK16 DC	3850 resuppressed	**M184V** H208Y E248K	**T66A** V72I	FTC-R >89 EVG-R 12
0111-7710-5649	E/C/F/TAF	WK48	322000	T200T/A Q207Q/R		NONE
0104-0031-4253	STB	WK16 DC	1130	V60I **K65R M184V**	**E92Q**	NO RT DATA EVG-R 21
0104-0783-4492	STB	WK16	1340 resuppressed	NONE	S24N	EVG 2.0
0104-1541-4150	STB	WK36 DC	364000	NONE	D6N, E35K, E69K, R107K **E138K**,E152K **M154I, E157K**	EVG-NO DATA
0104-1609-4268	STB	WK24 DC	1100 resuppressed	E36E/Q E44E/D K49K/Q **M184M/V**	NONE	FTC 1.5
0104-4735-4448	STB	WK12 DC	53400	A98G/R **M184V**	G70G/E V72I P90P/S **E92E/Q** H114H/Y **E138E/K Q148R** G190G/E	FTC-R >71 EVG-R >156
0111-0031-5129	STB	WK24	2820	V179V/I V245V/A	NO DATA	NO INSTI DATA

0111-0044-5800	STB	WK48	871	G196E		NO INSTI DATA
0111-0255-5134	STB	WK8 DC	603000	NO DATA	NO DATA	NO DATA
0111-0729-5836	STB	WK36	576	NONE	NONE	NONE
0111-0986-5527	STB	WK48	60400	NONE	NO INSTI DATA	NO INSTI DATA
0111-0994-5655	STB	WK48	1280	NONE	S17N L101L/I K127K/R L172L/F	NONE
0111-1480-5728	STB	WK24	1010	NONE	L45I	EVG 1.7
0111-1534-5156	STB	WK48	73	**M184V** T200I		FTC-R >64 EVG 1.9
0111-2106-5415	STB	WK48	11700	NONE	V249I/V	
0111-2348-5569	STB	WK36	803	NO DATA	NO DATA	NO DATA
0111-2348-5662	STB	WK36	1410	NO DATA	NO DATA	NO DATA
0111-2704-5132	STB	WK36	44800	**K65R M184V**	S17N **E138K Q148R**	FTC-R >82 TDF 1.3 EVG-R >95

Bolded subjects PIDs had emergent RT on IN substitutions.
Italicized subject PIDs were removed from denominator because they resuppressed and had no resistance data.

In Study 109, 4 subjects (Subjects 2838-6691, 2840-6507, 5083-6551 and 0959-6571) achieved HIV-1 RNA resuppression to <50 copies/mL with further treatment and were not included in the sponsor's final resistance analysis population. However, all these virologic failures subjects were included FDA resistance analysis because they have confirmed >400 copies/mL and resistance data (Table B). Subject 2838-6691 demonstrated genotypic phenotypic resistance to

FTC (M184M/I; FTC FC = 3.8) at Week 8 and the HIV-1 RNA level resuppressed to <50 copies/mL by early study drug discontinuation when the subject switched to a new drug regimen. The remaining 2 subjects (Subjects 2840-6507 and 5083-6551) did not have resistance to emtricitabine or TAF detected, and no IN resistance data was available. Subject 2824-6999 in the E/C/F/TAF group that was included in the sponsor's final resistance analysis set had no detectable resistance to any study drug.

Table B. Emergent Substitutions in Virologic Failures from Study 109

PID	ARM	Timepoint with Resistance Data	Viral Load	RT Emergent Substitutions	INSTI Emergent Substitutions	Phenotype Resistance
0109-2824-6999	E/C/F/TAF	WK24	4970	NONE	S17N S39C	NONE
0109-2838-6691	E/C/F/TAF	WK8 resuppressed	662	**M184M/I**	I84M	**FTC-R 3.8**
0109-2840-6507	E/C/F/TAF	WK12 resuppressed	438	NONE	NO DATA	NO INSTI DATA
0109-5083-6551	E/C/F/TAF	WK4 resuppressed	617	NONE	NO DATA	NO INSTI DATA
0109-0959-6571	STB	WK12 resuppressed	4760	NO DATA	NO DATA	NO DATA

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/s/

LISA K NAEGER
07/09/2015

JULIAN J O REAR
07/10/2015

MICROBIOLOGY FILING CHECKLIST FOR NDA or Supplement

NDA Number: 207561 **Applicant:** Gilead Sciences **Stamp Date:** 11/05/2014

Drug Name: Elvitegravir /cobicistat/emtricitabine/ tenofovir alafenamide (E/C/F/TAF) fixed-dose combination tablet

NDA Type: Original

On **initial** overview of the NDA application for filing:

	Content Parameter	Yes	No	Comments
1	Is the virology information (nonclinical and clinical) provided and described in different sections of the NDA organized in a manner to allow substantive review to begin?	x		
2	Is the virology information (nonclinical and clinical) indexed, paginated and/or linked in a manner to allow substantive review to begin?	x		
3	Is the virology information (nonclinical and clinical) legible so that substantive review can begin?	x		
4	On its face, has the applicant submitted cell culture data in necessary quantity, using necessary clinical and non-clinical strains/isolates, and using necessary numbers of approved current divisional standard of approvability of the submitted draft labeling?	x		
5	Has the applicant submitted any required animal model studies necessary for approvability of the product based on the submitted draft labeling?			n/a
6	Has the applicant submitted all special/critical studies/data requested by the Division during pre-submission discussions?	x		
7	Has the applicant submitted the clinical virology datasets in the appropriate format as described in the relevant guidance documents and are the datasets complete?	x		
8	Has the applicant used standardized or nonstandardized methods for virologic outcome measures? If nonstandardized methods were used, has the applicant included complete details of the method, the name of the laboratory where actual testing was done and performance characteristics of the assay in the laboratory where the actual testing was done?			standardized
9	Has the applicant submitted draft labeling consistent with current regulation, divisional and Center policy, and the design of the development package?	x		
10	Has the applicant submitted annotated microbiology draft labeling consistent with current divisional policy, and the	x		

MICROBIOLOGY FILING CHECKLIST FOR NDA or Supplement

	Content Parameter	Yes	No	Comments
	design of the development package?			
11	Have all the study reports, published articles, and other references been included and cross-referenced in the annotated draft labeling or summary section of the submission?	x		
12	Are any study reports or published articles in a foreign language? If yes, has the translated version been included in the submission for review?		x	

IS THE MICROBIOLOGY SECTION OF THE APPLICATION FILEABLE? __yes___

If the NDA is not fileable from the microbiology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

Lisa K. Naeger, Ph.D.	12/02/14
Reviewing Microbiologist	Date
Julian O'Rear, Ph.D.	12/02/14
Microbiology Team Leader	Date

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/s/

LISA K NAEGER
03/10/2015

JULIAN J O REAR
03/11/2015