CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

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MICROBIOLOGY REVIEW(S)

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)

VIROLOGY REVIEW

NDA: 203-100 **SDN:** 001 **DATE REVIEWED:** 06/27/12

Clinical Virology Reviewers: Sung S. Rhee, Ph.D. and Takashi E. Komatsu, Ph.D.

NDA #: 203-100 **Supporting Document Numbers:** 001

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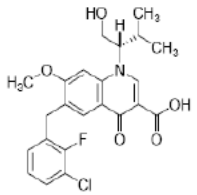
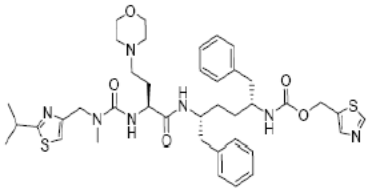
Product Name(s): STRIBILD tablet is a fixed-dose combination product containing four active pharmaceutical ingredients, elvitegravir (EVG), cobicistat (COBI), emtricitabine (FTC), and tenofovir disoproxil fumarate (TDF). Chemical characteristics of individual components of STRIBILD are listed below.

Proprietary: STRIBILD
Non-Proprietary/USAN: EVG/COBI/FTC/TDF tablet
Code Name/Number: STR (single-tablet regimen of EVG 150 mg, COBI 150 mg, FTC 200 mg, and TDF 300 mg)

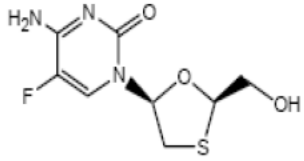
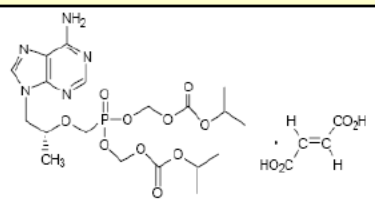
Individual Component	**EVG**	**COBI**
Structure		
Chemical Name	6-(3-chloro-2-fluorobenzyl)-1-[(2S)-1-hydroxy-3-methylbutan-2-yl]-7-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid	1,3-thiazol-5-ylmethyl[(2R,5R)-5-{[(2S)-2-[(methyl{[2-(propan-2-yl)-1,3-thiazol-4-yl]methyl}carbamoyl)amino]-4-(morpholin-4-yl)butanoyl]amino}-1,6-diphenylhexan-2-yl]carbamate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

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)

VIROLOGY REVIEW

NDA: 203-100 **SDN:** 001 **DATE REVIEWED:** 06/27/12

Clinical Virology Reviewers: Sung S. Rhee, Ph.D. and Takashi E. Komatsu, Ph.D.

Drug Class	INSTI	Pharmacoenhancer (No anti-HIV-1 activity in cell culture)
Supporting Document	IND (b) (4)	IND (b) (4)
Individual Component	**FTC**	**TDF**
Structure		
Chemical Names	5-fluoro-1-(2R,5S)-[2-(hydroxymethyl) -1,3-oxathiolan-5-yl]cytosine	9-[(R)-2[[bis[[(isopropoxycarbonyl)oxy] methoxy]phosphinyl]methoxy]propyl] adenine fumarate (1:1)
Molecular Formula	$C_8H_{10}FN_3O_3S$	$C_{23}H_{34}N_5O_{14}P$
Molecular Weight	247.24	635.52
Drug Class	NRTI	NRTI
Supporting Document	IND 53,971 and NDA 21-500	IND52,849 and NDA 21-356

Indication(s): Once-daily complete regimen for the treatment of HIV-1 infection in adults 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

Dosage Form(s): Tablet (EVG 150 mg, COBI 150 mg, FTC 200 mg, and TDF 300 mg)
Route(s) of Administration: Oral
Recommended Dosage: One tablet taken once daily with food
Dispensed: Rx _X_ **OTC** ___ **(Discipline relevant)**

Abbreviations: AAG, alpha-1-acid glycoprotein; 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; BACE-1, beta-site APP-cleaving enzyme 1; CC_{50}, 50% cytotoxic concentration; CI, combination index; COBI, cobicistat; ddI, didanosine; DHHS, Department of Health and Human Services; DRV, darunavir; d4T, stavudine; EC_{50}, effective concentration inhibiting viral replication by 50%; EC_{90}, effective concentration inhibiting viral replication by 90%; EC_{95}, effective concentration inhibiting viral replication by 95%; EFV, efavirenz; ELISA, enzyme-linked immunosorbent assay; ETR, etravirine; ETV, entecavir; EVG, elvitegravir; EVG^R, elvitegravir resistance-associated; EVG/r, ritonavir-boosted elvitegravir; FBS, fetal bovine serum; FTC, emtricitabine; GFP, green fluorescent protein; HAART, highly active antiretroviral therapy; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus (including HIV-1 and -2); HIV-1, human immunodeficiency virus type 1; HIV-2, human immunodeficiency virus type 2; HS, human

serum; HSA, human serum albumin; HTLV-1, human T-cell lymphotropic virus type 1; IC_{50}, 50% inhibitory concentration; IDV, indinavir; IL-2, interleukin 2; IN, HIV-1 integrase; INSTI, HIV-1 integrase strand transfer inhibitor; LAM, lamivudine; LPV, lopinavir; LTR, HIV-1 long terminal repeat; L-dT, telbivudine; L-FMAU, clevudine; MDR, multidrug-resistant; MOI, multiplicity of infection; mtDNA, mitochondrial DNA; MVC, maraviroc; NDA, new drug application; NFV, nelfinavir; NNRTI, HIV-1 non-nucleoside reverse transcriptase inhibitor; NR, virologic non-response; NRTI, HIV-1 nucleoside/nucleotide reverse transcriptase inhibitor; NVP, nevirapine; PBMC, peripheral blood mononuclear cell; PCR, polymerase chain reaction; PHA, phytohemagglutinin; PI, HIV-1 protease inhibitor; PI/r, ritonavir-boosted HIV-1 protease inhibitor; PIC, pre-integration complex; PK, pharmacokinetics; PR, HIV-1 protease; QD, once daily; RAL, raltegravir; RBV, ribavirin; RPV, rilpivirine; RT, HIV-1 reverse transcriptase; rt; HBV reverses transcriptase; RTE, resistance testing eligible; RTV, ritonavir; SD, standard deviation; SI, selective index; SQV, saquinavir; SR, suboptimal virologic response; TAM, thymidine analogue mutations; TDF, tenofovir disoproxil fumarate; TFV, tenofovir (active moiety of the diester prodrug TDF); TPV, tipranavir; TVD, Truvada; T-20, enfuvirtide; VF, virologic failure; VR, virologic rebound; VSV, vesicular stomatitis virus

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EXECUTIVE SUMMARY

This application was submitted in support of a new drug application (NDA) for STRIBILD tablets that contain a fixed-dose combination of two investigational compounds, 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 (FTC, 200 mg; Emtriva®) and tenofovir disoproxil fumarate (TDF, 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. Once daily single tablet regimens have been found to improve adherence and efficacy. To date there are two FDA-approved complete regimens in a single-pill, both NNRTI-based with FTC/TDF as a preferred NRTI backbone, for once-daily administration in the treatment of HIV-1 infection: Atripla® (efavirenz 600 mg + FTC 200 mg + TDF 300 mg; approved in July, 2006) and Complera® (rilpivirine 27.5 mg + FTC 200 mg + TDF 300 mg; approved in August, 2011). STRIBILD is thus the first single tablet complete regimen that combines an inhibitor targeting HIV-1 integrase.

Three antiviral components, EVG, FTC, and TDF, of the STRIBILD tablet 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. Upon entry of extracellular HIV-1 into susceptible host cells, mostly $CD4^+$ T cells, the viral single-stranded RNA genome is reverse transcribed into a linear double-stranded DNA in the cytoplasm by the HIV-1 RT. FTC and TDF inhibit this RT-catalyzed viral DNA synthesis by being incorporated into the newly synthesized DNA strand and thus resulting in chain termination. Linear double-stranded DNA then moves to the nucleus, where the molecule is covalently integrated into host chromosomal DNA by the HIV-1 IN to form the provirus. Proviral DNA is transcribed into RNA by cellular RNA polymerase II to serve as mRNA and spliced mRNAs that are translated to yield viral proteins, or genomic viral RNA that is encapsidated into progeny virus particles. EVG prevents the IN-catalyzed integration of unintegrated linear HIV-1 cDNA into the host cell genome by specifically inhibiting the strand transfer reaction of HIV-1 IN.

In a biochemical reaction, EVG was shown to inhibit the DNA strand transfer step of HIV-1 integrase (IN) where the viral DNA 3' ends are covalently linked to the cellular chromosomal DNA with an IC_{50} value of 8.8 nM. EVG dissociated from a wild-type IN-DNA complex with a binding half-life ($t_{½}$) of 11.1 hours. In HIV-1-infected MT-4 cells, EVG inhibited the integration of HIV-1 DNA into the host chromosomal DNA.

EVG exhibited antiviral activity against laboratory and clinical isolates of HIV-1 with EC_{50} values of 0.02-1.7 nM when assessed in human $CD4^+$ T cell lines and PHA/IL-2-activated primary peripheral blood mononuclear cells (PBMCs). The anti-HIV-1 activity of EVG was reduced in the presence of 50% human serum, resulting in a 7.5-fold increase in the EC_{50} value in activated PBMCs with HIV-1 infection. EVG was not antagonistic when evaluated in pair-wise combination antiviral activity assays with 22 FDA-approved antiretroviral drugs in HIV-1-infected cells: the INSTI raltegravir (RAL), 3 NNRTIs (efavirenz, etravirine, and nevirapine), 7 NRTIs abacavir, didanosine, emtricitabine, lamivudine, stavudine, tenofovir, and zidovudine), 9 PIs amprenavir, atazanavir, darunavir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir, and tipranavir), a fusion inhibitor enfuvirtide, and a CCR5 co-receptor antagonist maraviroc. The

combination of EVG, FTC, and tenofovir was not antagonistic in 3-drug combination studies, and was not impacted by the presence of COBI 25 μM.

In the cell-based resistance selection experiments, HIV-1 variants that were resistant to EVG were selected, in which 13 substitutions in HIV-1 IN were persistently detected: D10E, S17N, H51Y, T66A/I, E92G/Q, F121Y, S147G, Q148R, S153F/Y, E157Q, D232N, R263K, and V281M. Eight of these substitutions (excluding D10E, S17N, E157Q D232N, and V281M) conferred individually >3-fold reductions in susceptibility to EVG (up to 109-fold) with T66A/I, E92G/Q, F121Y, and Q148R resulting in >10-fold reduction in susceptibility to EVG.

Cross-resistance between EVG and RAL (only approved member of the INSTI class) should be expected with them sharing a common mechanism of action against HIV-1 IN. HIV-1 variants harboring IN substitution(s) selected by EVG in the resistance selection experiments showed varying degrees of cross-resistance to RAL depending on the type and number of IN substitutions. Three of the primary EVG resistance-associated substitutions, E92Q, Q148R, and N155H individually conferred reduced susceptibility both to elvitegravir (>32-fold) and RAL (>5-fold). The T66I substitution conferred >14-fold reduced susceptibility to EVG but <3-fold to RAL. Among the three primary RAL resistance-associated substitutions (Y143H/R, Q148H/K/R, and N155H), all but one (Y143H) conferred significant reductions in susceptibility to EVG (>5-fold). Thus, although there were unique resistance substitutions for EVG and RAL (i.e., T66I and Y143H, respectively), a high degree of overlapping resistance between EVG and RAL was observed.

The 4th component of the STRIBILD tablet, cobicistat (COBI), is a new chemical entity and structural analogue of ritonavir (RTV) devoid of anti-HIV activity in biochemical and cell based assays. It is a mechanism-based cytochrome P450 3A (CYP3A) inhibitor that enhances the exposure of CYP3A substrates, including EVG. The use of COBI is proposed as a pharmacoenhancer in the absence of a HIV-1 protease inhibitor (PI) in treatment-naïve subjects.

This NDA package includes clinical data from two pivotal Phase 3 trials (GS-US-236-0102 and GS-US-236-0103; Week-48 data included) and one supportive Phase 2 trial (GS-US-236-0104; Week-60 data included). All three studies are randomized, double-blind, non-inferiority trials of STRIBILD in HIV-1 infected, antiretroviral treatment-naive adult subjects, compared to Atripla® (ATR; a single-tablet complete regimen of EFV/FTC/TDF) in Studies 102 and 104 or to ritonavir-boosted atazanavir (ATV/r, 300/100 mg) plus TRUVADA® (TVD; fixed-dose combination tablet containing FTC 200 mg and TDF 300 mg) in Study 103.

In antiviral efficacy (potency and durability) analyses of STRIBILD in Studies 102 and 103, the rates of virologic failure (HIV-1 RNA ≥50 copies/mL) at Week 48 were numerically slightly lower or comparable for STRIBILD recipients, compared to those for ATR and ATV/r+TVD recipients, respectively: 7.8% versus 8.6% and 6.5% versus 6.6%. Virologic failure of the STRIBILD recipients was largely due to treatment-emergent virologic rebound (75% of the failures), rather than due to suboptimal response (including nonresponse) to the treatment (25% of the failures). Furthermore, 86.1% of the STRIBILD-treated virologic rebounders achieved virologic suppression initially but failed to maintain suppression as they experienced virologic breakthrough with HIV-1 RNA >50 copies/mL. Thus, these results indicate that STRIBILD once daily therapy can suppress HIV-1 replication but such antiviral response may not be durable in a

small number of virologic failures. Higher rates of virologic failure and rebound at Week 48 were observed in the subgroup of subjects with higher baseline viral load (negative correlation between baseline HIV-1 RNA levels and antiviral efficacy of STRIBILD). Subjects with baseline viral RNA levels ≥100,000 copies/mL experienced virologic failure and rebound at higher rates, 12% and 9.6%, respectively, while the virologic failure and rebound rates for subjects with baseline viral RNA levels <100,000 copies/mL were 4.1% and 2.7%, respectively. The majority of subjects in these two Phase 3 studies were infected with HIV-1 subtype B (94.3%). In STRIBILD-treated subjects with HIV-1 non-B subtype infection (n=35), a slightly lower rate of virologic failure was observed compared to those with subtype B infection (5.7% versus 7.3%). Thus, these limited data indicated that STRIBILD may be active against non-B subtypes as well as subtype B.

HIV-1 variants harboring EVG-treatment emergent substitutions in the HIV-1 IN were detected in failure virus samples from 20 of the 24 subjects with evaluable resistance data. A total of 42 amino acid residues (14.6% of the IN 288 residues) were found to be substituted in the STRIBILD treatment-failure viruses. To date many amino acid substitutions in HIV-1 IN have been reported to be detectable in EVG- or RAL-treatment failure subject isolates and/or selected by passage of virus in cell culture in the presence of EVG or RAL. Some substitutions (T66A/I/K, E92Q, Y143C/H/R, S147G, Q148H/K/R, and N155H) were shown to be the primary causes of EVG and/or RAL resistance, while others were frequently detected in treatment failure isolates but could confer only small or no decreases in EVG and/or RAL susceptibility. Currently, 36 IN substitutions have been associated with resistance to EVG and/or RAL (referred to as INSTI resistance-associated substitutions). Of the 42-reside changes observed in the STRIBILD-treatment failure isolates, 11 substitutions were previously identified as INSTI resistance-associated substitutions. The remaining 31 substitutions were not observed commonly, as these were detected only in one (n=27) or two (n=4) failures.

Four of the primary EVG resistance-associated substitutions (T66I, E92Q, Q148R, and N155H) were found in 11 (45.8%) of the 24 STRIBILD-treatment failures with evaluable genotypic data. These 11 isolates showed reduced susceptibility to EVG (5.6- to >198-fold compared to wild-type reference HIV-1). E92Q was most frequently found, occurring in 8 subjects. The other primary substitutions T66I, Q148R, and N155H were detected in 2, 3, and 3 subjects, respectively. S147G was not seen in these studies. Additional IN substitutions detected included previously observed IN substitutions associated with INSTI resistance, H51Y, L68I/V, G140C, S153A, E157Q, V165I, and H183P.

Of the 24 STRIBILD-treatment failures with evaluable IN genotypic data, failure variants collected from 9 subjects had one or more treatment-emergent IN substitutions in the absence of identified INSTI resistance-associated substitutions. The subjects-derived recombinant viruses displayed <2.1-fold reductions in EVG susceptibility (below the biological cutoff for EVG), and thus the relevance of these infrequently observed IN substitutions to EVG resistance is unclear at this time. Therefore, no new IN substitutions were identified that may be associated with virologic failure to EVG therapy and EVG resistance in these pooled studies, other than those previously found to be associated with EVG resistance.

In Studies 102, 103, and 104, all subjects received a TRUVADA® (FTC+TDF) background treatment. Genotypic resistance development to TRUVADA components of the regimen occurred more frequently in the STRIBILD-treatment failures, 54.5% (12/22) versus 13.3%

(1/15) and 0% of the ATR and ATV/r+TVD treatment failures with evaluable genotypic data, respectively.

In summary, the development of one or more primary substitutions associated with resistance to EVG, FTC, and/or TDF was observed in 54.2% (13/24) of the 24 STRIBILD-treatment virologic failure subjects with evaluable genotypic data. Eleven subjects developed the primary EVG resistance-associated IN substitutions: T66I (n=2), E92Q (n=8), Q148R (n=3), and N155H (n=3). Twelve subjects developed the primary FTC resistance-associated RT substitutions: K65R (n=4) and M184V/I (n=12). In phenotypic analyses, 50% (11/22) of the viremic subjects with evaluable data had HIV-1 isolates with reduced susceptibility to EVG, 57% (12/21) had reduced susceptibility to FTC, and 10% (2/21) had reduced susceptibility to tenofovir. Among these subjects, 2 subjects had HIV-1 isolates with reduced susceptibility to all 3 antiretrovirals of STRIBILD and 8 subjects to EVG and FTC.

All 11 isolates with emerging primary EVG resistance-associated substitutions were phenotypically resistant not only to EVG with >2.5-fold reduced susceptibility (above the biological cutoff for EVG) but also to RAL with >1.5-fold reduced susceptibility (above the biological cutoff for RAL). These observations with subject-derived treatment failure isolates confirmed the cell-based cross-resistance findings of broad cross-resistance between EVG and RAL. Thus, the sequential use of these INSTIs can not be a valid treatment option for HIV-1 infection.

To assess possible protease inhibitory activity of COBI *in vivo*, the protease sequences in failure isolates from the STRIBILD and Atripla (non-PI containing regimen) treatment arms of their clinical studies were compared. A disproportionate number of amino acid substitutions developed on-treatment in the STRIBILD arm (9 substitutions/14 subjects) compared to the Atripla arm (4 substitutions/15 subjects). The clinical relevance of this observation is unclear at this time as the numbers were small and not statistically significant. The sponsor will need to follow up on this observation.

1. Recommendations

1.1. Recommendation and Conclusion on Approvability: Approval of this original NDA for STRIBILD tablet (EVG 150 mg/COBI 150 mg/FTC 200 mg/TDF 300 mg) is recommended with respect to Clinical Virology. STRIBILD is a once-daily complete regimen for the treatment of HIV-1 infection in adult patients who are antiretroviral treatment-naïve or have no known substitutions associated with resistance to the individual components.

1.2. Recommendation on Phase 4 (Post-Marketing) Commitments, Agreements, and/or Risk Management Steps, If Approvable: The applicant needs to follow-up with the virologic failure subjects and sequence the protease for a longer period to see if the disproportionate number of amino acid substitutions in the STRIBILD treatment arm compared with the Atripla® continues. Also, the sponsor might require a clinical study of STRIBILD treatment failures treated with a PI-containing regimen against ATR failures.

2. Summary of OND Virology Assessments

2.1. Nonclinical Virology

Nonclinical virology data from studies of the individual drug components (elvitegravir, cobicistat, emtricitabine, and tenofovir disoproxil fumarate) of STRIBILD are summarized here.

In a biochemical reaction, elvitegravir (EVG) was shown to inhibit the DNA strand transfer step of HIV-1 Integrase (IN) where the viral DNA 3' ends are covalently linked to the cellular chromosomal DNA with an IC_{50} value of 8.8 nM. EVG dissociated from a wild-type IN-DNA complex with a binding half-life ($t_{½}$) of 11.1 hours, similar to that of 11 hours for an FDA-approved HIV-1 integrase strand transfer inhibitor (INSTI) raltegravir (RAL; ISENTRESS®). No inhibitory activities of EVG were observed in a biochemical reaction against the human DNA topoisomerases I and II with <10% inhibition at EVG concentrations up to 50 μM and 150 μM, respectively. DNA topoisomerases I and II, cellular enzymes that are found in all mammalian cells and involved in DNA replication, recombination, and transcription, display some analogous activities in DNA cleavage and transesterification reactions of the HIV-1 IN. In addition, EVG showed no significant off-target inhibition or stimulation effects on the specific ligand binding to the various receptors, and on the activities of various enzymes and cell-based assay systems including the immune cell functions of cell adhesion (ICAM-1/VCAM-1 mediated), IL-2 secretion, and mixed lymphocyte reaction (splenic lymphocytes).

EVG inhibited the integration of HIV-1 DNA into the host chromosomal DNA in HIV-1-infected MT-4 cells using an Alu-PCR assay at 18 hours after infection (94.6% reduction at 10 nM) and caused a dose-dependent increase in the accumulation of 2-LTR circular viral DNA representing unintegrated viral DNA in the nucleus (5.1-fold increase at 10 nM compared to that of the untreated control). Similar findings were observed using the single-cycle infection assay in SupT1 cells infected with VSV-G-pseudotyped HIV-1, where EVG had no effect on the production of full-length linear unintegrated viral DNA but prevented integration of viral DNA with an EC_{50} value of 0.3 nM.

EVG exhibited antiviral activity against laboratory and clinical isolates of HIV-1 with EC_{50} values of 0.02-1.7 nM when assessed in human $CD4^+$ T cell lines and PHA/IL-2-activated primary peripheral blood mononuclear cells (PBMCs). EVG was active against all B and non-B subtype HIV-1 clinical isolates tested with mean EC_{50} values ranging from 0.1 to 1.3 nM. A similar antiviral potency (EC_{50} value of 0.53 nM) was also observed against a single HIV-2 isolate. EVG exhibited low cytotoxicity in these tested cells with CC_{50} values ranging from >0.1 μM (activated primary monocytes/macrophages) to 9.7 μM (activated primary T lymphocytes). EVG inhibited viral replication during the first 10-12 hours after viral infection, coinciding with the time of viral integration in the infected cells, and the inhibitory effect of EVG was dependent on its presence in the extracellular medium at the time of viral integration. The anti-HIV-1 activity of EVG was not due to inhibition of two other HIV-1-encoded enzymes that are essential for viral replication, reverse transcriptase (RT) and protease, with IC_{50} values of >50 μM in biochemical reactions. EVG showed no antiviral activity against HBV (genotype D tested) and HCV (genotype 1b replicon tested) with EC_{50} values of >6.3 μM and 22.9 μM, respectively.

EVG retained anti-HIV-1 activity against 112 patient-derived HIV-1 recombinant clones harboring one or more substitutions in the HIV-1 protease- or RT-coding regions associated with resistant to NNRTIs, NRTIs, or PIs with the mean EC_{50} value of 1.06 nM (0.8- to 1.2-fold changes in EC_{50} values, compared to the reference clone of HIV-1_{NL4-3}) in the PhenoSense™ HIV assay (Monogram Biosciences). Thus, there appears to be no cross-class resistance between EVG and other classes of antiretrovirals, as expected with them having different

mechanisms of action against HIV-1.

EVG was highly bound to human plasma proteins (99.4%) with preferential binding to albumin (99.4%) over alpha-1-acid glycoprotein (up to 40.7%) in equilibrium dialysis experiments. The anti-HIV-1 activity of EVG was shown to be moderately reduced in the presence of 50% human serum, resulting in a 7.5-fold increase in the EC_{50} value (from 0.2 nM to 1.5 nM) in activated PBMCs with HIV-1 infection. The protein-adjusted EC_{90} value was estimated to be 9.8 nM (4.4 ng/mL). In the presence of 50% human serum EVG became less toxic (17.5-fold) to activated PBMCs with the CC_{50} value of 170.1 μM compared to that of 9.7 μM to the cells cultured in the absence of human serum. No significant differences were observed in the cytotoxicity of EVG between unstimulated and stimulated PBMCs. EVG is not a nucleoside analog and does not share chemical structure with DNA polymerase γ natural substrates, and therefore is not expected to have mitochondrial toxicity. No measurable depletion of mitochondrial DNA was observed at EVG 10 μM (highest concentration tested) in HepG2 liver cells.

EVG was not antagonistic when evaluated in pair-wise combination antiviral activity assays combinations with 22 FDA-approved antiretroviral drugs in HIV-1-infected cells: the INST raltegravir (RAL), 3 NNRTIs (efavirenz, etravirine, and nevirapine), 7 NRTIs abacavir, didanosine, emtricitabine, lamivudine, stavudine, tenofovir, and zidovudine), 9 PIs amprenavir, atazanavir, darunavir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir, and tipranavir),, a fusion inhibitor enfuvirtide, and a CCR5 co-receptor antagonist maraviroc. There was no evidence of antagonistic interactions against HIV-1 between EVG and HBV antivirals, 3 FDA-approved drugs (adefovir, entecavir, and telbivudine) and the investigational agent clevudine, in the two-drug combination studies. Thus, EVG could potentially be used in a regimen containing any of the tested HBV antiviral drugs for HIV-1 and HBV co-infected patients.

The combination of EVG, emtricitabine, and tenofovir (antiretroviral components of STRIBILD) was not antagonistic in 3-drug combination studies, and was not impacted by the presence of the pharmacoenhancer COBI 25 μM.

EVG represents 93% of all EVG-associated products in human plasma of EVG-treated subjects. Four main circulating metabolites of EVG in human plasma were identified in Phase 1 studies: M1 (<1%; hydroxylation of the chlorofluorophenyl group), M4 (3.5%, glucuronide conjugate of the carboxylic acid), M7 (<1%; glucuronide conjugate of M1), and HM1 (<1%; sulfate conjugate of M1). Compared to EVG, all four metabolites inhibited the HIV-1 IN-catalyzed strand transfer reaction in the biochemical mechanism-of-action assay with 5.2- to 425.7-fold lower potency. M1 and M4 retained relatively higher inhibitory activity with 5.2- and 38-fold decreases in potency, respectively, compared to the other two metabolites (>360-fold). Both M1 and M4 showed detectable anti-HIV-1 activity in HIV-1-infected MT-2 cells with EC_{50} values of 12.5-17.5 nM (10.4- to 12.5-fold reduction in potency compared to the parent drug EVG) and of 10.5-12.3 nM (8.8-fold reduction), respectively.

In the cell-based resistance selection experiments, HIV-1 variants that were resistant to EVG or its metabolites M1 and M4 were selected, in which 13 substitutions in HIV-1 IN were persistently detected: D10E, S17N, H51Y, T66A/I, E92G/Q, F121Y, S147G, Q148R, S153F/Y, E157Q, D232N, R263K, and V281M. No phenotypic resistance data were available for D10E, S17N, D232N, and V281M. Of the remaining 9 substitutions, all but one (E157Q) conferred individually >3-fold reduced susceptibility to EVG (up to 109-fold) with T66A/I, E92G/Q, F121Y,

and Q148R resulting in high-level resistance to EVG (>10-fold reduction in susceptibility). Overall, the resistance profiles of EVG and its metabolites M1 and M4 were overlapping. The magnitude of resistance of selected virus pools and site-directed mutant viruses harboring selected IN substitutions was similar to EVG, M1, and M4, with the exception of H51Y, which showed a higher magnitude of resistance to M1 than EVG or M4. Of the 8 substitutions conferring varying degrees of resistance to EVG (H51Y, T66A/I, E92G/Q, F121Y, S147G, Q148R, S153F/Y, and R263K), 4 substitutions (T66A, E92Q, F121Y, and Q148R) also conferred cross-resistance to RAL (3.8- to 37.6-fold reductions in susceptibility).

Of the 13 cell-selected IN substitutions, 5 substitutions H51Y, T66I, E92Q, Q148R, and E157Q were also observed in the STRIBILD-treatment virologic failure isolates evaluated in 3 clinical studies (GS-US-236-0102 [102], -0103 [103], and -0104 [104]). The H51Y, T66I, E92Q, Q148R, and E157Q substitutions individually conferred 2.9-, 30.8-, 78.1-, 107.6-, and 2.9-fold reduced susceptibility to EVG, respectively, in cell culture.

Cross-resistance between EVG and RAL (only approved member of the INSTI class) should be expected with them sharing a common mechanism of action against HIV-1 IN, involving metal chelation of the divalent metal cation Mg^{++} and interaction with the catalytic loop (residues 140-149). HIV-1 variants harboring IN substitution(s) selected by EVG (or its metabolites M1 and M4) in the cell-based resistance selection experiments showed varying degrees of cross-resistance to RAL depending on the type and number of IN substitutions. In contrast, these substitutions conferred no significant cross-class resistance to other antiretrovirals from different classes (≤2.5-fold reductions in susceptibility to tested inhibitors including the NNRTI efavirenz, the NRTIs FTC, tenofovir, and zidovudine, and the PI lopinavir), as they have different mechanisms of action against HIV-1.

The phenotypic impact of individual and combinations of IN substitutions occurring at 21 amino acid positions was evaluated on EVG susceptibility and cross-resistance to RAL. These substitutions included those detected in the resistance selection experiments, detected in the STRIBILD-treatment failure isolates in Studies 102, 103, and 104, and known to be associated with RAL resistance: H51Y, T66A/I/K, L68I/V, V72I, E92G/Q, T97A, F121Y, E138A/K, G140S, Y143H/R, P145S, Q146I/K/L/P/R, S147G, Q148H/K/R, S153F/Y, N155H/S, E157Q, G163K/R, S230R, N232D, and R263K. Overall, most viruses containing the examined IN substitution(s), individually and on combinations, exhibited significant reductions in susceptibility against EVG and/or RAL: only some substitutions H51Y, L68I/V, V72I, T97A, E138A/K, Q146K, E157Q, G163K/R, S230R, and N232D showed <3-fold loss of susceptibility to both EVG and RAL. The E92Q substitution, most frequently found in the STRIBILD-treatment virologic failure isolates in Studies 102, 103, and 104, conferred reduced susceptibility to EVG (39.4- to 78.1-fold) and RAL (5.8- to 11.2-fold). Among the remaining 3 primary EVG resistance-associated substitutions, Q148R and N155H detected in the STRIBILD-treatment virologic failure isolates conferred reduced susceptibility to EVG (>32-fold) and RAL (>5-fold), while T66I conferred >14-fold reduced susceptibility to EVG but <3-fold to RAL. Among the 3 primary RAL resistance-associated substitutions (Y143H/R, Q148H/K/R, and N155H tested), all but one (Y143H) conferred measurable reductions in susceptibility to EVG (4.8- to 107.6-fold). All HIV-1 variants expressing multiple IN substitutions in combination with E92Q, Q148H/K/R, or N155H showed high levels of resistance to EVG (42.7- to 868.7-fold) and RAL (6.1- to 755.8-fold). Thus, although there were unique resistance substitutions for EVG and RAL (i.e., T66I and Y143H, respectively), a high degree of overlapping resistance between EVG and RAL was observed for

key INSTI resistance-associated substitutions.

Cobicistat (COBI) is structurally similar to the HIV-1 protease inhibitor ritonavir, and, like ritonavir, is a specific inhibitor of CYP3A, the body's major drug-metabolizing enzyme. CYP3A inhibitors are used as PK enhancers to increase the systemic levels of co-administered agents metabolized by this enzyme system.

COBI was tested in a biochemical enzymatic assay for its inhibitory effect on recombinant HIV-1 protease. COBI is completely inactive in this enzymatic assay, up to a concentration of 30 μM. These enzyme inhibition data indicate that the structural differences introduced into COBI compared to ritonavir eliminate its ability to bind to the active site of HIV-1 protease and inhibit its enzymatic activity. The antiviral activity of COBI was tested in PBMCs against a panel of 17 HIV-1 and 2 HIV-2 primary isolates. Different groups of HIV-1 (M, N, O), as well as the most prevalent group M (subtypes A, B, C, D, E, F, and G), were represented among the tested isolates. After infection, the cells were incubated with or without drugs for 7 days. The endpoints were the RT activity assay or the p24 antigen ELISA assay. The cells were stained with MTS to evaluate cytotoxicity. COBI exhibited essentially no antiviral activity against any isolates tested in this assay system. Mild cytotoxicity was observed with this compound resulting in 81.2% (CC_{50} = 12.9 μM; experimental #1) and 41.6% (CC_{50} >30 μM; experimental #2) reduction in cell viability at the highest concentration tested, 30 μM. The anti-HBV activity of COBI was tested in WT-42 cells, a clone of HepG2 cells stably transfected with a wild-type recombinant HBV DNA clone (genotype A, serotype adw2) which expresses HBV transcripts and proteins. The cells were incubated with or without drug for 3 days. The endpoint was measuring HBV DNA from cell lysates by real-time PCR. The anti-HCV activity of COBI was tested in Huh-Luc cells, a clone of Huh-7, which stably replicates a subgenomic HCV genotype 1b replicon encoding a luciferase reporter gene. No anti-HCV activity or cytotoxicity was detected after a 3-day treatment with COBI at concentrations up to 30 μM.

Cell culture antiviral activity of selected HIV-1 drugs was tested in MT-2 cells infected with HIV-1, in the presence or absence of 5 μM COBI. Overall, no significant changes were observed in the activity of any of the tested antiretrovirals in the presence of 5 μM COBI. For all the drugs tested, the change in their EC_{50} values in the presence of COBI ranged from 0.7- to 1.9-fold. No cell culture resistance selection studies or cross-resistance studies with other approved drugs were conducted as cell culture antiviral activity was not demonstrated.

Nonclinical virology information on the two FDA-approved NRTIs emtricitabine (FTC) and tenofovir disoproxil fumarate (TDF) can be found in the Clinical Virology reviews of Nara Battula, Ph.D. (N021500.000 and N021356.000), and also in the US Prescribing Information for EMTRIVA® (revised in November, 2011) and VIREAD® (revised in January, 2012), respectively. EMTRIVA and VIREAD were initially approved for the treatment of HIV-1 infection in combination with other antiretroviral agents in July, 2003 (NDA 21-500) and October, 2001 (NDA 21-356), respectively. Nonclinical virology data of FTC and TDF are briefly summarized below.

FTC is a synthetic nucleoside analog of cytidine that is phosphorylated by cellular enzymes to form FTC 5'-triphosphate. FTC 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. FTC 5′-triphosphate is a weak inhibitor of mammalian DNA polymerases α, β, ε, and mitochondrial DNA polymerase γ. FTC exhibited antiviral activity against laboratory and clinical isolates of HIV-1 with EC_{50} values of 0.0013-0.64 μM in cell culture. The EC_{50} values against HIV-1 isolates from clades A, B, C, D, E, F, and G were in range 0.007-0.075 μM. In addition, FTC showed strain-specific antiviral activity against HIV-2 with EC_{50} values of 0.007-1.5 μM. In two-drug combination studies, FTC showed no antagonistic effects when combined with each of 14 antiretrovirals: NNRTIs delavirdine, efavirenz, nevirapine, and rilpivirine; NRTIs abacavir, lamivudine, stavudine, tenofovir, and zidovudine; PIs amprenavir, nelfinavir, ritonavir, and saquinavir; and the investigational integrase strand transfer inhibitor elvitegravir.

TDF is an acyclic nucleoside phosphonate diester analog of adenosine monophosphate. TDF requires initial diester hydrolysis for conversion to tenofovir (TFV) and subsequent phosphorylations by cellular enzymes to form TFV diphosphate. TFV diphosphate inhibits the activity of HIV-1 RT by competing with the natural substrate deoxyadenosine 5′-triphosphate and, after incorporation into DNA, by DNA chain termination. TFV diphosphate is a weak inhibitor of mammalian DNA polymerases α, β, and mitochondrial DNA polymerase γ. TFV exerted antiviral activity against laboratory and clinical isolates of HIV-1 with EC_{50} values of 0.04-8.5 μM in cell culture. The EC_{50} values against HIV-1 isolates from clades A, B, C, D, E, F, G, and O ranged from 0.5 to 2.2 μM. In addition, TFV showed strain-specific activity against HIV-2 with EC_{50} values of 1.6-5.5 μM. No antagonistic effects were observed in two-drug combination studies of TFV with an NNRTI (delavirdine, efavirenz, nevirapine, or rilpivirine), an NRTI (abacavir, didanosine, emtricitabine, lamivudine, stavudine, or zidovudine), a PI (amprenavir, indinavir, nelfinavir, ritonavir, or saquinavir), or an investigational integrase strand transfer inhibitor elvitegravir.

2.2. Clinical Virology

Clinical virology analyses were conducted to evaluate the antiviral efficacy (potency and durability) and virological resistance of the single-tablet complete regimen of EVG 150 mg/COBI 150 mg/FTC 200 mg/TDF 300 mg (referred to E/C/F/T through this review) in HIV-1-infected, antiretroviral treatment-naïve (ARV-naïve) adult subjects in 3 clinical trials, two Phase 3 Studies GS-US-236-0102 and GS-US-236-0103 (Studies 102 and 103, respectively) and one Phase 2 Study GS-US-236-0104 (Study 104). In these studies, the comparator groups were treated with a single-tablet complete regimen of EFV 600 mg/FTC 200 mg/TDF 300 mg (Atripla®; ATR; Studies 102 and 104) or 100 mg ritonavir-boosted 300 mg atazanavir (ATV/r) plus TRUVADA® (fixed-dose combination tablet containing FTC 200 mg and TDF 300 mg; TVD) in Study 103.

Clinical virology analyses were conducted using the censored, as-treated subject population (n=1392, pooled from the three studies) including all randomized subjects who received at least one dose of study medication with at least one virologic observation after Baseline but excludes subjects who discontinued their assigned treatment before the primary efficacy assessment while they had a suppressed viral load (HIV-1 RNA <50 copies/mL).

A total of 105 virologic failures, 7.5% of the 1392 treated-subjects in the three pooled studies, were identified. Of subjects receiving E/C/F/T, ATR, and ATV/r+TVD treatment, 7.3% (52/715), 9% (31/346), and 6.6% (22/331) experienced virologic failure, respectively. Virologic failures

were identified as subjects who failed to achieve HIV-1 RNA <50 copies/mL either at Week 48 or at the time of early discontinuation in Studies 102 and 103; and subjects who failed to achieve HIV-1 RNA <50 copies/mL either at the time of study unblinding (Week 60) or at the time of early discontinuation in Study 104.

In antiviral efficacy analyses of E/C/F/T in Studies 102 and 103, the rates of virologic failure (HIV-1 RNA ≥50 copies/mL) at Week 48 were numerically slightly lower or comparable for E/C/F/T recipients, compared to those for ATR and ATV/r+TVD recipients, respectively: 7.8% versus 8.6% and 6.5% versus 6.6%. Plasma HIV-1 RNA levels at Baseline were well matched among the treatment groups with a median baseline HIV-1 RNA level of 4.8 $\log_{10}$ copies/mL for all 3 treatment groups.

Virologic failure of the E/C/F/T recipients was largely due to treatment-emergent virologic rebound (75% of the failures [36/48], pooled from the two studies), rather than due to suboptimal response (including nonresponse) to the treatment (25% of the failures [12/48], pooled from the two studies). Furthermore, of the 36 E/C/F/T-treated virologic rebounders, 31 subjects achieved virologic suppression initially but failed to maintain suppression as they experienced virologic breakthrough with HIV-1 RNA >50 copies/mL. Thus, these results indicate that E/C/F/T QD therapy can suppress HIV-1 replication but such antiviral response may not be durable in a small number of virologic failures. Similarly, in control-drug recipients (ATR or ATV/r+TVD), virologic rebound was also a primary cause of the virologic failure, as 60.7% (17/28) and 50% (11/22) of the virologic failures being virologic rebounders, respectively.

Higher rates of virologic failure and rebound at Week 48 were observed in the subgroup of subjects with higher baseline viral load (negative correlation between baseline HIV-1 RNA levels and antiviral efficacy of E/C/F/T). Compared to overall virologic failure and rebound rates of 7.2% and 5.4%, respectively, in E/C/F/T-treated subjects (pooled from the two studies), subjects with baseline viral RNA levels ≥100,000 copies/mL experienced virologic failure and rebound at higher rates, 12% and 9.6%, respectively. The virologic failure and rebound rates for subjects with baseline viral RNA levels <100,000 copies/mL were 4.1% and 2.7%, respectively. A similar negative correlation, however less prominent, was also observed in subjects who received ATR or ATV/r+TVD treatment.

The majority of subjects in these two Phase 3 studies were infected with HIV-1 subtype B (94.3%). In E/C/F/T-treated subjects with HIV-1 non-B subtype infection (n=35), a slightly lower rate of virologic failure was observed compared to those with subtype B infection (5.7% versus 7.3%). Baseline HIV-1 RNA levels were comparable between the two subgroups of subjects with median baseline HIV-1 RNA levels of 4.8 $\log_{10}$ copies/mL for subtype B-infected subjects and of 4.9 $\log_{10}$ copies/mL for non-B subtype-infected subjects. Thus, these limited data indicated that E/C/F/T may be active against non-B subtypes as well as subtype B.

In virological resistance analyses of E/C/F/T in Studies 102, 103, and 104, drug resistance testing was performed for viruses (pre-treatment and on-treatment) collected from virologic failure subjects with HIV-1 RNA ≥400 copies/mL at Week 48 or at the time of early study drug discontinuation to assess whether these subjects developed during therapy amino acid substitutions associated with resistance to the individual antiretroviral components of the regimens. Of the 52 E/C/F/T recipients with evidence of virologic failure (HIV-1 RNA ≥50 copies/mL) by Week 48 (Studies 102 and 103) or at Week 60 (Study 104), 32 of them were

eligible for resistance testing with plasma HIV-1 RNA levels ≥400 copies/mL at the time of virologic failure (or later while still on treatment). Evaluable genotypic and/or phenotypic resistance data were obtained from 24 subjects (46.2% of E/C/F/T-treatment virologic failures).

HIV-1 variants harboring EVG-treatment emergent substitutions in the HIV-1 integrase (IN) were detected in failure virus samples from 20 of the 24 subjects with evaluable resistance data. These failure isolates with IN genotypic changes had reductions in susceptibility to EVG ranging from 1- to >198-fold that of wild-type reference HIV-1. Failure isolates from 4 subjects with no detectable IN genotypic changes had fold-change values in EVG susceptibility below the biological cutoff (ranging from 1.1- to 1.8-fold).

A total of 42 amino acid residues (14.6% of the IN 288 residues) were found to be substituted in the E/C/F/T treatment-failure viruses with the average number of substitutions per isolate of 2.2 ± 2.0. To date, many amino acid substitutions in HIV-1 IN have been reported to be detectable in EVG- or RAL-treatment failure subject isolates and/or selected by passage of virus in cell culture in the presence of EVG or RAL. Some substitutions were shown to be the primary causes of EVG (T66A/I/K, E92Q, S147G, Q148H/K/R, and N155H) and/or RAL (Y143C/H/R, Q148H/K/R, N155H) resistance, while others were frequently detected in treatment failure isolates but could confer only small or no decreases in EVG and/or RAL susceptibility. Currently, 36 IN substitutions were identified to be associated with resistance to EVG and/or RAL (referred to as INSTI resistance-associated substitutions). Of the 42-reside changes observed in the E/C/F/T-treatment failure isolates, 11 substitutions were previously identified as INSTI resistance-associated substitutions. The remaining 31 substitutions were not observed commonly, as these were detected only in one (n=27) or two (n=4) failures.

The primary EVG resistance-associated substitutions (T66I, E92Q, Q148R, and N155H) were found in 11 (45.8%) of the 24 E/C/F/T-treatment failures with evaluable genotypic data in Studies 102, 103, and 104. These 11 isolates showed reduced susceptibility to EVG (5.6- to >198-fold compared to wild-type reference HIV-1). Of the previously identified 5 primary EVG resistance-associated substitutions, S147G was not detectable in these studies. E92Q was most frequently found, occurring in 8 subjects: 5 subjects had a single E92Q change and 3 subjects had mixtures of E92Q+T66I, E92Q+T66I+N155H, or E92Q+Q148R+N155H in their virus samples (one subject each). The other primary substitutions T66I, Q148R, and N155H were detected in 2, 3, and 3 subjects, respectively. Both subjects with T66I had mixtures of T66I+E92Q or T66I+E92Q+ N155H in their virus samples. A single Q148R change was detected in 2 subjects and one subject had mixtures of Q148R+E92Q+N155H in his virus samples, while a single N155H change was observed in one subject and mixtures of N155H+T66I+E92Q or N155H+E92Q+ Q148R in 2 subjects. Additional IN substitutions were detected, in addition to the primary EVG resistance-associated substitutions, including previously observed IN substitutions associated with INSTI resistance, H51Y, L68I/V, G140C, S153A, E157Q, V165I, and H183P.

Of the 24 E/C/F/T-treatment failures with evaluable genotypic data, failure variants collected from 9 subjects had one or more treatment-emergent IN substitutions (n=18) in the absence of detectable INSTI resistance-associated substitutions. The subjects-derived recombinant viruses displayed <2.1-fold reductions in EVG susceptibility (below the biological cutoff for EVG), and thus the relevance of these infrequently observed IN substitutions to EVG resistance is unclear at this time. Therefore, no new IN substitutions were identified that may be

associated with virologic failure to EVG therapy and EVG resistance in these pooled studies, other than those previously identified to be associated with EVG resistance.

With a common mechanism of action against HIV-1 IN and overlapping genetic pathways of resistance to EVG and RAL, cross-resistance between EVG and RAL should be expected. All E/C/F/T-treatment failure isolates genotypically resistant to EVG with evidence of emerging primary EVG resistance-associated substitutions were phenotypically resistant not only to EVG with >2.5-fold reduced susceptibility (above the biological cutoff for EVG) but also to RAL with >1.5-fold reduced susceptibility (above the biological cutoff for RAL). Furthermore, isolates with no genotypic changes or only with substitutions not associated with EVG resistance in the IN protein appeared to remain susceptible to EVG and to RAL with <2.5-fold (ranging from 1- to 2.1-fold) and <1.5-fold (ranging from 0.8- to 1.4-fold) reductions in susceptibility, respectively. These results observed with subject-derived treatment failure isolates confirmed the cell-based cross-resistance findings with IN substitutions generated by site-directed mutagenesis of broad cross-resistance between EVG and RAL. The sequential use of these INSTIs thus can not be a valid treatment option for HIV-1 infection.

In Studies 102, 103, and 104, all subjects received a TRUVADA® background treatment. At screening, all subjects were tested for genotypic sensitivity to FTC and TDF, and none had baseline HIV-1 harboring RT substitutions associated with primary resistance to FTC and TDF (K65R, K70E, M184I/V), while one or two TAMs (thymidine-associated mutations) were detectable in 39 subjects' baseline isolates (14, 14, and 11 subjects in the E/C/F/T, ATR, and ATV/r+TVD treatment groups, respectively). All but one subjects with baseline TAM(s) achieved virologic suppression by Week 48.

Of the 105 virologic failures in the virology analyses in the 3 pooled studies, 43 subjects' RT resistance data were obtained. The emergence of RT substitutions associated with FTC and TDF resistance were observed in 32.6% (14/43) of the evaluated failures. The remaining 29 subjects had failure isolates with no genotypic changes (n=7) in RT or treatment-emergent RT substitutions that were not associated with resistance to any NRTIs with <1.7-fold and <1.3-fold reduced susceptibility to FTC and TFV, respectively. Overall, genotypic resistance development to TRUVADA components of the regimen occurred more frequently in the E/C/F/T-treatment failures, 54.5% (12/22) versus 13.3% (1/15) and 0% of the ATR and ATV/r+TVD treatment failures with evaluable genotypic data, respectively.

All 14 subjects with emerging RT substitutions associated with FTC and TDF resistance developed M184I/V, a primary FTC resistance-associated substitution, and their failure isolates became resistant to FTC with >42-fold reductions in FTC susceptibility (above the clinical cutoff of 3.5-fold). In 6 of these 14 subject isolates (including 4 E/C/F/T recipients), K65R associated with both FTC and TDF resistance was also detectable. Three of the 6 isolates with the K65R substitution in addition to M184I/V demonstrated reduced susceptibility both to FTC (>84-fold) and tenofovir (1.5- to 1.8-fold, above the clinical cutoff of 1.4-fold).

All 14 isolates harboring the M184I/V RT substitution exhibited cross-resistance to lamivudine with >43.7-fold reduced susceptibility (above the clinical cutoff of 3.5-fold), and some to didanosine with 1.4- to 3.7-fold reduced susceptibility (n=11; above the clinical cutoff of 1.3-fold). In addition, 3 of the 6 isolates harboring the K65R RT substitution in addition to M184V/I showed cross-resistance to multiple NRTIs, abacavir, didanosine, emtricitabine, and tenofovir,

demonstrating reduced susceptibility to abacavir (4.7- to 6.8-fold, above the clinical cutoff of 4.5-fold), didanosine (2.6- to 3.7-fold), FTC (84.1- to 129.9-fold) and TFV (1.5- to 1.8-fold). No cross-resistance was observed to stavudine and zidovudine (below the clinical and biological cutoffs of 1.7-fold and 1.9-fold for stavudine and zidovudine, respectively).

In the EVG resistance-associated substitution-harboring virus populations of all 10 E/C/F/T-treatment failures with evaluable data, M184I/V was always detectable, conferring reduced susceptibility both to EVG and FTC. Preliminary linkage analysis of failure isolates from 2 of these 10 subjects by sequencing approximately 20 clones per sample revealed that these IN and RT substitutions were present in the same virus genome. In virologic failures in the ATR treatment group, concurrent emergence of M184I/V with RT substitutions associated with resistance to EFV (predominantly the K103N substitution), thus conferring reduced susceptibility both to EFV and FTC, were less frequently observed (25% [2/8] of the failures). With similar observations made with RAL-treatment virologic failures in clinical studies of RAL in combination with TRUVADA (STARTMRK and QDMRK studies), concurrent emergence of INSTI resistance-associated IN substitutions with the FTC resistance-associated RT substitution M184V/I may be an INSTI class-specific effect when used in combination with TRUVADA. In those two RAL studies, RAL genotypic resistance (by Y143C/H/R, Q148H, and N155H substitutions) was always noted in association with FTC resistance (by detectable M184I/V RT substitution) in 11 evaluated RAL-treatment failures. In contrast, only 2 of the 5 failures on EFV (as a comparator in the two studies) with evaluable genotypic data developed resistance both to EFV and FTC.

Since COBI is structurally similar to ritonavir (RTV), an HIV-1 protease inhibitor (PI), the question was whether the *in vivo* data support COBI not having antiviral activity. The sponsor was asked to evaluate the viral protease sequences in their clinical studies. There was a disproportionate number of amino acid substitutions in the HIV-1 protease (PR) that developed on-treatment in the E/C/F/T treatment arm (9 substitutions/14 subjects) compared to the ATR treatment arm (4 substitutions/15 subjects). Three of the 9 PR substitutions in isolates from the E/C/F/T arm have been associated with resistance to PIs (M36I, D60E, and V77I) compared to 1 out of 4 in the ATR arm (K20R). The only amino acid substitution that developed in more than one subject was the PR R57K/R substitution. This amino acid substitution has not been associated with resistance to any of the approved HIV-1 PIs. Furthermore, the drug susceptibility to HIV-1 PIs (APV, ATV, DRV, IDV, LPV, NFV, RTV, SQV, and TPV) ranged from 0.4- to 2.5 fold shift in susceptibility for all of the isolates tested. The clinical relevance of this observation is unclear at this time as the number of subjects was small; however, this issue will require careful follow up.

3. Administrative

3.1. Reviewer's Signatures

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Clinical Virology Reviewer

_____

Takashi E. Komatsu, Ph.D.
Clinical Virology Reviewer

3.2. Concurrence

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HFD-530/NDA # 203100
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OND VIROLOGY REVIEW

1. Introduction and Background

Approximately 33.3 million people worldwide are estimated to be living with human immunodeficiency virus type 1 (HIV-1) infection worldwide (UNAIDS Global Report, 2010). The infection, if left untreated or suboptimally treated, is characterized by deterioration in immune function, the subsequent occurrence of opportunistic infections and malignancies, ultimately resulting in death. Standard-of-care for the treatment of HIV-1 infection involves the use of a combination of antiretroviral drugs to suppress viral replication to below detectable limits (40-50 copies/mL), increase $CD4^+$ T cell counts, and delay disease progression. Current treatment guidelines (DHHS, updated in March, 2012) for treatment-naïve HIV-1 infected patients recommend that initial therapy generally consists of two nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) in combination with a non-nucleoside reverse transcriptase inhibitor (NNRTI), a protease inhibitor (PI, preferably boosted with ritonavir [RTV]), an integrase stand transfer inhibitor (INSTI, namely raltegravir [RAL]), or a CCR5 co-receptor antagonist (namely maraviroc [MVC]). For treatment-experienced HIV-1 infected patients, a new regimen contains at least two (preferably three) fully active antiretrovirals (ARVs) to combine with an optimized background antiretroviral regimen, based on the patient's treatment history, and past and current resistance test results.

The use of antiretroviral combination therapy, introduced in the early 1990s, greatly reduced the morbidity and mortality associated with AIDS, but increased adherence challenges for patients due to the regimen-associated factors such as dosing frequency, increased pill burden, dietary restrictions, and safety concerns (Chesney, 2000; Chesney *et al*., 2000). Poor adherence increases the risk of incomplete viral suppression, development of drug-resistant virus, and disease progression. Clinical studies have demonstrated high levels of adherence and treatment success with simple, once-daily, highly active antiretroviral therapy (HAART) regimens, resulting in persistent suppression of HIV-1 viral load (Arribas *et al*., 2004; Felizarta *et al*., 2004; Maggiolo *et al*., 2001). Recently, a meta-analysis of 11 randomized, controlled trials involving 3,029 subjects also demonstrated that the adherence rate was better with once-daily regimens (+2.9%; 95% confidence interval, 1.0-4.8%) than with twice-daily regimens (Jean-Jacques *et al*., 2009). To reduce pill burden, a number of ARVs are available in fixed-dose combinations; however, with some combinations patients may take an incomplete regimen, leading to increased risk of resistance development. This risk could be reduced if a complete treatment regimen (i.e., 2 NRTIs plus one ARV from another class) is available in a once-daily, single pill. To date, there are two FDA-approved complete regimens in a single-pill, both NNRTI-based with FTC/TDF as a preferred NRTI backbone, for once-daily administration in the treatment of HIV-1 infection: Atripla® (ATR; 600 mg EFV/200 mg FTC/300 mg TDF; approved in July, 2006) and Complera® (27.5 mg rilpivirine [RPV]/200 mg FTC/300 mg TDF; approved in August, 2011).

The applicant co-formulated an investigational HIV-1 INSTI elvitegravir (EVG 150 mg) and an investigational pharmacoenhancer cobicistat (COBI 150 mg) devoid of anti-HIV activity with the FTC 200 mg/TDF 300 mg standard-of-care NRTI backbone into a single fixed-dose combination tablet (referred to as E/C/F/T tablet through this review). This original new drug application (NDA) is submitted for U.S. marketing approval of E/C/F/T tablets. The proposed indication for the E/C/F/T 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. The NDA package includes clinical study reports and datasets from the two pivotal Phase 3 trials (GS-US-236-0102 and GS-US-236-0103; Week-48 data included) and one supportive Phase 2 trial (GS-US-236-0104; Week-60 data included). All three studies are randomized, double-blind, non-inferiority trials of a single-tablet, E/C/F/T regimen in HIV-1 infected, antiretroviral treatment-naive adult subjects, compared to Atripla® (a single-tablet complete regimen of EFV/FTC/TDF) in Studies GS-US-236-0102 and -0104 or to ritonavir-boosted atazanavir (ATV/r, 300/100 mg) plus TRUVADA® (fixed-dose combination tablet containing FTC 200 mg and TDF 300 mg) in Study GS-US-236-0103.

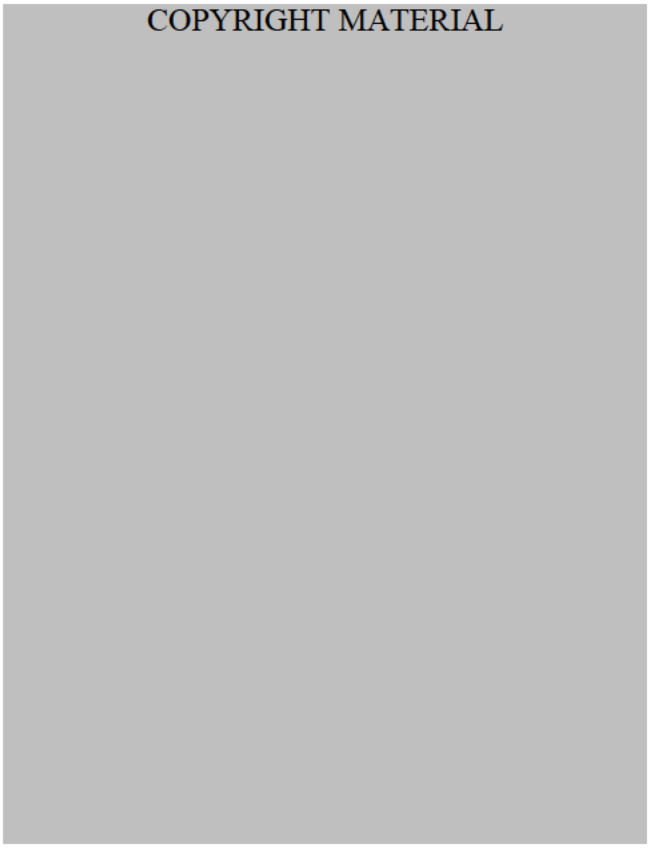
Three antiviral components of the E/C/F/T regimen target independently two essential viral enzymes, integrase (IN) and reverse transcriptase (RT), that are required during the early stages of the HIV-1 life cycle (reviewed by Freed, 2001). Upon entry of extracellular HIV-1 into susceptible host cells, mostly $CD4^+$ T cells, the viral single-stranded RNA genome is reverse transcribed into a linear double-stranded DNA in the cytoplasm by the HIV-1 reverse transcriptase (RT; see below Figure 1, from Telesnitsky and Goff, 1997). Linear double-stranded DNA then moves to the nucleus, where the molecule is covalently integrated into host chromosomal DNA by the HIV-1 integrase (IN) to form the provirus (see below Figure 2, from Van Maele *et al*., 2006). Having established itself as part of the host chromosomes, the provirus is replicated and transmitted to daughter cells, and transcribed into RNA by cellular RNA polymerase II to serve as mRNA and spliced mRNAs that are translated to yield viral proteins, or genomic viral RNA that is encapsidated into progeny virus particles.

Both the RT and IN proteins are encoded by the *pol* gene of the virus, expressed as part of a large Gag-Pol polyprotein, and cleaved by the viral protease into their biologically active forms, a heterodimeric form of RT consisting of p66 and p66-derived p51 subunits and a homodimeric form of IN consisting of two 32-kDa IN subunits. The catalytically active p66 subunit (560 amino acids) of RT comprises the DNA polymerase (residues 1-315), connector (residues 316-437), and ribonuclease H (RNase H; residues 438-560) domains (Jacobo-Molina *et al*., 1993; Kohlstaedt *et al*., 1992). The polymerase domain can be further divided into the fingers, palm, and thumb subdomains. The p51 RT subunit (440 amino acids) is created by a post-translational modification where the C-terminus of the protein (p66/p66 homodimer) is cleaved by the HIV-1 protease, thus lacking the RNaseH domain (Rodgers *et al*., 1995; Schultz *et al*., 1991; Shafter *et al*., 1999). HIV-1 IN protein (288 amino acids) consists of 3 distinct functional domains, an N-terminal domain of 50 amino acids, a central core domain of 160 amino acids, and a C-terminal domain of 80 amino acids. The N-terminal domain contains a putative zinc-binding motif (H12, H16, C40, and C43) and is believed to be involved in multimerization of IN (Pommier *et al*., 2005; Van Maele and Debyser, 2005). The central core domain contains an absolutely conserved DDE catalytic motif (D64, D116, and E152) that forms an active site, coordinating a divalent metal ion, either Mg^{++} or Mn^{++} for the IN catalytic activity (Grobler *et al*., 2002; Pommier *et al*., 2005; Van Maele and Debyser, 2005). The less conserved C-terminal domain appears to play a role in binding to viral and host DNA (Pommier *et al*., 2005; Van Maele and Debyser, 2005). Nuclear localization signals (NLS), which facilitate the entry of preintegration complexes (PIC) into the nucleus, have been mapped to the C-terminal IN domain of several retroviruses.

HIV-1 cDNA synthesis is a relatively complex process that requires at least three elements

(reviewed by Matamoros *et al*., 2011): (i) the viral genomic RNA (that serves as template); (ii) a specific primer (i.e., tRNA; in HIV-1, $tRNA^{Lys3}$); and (iii) the viral RT. RT possesses two distinct enzymatic activities: (i) a DNA polymerase activity that uses either RNA or DNA as template, and (ii) an RNase H activity, which degrades RNA from RNA/DNA hybrids. As summarized in Figure 1 (from Telesnitsky and Goff, 1997), viral cDNA synthesis is initiated from a cellular tRNA base-paired to the viral RNA at the primer binding site (PBS) near the 5' end of the genome. As the (-)strand DNA is synthesized, the genomic RNA is digested by RNase H, which allows DNA synthesis to be transferred to the 3' end of the RNA by annealing to the terminally redundant R sequence. After strand transfer, (-)strand DNA synthesis can continue with simultaneous degradation of the viral RNA. The purine-rich polypurine tract (PPT) that is resistant to RNase H cleavage serves as the primer for (+)strand DNA synthesis. The second strand transfer reaction involves the annealing of the (+)strand DNA to the 3'-end of the full length (-)strand DNA. This strand transfer reaction is usually intra-molecular (Yu *et al*., 1998), and renders a circular intermediate. The strand displacement activity of the RT is required to complete DNA synthesis and for the generation of a proviral DNA with duplicated long terminal repeats (LTR) at both ends. FTC and TDF in the E/C/F/T regimen inhibit this RT-catalyzed viral DNA synthesis during either first (-) or second (+) strand synthesis by being incorporated into the newly synthesized DNA strand and thus resulting in chain termination.

Figure 1: Reverse Transcription of the Viral Genome

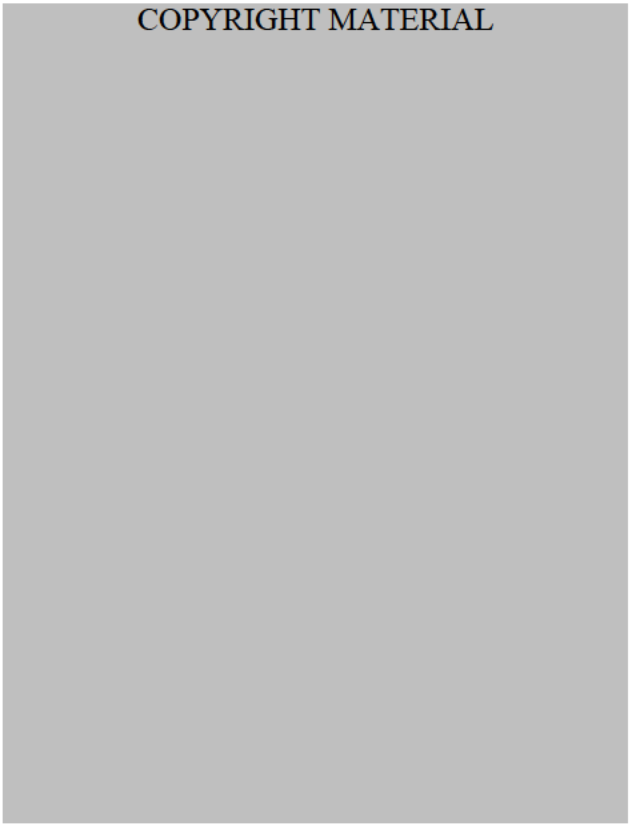


HIV DNA integration is mediated by a complicated series of reactions that includes two catalytic reactions (Figure 2, from Van Maele *et al*., 2006): 3' endonucleolytic processing of the viral DNA ends and strand transfer or joining of the viral and cellular DNAs (Asante-Appiah and Skalka, 1999; Esposito and Craigie, 1999; reviewed by Nair, 2002 and Van Maele *et al*., 2006). These 2 catalytic reactions are mediated by the HIV-1-encoded integrase (IN). Although purified recombinant HIV-1 IN sufficiently carries out 3' endonucleolytic processing and strand transfer reactions in a biochemical reaction, several studies indicated that a variety of viral (such as RT) and cellular proteins, including a lens-epithelium-derived growth factor (LEDGF; also known as p75) that tethers HIV-1 integrase to the chromosomes, may play a role in efficiently establishing the integrated provirus in the HIV-1-infected cell (reviewed by Van Maele *et al*., 2006).

In the first catalytic step, HIV-1 IN recognizes short sequences within the LTR (approximately 20 bp; Sherman and Fyfe, 1990) and removes a terminal GT dinucleotide from the 3'-OH end of

each LTR to produce CA-OH-3 that are thus recessed by two nucleotides (Figure 2, 3' processing). This 3'-endonucleolytic processing takes place in the cytoplasm within the pre-integration complex (PIC) where linear viral DNA and several viral proteins including MA (matrix protein; Bukrinsky *et al*., 1993) and NC (nucleocapsid protein; Lapadat-Tapolsky *et al*., 1993), IN (Farnet and Haseltine, 1990), and RT (Lee and Coffin, 1991) are found.

Figure 2: Outline of the Integration Reaction *In Vivo*



Following the nuclear import of the PIC, the compact structure of the PIC disintegrates and the linear viral DNA is joined to the cellular DNA. This strand-transfer step involves a nucleophilic attack of the phosphodiester bond of the host cellular DNA by the 3'-OH group of the viral DNA to yield a staggered cleavage (4–6 bp depending upon the retrovirus type) in the cellular DNA, and a subsequent ligation of the processed CA-OH-3' viral DNA ends to the 5'-phosphate ends of the cellular DNA. The applicant identified EVG to specifically inhibit this strand transfer reaction of HIV-1 IN and consequently suppresses HIV-1 replication.

The 3'-ends of the target cellular DNA remain unjoined after the strand transfer. The integration reaction is completed by the removal of the 2 unpaired nucleotides at the 5'-end of the viral DNA and the repair of the single-stranded gaps created by the staggered cleavage of the cellular DNA, resulting in the duplication of host cell sequences immediately flanking the inserted proviral DNA. This repair is likely accomplished by host cellular DNA-repair enzymes (Yoder and Bushman, 2000).

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.

2. Nonclinical Virology

Numerous nonclinical virology study results were submitted in support of the NDA for E/C/F/T tablets. As the EVG and COBI components are new chemical entities, the applicant included full data on these two components in the submission, which are reviewed in this section. Nonclinical virology information on the two FDA-approved NRTIs FTC and TDF can be found, individually and in combination, in the US Prescribing Information for EMTRIVA® (revised in November, 2011), VIREAD® (revised in January, 2012), and TRUVADA® (revised in July, 2011), respectively. See the Clinical Virology reviews of Nara Battula, Ph.D. for detailed descriptions of FTC and TDF. EMTRIVA, VIREAD, and TRUVADA were initially approved for the treatment of HIV-1 infection in combination with other antiretroviral agents in July, 2003 (NDA 21-500), October, 2001 (NDA 21-356), and August, 2004 (NDA 21-752), respectively.

2.1. Mechanism of Action

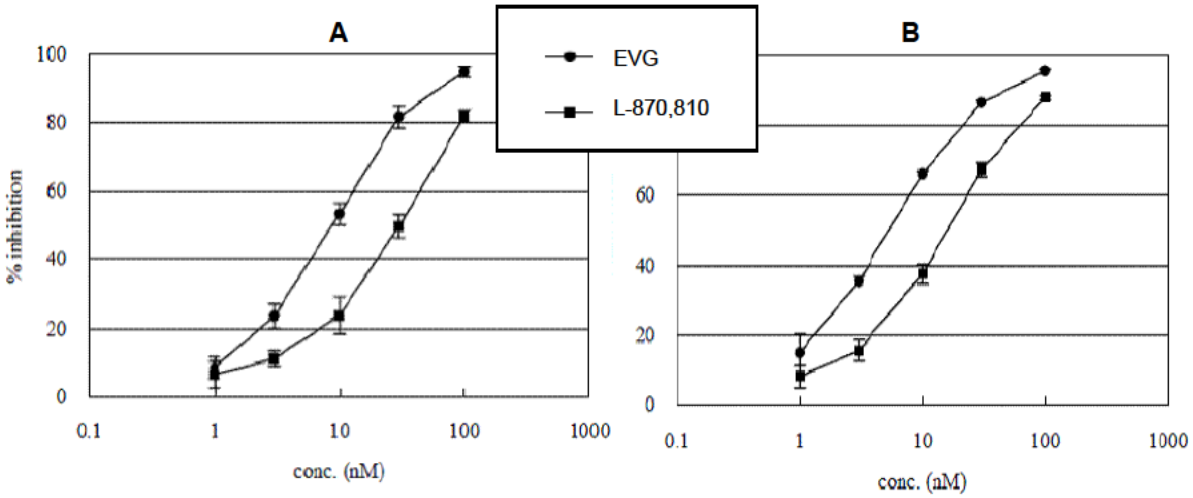
2.1.1. Mechanism of Action of EVG

HIV-1 integrase (IN) catalyzes integration of the unintegrated linear viral DNA, made by reverse transcription of the viral genomic RNA, into the host chromosome. Integration is essential for HIV-1 replication. The integration reaction requires at least 3 steps: (1) assembly of a stable preintegration complex (PIC) at the termini of the viral DNA; (2) 3'-end endonucleolytic processing to remove the terminal dinucleotide from each 3' end of viral DNA; (3) strand transfer in which the viral DNA 3' ends are covalently linked to the cellular DNA (Esposito and Craigie, 1999; Asante-Appiah and Skalka, 1999) and thereby forming the provirus.

The applicant showed that elvitegravir (EVG), discovered by Japan Tobacco Inc., inhibited the HIV-1 IN-catalyzed strand transfer and overall integration in a dose-dependent manner with IC_{50} values of 8.8 ± 0.9 and 5.3 ± 0.2 nM, respectively, in a biochemical reaction (Figure 3; Study Report JTK303-PH-002). In this study, the inhibitory activity of EVG was evaluated in a microtiter plate strand transfer and integration assay using the recombinant HIV-1_{NL4-3} IN protein produced in *Escherichia coli* and purified by column chromatography (Study JTK303-PH-001), as previously described by Hazuda *et al*. (1994) with some modifications. As substrates for the enzymatic reaction, a double-stranded "donor" oligonucleotide representing HIV-1 U5 long terminal repeat (LTR) immobilized onto microtiter plates and a double-strand "target" oligonucleotide labeled with digoxigenin were included to measure the amount of digoxigenin-labeled target DNA ligated to donor DNA. Inhibitory activity of EVG was tested against the DNA strand transfer step of integrase with 3'-end processed donor DNA, and against the overall integration steps (assembly, 3'-processing, and strand transfer) with unprocessed donor DNA. As a reference control, L-870,810, developed by Merck & Co., was included. Previously, L-870,810 was reported to be an HIV-1 integrase strand transfer inhibitor (INSTI) with IC_{50} values of 8-15 nM (Hazuda *et al*., 2004), while it exhibited reduced activity with respect to assembly and 3-end processing (IC_{50} values of 85-250 nM). In this present study, the applicant showed L-

870,810 also inhibited DNA strand transfer and overall integration in a dose-dependent manner but slightly less effectively than EVG with IC_{50} values of 15.9 ± 1.4 nM and 30.2 ± 4.0 nM, respectively (Figure 3).

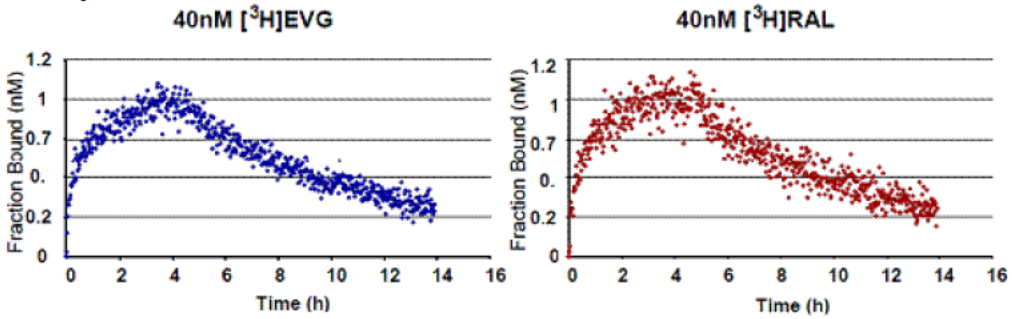
Figure 3: Inhibition of Strand Transfer (A) and Overall Integration (B) of HIV-1 IN by EVG



Error bars indicate standard deviations of mean values of three separate experiments.
Concentrations of compounds are expressed on a logarithmic scale.

The binding and dissociation kinetics of EVG (and RAL, an FDA-approved INSTI) were determined in a binding assay with [^{3}H]-labeled EVG (or [^{3}H]-labeled RAL) and recombinant IN-DNA complexes (Study Report PC-183-2017). When the binding of [^{3}H]EVG or [^{3}H]RAL to the IN-DNA complexes was measured over time, the overall binding and dissociation profiles were similar (Figure 4). Both EVG and RAL bound to the IN-DNA complexes with similar binding affinities with k_{on} values of $1.1x10^{-3}$ and $0.7x10^{-3}$ $\mu M^{-1}s^{-1}$ and dissociated from the complexes with similar efficiencies with k_{off} values of $0.17x10^{-4}$ and $0.18x10^{-4}$ s^{-1}, respectively. Overall, both EVG and RAL exhibited nearly identical half-lives ($t_{½}$) of 11.1 and 11 hours, respectively, when bound to IN-DNA complexes.

Figure 4: Binding and Dissociation of EVG and RAL from HIV-1 Integrase-DNA Complexes



Phase 1 studies of EVG conducted in Japan identified four main circulating metabolites in human plasma, M1 (<1%; hydroxylation of the chlorofluorophenyl group), M4 (3.5%,

glucuronide conjugate of the carboxylic acid), M7 (<1%; glucuronide conjugate of M1), and HM1 (<1%; sulfate conjugate of M1). EVG represents 93% of all EVG-associated products in human plasma of EVG-treated subjects. The microtiter plate strand transfer assay with 3'-end processed donor DNA was utilized to determine whether these EVG metabolites retained the inhibitory activity on HIV-1 IN-catalyzed DNA strand transfer reaction in Study JTK303-PH-013. Compared to the parent drug EVG, all four metabolites inhibited the reaction with 5.2- to 425.7-fold lower potency (Table 1): mean IC_{50} values for M1, M4, M7, and HM1 were 38.8 ± 4.9, 281.2 ± 16.5, 3,150.2 ± 1,364.0, and 2,689.1 ± 567.4 nM, respectively. M1 retained relatively higher inhibitory activity compared to the other three metabolites and the decrease in potency was only 5.2-fold.

Table 1: Inhibition of Strand Transfer of HIV-1 IN by EVG and 4 Main metabolites

	EVG	M1	M4	M7	HM1	L-870,810
IC_{50}[1] value, nM (Potency ratio[2])	7.4 ± 1.2 (1)	38.8 ± 4.9 (5.2)	281.2 ± 16.5 (38)	3,150.2 ± 1,364 (425.7)	2,689.1 ± 567.4 (363.4)	18 ± 1.1

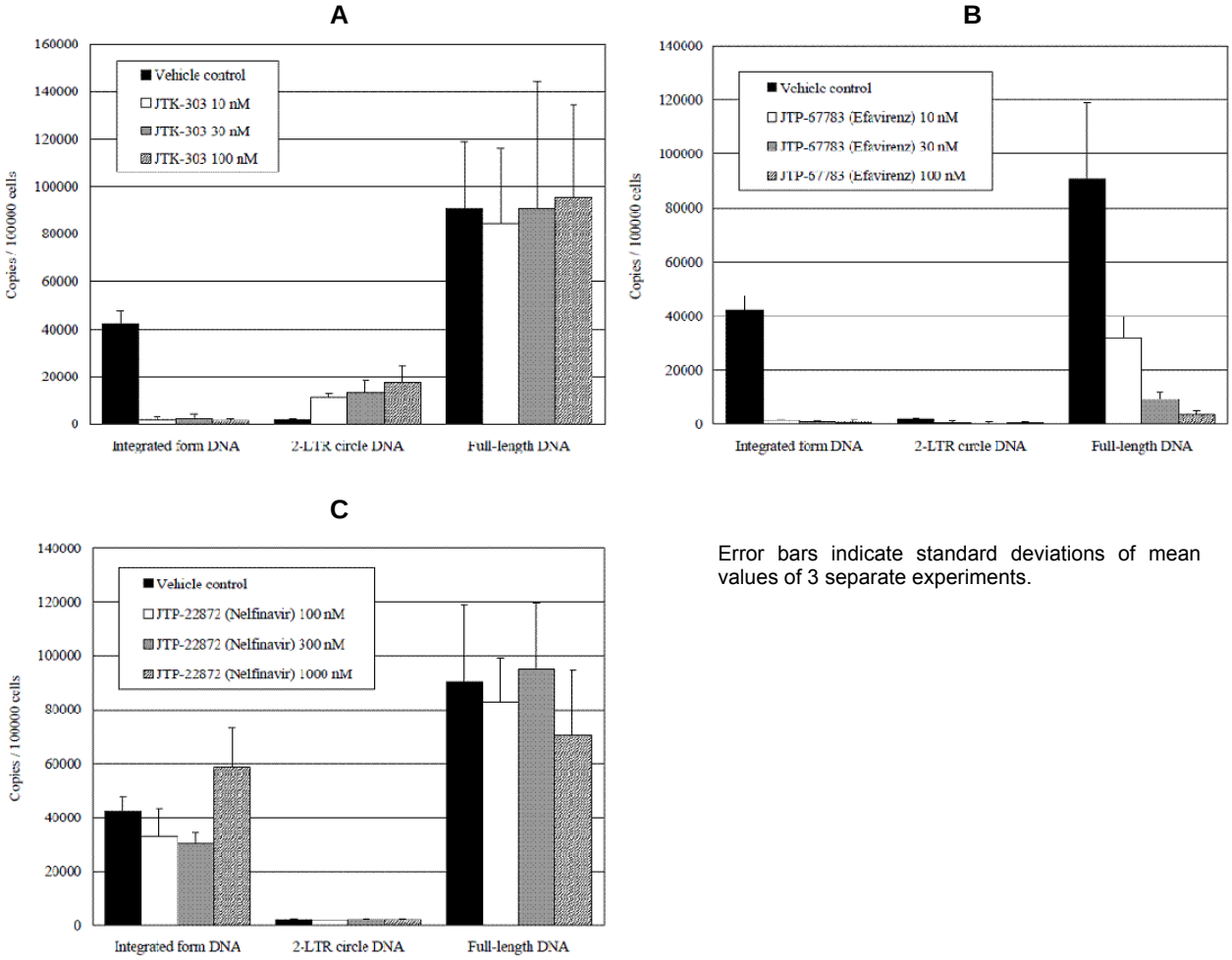
[1] Mean and standard deviations of three independent experiments
[2] Ratio of mean IC_{50} value for EVG metabolite/mean EC_{50} value for EVG

Under standard HIV-1 infection conditions in cells, approximately 90% of the linear viral DNA migrates to the nucleus (Zennou *et al*., 2000). The authors noted that at completion of a single cycle of infection, viral DNA from wild-type virus was processed into provirus (55%), 1-LTR circular viral DNA (35%), and 2-LTR circular viral DNA (<5%), while a small fraction remained as linear unintegrated viral DNA (<10%). Non-integrated circular forms of the viral cDNA are produced primarily by homologous recombination between the two LTRs to yield 1-LTR circles or by the cellular non-homologous DNA end-joining pathway at the cDNA ends to yield 2-LTR circles (Li *et al*., 2001). Circularization is believed to represent an unproductive pathway leading to loss of the viral genome and to increase in abundance when viral IN activity is genetically absent (Wiskerchen and Muesing, 1995) or pharmacologically inhibited (Hazuda *et al*., 2000). The effect of EVG on the production of full-length linear unintegrated viral DNA, proviral DNA, and 2-LTR circles was quantified in HIV-1 infected human T-cell lines, MT-4 or SupT1 (Studies JTK303-PH-005 and PC-186-2006, respectively).

Consistent with the observations in the biochemical studies, EVG inhibited the integration of HIV-1 DNA into the host chromosomal DNA in HIV-1_{NL4-3}-infected MT-4 cells ($CD4^+$ human T-cell line that has been transformed with human HTLV-1; Study Report JTK303-PH-005). MT-4 cells were infected with HIV-1_{NL4-3} for two hours and the virus-infected cells were subsequently cultured in medium containing various concentrations of EVG. Total DNA was extracted 18 hours after infection, and levels of each form of HIV-1 DNA were measured using a real-time PCR assay. EVG treatment showed a 94.6% reduction in proviral DNA at 10 nM (Figure 5), while an accumulation of the 2-LTR circular form that represents unintegrated viral DNA in the nucleus was observed in a dose-dependent manner (5.1-fold at 10 nM compared to that of the vehicle control). It should be noted that there was no effect of EVG on the generation of full-length linear unintegrated viral DNA synthesized by HIV-1 reverse transcriptase (RT), in agreement with the findings of no biologically meaningful inhibitory effect of EVG against HIV-1 RT in a biochemical reaction up to the highest concentration tested (50 μM) in Study JTK303-PH-003 (see below Table 10 in Section 2.2.1). Of note, since HIV-1 linear unintegrated viral DNA can integrate into multiple sites in the human genome, integrated HIV-1 DNAs were quantified using quantitative Alu-PCR with primers recognizing HIV-1 LTR sequence and the

human DNA repetitive element Alu sequence as previously described by Suzuki *et al*. (2003). These results strengthened the findings in biochemical studies that EVG does not inhibit the process of reverse transcription of the HIV-1 RNA genome but specifically inhibits the HIV-1 integration of linear unintegrated viral DNA. In these assays, the applicant included efavirenz (EFV), an NNRTI and nelfinavir (NFV), a PI, as controls, and, as expected, no accumulation of 2-LTR circle DNA was observed in cells treated with either drug (Figure 5, Panels B and C, respectively). EFV significantly suppressed the generation of full-length DNA (64.8% inhibition at 10 nM) because of its strong inhibitory activity against RT and thus integrated viral DNA (96.5% inhibition at 10 nM), while NFV did not significantly influence the quantity of any forms of HIV-1 DNA.

Figure 5: Effects of EVG (A), EFV (B), and NFV (C) on the Generation of Intracellular HIV-1 cDNA Forms in MT-4 Cells Infected with HIV-1_{NL4-3}



Error bars indicate standard deviations of mean values of 3 separate experiments.

Similar findings of EVG being an inhibitor of viral cDNA integration was observed in Study PC-186-2006 using the single-cycle infection assay in SupT1 cells (human lymphocytic leukemia cell line) infected with VSV G glycoprotein-pseudotyped, GFP-expressing reporter viruses (Kafri *et al*., 1999). The applicant noted accumulation of virus-specific late-RT products (to detect completion of reverse transcription of the incoming viral RNA genome) peaked at 12 hours post-

infection and the Alu-PCR signal peaked at 48 hours post-infection. As summarized in Table 2, EVG had no effect on the production of full-length linear unintegrated viral DNA (Late-RT product signal at 12 hours post-infection; EC_{50} value of >1,000 nM) but was able to inhibit the integration of viral DNA with an EC_{50} value of 0.3 nM (Alu-PCR product signal at 48 hours post-infection). As observed in the biochemical mechanism-of-action assay (Figure 3), L-870,810, an investigational INSTI, also inhibited the integration process with an EC_{50} value of 1.3 nM, slightly less potent than EVG (Table 2). As expected, EFV (NNRTI) decreased both the Late-RT and the Alu-PCR products, consistent with inhibition at the reverse transcription step, and APV (amprenavir; PI) which acts after the integration step, had no effect on either the Late-RT or the Alu-PCR signals.

Table 2: Effects of EVG on the Accumulation of Late-RT and Alu-PCR Products in a Single-Cycle Infection Assay

HIV-1 DNA	EC_{50} (nM)[a]			
	EVG	L-870,810	EFV	APV
Late-RT Signal 12 h Post Infection	> 1000	> 1000	5.0	> 1000
Alu-PCR Signal 48 h Post Infection	0.3	1.3	0.5	> 1000

[a] Mean from 2 separate experiments

In the HIV-1 IN-catalyzed DNA strand transfer reaction, cleavage of the host DNA is followed by covalent joining of processed 3'-OH ends of viral DNA to the 5'-phosphoryl ends of the host DNA in a one-step transesterification process (Engelman *et al.*, 1991). Cellular DNA topoisomerases I and II that are found in all mammalian cells and involved in DNA replication, recombination, and transcription display some analogous activities in DNA binding, DNA cleavage, and transesterification reactions (reviewed by Champoux, 2001). In Study JTK303-PH-004, the effect of EVG was investigated on human recombinant DNA topoisomerases I and II (TopoGen) activities in a DNA relaxation assay (Spitzner *et al.*, 1990; Trask *et al.*, 1984). As summarized in Table 3, no inhibitory activities (<10% inhibition) of EVG were observed against the human DNA topoisomerases I and II at concentrations up to 50 µM and 150 µM, respectively. In contrast, the known inhibitors of human DNA topoisomerases I and II, camptothecin and amsacrine, respectively, inhibited the topoisomerase activity in a dose-dependent manner with IC_{50} values of 9.8 ± 2.2 µM and 105.1 ± 8.0 µM.

Table 3: Effects of EVG on the Activity of Human DNA Topoisomerases I and II

Human DNA Topoisomerase	IC_{50}[1] value (µM)		
	EVG	**Camptothecin**	**Amsacrine**
Topoisomerase I	>50 (-3.2 ± 2.1%)[2]	9.8 ± 2.2	-
Topoisomerase II	>150 (2.4 ± 2.5%)[3]	-	105.1 ± 8.0

[1] Mean and standard deviation of three independent experiments
[2] % inhibition at EVG 50 µM
[3] % inhibition at EVG 150 µM

According to the applicant, EVG showed no significant off-target inhibition or stimulation effects on the specific ligand binding to the various receptors (n=22), and on the activities of various

enzymes (n=7) and cell-based assay systems (n=3) including the immune cell functions of cell adhesion (ICAM-1/VCAM-1 mediated), IL-2 secretion, and mixed lymphocyte reaction (splenic lymphocytes; JTK303-PH-008). IC_{50} values were not determined because EVG did not show greater than 50% inhibition at 10 μM in any of these systems.

2.1.2. Mechanism of Action of COBI

COBI (GS-9350) is a specific inhibitor of CYP3A, the body's major drug-metabolizing enzyme, for use as a PK enhancer to increase the systemic levels of coadministered agents metabolized by this enzyme system.

COBI and RTV were tested in biochemical enzymatic assay for its inhibitory effect on recombinant HIV-1 protease. COBI is completely inactive in this enzymatic assay, up to a concentration of 30 μM (Table 4; Study Report PC-216-2001, page 7). In contrast, the IC_{50} value for RTV was 0.6 nM when tested in parallel under the same assay conditions. These enzyme inhibition data indicate that the structural changes introduced into COBI molecule eliminate its ability to productively bind to the active site of HIV-1 protease and inhibit its activity.

Table 4: Activity of COBI against HIV-1 Protease

Compound	$IC_{50} \pm SD$ (μM)[a]
GS-9350	> 30
Ritonavir	0.0006 ± 0.0001

GS-9350=COBI; SD, standard deviation
[a] Data represent mean ± SD values from at least 3 independent experiments.

RTV has been shown to inhibit certain host proteases in addition to HIV-1 protease (Andre *et al.*, 1998). The sponsor evaluated the activity of RTV against selected human enzymes including cathepsin D, renin and beta-site APP-cleaving enzyme 1 (BACE-1). RTV did not show any inhibition of renin and BACE-1 at concentrations up to 30 μM (data not shown), but inhibited cathepsin D activity with an IC_{50} value of 870 nM (Table 5; Study Report PC-216-2001, page 7). Therefore, COBI was tested against cathepsin D. COBI did not exhibit any activity towards this enzyme, showing an IC_{50} value of >30 μM.

Table 5: Activity of COBI against Cathepsin D

Compound	$IC_{50} \pm SD$ (μM)[a]
GS-9350	> 30
Ritonavir	0.87 ± 0.37

GS-9350=COBI; SD, standard deviation
[a] Data represent mean ± SD values from at least 3 independent experiments.

RTV has been shown to reduce proteasome-mediated degradation of several proteins (Tran *et al*., 2003). RTV inhibited the proteasome with an IC_{50} value of 7.9 μM (Table 6; Study Report PC-216-2001, page 8). In comparison, COBI and atazanavir showed somewhat less inhibition of proteasome activity with mean IC_{50} values of 12.8 and 17.7 μM, respectively.

Table 6: Activity of COBI against Proteasome

Compound	$IC_{50} \pm SD$ (μM)[a]
GS-9350	12.8 ± 3.7
Ritonavir	7.9 ± 1.0
Atazanavir	17.7 ± 3.0

GS-9350=COBI; SD, standard deviation
[a] Data represent mean ± SD values from at least 3 independent experiments.

2.2. Antiviral Activity in Cell Culture

2.2.1. EVG against Wild-Type Laboratory and Clinical Isolates of HIV (HIV-1 and HIV-2)

EVG was evaluated for antiviral activities against representative laboratory strains of T-tropic (IIIB) and M-tropic (ADA. BaL, and JR-CSF) HIV-1 (Table 7; Microbiology review I072177.000 and Study Report JTK303-PH-010). Assays for the T-tropic and the M-tropic viruses were performed in PHA/IL-2-activated, monocyte-depleted human PBMCs and in a human monocyte/macrophage culture, respectively, at 7 days post-infection. Cell-free virus in the cell-culture supernatant was quantified using the HIV-1 p24 ELISA kit (Beckman Coulter). Cytotoxicity was measured with the soluble tetrazolium-based dye MTS (Promega). EVG demonstrated anti-HIV-1 activity against these four laboratory strains of T-tropic and M-tropic HIV-1 with EC_{50} values in the sub-nM range (0.07 to 0.67 nM). The selectivity indices (SI; CC_{50} value/EC_{50} value) for EVG were >746. Compared to the other tested FDA-approved antiretrovirals (AZT, EFV, and NFV), EVG appeared to have greater or equivalent potency.

Table 7: Anti-HIV-1 Activity of EVG against T- and M-tropic Laboratory Strains of HIV-1 in PBMCs and Monocytes/Macrophages, Respectively

HIV-1 strain (tropism)	EVG			AZT			EFV			NFV		
	EC_{50}, nM	CC_{50}, μM	SI[1]	EC_{50}, nM	CC_{50}, μM	SI[1]	EC_{50}, nM	CC_{50}, μM	SI[1]	EC_{50}, nM	CC_{50}, μM	SI[1]
IIIB[2] (T)	0.2	9.7	48,500	-	-	-	0.2	24.4	122,000	2.6	10.9	4192
ADA[3] (M)	0.07	>0.5[4]	>7,143	0.18	>1[4]	>5,556	3.58	>10[4]	>2,793	45.2	>10[4]	>221
BaL[3] (M)	0.67	>0.5[4]	>746	0.2	>1[4]	>5,000	2.12	>10[4]	>4,717	36.3	>10[4]	>275
JR-CSF[3] (M)	0.31	>0.1[4]	>323	3.82	>1[4]	>262	-	-	-	-	-	-

"-", not determined.
[1] SI, selective index (CC_{50}/EC_{50})
[2] Mean values from three experiments performed in triplicate
[3] Value determined from a single experiment performed in triplicate
[4] CC_{50} values were higher than the maximum concentrations of these compounds in each assay.

In Studies PC-186-2002 and JTK303-PH-016, two T-tropic laboratory strains of HIV-1, NL4-3 and IIIB, were evaluated to determine the antiviral activity of EVG in MT-2 and/or MT-4 cells (both $CD4^+$, $CXCR4^+$, $CCR5^-$ human T-cell lines that has been transformed with human HTLV-1). Using the 5-day cytopathic assay, EVG showed an EC_{50} value of 0.38 nM against HIV-1_{NL4-3} in MT-4 cells (Table 8). The selectivity index (SI) for EVG was >14,000. Against HIV-1_{IIIB}, EVG showed EC_{50} values of 0.17 nM in MT-4 cells and 0.6 nM in MT-2 cells, and SI values of >23,000 and >13,000, respectively. The reference inhibitor L-870,810 inhibited tested viruses with 3- to 24-fold higher EC_{50} values.

Table 8: Anti-HIV-1 Activity of EVG against T-tropic Laboratory Strains of HIV-1, NL4-3 and IIIB, in MT-2 and MT-4 cells

HIV-1 Strain	Cell Line	EVG			L-870,810		
		EC_{50} (nM)[a]	CC_{50} (nM)	SI[b]	EC_{50} (nM)	CC_{50} (nM)	SI
NL4-3	MT-4	0.38[c]	5623[c]	14,797	—	—	—
III_B	MT-4	0.17 ± 0.03	4000 ± 2000	23,529	4 ± 3	1600 ± 400	400
III_B	MT-2	0.6 ± 0.1	8000 ± 1000	13,333	2 ± 1	3000 ± 2000	1500
Mean	—	0.38 ± 0.22	5900 ± 2000	15,500	3 ± 1.3	2300 ± 990	750

[a] Mean and standard deviation of three independent experiments unless otherwise indicated.
[b] Selectivity index = CC_{50} value/EC_{50} value
[c] Value determined from a single experiment

The applicant also evaluated in Study JTK-PH-010 EVG activity against clinical isolates of HIV-1 (n=12) and HIV-2 (n=1) in human PHA/IL-2-activated PBMCs at 7 days post-infection using the HIV-1 p24 ELISA kit (Beckman Coulter) for HIV-1 and the RT activity assay for HIV-2 (Buckheit *et al.*, 1991). All HIV-1 and HIV-2 virus isolates were obtained from the NIH AIDS Research and Reference Reagent Program. As summarized in Table 9, EVG was shown to be active against all B and non-B subtype HIV-1 clinical isolates tested in this study with mean EC_{50} values ranging from 0.1 to 1.26 nM. A similar antiviral potency (EC_{50} value of 0.53 nM) was also observed against a single HIV-2 isolate. Compared with AZT, EFV, and NFV, EVG was highly active against the range of viruses used in this study.

Table 9: Anti-HIV-1 Activity of EVG against Clinical Isolates of HIV-1 and HIV-2 in PBMCs

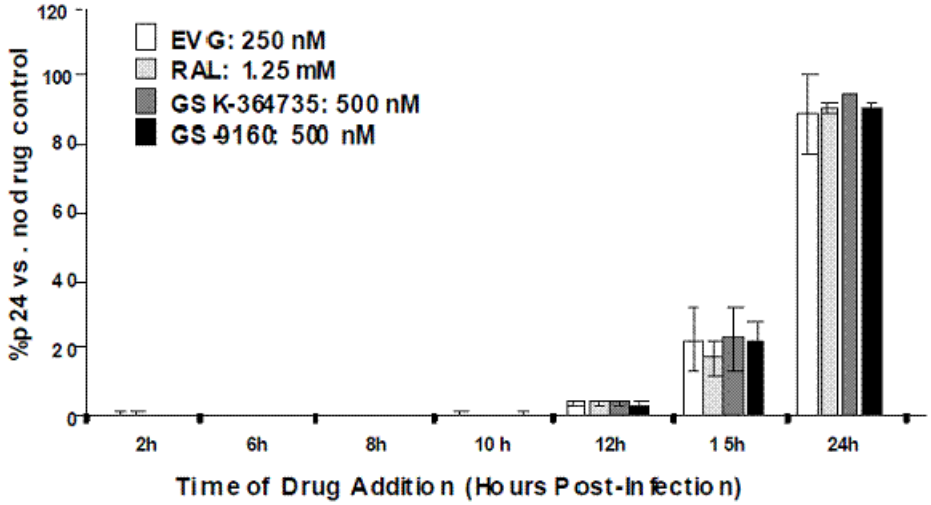
Type/Group/Subtype			Isolate	Co-receptor tropism	Mean EC_{50}[1], nM			
					EVG	AZT	EFV	NFV
HIV-1	Group M	A	RW/92/016	R5	0.41	7.91	0.61	13.4
		B	96USHIPS7	R5	0.26	8.41	0.65	25.8
			BR/92/014	R5/X4 (SI)	0.53	0.60	30.9	32.5
			BR/92/021	R5 (NSI)	0.76	2.13	47.0	70.5
			BR/93/017	R5	0.18	1.10	0.58	2.05
			BR/93/022	R5	1.13	11.7	0.62	20.1
		C	BR/92/025	R5 (NSI)	0.10	2.84	0.30	<0.10
		D	UG/92/046	X4 (SI)	0.50	7.26	1.19	27.7
		E	CMU02	X4 (SI)	1.26	9.07	1.82	22.7
		F	BR/93/020	R5/X4 (SI)	0.74	25.3	0.32	16.4
		G	JV1083	R5 (NSI)	0.35	11.1	0.65	14.0
	Group O		BCF01	R5 (NSI)	1.17	1.52	0.69	0.99
HIV-2			CDC310319	-	0.53	1.14	>1,000	16.1

"-", not determined; NSI, non-syncytium-inducing; R5, CCR5, SI, syncytium-inducing; X4, CXCR4
[1] Mean values from a single experiment performed in triplicate

In Study PC-183-2027, the applicant showed that (1) EVG acts in the first 12 hours of the viral lifecycle and at the time of viral integration (Figure 6) and (2) EVG does not persist intracellularly

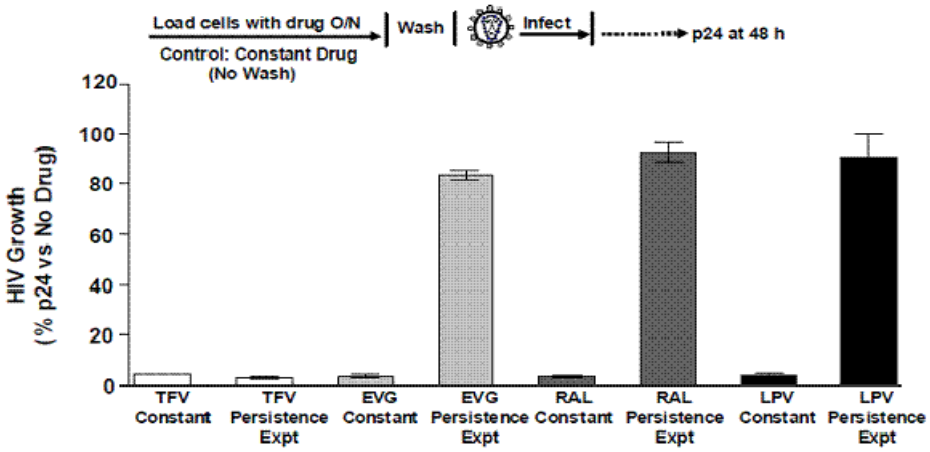
(Figure 7). In Figure 6, the applicant established that EVG inhibits viral replication during the first 10-12 hours after viral infection, coinciding with the time of viral integration in the infected cells. In a time-of-addition assay, MT-2 cells were infected with HIV-1_{IIIB} (MOI=0.1) for 2 hours and then the virus was removed. Infected cells were then exposed to EVG (250 nM, 500 times the EC_{50} value) at different time points post-infection and continuously thereafter until the 48-hour time point. Virus-containing supernatant was harvested 48 hours post-infection and virus replication was measured by quantifying HIV-1 p24 antigen (HIV-1 p24 ELISA assay kit, PerkinElmer). Complete inhibition of viral replication was observed when EVG was added within 10 hours after viral infection (Figure 6), while EVG addition at 12, 15, or 24 hours post-infection resulted in progressively less inhibition of viral replication. EVG addition at 24 hours post-infection showed little effect on HIV-1 replication, indicating that viral integration was completed by this time. Similar observations were made with other INSTIs including RAL and two investigational compounds GSK-364735 (Garvey *et al*., 2008) and GS-9160 (Jones *et al*., 2009) at concentrations above their respective EC_{95} values.

Figure 6: Antiviral Inhibition by EVG by Time of Drug Addition



In Figure 7, the applicant assessed the intracellular antiviral persistence of EVG. MT-2 cells were pre-exposed to EVG (250 nM, 500 times the EC_{50} value) for 15 hours and washed to remove extracellular EVG. Cells were then infected with HIV-1_{IIIB} (MOI=0.3) for 3 hours. Following a subsequent round of washing to remove input virus, cells were cultured for 48 hours in the absence of EVG, and then virus-containing supernatant was harvested for p24 quantification. Cells continuously exposed to EVG (no wash control) exhibited the maximal inhibition of viral replication (approximately 95% inhibition), while cells pre-treated with EVG but removed prior to infection showed only weak antiviral activity (<20% inhibition). Similar results were observed with RAL (2 mM, 500 times the EC_{50} value). Thus, the intracellular antiviral activities of tested INSTIs, EVG and RAL, do not appear to be persistent, and the protective effects of EVG and RAL are dependent on its presence in the extracellular medium at the time of viral integration. In contrast, TFV (tenofovir, NRTI; 350 mM, 100 times the EC_{50} value) retained antiviral potency after cell washing, which is consistent with the persistence of the TFV diphosphate active metabolite within cells (median half-life of 150 hours, ranging from 60 to >175 hours; Hawkins *et al*., 2005).

Figure 7: Inhibition of Viral Infection by Intracellular and Extracellular EVG at the Time of Infection



As described in Section 2.1.1., the inhibitory activity of EVG against HIV-1 IN-catalyzed strand transfer was retained with decreased potency (5.2- and 38-fold, respectively) in two EVG metabolites, M1 and M4, compared to the parent drug EVG in the biochemical mechanism-of-action study (Table 1). Both M1 and M4 showed detectable anti-HIV-1 activity in HIV-1_{HXB2}-infected MT-2 cells with EC_{50} values of 12.5-17.5 nM (10.4- to 12.5-fold reduction in potency compared to the parent drug EVG) and of 10.5-12.3 nM (8.8-fold reduction), respectively (see below Tables 38 and 42 in Section 2.6.1). The applicant reasoned the increased potency of M4 observed in the cell-based assay compared to that in the biochemical assay may be due to EVG contaminating M4, or generated chemically or enzymatically by hydrolysis.

The applicant showed the anti-HIV-1 activity of EVG was not due to inhibition of two other HIV-1-encoded enzymes that are essential for viral replication, RT and protease. No biologically meaningful inhibitory effects of EVG against HIV-1 RT (<5% inhibition) and protease (<30% inhibition) were observed in biochemical reactions at concentrations up to 50 μM (Table 10; Studies JTK303-PH-003 and -008, respectively). In contrast, the positive control compounds 5'-triphosphate of ZDV (ZDV-TP or AZT-TP; NRTI) and pepstatin A (aspartic protease inhibitor) inhibited HIV-1 RT and protease activities with IC_{50} values of 0.01 μM and 1.2 μM, respectively. Of note, EVG inhibited the HIV-1 IN-catalyzed strand transfer with an IC_{50} value of 8.8 nM and HIV-1 replication with EC_{50} values of 0.1 to 1.3 nM in the biochemical and cell-based assays, respectively.

Table 10: Effects of EVG on the Activity of HIV-1 RT and Protease

Compound	HIV-1 RT	HIV-1 Protease
	IC_{50} (μM)[a]	IC_{50} (μM)
EVG	> 50 (3.9 ± 1.4%)[b]	> 50 (29%)[b]
ZDV-TP	0.0104 ± 0.0013	—
Pepstatin A	—	1.2

[a] Mean and standard deviation of three independent experiments
[b] % inhibition at EVG 50 μM

2.2.2. EVG against Other Viruses

Since HIV-1-positive patients are frequently co-infected with hepatitis B virus (HBV) or hepatitis C virus (HCV), the antiviral activity of EVG against HBV and HCV was evaluated in Study PC-183-2024. Of note, HBV, a hepadnavirus, and HCV, a flavivirus, do not form proviruses.

The anti-HBV activity of EVG was tested in AD38 cells, a clone of HepG2 cells (human hepatoma cell line) stably expressing wild-type HBV (genotype D subtype ayw) under the control of a tetracycline-responsive promoter (Ladner *et al*., 1997). Cells were treated with EVG or lamivudine (LAM; as a positive control) at various concentrations for 4 days, and then the intracellular HBV DNA was extracted and quantified with a TaqMan PCR assay (Roche Molecular Systems). The cytotoxicity of EVG and LAM were determined using an XTT/PMA assay (Sigma). EVG was found to have no anti-HBV activity at concentrations up to 6.25 μM with no detectable cytotoxicity (Table 11). As expected, LAM showed anti-HBV activity with an EC_{50} value of 0.43 ± 0.28 μM and had a CC_{50} value >100 μM.

Table 11: Anti-HBV Activity and Cytotoxicity of EVG in AD38 Cells

	EC_{50} ± SD (μM)[a]	CC_{50} ± SD (μM)[a]
EVG	> 6.25	> 6.25
Lamivudine	0.43 ± 0.28	> 100

[a] Mean and standard deviation of at least two independent experiments

The anti-HCV activity of EVG was tested in Huh-Luc replicon cells, derived from Huh-7 cells (human hepatocellular carcinoma cell lines) containing a genotype 1b subgenomic replicon encoding a luciferase reporter gene for 3 days and luciferase expression was quantified using a luciferase assay kit (Promega). Cytotoxicity in the Huh-Luc cells was determined using the CellTiter-Glo Luminescent Cell Viability assay (Promega). EVG had no anti-HCV activity against HCV genotype 1b replicon with an EC_{50} value of 22.9 μM. Of note, the observed EC_{50} value was above the CC_{50} value for EVG in this cell line (16.3 μM) and thus inhibition by EVG is likely attributable to cytotoxicity rather than EVG-associated antiviral activity. In contrast, the positive control compound, BILN-2061, an investigational HCV NS3 protease inhibitor (Lamarre *et al*., 2003), showed anti-HCV activity with an EC_{50} value of 0.3 nM, and had a CC_{50} value 43.1 μM.

Table 12: Anti-HCV Activity and Cytotoxicity of EVG in Huh-7 Cells

	EC_{50} ± SD (μM)[a]	CC_{50} ± SD (μM)[a]
EVG	22.9	16.3 ± 7.07
BILN-2061	0.0003	43.1[b]

[a] Mean and standard deviation of at least two independent experiments unless otherwise indicated
[b] Data was obtained from a single experiment.

2.2.3. COBI against HIV (HIV-1 and HIV-2)

The cell culture antiviral activity of COBI was evaluated in a standard 5-day $HIV\text{-}1_{IIIB}$ cytopathic assay in MT-2 cells. HIV-1 replication was not inhibited by COBI at concentrations of up to 30 μM relative to untreated control (Table 13; Study Report PC-216-2002, page 7). According to

the sponsor, higher concentrations of COBI could not be tested because of compound solubility limitations. In contrast, RTV displayed antiviral activity as previously reported, with an EC_{50} value of 13.3 nM.

Table 13: Antiviral Activity of COBI against HIV-1_{IIIB} in the Presence of HS, HSA, and AGP

Compounds	EC50 ± SD (μM)[a]		
	No Serum Proteins	40% HS	HSA + AGP
GS-9350	> 30	> 90	> 90
Ritonavir	0.0133 ± 0.0047	0.06 ± 0.01	0.400 ± 0.0

GS-9350=COBI
[a] Data shown represent the mean and standard deviation from at least two independent experiments.

The antiviral activity of COBI was tested in PBMCs against a panel of 17 HIV-1 and 2 HIV-2 primary isolates. Different groups of HIV-1 (M, N, O), as well as the most prevalent group M (subtypes A, B, C, D, E, F, and G), were represented among the tested isolates. After infection, the cells were incubated with or without drugs for 7 days. The endpoints were the RT activity assay or the p24 antigen ELISA assay. The cells were stained with MTS to evaluate cytotoxicity. COBI exhibited essentially no antiviral activity against any isolates tested in this assay system (Table 14; Study Report 12148.11, pages 6-9). Mild cytotoxicity was observed with this compound resulting in 81.2% (CC_{50} value of 12.9 μM; experimental #1) and 41.6% (CC_{50} value >30 μM; experimental #2) reduction in cell viability at the high test concentration of 30 μM. RTV was used as a control and exhibited the expected level of antiviral activity across all tested isolates. The median EC_{50} value of RTV against the 17 tested HIV-1 isolates was 0.043 μM (range of 0.005 to 0.406 μM; mean 0.068 μM). The CC_{50} value of RTV was >10 μM.

Table 14: Activity of COBI against HIV-1 and HIV-2 Isolates in Human PBMCs

Virus	Test or Reference Article	Endpoint	IC_{50} (nM)	IC_{90} (nM)	TC_{50} (nM)	Therapeutic Index
HIV-1 92UG029 Subtype A	GS-9350	RT	20,498	>30,000	12,901	<1.00
		p24	17,526	28,978		<1.00
	AZT	RT	4.90	38.4	>1,000	>204
		p24	5.69	29.0		>176
	RTV	RT	34.3	84.5	>10,000	>292
		p24	42.4	84.2		>236
HIV-1 92UG037 Subtype A	GS-9350	RT	11,593	25,008	12,901	1.11
		p24	14,595	>30,000		<1.00
	AZT	RT	4.48	12.8	>1,000	>223
		p24	9.45	668		>106
	RTV	RT	17.7	69.3	>10,000	>565
		p24	81.1	5,076		>123
HIV-1 93BR021 Subtype B	GS-9350	RT	12,154	25,148	12,901	1.06
		p24	15,745	27,601		<1.00
	AZT	RT	3.41	13.2	>1,000	>293
		p24	5.26	41.4		>190
	RTV	RT	26.3	76.9	>10,000	>380
		p24	40.6	83.5		>246

Virus	Test or Reference Article	Endpoint	IC_{50} (nM)	IC_{90} (nM)	TC_{50} (nM)	Therapeutic Index
HIV-1 JR-CSF Subtype B	GS-9350	RT	16,552	26,795	12,901	<1.00
		p24	15,344	27,109		<1.00
	AZT	RT	0.65	1.52	>1,000	>1,536
		p24	0.84	17.8		>1,193
	RTV	RT	14.1	28.4	>10,000	>709
		p24	15.9	28.2		>629
HIV-1 92BR025 Subtype C	GS-9350	RT	13,808	26,664	12,901	<1.00
		p24	12,074	25,101		1.07
	AZT	RT	6.86	25.5	>1,000	>146
		p24	6.59	25.7		>152
	RTV	RT	48.2	111	>10,000	>207
		p24	52.5	120		>190
HIV-1 93IN101 Subtype C	GS-9350	RT	17,766	27,476	12,901	<1.00
		p24	22,514	>30,000		<1.00
	AZT	RT	2.03	9.89	>1,000	>491
		p24	2.26	8.96		>443
	RTV	RT	38.0	91.2	>10,000	>263
		p24	45.8	98.8		>218
HIV-1 92UG001 Subtype D	GS-9350	RT	13,315	25,525	12,901	<1.00
		p24	26,906	>30,000		<1.00
	AZT	RT	3.96	20.4	>1,000	>253
		p24	1.60	>1,000		>623
	RTV	RT	27.1	205	>10,000	>369
		p24	23.9	203		>419
HIV-1 92UG024 Subtype D	GS-9350	RT	7,236	25,612	12,901	1.78
		p24	11,812	>30,000		1.09
	AZT	RT	0.79	14.2	>1,000	>1,263
		p24	0.61	8.87		>1,650
	RTV	RT	17.1	30.9	>10,000	>584
		p24	16.2	30.4		>616
HIV-1 93TH073 Subtype E	GS-9350	RT	16,206	29,636	12,901	<1.00
		p24	18,314	27,180		<1.00
	AZT	RT	2.87	>1,000	>1,000	>348
		p24	2.27	828		>440
	RTV	RT	8.97	>10,000	>10,000	>1,114
		p24	5.54	9.31		>1,804
HIV-1 CMU08 Subtype E	GS-9350	RT	21,134	28,755	12,901	<1.00
		p24	24,028	>30,000		<1.00
	AZT	RT	2.93	8.46	>1,000	>341
		p24	4.36	>1,000		>230
	RTV	RT	22.2	57.6	>10,000	>450
		p24	19.7	35.7		>507

Virus	Test or Reference Article	Endpoint	IC_{50} (nM)	IC_{90} (nM)	TC_{50} (nM)	Therapeutic Index
HIV-1 93BR019 Subtype F	GS-9350	RT	5,307	19,307	>30,000	>5.65
		p24	4,439	19,163		>6.76
	AZT	RT	3.88	52.1	>1,000	>257
		p24	5.97	44.0		>168
	RTV	RT	34.1	151	>10,000	>294
		p24	45.7	180		>219
HIV-1 93BR019 Subtype F **Repeat Experiment**	GS-9350	RT	17,228	28,124	>30,000	>1.74
		p24	14,975	28,067		>2.00
	AZT	RT	8.69	29.1	>1,000	>115
		p24	9.28	38.6		>108
	RTV	RT	49.4	129	>5,000	>101
		p24	73.9	150		>67.7
HIV-1 93BR020 Subtype F	GS-9350	RT	19,835	>30,000	12,901	<1.00
		p24	20,705	>30,000		<1.00
	AZT	RT	4.93	16.8	>1,000	>203
		p24	5.63	9.92		>178
	RTV	RT	48.0	88.1	>10,000	>208
		p24	43.2	84.6		>231
HIV-1 G3 Subtype G	GS-9350	RT	12,250	25,577	12,901	1.05
		p24	12,523	27,782		1.03
	AZT	RT	3.53	21.1	>1,000	>284
		p24	8.27	134		>121
	RTV	RT	28.1	164	>10,000	>356
		p24	24.7	1,677		>405
HIV-1 JV1083 Subtype G	GS-9350	RT	10,670	26,411	>30,000	>2.81
		p24	9,252	24,136		>3.24
	AZT	RT	40.9	556	>1,000	>24.5
		p24	23.9	380		>41.8
	RTV	RT	50.3	99.6	>10,000	>199
		p24	53.9	203		>186
HIV-1 JV1083 Subtype G **Repeat Experiment**	GS-9350	RT	16,366	28,608	>30,000	>1.83
		p24	15,971	>30,000		>1.88
	AZT	RT	22.6	105	>1,000	>44.2
		p24	33.5	422		>29.8
	RTV	RT	47.0	127	>5,000	>106
		p24	73.5	153		>68.1
HIV-1 BCF02 Group O*	GS-9350	RT	23,693	>30,000	12,901	<1.00
		p24	NA	NA		NA
	AZT	RT	7.84	141	>1,000	>128
		p24	NA	NA		NA
	RTV	RT	406	2,585	>10,000	>24.6
		p24	NA	NA		NA

Virus	Test or Reference Article	Endpoint	IC_{50} (nM)	IC_{90} (nM)	TC_{50} (nM)	Therapeutic Index
HIV-1 BCF03 Group O*	GS-9350	RT	14,217	27,457	12,901	<1.00
		p24	NA	NA		NA
	AZT	RT	3.99	11.3	>1,000	>251
		p24	NA	NA		NA
	RTV	RT	136	277	>10,000	>73.4
		p24	NA	NA		NA
HIV-1 YBF30 Group N	GS-9350	RT	11,742	25,205	12,901	1.10
		p24	12,380	25,415		1.04
	AZT	RT	2.36	22.4	>1,000	>423
		p24	3.48	25.5		>287
	RTV	RT	47.6	124	>10,000	>210
		p24	57.3	219		>174
CDC 310319 HIV-2*	GS-9350	RT	6,582	16,190	>30,000	>4.56
		p24	NA	NA		NA
	AZT	RT	40.2	590	>1,000	>24.9
		p24	NA	NA		NA
	RTV	RT	227	711	>10,000	>44.0
		p24	NA	NA		NA
CDC 310319 HIV-2* **Repeat Experiment**	GS-9350	RT	6,471	17,744	>30,000	>4.64
		p24	NA	NA		NA
	AZT	RT	5.17	65.8	>1,000	>193
		p24	NA	NA		NA
	RTV	RT	108	387	>5,000	>46.4
		p24	NA	NA		NA
CDC 310342 HIV-2*	GS-9350	RT	11,921	25,037	12,901	1.08
		p24	NA	NA		NA
	AZT	RT	0.43	21.7	>1,000	>2,329
		p24	NA	NA		NA
	RTV	RT	58.6	92.9	>10,000	>171
		p24	NA	NA		NA

GS-9350=COBI
*Activity determined by virion-associated RT assay

The sponsor identified three major metabolites of COBI in human hepatocytes. These metabolites designated E1 (GS-342006), E3 (GS-364751), and E5 (GS-341842) were tested for their antiviral activity in MT-2 cells infected with HIV-1. Like COBI, the three identified metabolites did not show any antiretroviral activity at concentrations of up to 30 μM (Table 15; Study Report PC-216-2002, page 8).

Table 15: Anti-HIV-1 Activity of COBI Metabolites

Compounds	EC50 (μM)[a]
GS-341842	> 30
GS-342006	> 30
GS-364751	> 30

[a]Data shown represent the mean and standard deviation from at least two independent experiments.

2.2.4. COBI against Other Viruses

The anti-HBV activity of COBI was tested in WT-42 cells, a clone of HepG2 cells stably transfected with the wild-type HBV (genotype A, serotype adw2). The cells were incubated with or without drug for 3 days. The endpoint was measuring HBV DNA by real-time PCR. In contrast to tenofovir, a compound with demonstrated anti-HBV activity, COBI had no antiviral activity against HBV or cytotoxicity at the tested concentrations up to 12.5 μM (Table 16; Study Report PC-216-2006, page 7).

Table 16: Anti-HBV Activity of COBI in WT-42 Cells

Compounds	$CC_{50} \pm SD$ (μM)[a]	$EC_{50} \pm SD$ (μM)[a]
GS-9350	> 12.5	> 12.5
Tenofovir	> 50	0.63 ± 0.02

GS-9350=COBI
[a] Data shown represent the mean and standard deviation from at least two independent experiments.

The anti-HCV activity of COBI was tested in Huh-Luc cells, a clone of Huh-7, which stably replicates a subgenomic HCV genotype 1b replicon encoding a luciferase reporter gene. No anti-HCV activity or cytotoxicity was detected after a 3-day treatment with COBI at concentrations of up to 30 μM (Table 17; Study Report PC-216-2006, page 7).

Table 17: Anti-HCV Activity of COBI in Huh-Luc Replicon Cells

Compounds	$CC_{50} \pm SD$ (μM)[a]	$EC_{50} \pm SD$ (μM)[a]
GS-9350	> 30	> 30
BILN-2061	> 30	0.0006 ± 0.0001

GS-9350=COBI
[a] Data shown represent the mean and standard deviation from at least two independent experiments.

2.3. Effect of Human Serum Protein on Antiviral Activity in Cell Culture

2.3.1 Effect on EVG Anti-HIV-1 Activity

Using [^{14}C]-labeled EVG, the applicant determined the degree of protein binding of the drug by equilibrium dialysis in Study JTK303-AD-014. [^{14}C]EVG was added to final concentrations of 0.1, 1, and 10 μg/mL to the plasma of humans, dogs, monkeys, and rats. [^{14}C]EVG was highly bound to plasma proteins in those tested species with unbound fractions in plasma in the range of 0.07 to 1.2% (Table 18). Thus, binding of EVG to plasma was high, and species- and EVG concentration-independent. In addition, EVG was 99.4% bound to human serum albumin (5% HSA), similar to that with human plasma, and 39.1-40.7% to human alpha-1-acid glycoprotein (0.07% AAG) at their physiological concentrations. These results demonstrated EVG is highly bound to human plasma proteins with preferential binding to albumin over AAG. These biochemical findings were confirmed in the *in vivo* Phase 1 PK studies of EVG (GS-US-183-0133 and GS-US-216-0124). The mean (SD) % free fraction (plasma unbound concentration) for EVG ranged from 1.15 (0.14) to 1.16 (0.16) in healthy subjects who were treated daily with EVG 150 mg and COBI 150 mg. Thus, the effect of human serum (HS) was evaluated on the

anti-HIV-1 activity of EVG with HS in HIV-1-infected PBMCs and T cells (see below Tables 19 - 21).

Table 18: Rate of the Plasma Protein Binding of [^{14}C]EVG in Humans, Dogs, Monkeys, and Rats

[^{14}C]EVG Concentration, µg/mL	Protein binding rate[1] (%)					
	Human			Dog	Monkey	Rat
	plasma	5% HSA	0.07% AGG	plasma	plasma	plasma
0.1	99.35 ± 0.05	99.40 ± 0.02	39.25 ± 1.04	99.23 ± 0.17	98.83 ± 0.11	99.89 ± 0.01
1	99.34 ± 0.07	99.39 ± 0.01	39.05 ± 0.93	99.22 ± 0.15	98.81 ± 0.09	99.93 ± 0.01
10	99.31 ± 0.04	99.38 ± 0.01	40.68 ± 1.99	99.19 ± 0.16	98.80 ± 0.09	99.93 ± 0.00

[1] Mean and standard deviation of three independent experiments

In Study JTK303-PH-006, the anti-HIV-1 activity of EVG was shown to be moderately reduced by the addition of 50% HS to the human PBMC culture medium (already supplemented with 15% fetal bovine serum [FSB]), resulting in a 7.5-fold increase in the EC_{50} value (from 0.2 nM to 1.5 nM; Table 19). This experiment was carried out in human PHA/IL-2-activated PBMCs infected with HIV-1_{IIIB} and the antiviral activity was measured at 7 days post-infection using a HIV-1 p24 ELISA (RETRO-TEK HIV-1 p24 Antigen ELISA kit, ZeptoMetrix). Cytotoxicity of EVG was also decreased in the presence of HS: the CC_{50} values were 9.7 µM in 0% HS and 170.1 µM in 50% HS. The EC_{90} value of EVG was estimated to be 9.8 nM in the presence of 50% HS, similar to that of EFV (10.8 nM).

Table 19: Effects of Human Serum on the Anti-HIV-1 Activity and Cytotoxicity of EVG in Human PBMCs Infected with HIV-1_{IIIB}

	EVG			EFV		
	EC_{50} (nM)	EC_{90} (nM)	CC_{50} (µM)	EC_{50} (nM)	EC_{90} (nM)	CC_{50} (µM)
0% HS[a]	0.2 ± 0.1[b]	1.2 ± 0.4	9.7 ± 2.5	0.2 ± 0.1	1.2 ± 0.4	24.4 ± 11.4
50% HS	1.5 ± 0.4 (7.5)[c]	9.8 ± 4.6 (8.2)	170 ± 12	2.2 ± 1.0 (11.0)[c]	10.8 ± 5.2 (9.0)	> 300

[a] Cells were maintained in media complemented with 15% fetal bovine serum (FBS) for all studies, and HS was added to 0% or 50%.
[b] Mean and standard deviation of independent experiments with PBMCs from 5 to 6 donors
[c] Fold-reduction in potency (mean EC_{50} value in the presence of 50% HS/mean EC_{50} value in the absence of HS)

The effect of HS on the activity of EVG was further evaluated separately by its major components, serum albumin (35 mg/mL HSA) and alpha-1-acid glycoprotein (1.5 mg/mL AAG), in human PHA/IL-2-activated, monocyte-depleted PBMCs (Study Report PC-186-2004). Cells were infected with HIV-1_{BaL} that were previously passaged in PBMC and subjected to the 5-day cytopathic assay. Using a HIV-1 p24 ELISA (Beckman Coulter), the applicant observed a significant reduction in EVG potency in the presence of 35 mg/mL HSA (80-fold), while the addition of 1.5 mg/mL AAG resulted in only a 3-fold increase in the EC_{50} value (Table 20). The percentage of protein binding for EVG was calculated from these values as 99.0% and 74.5% for HSA and AAG, respectively. The combined protein binding of EVG was calculated as

99.0%, resulting in a combined protein-adjusted EC_{50} value of 16 nM and an overall 80-fold reduction in the anti-HIV-1 potency of EVG. The combined protein-adjusted EC_{95} value was estimated to be 100 nM (45 ng/mL).

Table 20: Effects of Human Plasma Components on the Anti-HIV-1 Activity of EVG in Human PBMCs Infected with HIV-1_{BaL}

Compound	Control EC_{50} (nM)[a]	HSA EC_{50} (nM)[a]	HSA Protein Binding (%)	AAG EC_{50} (nM)[a]	AAG Protein Binding (%)	Combined Protein Binding (%)	Combined Protein-Adjusted EC_{50} (nM)	Combined Protein-Adjusted EC_{95} (nM)
EVG[b]	0.2 ± 0.1	16 ± 17	99.0	0.6 ± 0.3	74.5	99.0	16 ± 18	100 ± 117
L-870,810[c]	3 ± 2	54 ± 55	93.7	9 ± 10	62.2	94.3	60 ± 76	372 ± 477

[a] The EC_{50} values for Control (10% FBS only), HSA (10% FBS + HSA 35 mg/mL), and AAG (10% FBS + AAG 1.5 mg/mL) were run in parallel.
[b] Mean and standard deviation of independent experiments with PBMCs from at least 4 donors.
[c] Mean and standard deviation of independent experiments with PBMCs from at least 3 donors.

As observed in freshly isolated primary human PBMCs with HIV-1_{BaL} infection, Study JTK303-PH-007 also demonstrated the anti-HIV-1 activity of EVG was negatively influenced by addition of HSA (4%; 14.1-fold reduction) but not by AGG (0.08% and 0.12%; <2.5-fold reduction) in MT-4 cells infected with HIV-1_{IIIB} (Table 21). Similarly, the addition of 4% HSA also decreased the anti-HIV-1 activity of EFV (10.5-fold) but not AAG (1.2- to 1.8-fold increases in EC_{50} values). In contrast, in the presence of either 0.08% or 0.12% AAG, NFV (nelfinavir, PI) showed no anti-HIV-1 activity at concentrations up to 100 nM. The amount of viral particles in culture supernatant was quantified at 5 days post-infection using a HIV-1 p24 ELISA (RETRO-TEK HIV-1 p24 Antigen ELISA kit, ZeptoMetrix).

Table 21: Effects of Human Plasma Components on the Anti-HIV-1 Activity of EVG in HIV-1_{IIIB}-Infected MT-4 Cells

Plasma Component	EC_{50} (nM)[a] (Fold Reduction)[b]		
	EVG	EFV	NFV
MT-4 Cells			
None	1.7 ± 0.2	1.6 ± 0.1	14.9 ± 1.2
+ 40 mg/mL HSA	23.9 ± 3.2 (14.1)	16.8 ± 2.3 (10.5)	55.2 ± 9.2 (3.7)
+ 0.8 mg/mL AAG	3.2 ± 0.3 (1.9)	1.9 ± 0.1 (1.2)	> 100 (> 6.7)
+ 1.2 mg/mL AAG	3.9 ± 0.4 (2.3)	2.9 ± 0.3 (1.8)	> 100 (> 6.7)

[a] Mean and standard deviation of 3 separate experiments. All experiments were performed in the presence of 10% FBS.
[b] Fold-reduction in potency (mean EC_{50} value in the presence of plasma component/mean EC_{50} value in the absence of plasma component)

2.3.2. Effect on COBI Activity

Majority of PIs bind extensively to human serum proteins so the sponsor evaluated the effect of human serum on the cell culture antiviral activity of COBI and RTV in MT-2 cells in the presence of 40% human serum. In addition, cell culture antiviral activity in the presence of physiological

concentrations of human serum albumin (35 mg/mL) combined with alpha 1-acid glycoprotein (1.0 mg/mL), the 2 major human serum components responsible for binding of PIs, were determined.

The EC_{50} values of COBI in the presence of 40% human serum or human serum albumin combined with alpha 1-acid glycoprotein were both >90 μM (see above Table 13; Study Report PC-216-2002, page 7). In the presence of human serum or human serum albumin + alpha 1-acid glycoprotein, RTV retained significant ARV activity (EC_{50} values of 60 and 400 nM, respectively), although reduced compared to the activity without human serum. These observations are consistent with the lack of inhibition of the HIV-1 protease enzymatic activity by COBI, and indicate that, even at concentrations exceeding maximal unbound clinical exposure levels by over 300-fold (0.09 μM free COBI concentration; using a maximal plasma concentration of approximately 1.4 μM [C_{max} 1150 ng/mL]), COBI does not exert any ARV activity.

2.4. Cytotoxicity

2.4.1. Cytotoxicity of EVG

The cytotoxicity of EVG was evaluated in PHA/IL-2-activated human PBMCs (monocyte-depleted T lymphocytes and monocytes/macrophages) and $CD4^+$ T cell lines (MT-2 and MT-4) in parallel with the assays to determine the anti-HIV-1 activity of EVG in a number of studies (see above Tables 7, 8, and 19). Overall, EVG appears to have low cytotoxicity in the absence of human serum in these tested cells with CC_{50} values ranging from >0.1 μM (activated primary monocytes/macrophages) to 9.7 μM (activated primary T lymphocytes). In the presence of 50% human serum EVG became less toxic (17.5-fold) to activated primary T lymphocytes with the CC_{50} value of 170.1 μM.

In Study PC-183-2001, the cytotoxicity of EVG in unstimulated human PBMCs was evaluated in the absence of human serum after 5 days of drug exposure using the CellTiter-Glo Luminescent Cell Viability assay (Promega). The results were compared to that of PHA/IL-2-stimulated PBMCs conducted in parallel. As a control compound, the Merck HIV-1 integrase inhibitor L-870,810 was included in the assay. EVG demonstrated dose-dependent cytotoxicity in both unstimulated and stimulated PBMC cultures with mean CC_{50} values of 16.60 ± 5.61 μM and 10.79 ± 4.61 μM, respectively, and no significant differences in the cytotoxicity between unstimulated and stimulated PBMCs (Table 22). By comparison, under identical culture conditions and using the same set of purified PBMC, CC_{50} values for L-870,810 were 4.36 ± 1.82 μM and 2.86 ± 1.47 μM in unstimulated and stimulated PBMC, respectively.

Table 22: Cytotoxicity of EVG in Unstimulated and Stimulated Human PBMCs

Compound	EVG		L870,810	
PBMCs	Unstimulated	Stimulated with PHA/IL-2	Unstimulated	Stimulated with PHA/IL-2
Mean CC_{50}[1] value, μM	**16.60 ± 5.61**	**10.79 ± 4.61**	4.36 ± 1.82	2.86 ± 1.47

[1]Mean and standard deviation of 5 separate experiments. All experiments were performed in the presence of 15% FBS.

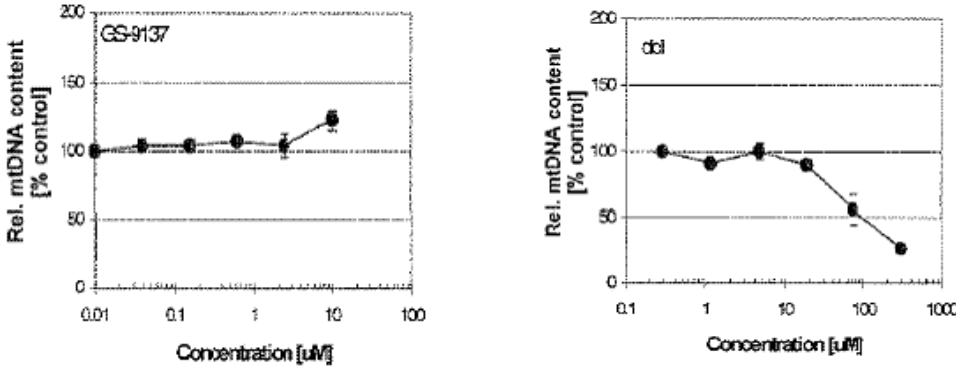
The HIV-1 IN catalytic core is composed of an absolutely conserved DDE active site motif (D64, D116, and E152) and may be structurally related to others members of the superfamily of polynucleotidyl transferases known as DDE recombinases (including transposases, recombinases, and RNases; reviewed by Mizuuchi, 1997). One cellular member of this family is the RAG1/2 recombinase.

The RAG1/2 complex is a lymphoid-specific recombinase that is involved in V(D)J recombination, a specialized DNA rearrangement used by cells of the immune system to assemble immunoglobulin and T cell receptor genes from the preexisting gene segments (reviewed by Gellert, 1997). In the absence of functional RAG1/2 recombinase complex, no T or B cells can be produced, as is noted in patients and mice with severe combined immunodeficiency. Humans with reduced RAG1/2 functioning (Omenn syndrome) have been identified, and are characterized by a paucity of B cells but virtually normal numbers of T cells with a distinctly oligoclonal (limited) repertoire. Clinically, these patients exhibit failure to thrive, erythroderma, hepatosplenomegaly, eosinophilia, and increased susceptibility to infection. Mice with either a spontaneously appearing or genetically engineered mutation decreasing RAG functioning have a very similar spectrum of pathology. Collectively, these data highlight the potential toxicologic consequences of RAG1/2 inhibition, particularly in the case where the immune system is not fully developed. Thus, it was suggested that inhibitors targeting HIV-1 IN may inhibit RAG1/2 and may possibly interfere with its role in immune development. Melek *et al*. (2002) showed that two HIV-1 integrase inhibitor compounds of the diketo acid class, p8 (5CITEP; Goldgur *et al*., 1999) and p10 (L-708,906; Hazuda *et al*., 2000), interfered with the DNA cleavage and disintegration activities of RAG1/2 at a high micromolar range (IC_{50} values of 200 and 20 μM, respectively) in a cell-free system. These results implied that HIV-1 IN strand transfer inhibitors such as EVG and RAL may have the potential to interfere with T-cell receptor and immunoglobulin gene rearrangement in human. However, the authors were not able to determine whether IN inhibitors are able to interfere with RAG1/2 activity in cells.

According to the applicant, EVG showed no evidence of impairment to the immune system in an immunotoxicity study in rats (Study JTK303-TX-011) and in repeat-dose toxicity studies in mice, rats (including a juvenile toxicity study), and dogs at doses up to 2,000 mg/kg (Studies TX-183-2006, TX-183-2004, JTK303-TX-022, and JTK303-TX-023). In addition, 2-year carcinogenicity studies in mice and rats (Studies TX-183-2011 and TX-183-2012, respectively) also showed no significant decrease in lymphocytes or lymphoid organ changes, no increase in opportunistic infections in treated animals, and no increase in tumors. Based on these data, the applicant concluded the immunotoxic potential for EVG is considered low.

A variety of clinical symptoms observed in patients with HIV-1 infection who have been treated with prolonged NRTI therapy appear to be linked to mitochondrial toxicity (reviewed by Kohler and Lewis, 2007). These clinical adverse events have been attributed to inhibition of mitochondrial function due to nucleoside analog incorporation into mitochondrial DNA (mtDNA) and reduction in mtDNA content. EVG is not a nucleoside analog, and does not share chemical structure with DNA polymerase γ natural substrates, and therefore is not expected to have this activity. Study TX-183-2009 showed no measurable changes in the content of mtDNA at EVG 10 μM (highest concentration tested) as determined by a dot-blot hybridization following a 14-day exposure of the drug in HepG2 liver cells (Figure 8). The positive control ddI (Birkus *et al*., 2002) showed an expected depletion of mtDNA in HepG2 cells.

Figure 8: Effect of EVG on the Relative Levels of mtDNA in HepG2 Cells



GS-9137=EVG
Error bars indicate standard deviations of mean values of 3 separate experiments.

2.4.2. Cytotoxicity of COBI

The cell culture cytotoxicity of COBI and RTV was evaluated in MT-2 lymphoblastoid cells, following a 5-day incubation. The CC_{50} value, using the XTT assay as endpoint, was 88.6 μM for COBI (Table 23; Study Report PC-216-2003, page 7).

Table 23: Cytotoxicity of COBI in MT-2 Cells

Compounds	CC50 ± SD (μM)[a]
GS-9350	88.6 ± 13.2
Ritonavir	37.6 ± 18.5

GS-9350=COBI
[a] Data shown represent the mean and standard deviation from three independent experiments.

The cell culture cytotoxicity of COBI and RTV was evaluated in HepG2 hepatoblastoma cells, following a 3-day incubation. The CC_{50} value, using the CellTiter Glo cell viability kit (Promega) as endpoints, was 44 μM for COBI (Table 24; Study Report PC-216-2003, page 7).

Table 24: Cytotoxicity of COBI in HepG2 Cells

Compounds	CC50 ± SD (μM)[a]
GS-9350	44 ± 7
Ritonavir	64 ± 25

GS-9350=COBI
[a] Data shown represent the mean and standard deviation from three independent experiments.

2.5. Anti-HIV-1 Activity in Drug Combination

2.5.1. Combination Activity of EVG, COBI, FTC, and TDF

In Study PC-236-2004, the combination anti-HIV-1 effects of the three antiretroviral components of the EVG/COBI/FTC/TDF fixed-dose combination tablet were evaluated using the 5-day

cytopathic assay in MT-2 cells acutely infected with HIV-1_{xxLAI}, a chimeric laboratory strain containing an HIV-1_{HXB2} RT sequence spanning amino acids 14 to 491 cloned into a RT-defective HIV-1 proviral vector (xxLAI-np; Shi and Mellors, 1997). This drug combination study was conducted in two different experimental set-ups, in the absence and presence of 25 μM COBI (pharmacoenhancer that lacks antiviral activity). EC_{50} values of each drug (EVG, FTC, and TFV), individually and in combinations (with or without COBI), were determined and the presence of drug-drug interaction was analyzed using the combination index (CI) method of the CalcuSyn software (Biosoft). The results were expressed as the mean CI value from CI values at 50%, 75%, and 90% inhibition from at least three separate experiments. As summarized in Table 25, the three-drug combination of EVG+FTC+TFV displayed antiviral synergy with CI scores of 0.45 ± 0.10 and 0.45 ± 0.06 in the absence and presence of COBI, respectively. Moreover, the applicant observed no evidence of antagonism and of cytotoxicity in the absence of HIV-1 infection in these tested drug combinations. As a control for synergy, the three-drug combination of EFV+FTC+TFV was evaluated in parallel and yielded the expected synergy (CI value of 0.56 ± 0.08; Feng *et al*., 2009), while the control combination for additivity of FTC+FTC+FTC showed additive antiviral activity (CI = 0.92 ± 0.06). The control combination for antagonism of AZT+d4T+RBV showed strong antagonism (CI > 5.91 ± 2.62). In another set of triple combination experiments in HIV-1_{IIIB}-infected MT-2 cells, EVG in combination with FTC+TFV demonstrated also synergy with a mean CI value of 0.53 ± 0.01 when evaluated using the 5-day cytopathic assay (Study Report PC-183-2004).

Table25: Combination Anti-HIV-1 Effects of EVG/COBI/FTC/TDF in MT-2 Cells

Drug Combination[a]	CI ± SD[b]	Combination Result
EVG + FTC + TFV	0.45 ± 0.10	Synergy
EVG + COBI + FTC + TFV	0.45 ± 0.06	Synergy
EFV + FTC + TFV	0.56 ± 0.08	Synergy
FTC + FTC + FTC	0.92 ± 0.06	Additive
AZT + d4T + RBV	>5.91 ± 2.62	Strong Antagonism

CalcuSyn provides the following guideline for interpretation of the CI values: <0.1, very strong synergy; 0.1–0.3, strong synergy; 0.3–0.7, synergy; 0.7–0.85, moderate synergy; 0.85–0.9, slight synergy; 0.9–1.1, additive; 1.1–1.2, slight antagonism; 1.2–1.45, moderate antagonism; 1.45–3.3, antagonism; 3.3–10, strong antagonism; >10, very strong antagonism.
[a] AZT, zidovudine; COBI, cobicistat; d4T, stavudine; EFV, efavirenz; EVG, elvitegravir; FTC, emtricitabine; RBV, ribavirin; TFV, tenofovir
[b] Mean CI values and standard deviations (SD) were calculated from 3-7 experiments for all combinations.

2.5.2. Combination Activity Relationships of EVG with Other Antiviral Drugs

The antiviral activity of EVG was tested in pair-wise combinations with FDA-approved HIV-1 inhibitors: an INSTI RAL, a fusion inhibitor T-20, 3 NNRTIs (EFV, ETR, and NVP), 7 NRTIs (ABC, AZT, ddI, d4T, FTC, LAM, and TFV), and 9 PIs (APV, ATV, DRV, IDV, LPV, NFV, RTV, SQV, and TPV) using the 5-day cytopathic assay in MT-2 cells acutely infected with HIV-1_{IIIB} (Study Report PC-183-2006). The combination effects of EVG were analyzed by the Prichard and Shipman method using the MacSynergy™ II software (Prichard and Shipman, 1990; Prichard *et al*., 1993) and expressed as the mean synergy/antagonism volume values (nM^2%) that were calculated at the 95% confidence interval from at least 3 separate experiments performed in triplicate. Combination effects were defined as:

Strong synergy:	>100 nM^2%
Moderate synergy:	>50 to 100 nM^2%
Minor synergy:	>25 to 50 nM^2%
Additive:	25 to and >-25 nM^2%
Minor antagonism:	-25 to >-50 nM^2%
Moderate antagonism:	-50 and >-100 nM^2%
Strong antagonism:	≤-100 nM^2%

No antagonism was observed between EVG and all tested approved antiretroviral drugs. EVG displayed additive to synergistic interactions with mean synergy/antagonism volumes in the range of 9 to 80.3 nM^2% (Tables 26 - 29; Study Report PC-183-2006). EVG combined with itself resulted in an additive interaction (Table 26). As a positive antagonism control, the combination of d4T with RBV was tested in parallel, and, as reported previously (Margot and Miller, 2005), the interaction between RBV and d4T was strongly antagonistic with a mean synergy/antagonism volume of -481 ± 197 nM^2% (Table 26).

In addition, EVG exhibited additive antiviral activity when combined with maraviroc (MVC), a CCR5 co-receptor antagonist, in HIV-1_{BaL}-infected PM-1 cells ($CD4^+$/$CXCR4^+$/$CCR5^+$; clonal derivative of Hut 78, human T lymphoblastic leukemia cell line) and no evidence of antiviral antagonism was observed (Table 30; Study Report PC-183-2017). Together, these two-drug combination study results thus suggest that EVG could potentially be used in a regimen containing any of the tested antiretroviral drugs. Of note, mean synergy/antagonism volumes for EVG combined with either FTC or TFV in Table 28 represent data derived from at least 5 independent experiments including 3 EVG/FTC and EVG/TFV combination experiments conducted in parallel with the other EVG/NRTI combinations in this study. In a separate study (Study PC-183-2004), the remaining two experiments were conducted also using the 5-day cytopathic assay in HIV-1_{IIIB}-infected MT-2 cells, and the combination effect of the two drugs was analyzed by the Prichard and Shipman method (MacSynergy II) and the combination index method (CalcuSyn). The two methods yielded very similar results: the interaction of EVG with FTC and TFV is synergistic.

Table 26: Anti-HIV-1 Effects of EVG in Combination with RAL or T-20 in HIV-1_{IIIB}-Infected MT-2 Cells

	Combination with EVG	Synergy (nM^2%) ± SD[a,b]	Antagonism (nM^2%) ± SD[a,b]	Description
Fusion Inhibitor[c]	T-20	26.3 ±14.5	–2.6 ± 3.3	Minor Synergy
Integrase Inhibitors[c]	RAL	9.0 ± 5.9	–19.7 ± 12.8	Additive
	EVG	14.5 ± 14.2	–2.3 ± 2.9	Additive
Antagonism Control[c]	d4T + RBV	13.2 ± 27.8	–481 ± 197	Strong Antagonism

[a] Synergy/antagonism volumes represent the mean and standard deviations of at least three independent experiments performed in triplicate.
[b] Synergy/antagonism volumes are calculated at the 95% confidence interval.
[c] RAL, raltegravir; RBV, r bavirin; T-20, enfuvirtide; d4T, stavudine

Table 27: Anti-HIV-1 Effects of EVG in Combination with NNRTIs in HIV-1_{IIIB}-Infected MT-2 Cells

	Combination with EVG	Synergy (nM^2%) ± SD[a,b]	Antagonism (nM^2%) ± SD[a,b]	Description
NNRTI[c]	EFV	80.3 ± 46.4	–3.9 ± 5.0	Moderate Synergy
	NVP	63.3 ± 20.0	–22.1 ± 20.5	Moderate Synergy
	Etravirine	35.9 ± 27.0	–16.0 ± 15.2	Minor Synergy

[a] Synergy/antagonism volumes represent the mean and standard deviations of at least three independent experiments performed in triplicate.
[b] Synergy/antagonism volumes are calculated at the 95% confidence interval.
[c] EFV, efavirenz; NVP, nevirapine

Table 28: Anti-HIV-1 Effects of EVG in Combination with NRTIs in HIV-1_{IIIB}-Infected MT-2 Cells

	Combination with EVG	Synergy (nM^2%) ± SD[a,b]	Antagonism (nM^2%) ± SD[a,b]	Description
NRTIc	FTC	80.1 ± 58.6	–7.0 ± 4.5	Moderate Synergy
	ddI	66.0 ± 73.6	–3.5 ± 4.4	Moderate Synergy
	TFV	61.5 ± 49.1	–2.2 ± 3.6	Moderate Synergy
	AZT	59.4 ± 64.5	–3.1 ± 6.1	Moderate Synergy
	3TC	52.7 ± 47.9	–4.6 ± 23.6	Moderate Synergy
	ABC	17.6 ± 14.0	–17.9 ± 12.8	Additive
	d4T	12.1 ± 15.7	–6.5 ± 3.9	Additive

[a] Synergy/antagonism volumes represent the mean and standard deviations of at least three independent experiments performed in triplicate.
[b] Synergy/antagonism volumes are calculated at the 95% confidence interval.
[c] ABC, abacavir; AZT, zidovudine; ddI, didanosine; d4T, stavudine; FTC, emtricitabine; TFV, tenofovir; 3TC, lamivudine

Table 29: Anti-HIV-1 Effects of EVG in Combination with PIs in HIV-1_{IIIB}-Infected MT-2 Cells

	Combination with EVG	Synergy (nM^2%) ± SD[a,b]	Antagonism (nM^2%) ± SD[a,b]	Description
PI[c]	DRV	49.0 ± 8.9	–1.6 ± 2.7	Minor Synergy
	RTV	35.7 ± 43.2	–4.8 ± 7.2	Minor Synergy
	NFV	33.1 ± 7.6	–1.9 ± 5.9	Minor Synergy
	ATV	32.2 ± 6.6	–13.9 ± 14.2	Minor Synergy
	IDV	31.7 ± 9.1	–7.7 ± 5.4	Minor Synergy
	LPV	29.3 ± 30.2	–18.2 ± 14.4	Minor Synergy
	APV	29.3 ± 10.2	–13.3 ± 2.0	Minor Synergy
	TPV	23.2 ± 20.4	–6.5 ± 6.9	Additive
	SQV	15.1 ± 14.3	–11.4 ± 10.3	Additive

[a] Synergy/antagonism volumes represent the mean and standard deviations of at least three independent experiments performed in triplicate.
[b] Synergy/antagonism volumes are calculated at the 95% confidence interval.
[c] APV, amprenavir; ATV, atazanavir; DRV, darunavir; IDV, indinavir; LPV, lopinavir; NFV, nelfinavir; RTV, ritonavir; SQV, saquinavir; TPV, tipranavir

Table 30: Anti-HIV-1 Effects of EVG in Combination with Maraviroc in HIV-1_{BaL}-Infected PM-1 Cells

Drug 1 [a]	Drug 2 [a]	Synergy (nM^2%) ± SD[b]	Antagonism (nM^2%) ± SD[b]	Description
EVG	MVC	7.8 ± 15.1	–2.6 ± 3.3	Additive
d4T [c]	RBV	0.2 ± 0.4	–180 ± 92.6	Strong Antagonism

[a] MVC, maraviroc; RBV, ribavirin; d4T, stavudine
[b] Synergy/antagonism volumes represent the mean and standard deviations of four independent experiments performed in triplicate. Synergy/antagonism volumes are calculated at the 95% confidence interval.
[c] Antagonism control.

There was no evidence of antagonistic interactions against HIV-1 between EVG and HBV antivirals, 3 FDA-approved (ADV, ETV, and L-dT) and the investigational agent clevudine (L-FMAU), in the two-drug combination studies (Table 31; Study Report PC-183-2032). Thus, EVG could potentially be used in a regimen containing any of the tested HBV antiviral drugs for HIV-1 and HBV co-infected patients. The antiviral activity of EVG in combination with HBV antiviral drugs was evaluated using the 5-day cytopathic assay in HIV-1_{IIIB}-infected MT-2 cells, and the combination effect of the two drugs was analyzed by the Prichard and Shipman method with MacSynergy II. Additive interactions were expected for L-dT and L-FMAU in combination with EVG as these compounds have no HIV-1 activity on their own at the concentrations tested. ADV with measurable anti-HIV activity (EC_{50} value of 6 μM; Balzarini *et al*., 1991) displayed minor synergy in combination with EVG. ETV also has been shown to have detectable activity against HIV-1 *in vivo* and can select the M184V substitution in HIV-1 RT (McMahon *et al*., 2007). As expected, minor anti-HIV-1 activity was observed for ETV alone when the drug dilution series was started at a significantly higher concentration of 5 μM, and the overall dilution series resulted in a score of moderate synergy in combination with EVG.

Table 31: Anti-HIV-1 Effects of EVG in Combination with HBV rt Inhibitors in HIV-1_{IIIB}-Infected MT-2 Cells

Combination with EVG	Synergy (nM^2%) ± SD[a,b]	Antagonism (nM^2%) ± SD[a,b]	Description
ADV[c] (8x)	44.1 ± 10.8	-4.13 ± 4.05	Minor Synergy
ETV (8x)	14.8 ± 22.6	-10.0 ± 16.8	Additive
ETV (5 μM)[d]	76.7 ± 56.2	-4.83 ± 8.00	Moderate Synergy
L-dT (8x)	15.2 ± 9.08	-12.3 ± 8.25	Additive
L-FMAU (8x)	7.86 ± 10.9	-21.6 ± 2.51	Additive
L-FMAU (10 μM)[d]	5.59	-2.01	Additive

[a] Synergy/antagonism volumes represent the arithmetic mean and standard deviations of at least three independent experiments performed in triplicate.
[b] Synergy/antagonism volumes are calculated at the 95% confidence interval.

[c] ADV, adefovir; ETV, entecavir; L-dT, telbivudine; L-FMAU, clevudine
[d] ETV and L-FMAU were also tested at high concentrations.

2.5.3. Combination Activity Relationships of COBI with Antiretroviral Drugs

Cell culture antiviral activity of selected HIV-1 drugs was tested in MT-2 cells infected with HIV-1, in the presence or absence of 5 μM COBI. Overall, no significant changes were observed in the activity of any of the tested antiretrovirals in the presence of 5 μM COBI (Table 32; Study Report PC-216-2005, page 6). For all the drugs tested, the change in their EC_{50} values in the presence of COBI ranged from 0.7- to 1.9-fold.

Table 32: Cell Culture Antiviral Activity of HIV-1 Drugs in Presence of COBI

Drug	Class	Fold Change in $EC_{50} \pm SD$[a] with 5 μM GS-9350	$EC_{50} \pm SD$ (nM)[a] without GS-9350
tenofovir	NRTI	1.0 ± 0.5	5467 ± 661
emtricitabine	NRTI	1.9 ± 0.3	903 ± 359
abacavir	NRTI	1.5 ± 1.1	927 ± 79
lamivudine	NRTI	1.0 ± 0.7	5117 ± 1147
zidovudine	NRTI	1.7 ± 0.4	82 ± 52
efavirenz	NNRTI	1.0 ± 0.0	0.5 ± 0.4
nevirapine	NNRTI	1.1 ± 0.3	44 ± 18
raltegravir	INI	1.3 ± 0.3	5.6 ± 2.9
elvitegravir	INI	1.1 ± 0.2	1.4 ± 1.4
atazanavir	PI	0.9 ± 0.8	8.8 ± 3.3
darunavir	PI	0.7 ± 0.6	8.1 ± 5.0

GS-9350=COBI
[a] Data shown represent the mean and standard deviation from three independent experiments.

2.6. Resistance Development in Cell Culture

2.6.1. Development of Resistance to EVG

In order to obtain predictive information about the potential for development of resistance to EVG in antiviral therapy, cell-based resistance selection experiments by serial passage of culture supernatants containing virus or virus-infected cells in the presence of increasing concentrations of EVG.

In Study JTK303-PH-012, HIV-1_{IIIB} variants growing at EVG concentrations from 1 nM to 2560 nM were selected in MT-2 cells and the IN-coding region of the viruses was sequenced at approximately every 10 passages. Results are shown in Table 33. Selection was continued until the concentration of EVG reached approximately its 50% cytotoxic concentration (CC_{50}). An E92Q amino acid substitution in the HIV-1 IN protein emerged in the virus population at passage 30 (EVG 10 nM) followed by H51Y and S147G at passage 60 (640 nM), and these substitutions persisted until the end of the selection (passage 90 at 2,560 nM). An E157Q substitution was also observed in part of the virus population at passage 70 (1,280 nM) and

present as a mixture with wild-type E157 up to passage 90. The resulting final virus from this selection experiment had four IN substitutions H51Y, E92Q, S147G, and E157E/Q: three conserved-site substitutions H51Y, E92Q, and S147G; one polymorphic-site substitution E157Q. These selected substitutions, with the exception of S147G, were detected in the E/C/F/T-treatment virologic failure isolates evaluated in Studies 102, 103, and 104, and E92Q was most frequently found, occurring in 8 of the 24 evaluated subjects (see below Section 3.2.1. for details and Tables 56 and 57).

Table 33: Genotypic Analysis of the Integrase Genes from HIV-1_{IIIB} Variants Selected in the Presence of EVG in Study JTK303-PH-012

HIV-1 IIIB Variants [a]	Amino Acid at Position			
	51	92	147	157
IIIB (#0)	H	E	S	E
IIIB / JTK-303 (#10, 0.5 nM)	H	E	S	E
IIIB / JTK-303 (#22, 2.5 nM)	H	E	S	E
IIIB / JTK-303 (#30, 10 nM)	H	Q/E	S	E
IIIB / JTK-303 (#38, 20 nM)	H	Q	S	E
IIIB / JTK-303 (#50, 160 nM)	H	Q	S	E
IIIB / JTK-303 (#60, 640 nM)	H/Y	Q	G/S	E
IIIB / JTK-303 (#70, 1280 nM)	Y/H	Q	G	E/Q
IIIB / JTK-303 (#80, 1280 nM)	Y	Q	G	Q/E
IIIB / JTK-303 (#90, 2560 nM)	Y	Q	G	E/Q
DNA Sequence	CAT→TAT 151	GAA→CAA 274	AGT→GGT 439	GAA→CAA 469

JTK-303=EVG
[a] HIV-1_{IIIB} variants selected in MT-2 cells in the presence of EVG after the indicated number of passages (10, 22, 30, 38, 50, 60, 70, 80, or 90 passages).

The susceptibilities to EVG of the selected four virus populations at passages 30, 60, 70, and 80 containing IN substitutions E92E/Q, H51H/Y+E92Q+S147S/G, H51H/Y+E92Q+S147G+ E157E/Q, and H51Y+E92Q+S147G+E157E/Q, respectively) were evaluated in MT-2 cells using a HIV-1 p24 antigen ELISA (RETRO-TEK HIV-1 p24 Antigen ELISA kit, ZeptoMetrix) to quantify viral replication and in MT-4 cells using an MTT assay to quantify HIV-mediated cell-killing (Bergamini *et al*., 1992). In MT-2 cells, the susceptibility to EVG decreased by 8.7-, 63.3-, 212-, and 449-fold after 30, 60, 70, and 80 passages, respectively (Table 34) reflecting the concentrations of EVG at each passage step and the accumulation of IN substitutions. Similarly, in MT-4 cells (Table 35), the EC_{50} values also increased from 3.3 nM of wild-type virus to 49.1 nM (14.9-fold), 100.6 nM (30.5-fold), 210.4 nM (63.8-fold), and 319.3 nM (96.8-fold), respectively. These EVG-resistant variants retained susceptibility to AZT (NRTI), EFV (NNRTI), and NFV (PI) with ≤1.1-fold reductions in susceptibility (Table 35), whereas the susceptibility to L-870,810, an investigational HIV-1 INSTI (Hazuda *et al*., 2004), decreased by 5.2-, 7.9-, 11.7-, and 11.1-fold after 30, 60, 70 or 80 passages, respectively. Thus, these results indicated the lack of cross-resistance to the antiretrovirals other than those in the INSTI class. These EVG-selected viruses also exhibited similarly reduced levels of susceptibility (18.8- to >235.4-fod) to one of the main EVG metabolites in human plasma, M1, as observed with EVG (Table 36; Study Report JTK303-PH-014). The differences in potency between M1 and EVG against selected resistance viruses were comparable to that against wild-type HIV-1_{IIIB} (Table 36, "Potency ratio").

Table 34: EVG Susceptibilities of 4 EVG-Selected HIV-1_{IIIB} Populations at Passages 30, 60, 70, and 80 in MT-2 Cells

Test Compound	Test virus	EC_{50} (nM) (Fold-resistance)[a]					CC_{50} (nM)
		Wild-type	IIIB/ (#30)[b]	IIIB/ (#60)[b]	IIIB/ (#70)[b]	IIIB/ (#80)[b]	
GS-9137	Mean ± SD[c]	0.7±0.5	6.1±3.6 (8.7)	44.3 ± 16.7 (63.3)	148.4 ± 17.2 (212.0)	314.3 ± 55.7 (449.0)	5843.8 ± 352.9

GS-9137=EVG
[a] Ratio of mean EC_{50} value against EVG resistant variants/mean EC_{50} value against wild-type virus
[b] Passage number of EVG resistant variants selected
[c] Mean and standard deviation of three separate experiments

Table 35: Resistance and Cross-Resistance Profiles of EVG-Selected HIV-1_{IIIB} Populations at Passages 30, 60, 70, and 80 in MT-4 Cells

Test or Reference Compounds	EC_{50} (nM)[a] (Fold-resistance)					CC_{50} (nM)[a]
	Wild-type IIIB[b]	IIIB/GS-9137 (#30)[c] E92Q	IIIB/GS-9137 (#60)[c] E92Q, S147G	IIIB/GS-9137 (#70)[c] H51Y, E92Q, S147G, E157E/Q	IIIB/GS-9137 (#80)[c] H51Y, E92Q, S147Q, E157Q/E	
GS-9137	3.3 ± 1.4	49.1 ± 12.2 (14.9)	100.6 ± 35.5 (30.5)	210.4 ± 111.8 (63.8)	319.3 ± 129.8 (96.8)	2785.3 ± 563.0
AZT (NRTI)	9.1 ± 3.7	5.0 ± 1.6 (0.5)	4.3 ± 1.2 (0.5)	6.4 ± 3.9 (0.7)	4.3 ± 1.3 (0.5)	1386.5 ± 184.0
EFV (NNRTI)	1.5 ± 0.2	1.6 ± 0.2 (1.1)	1.2 ± 0.2 (0.8)	1.2 ± 0.2 (0.8)	0.9 ± 0.4 (0.6)	9813.5 ± 1587.8
NFV (PI)	34.9 ± 12.7	22.3 ± 5.6 (0.6)	21.1 ± 9.7 (0.6)	28.9 ± 8.1 (0.8)	21.1 ± 5.3 (0.6)	8287.1 ± 763.4
L-870,810 (INSTI)	**8.7 ± 3.0**	**45.2 ± 22.0 (5.2)**	**68.4 ± 40.1 (7.9)**	**101.9 ± 59.0 (11.7)**	**97.0 ± 31.0 (11.1)**	**856.8 ± 41.5**

GS-9137=EVG
[a] Mean and standard deviation of three separate experiments; Fold-resistance is the ratio of mean EC_{50} value against EVG resistant variants/mean EC_{50} value against wild-type virus.
[b] HIV-1_{IIIB}
[c] Passage number of EVG resistant virus variant

Table 36: Susceptibilities of EVG-Selected Four HIV-1_{IIIB} Populations at Passages 30, 60, 70, and 80 to M1 Metabolite in MT-4 Cells

Test or Reference Articles	EC_{50}[a] value, nM (Fold-reduction in suscept bility[b])					CC_{50}[a] (nM)
	HIV-1_{IIIB} (Wild-type)	EVG-resistant virus population at passage				
		#30	#60	#70	I#80	
EVG	7.0 ± 2.4	150.2 ± 17.5 (21.5)	561.7 ± 78.9 (80.2)	>3,000 (>428.6)	>3,000 (428.6)	2811.1 ± 853.4
M1	**42.5 ± 8.2**	**797.3 ± 272.0 (18.8)**	**>2,744.7 (>64.6)**	**>10,000 (>235.3)**	**>10,000 (>235.3)**	10,034.3 ± 1,672.7
Potency ratio[c]	6.1	5.3	>4.9	-	-	-

EFV	4.1 ± 1.7	4.4 ± 1.1 (1.1)	4.5 ± 1.0 (1.1)	4.7 ± 0.9 (1.1)	5.1 ± 0.3 (1.2)	13,877.3 ± 953.0

[a] Mean and standard deviation (SD) of three separate experiments
[b] Ratio of EC_{50} value against EVG-resistant variants/EC_{50} value against wild-type virus
[c] Ratio of mean EC_{50} value for M1/mean EC_{50} value for EVG

The contribution to EVG phenotypic resistance of each of the four IN substitutions observed in the EVG-selected virus populations described above was determined using site-directed mutagenesis in MT-4 cells (Study JTK303-PH-016). Seven infectious molecular clones of HIV-1_{NL4-3} with IN substitutions were generated: 4 clones with a single IN substitution, H51Y, E92Q, S147G, or E157Q; and 3 clones with combinations of the substitutions as observed in the selected virus populations, E92Q+S147G, H51Y+E92Q+S147G, and H51Y+E92Q+S147G+ E157Q. As summarized in Table 37, three single substitutions H51Y, S147G, and E157Q did not confer significantly reduced susceptibility to EVG (all <3-fold changes). Of note, the assay variability normally observed in EC_{50} values was typically 3- to 5-fold in the HIV-1 cytoprotection assay. In contrast, the E92Q single IN substitution conferred 79.2-fold reduced susceptibility to EVG. The level of resistance to EVG increased to 358-, 405-, and 437-fold with sequential addition of S147G, H51Y, and E157Q to the E92Q substitution. E92Q was the first IN substitution to emerge during the EVG selection (Table 33). Thus, E92Q appears to be the primary substitution in this EVG resistance pathway. The H51Y, S147G, and E157Q substitutions also appear to contribute to EVG resistance but possibly not as a primary cause, since those substitutions individually conferred only 1.5-, 2.1-, and 0.6-fold reduced susceptibility to EVG, respectively, but enhanced EVG resistance to up to 437-fold when added to E92Q as double, triple, and quadruple substitutions (Table 37). No cross-resistance was observed for the control HIV-1 inhibitors, AZT, EFV, and lopinavir (LPV) with <2-fold reductions in drug susceptibility.

Table 37: Antiviral Activity of EVG against HIV-1_{NL4-3} Variants Harboring EVG-Selected IN Substitutions in MT-4 Cells

HIV-1_{NL4-3} with IN substitution	Drug Susceptibility (Fold-reduction[1])			
	EVG	AZT	EFV	LPV
Wild-type	**1**	1	1	1
H51Y	**1.53**	1.32	1.01	1.81
E92Q	**79.2**	1.70	0.84	1.58
S147G	**2.08**	1.30	0.82	1.36
E157Q	**0.55**	1.09	0.40	1.04
E92Q+S147G	**358**	1.48	0.99	0.88
H51Y+E92Q+S147G	**405**	1.33	0.75	0.77
H51Y+E92Q+S147G+E157Q	**437**	0.54	0.29	0.28

[1] Fold-reduction in drug susceptibility of mutant viruses compared to wild-type HIV-1. Mean EC_{50} values for wild-type HIV-1_{NL4-3} calculated from 2 independent experiments performed in triplicate are: 0.38 nM for EVG, 10.3 nM for AZT, 2.58 nM for EFV, and 21.3 nM for LPV.

All 4 HIV-1 variants harboring the E92Q substitution listed in Table 37 also had significant reductions in susceptibility against active metabolites of EVG, M1 and M4 (Table 38; Study Report PC-183-2002): 28.7- to >286-fold and 13.9- to 90-fold reduced susceptibilities to M1 and M4, respectively. This study was carried out in MT-2 cells with 7 infectious molecular clones of HIV-1_{HXB2} harboring EVG-selected IN substitutions generated by site-directed mutagenesis. Cross-resistance with other INSTIs (FDA-approved RAL, and investigational compounds GS-9160, GS-9224, GS-335309, GSK-364735, and L-870,810) was observed with all E92Q-containing viruses, though to a lesser degree than with EVG (ranging from 3- to 34.3-fold reductions in susceptibility). In contrast, these E92Q-containing mutant viruses remained fully susceptible (0.6- to 1.3-fold reductions in susceptibility; Table 38) to the NRTIs AZT, FTC, and tenofovir (TFV), the NNRTI EFV, and the PI LPV. Thus, these results indicate significant phenotypic cross-resistance between EVG and other members of the INSTI class. However, there appears to be no cross-class resistance between EVG and other classes of antiretrovirals.

Table 38: Drug Susceptibility to EVG Metabolites and INSTIs of HIV-1_{HXB2} Variants Harboring EVG-Selected IN Substitutions in MT-2 Cells

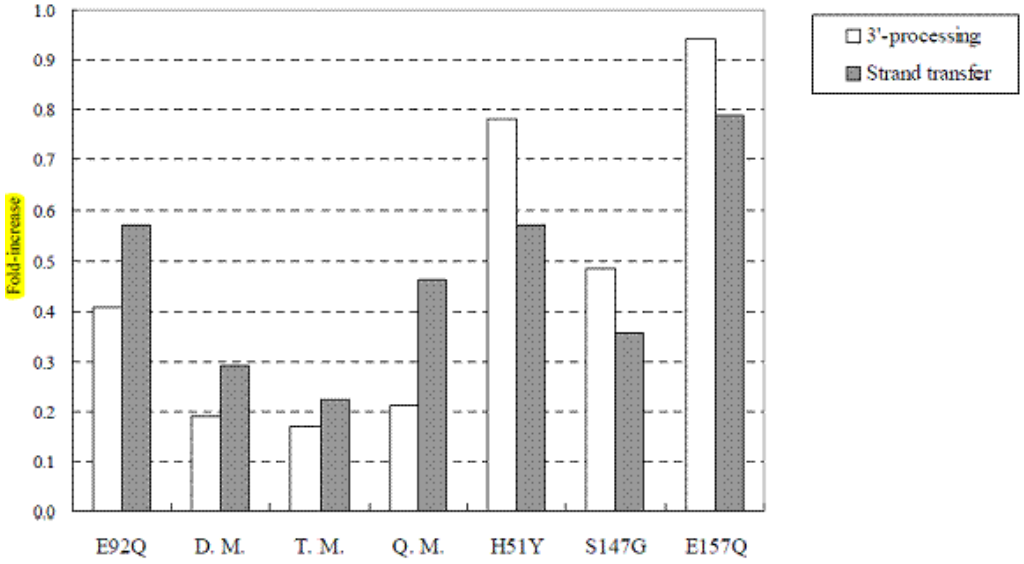
Fold Change in EC_{50}	E92Q	E92Q S147G	E92Q S147G H51Y	E92Q S147G H51Y E157Q	S147G	H51Y	E157Q
EVG [a]	32.7	69.6	143	154	7.59	3.60	2.33
M1 [a]	28.7	59	>286	>286	n/a	n/a	n/a
M4 [a]	13.9	45.0	72.3	90.0	n/a	n/a	n/a
RAL [b]	5.26	8.44	7.00	6.24	1.01	0.83	1.64
GSK-364735 [b]	2.99	7.12	7.19	6.99	n/a	n/a	n/a
L-870,810 [b]	14.9	28.4	22.4	34.3	2.29	0.64	1.76
GS-9224 [b]	9.58	19.9	13.2	20.4	n/a	n/a	n/a
GS-9160 [b]	12.3	32.3	21.0	25.2	n/a	n/a	n/a
GS-335309 [b]	9.96	20.7	14.0	19.9	n/a	n/a	n/a
TFV [c]	0.94	0.68	0.78	0.97	0.84	1.04	1.01
FTC [c]	1.03	1.05	0.91	0.95	n/a	n/a	n/a
AZT [c]	0.94	0.60	0.69	0.75	n/a	n/a	n/a
EFV [c]	1.02	1.18	1.14	1.31	0.99	0.75	0.98
LPV [c]	1.00	0.76	0.88	0.88	0.75	1.16	0.98

[a,b,c] Fold-changes are calculated using the mean EC_{50} values from ≥3 experiments. Mean EC_{50} values against wild-type HIV-1_{HXB2} were 1.4 nM for EVG, 17.5 nM for M1, 12.3 nM for M4, 5.4 nM for RAL, 2.8 nM for GSK-364735, 0.6 nM for L870,810, 3.7 nM for GS-9224, 2.3 nM for GS-9160, 4.1 nM for GS-335309, 3.4 μM for TFV, 0.6 μM for FTC, 130 nM for AZT, 1.3 nM for EFV, and 12.3 nM for LPV.

Effects of these IN substitutions on enzymatic activities of IN, 3'-processing and strand transfer, were assessed biochemically using the partially purified, bacterially expressed recombinant IN proteins containing the substitutions (Study JTK303-PH-015). All seven mutant IN enzymes exhibited weaker enzymatic activities than wild-type IN (Figure 9). The INs containing E92Q, E92Q+S147G (D.M.), and H51Y+E92Q+S147G (T.M.) showed 57%, 29%, and 22% of HIV-1 DNA strand transfer activity of wild-type IN, respectively. Addition of E157Q to H51Y+E92Q+ S147G (T.M.) recovered the activity up to 46% of wild-type IN. Other INs containing single substitutions H51Y, S147G, or E157Q also showed decreased strand transfer activity (57%,

36%, or 79%, respectively). The 3'-end endonucleolytic processing activity of mutant INs showed a decrease in a manner similar to the DNA strand transfer activity (Figure 9): INs containing E92Q, single, double (D.M.), triple (T.M.), and quadruple (Q.M.) substitutions, showed 41%, 19%, 17%, and 21% of activity of wild-type IN, respectively. Other INs with single substitutions H51Y, S147G, or E157Q showed 78%, 48%, and 94% of wild-type IN-associated 3'-processing activity, respectively. Decreased enzymatic activities of these mutant INs likely contribute to the decreased replication capacity of HIV-1 harboring these substitutions.

Figure 9: 3'-Processing and Strand Transfer Activities of HIV-1 IN Proteins Containing EVG-Selected Substitutions



D.M., double substitutions E92Q/S147G; T.M., triple IN substitutions H51Y/E92Q/S147G; and Q.M., quadruple substitutions H51Y/E92Q/S147G/E157Q.

Fold-increase as a percentage of 3'-processing and strand transfer activities of each mutant IN (1 μM) was calculated by comparing the activities to those of WT IN.

As observed with their contribution to EVG resistance in the cell-based assays (Tables 37 and 38), the IN enzymes containing E92Q were 4.3- to 7.6-fold less sensitive to EVG in the strand-transfer assay (Table 39). It should be noted that the addition of H51Y and E157Q to E92Q+ S147G did not cause further decreases in the susceptibility to EVG. Susceptibility to L-870,810 also decreased by 4.3- and 8.5-fold for INs with E92Q and E92Q+S147G (D.M.), respectively, and third and fourth substitution had little influence on the susceptibility (10.2- and 8.2-fold, respectively). The remaining single substitutions, H51Y, S147G, and E157Q, had little to no effect on EVG susceptibility (0.7- to 2.2- fold).

Table 39: Inhibitory Effects of EVG on Strand Transfer Activity of HIV-1 INs with EVG-Selected Substitutions

IN with substitution	IC_{50}[a] value, nM (Fold-reduction in susceptibility[b])	
	EVG	L-870,810[c]
Wild-type	53.5 ± 13.8	117.8 ± 24.1

E92Q	231.5 ± 17.6 (4.3)	507.0 ± 80.2 (4.3)
E92Q+S147G (D.M.)	404.5 ± 63.9 (7.6)	996.6 ± 243.6 (8.5)
H51Y+E92Q+S147G (T.M.)	407.8 ± 27.7 (7.6)	1206.6 ± 173.6 (10.2)
H51Y+E92Q+S147G+E157Q (Q.M.)	395.7 ± 99.7 (7.4)	970.3 ± 107.9 (8.2)
H51Y	53.0 ± 16.0 (1.0)	78.9 ± 12.4 (0.7)
S147G	83.0 ± 14.3 (1.6)	249.5 ± 33.5 (2.1)
E157Q	65.4 ± 17.0 (1.2)	140.0 ± 26.5 (1.2)

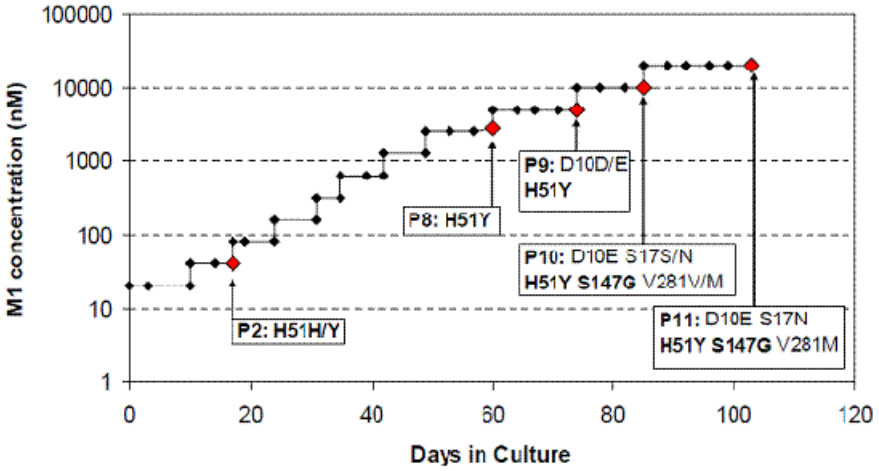
[a] Mean and standard deviation of three to five separate experiments
[b] Ratio of IC_{50} value against INs with substitutions/IC_{50} value against wild-type IN
[c] L-870,810, an investigational HIV-1 INSTI developed by Merck (Hazuda *et al*., 2004),

Two EVG metabolites M1and M4 were shown to have antiviral activity against HIV-1_{HXB2} with EC_{50} values of 17.5 nM and 12.3 nM, 12.5-fold and 8.8-fold less potent, respectively, compared to the parent EVG (Table 38). Although the prevalence of the M1 and M4 metabolites in plasma is low (3-4% of the EVG-associated plasma products), the applicant determined whether M1 and M4 may induce alternate resistance pathways compared to EVG. Cell-based resistance selection experiments in the presence of increasing concentrations of M1 and M4 were performed using HIV-1_{IIIB} in MT-2 cells.

In Study PC-183-2021, as shown in Figure 10, HIV-1 resistance selection experiments using M1 commenced at a concentration of 20 nM and proceeded in 2-fold steps. At each passage, the resulting viral pool was subjected to population-based nucleotide sequencing. At passage 2 (P2, 40 nM), an H51H/Y IN substitution was observed to develop with H51Y fully emerging by passage 8 (P8, 2,560 nM), which remained present through passage 11 (P11, 20,480 nM). In addition, D10E was noted in the viral pool at passage 9 (P9, 5,120 nM), and S17N, S147G, and V281M at passage 10 (P10, 10,240 nM). All these selected substitutions remained present along with H51Y for the duration of the selection (P11).

The resulting final virus from this selection experiment had five IN substitutions D10E, S17N, H51Y, S147G, and V281M: two conserved-site substitutions H51Y and S147G; three polymorphic-site substitution D10E, S17N, and V281M. The two conserved-site substitutions H51Y and S147G IN substitutions were also selected with EVG (see above Table 33). The H51Y substitution was also detectable in the E/C/F/T-treatment virologic failure isolates evaluated in Studies 102, 103, and 104 (see below Section 3.2.1. for details and Table 57), while S147G and the three polymorphic-site substitutions D10E, S17N, and V281M were not detected. Of note, a different amino acid substitution at residues 281 (V281A) was however seen in one of the 24 evaluated E/C/F/T-treatment virologic failure subjects (Subject 1925-7108 in Study 103, Appendix 2 Table A2-1). In addition to V281A, an additional IN substitution V31A was also detected in the subject's failure isolate that displayed 1.3-fold reduced susceptibility to EVG compared to wild-type HIV-1.

Figure 10: Kinetics of Selection of HIV-1_{IIIB} Variants Resistant to M1 in MT-2 cells



M1-selected virus pools at P2, P8, P10, and P11 showed decreased susceptibilities to M1 and also EVG (Table 40), and fold-changes in susceptibilities to M1 were greater than those to EVG at all passages, with the final passage P11 showing 245-fold and 54-fold reduced susceptibilities to M1 and EVG, respectively. The low frequency (34% [10/29] based on the clonal sequence analysis) of the H51Y substitution at P2 produced 2.3-fold reduced susceptibility to M1 and no reduction to EVG, but, as H51Y became the predominant genotype at P8 (95%, 25/27), viral susceptibility to M1 was reduced 32-fold while susceptibility to EVG decreased 3.5-fold. Clonal sequence analysis (linkage analysis) revealed one (5%, 1/21) of the clones harboring H51Y also had S147G at P8. The S147G substitution frequency increased at P10 to 86% (25/28) and all were in combination with H51Y. At P11 (terminal passage), H51Y combined with S147G was detected in 100% of viral clones examined (n=29). Thus, H51Y-conferred resistance to M1 and EVG appeared to be enhanced through the acquisition of S147G (to 326- and 28-fold at P10, respectively, from 32- and 3.5- fold at P8). Viral pools from all passages remained fully susceptible to the control compound EFV.

Table 40: Drug Susceptibilities of M1-Selected HIV-1_{IIIB} Viral Pools at Passages P2, P8, P10, and P11 in MT-2 Cells

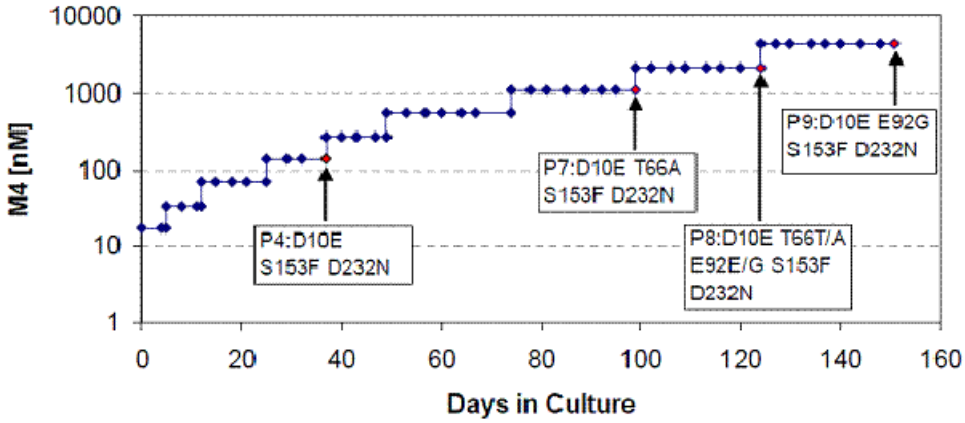
	EC_{50} Fold Change from Wild-type[a]		
Viral Pool	M1[b]	EVG	EFV
M1 P2	2.3	1.1	ND
M1 P8	32	3.5	1.5
M1 P10	326	28	ND
M1 P11	245	54	1.1

ND, not determined
[a] Values represent fold change in drug susceptibility compared to the control wild-type HIV-1_{IIIB}. Mean EC_{50} values were calculated from ≥3 replicates.
[b] Color coding by drug susceptibility: Sensitive (≤2.5-fold change, no shading), reduced susceptibility (>2.5- but ≤10-fold change, grey shading), and resistance (>10-fold change, black shading).

Figure 11 shows M4-selection results from Study PC-183-2008. HIV-1 resistance selection experiments using M4 commenced at a concentration of 17 nM and proceeded in 2-fold steps.

Three IN substitutions, D10E (also observed with M1; Figure 10), S153F, and D232N, were first observed to develop in the passage-4 virus pool (P4, 136 nM). All these 3 substitutions were subsequently present for the duration of the selection. Clonal sequence analysis revealed these 3 substitutions, D10E, S153F, and D232N, were always detected together. A T66A substitution was first detected by population-based sequencing by passage 7 (P7, 1,088 nM), was present as a mixture by passage 8 (P8, 2,176 nM), and was undetectable at the completion of passage 9 (P9, 4,352 nM). An E92G was detected as a mixture in P8 and remained present through P9. The resulting final virus from this selection experiment had four IN substitutions D10E, E92G, S153F, and D232N: three conserved-site substitutions E92G, S153F, and D232N; one polymorphic-site substitution D10E. Substitutions at IN amino acid positions T66, E92, and S153 were noted in the E/C/F/T-treatment virologic failure isolates evaluated in Studies 102, 103, and 104, but in each case the amino acid substituted was different (T66I, E92Q, and S153A; see below Section 3.2.1. for details and Table 57). Thus, residues T66, E92, and S153 appear to be involved in HIV-1 IN sensitivity to M4 as well as EVG.

Figure 11: Kinetics of Selection of HIV-1_{IIIB} Variants Resistant to M4 in MT-2 cells



Passages P4, P7, P8, and P9 viruses were characterized with respect to their susceptibilities to M4, EVG, and RAL, and two control compounds, the NRTI tenofovir (TFV) and the NNRTI efavirenz (EFV). The resulting phenotypes of the viral pools with respect to their fold-change in susceptibilities to M4 and EVG indicated that the substitutions selected by M4 reduced susceptibility to both M4 and EVG to a similar degree (Table 41). The presence of the S153F substitution along with D10E and D232N in P4 produced low-level reductions in susceptibility to both M4 and EVG (5- to 7-fold), as the 3 substitutions being detected in 83% (25/30) of viral clones examined at P4. At P7, all viral clones examined (n=30) had these 3 substitutions and 76.7% of them had an additional substitution T66A. Thus, the addition of T66A to these 3 substitutions appeared to increase resistance to both M4 and EVG (approximately 56- to 60-fold; Table 41). The emergence of the E92G substitution (combined with D10E+S153F+D232N) in P8 and P9 viral pools (43.3% [13/30] and 80% [24/30] of the examined viral clones, respectively) again further reduced susceptibility to M4 and EVG (80- to 115-fold). Since T66A declined in frequency to 7% (2/30) of the examined clones at P9, undetectable by population-based sequencing (Figure 11), the T66A-containing mutant viruses appeared to be rapidly outgrown by the mutant viruses harboring D10E+E92Q+S153F+D232N in the presence of higher M4 concentration (4,352 nM). Susceptibility to RAL was only minimally reduced in all viral pools tested and was <3.0-fold reduced until emergence of the E92G substitution in

combination with D10E+S153F+D232N in P8 and P9 at which point a 4-fold reduction in RAL susceptibility was observed. As expected, viral pools from all passages remained susceptible to the two control compounds, TFV and EFV.

Table 41: Drug Susceptibilities of M4-Selected HIV-1_{IIIB} Viral Pools at Passages P4, P7, P8, and P9 in MT-2 Cells

Virus pool	Drug Susceptibility (Fold-reduction[1])				
	M4	EVG	RAL	EFV	TFV
M4 P4	5.5	7.3	2.1	1.9	1.2
M4 P7	60	56.1	2.3	1.6	1
M4 P8	79.5	90.2	3.7	1.6	1.4
M4 P9	115	93.1	4	1.3	1.3

[1] Values represent fold change in drug susceptibility compared to the control wild-type HIV-1_{IIIB}. Mean EC_{50} values were calculated from 5 replicates for wild-type HIV-1_{IIIB} and 3 replicates for each virus pools at passages P4, P7, P8, and P9.

In Study PC-183-2021, a total of 8 infectious molecular clones of HIV-1_{HXB2} harboring IN substitutions, singly and in combination, selected by M1 and M4 were generated by site-directed mutagenesis (Table 42): M1-selected H51Y, S147G, and H51Y+S147G; and M4-selected T66A, E92G, S153F, T66A+S153F, and E92G+S153F. The mutant HIV-1_{HXB2} variants were characterized in MT-2 cells with respect to their susceptibilities to M1, M4, EVG, RAL, and two control compounds, the NRTI tenofovir (TFV) and the PI lopinavir (LPV).

Table 42: Drug Susceptibilities of HIV-1_{HXB2} Variants Harboring IN Substitutions Selected with M1 and M4 in MT-2 Cells

Virus	EC_{50} Fold Change from Wild-type[a]					
	M1[b]	M4	EVG	RAL	TFV	LPV
H51Y	17.8	0.8	2.9	1.2	1.5	1.1
S147G	6.8	2.7	9.5	1.2	1.6	1.0
H51Y+S147G	144	12.9	33	2.1	2.5	1.4
T66A	28.8	14.1	33.1	3.8	1.8	1.6
S153F	3.4	2.2	5.2	1.9	2.4	1.3
T66A+S153F	29.4	19.3	54.1	1.0	1.7	1.6
E92G	22.1	6.5	38.4	1.7	1.9	1.3
E92G+S153F	15.5	8.0	30.1	1.4	1.8	0.9

[a] Values represent fold-reduction in drug susceptibility compared to the control wild-type HIV-1_{HXB2}. Mean EC_{50} values were calculated from ≥13 replicates for wild-type HIV-1_{HXB2} and ≥3 replicates for HIV-1_{HXB2} variants with IN substitutions. Mean EC_{50} values against wild-type HIV-1_{IIIB} were 12.5 nM for M1, 10.5 nM for M4, 1.2 nM for EVG, 5.6 nM for RAL, 3.6 µM for TFV, and 11.2 nM for LPV.
[b] Color coding by drug susceptibility: Sensitive (≤2.5-fold change, no shading), reduced susceptibility (>2.5- but ≤10-fold change, grey shading), and resistance (>10-fold change, black shading). These definitions of susceptibility are arbitrary and not based on clinical cutoffs.

As summarized in Table 42, the H51Y+S147G double substitutions, representing the final M1

selection virus (Figure 10), displayed a larger fold reduction in susceptibility to M1 than to M4 and EVG (144-fold verses 12.9- and 33-fold, respectively). Similar reductions in susceptibility (245-fold for M1 and 54-fold for EVG in Table 40) were observed in phenotypic analysis of the terminal passage (P11) of the M1 selection, which consisted of viruses with H51Y+S147G and additional 3 polymorphic-site substitutions D10E, S17N, and V281M. Analysis of the H51Y substitution showed a 17.8-fold reduction in susceptibility to M1, and no or minimal reductions (0.8- to 2.9-fold) in susceptibilities to M4 and EVG, respectively. The S147G substitution alone conferred 6.8-, 2.7-, and 9.5-fold reduced susceptibilities to M1, M4, and EVG, respectively. Thus, these 2 substitutions H51Y and S147G when combined exerted greater than additive effects on susceptibility to EVG and its 2 metabolites M1 and M2. Of note, there were no E/C/F/T-treatment virologic failures who developed this combination of IN substitutions (H51Y+S147G): S147G was never detected in the examined failure isolates and H51Y detectable together with E92Q in only one failure subject conferring 148.6-fold reduced susceptibility to EVG (Subject 0698-6012 in Study 102, Appendix 2 Table A2-1). These 2 substitutions, singly and in combination, had little to no effect (1.1- to 2.5-fold) on susceptibility to RAL and the 2 controls TFV and LPV.

Viruses harboring IN substitutions T66A or E92G, selected by M4, showed significant phenotypic resistance to M1, M4, and EVG with ≥6.5-fold reductions in drug susceptibility (Table 42). Small increases in drug resistance to these compounds (overall >8-fold reduced susceptibility) were observed when these substitutions were combined with S153F, which by itself conferred a moderate reduction in susceptibility to EVG and both metabolites (2.2- to 5.2-fold). There was one E/C/F/T-treatment virologic failure isolate harboring E92Q and S153A with 111.2-fold reduced susceptibility to EVG (Subject 0031-6257 in Study 102, Appendix 2 Table A2-1). Only T66A showed moderate cross-resistance to RAL with 3.8-fold reductions in susceptibility. As expected, all mutant viruses with M4-selected IN substitutions were ≤2.4-fold less susceptible to control compounds, TFV and LPV.

Overall, the resistance profiles of EVG and its metabolites M1 and M4 were overlapping. The magnitude of resistance of selected virus pools and site-directed mutant viruses harboring selected IN substitutions was similar to EVG, M1, and M4, with the exception of H51Y, which showed a higher magnitude of resistance to M1 than EVG or M4 (Table 42). EVG itself is the dominant plasma species among EVG and its metabolic products, and thus, HIV-1 variants selected *in vivo* by EVG itself will likely predominate if virologic failure occurs on EVG.

In previous 3 studies JTK303-PH-012, PC-183-2008, and PC-183-2021, the development of resistance IN substitutions, selected by EVG and its metabolites M1 and M4, was assessed using a dose-escalation assay where the drug concentration was increased 2-fold from one passage to the next when viral replication was detected based on observed cytopathic effect. In Study 183-2007, the applicant utilized an alternate method 'Breakthrough selection' to select drug-resistance virus pools where infected cells were subjected to drug selective pressure at constant drug concentrations throughout the culture period. SupT1 cells were infected with HIV-1_{HXB2} at an MOI of 0.1 in the presence of constant drug concentrations for up to 45 days (until extensive cytopathic effects were observed). Five different drug concentrations corresponding to 5-, 10-, 20-, 40-, and 80-fold the EC_{50} values for EVG and RAL (as a control) were used in these experiments and the results are summarized in Table 43. Virologic breakthrough occurred at EVG concentrations corresponding to 10-, 40-, and 80-fold the EC_{50} values, and the emergence of T66I/T, Q148R, and Q148R IN substitutions were noted in the breakthrough virus

pools by population-based nucleotide sequencing, respectively. T66I and Q148R conferred 14.5- and 109-fold reduced susceptibilities to EVG, respectively (Table 44). RAL-resistance breakthrough viruses harboring primary RAL resistance-associated IN substitutions were also obtained at RAL concentrations corresponding to 5- (N155H) and 40-fold (Q148K) the EC_{50} values. These 2 substitutions conferred phenotypic resistance to both RAL and EVG (23- to 26-fold for RAL and 4- to -50-fold for EVG; Table 44).

Table 43: IN Genotypic Results from the Breakthrough Selections

Selecting Compound[a, b]	Drug Concentrations (Fold over EC_{50}[c])				
	5	10	20	40	80
EVG	Wild-type	T66I/T	Wild-type	Q148R	Q148R
RAL	N155H	Wild-type	Wild-type	Q148K	Wild-type
EFV	nd	L100I D237N/D	L100I D237N	L100I	L100I V108I

nd: not done; EVG
[a,b] IN substitutions detected by population-based nucleotide sequencing in the virology breakthrough virus pools selected by EVG and RAL, and RT substitutions by EFV, compared to wild-type HIV-1_{HXB2}.
[c] The EC_{50} values for EVG, RAL, and EFV against HIV-1_{HXB2} were 1.2 nM, 5.6 nM, and 1 nM, respectively.

Table 44: Drug Susceptibilities of HIV-1_{HXB2} Variants Harboring EVG-Selected IN Substitutions in MT-2 Cells

Selection Experiments and Associated Site-Directed Mutant Viruses	EC_{50} Fold Change Compared To Wild-Type[a, b]				
	M1	M4	EVG	RAL	TFV
EVG Selection #1					
T66I[c]	23.1	10.7	14.5	1.3	1
R263K	18.1	3.3	6.3	1.4	1.2
T66I R263K	297	58.3	105	1	0.5
EVG Selection #2					
F121Y	19.1	12.5	11.8	7.2	1
S153Y	2.1	1.6	4.9	1.7	1.1
T66I F121Y	41.3	22.6	37.2	10.2	0.6
T66I S153Y	43.7	24.8	41.3	0.9	0.6
T66K	-	-	39.9	19.1	1.2
RAL Selection					
Q148R[d]	-	-	109	37.6	0.7
RAL Breakthrough Selections					
N155H	-	-	41.4	23.8	1.2
Q148K	-	-	49.6	26.1	0.7

'-': not determined
[a] EC_{50} values against wild-type HIV-1_{HXB2} were 12.5 nM for M1, 10.5 nM for M4, 1.2 nM for EVG, 5.6 nM for RAL, and 3.6 μM for TFV.

[b] Fold-change values ≤2.5 reflect assay variation. All fold-change values >2.5 demonstrated a statistically significant difference compared to wild-type HIV-1 using the Student's t-test ($p < 0.01$).
[c] The T66I substitution was also observed in the EVG selection #2 (Table 45).
[d] The Q148R substitution was also observed in the breakthrough selections using EVG (Table 43).

In parallel, the applicant also utilized dose-escalation to select drug-resistant viruses in two independent experiments (Table 45) in this study (Study 183-2007). In the first experiment (EVG #1), D10E, S17N, T66I, and R263K were observed: all 4 substitutions were present in almost 100% of the examined clones at passage 11. In the previous dose-escalation experiments, D10E and S17N were found in the viruses selected by EVG metabolites M1 and M4 (Figures 10 and 11) with no EVG susceptibility data of site-direct mutants with individual substitutions, whereas the emergence of R263K was not noted in the previous selection experiments. The R263K substitution conferred 6.3-fold reduced susceptibility to EVG (Table 44) and no E/C/F/T treatment-failure subjects developed this substitution. T66I *per se* was not noted in the previous selection experiments, either, but a threonine-to-alanine change at 66 (T66A) that conferred 33.1-fold reduced susceptibility to EVG was detected in the M1-selected virus pools (Figure 11). The T66I substitution conferred 14.5-fold reduced susceptibility to EVG and EVG resistance conferred by T66I was enhanced to 105-fold when R263K was added (Table 44). In agreement with this, the R263K substitution developed at the end of passaging (passages 8 and 11): 3 IN substitutions D10E, S17N, and T66I were present in almost 100% of the clones at passage 8, while R263K frequency increased from 4.2% (1/24) of the examined clones at passage 8 to 100% of the clones by passage 11.

In the second experiment (EVG #2, Table 45), in addition to D10E, S17N, and T66I substitutions observed in EVG #1, F121Y, S153Y, and D232N were shown in the selected virus. T66I was first detected as a mixture with wild-type T66 in the virus pool at passage 3 and persisted until the end of the selection (passage 12). The passage-3 viruses had 7.8-fold reduced susceptibility to EVG and acquired sequentially D232N, S153Y, D10E, S17N, and F121Y by passage 12, which further increased the resistance of the selected virus pool to 585-fold. Nearly 100% of the examine clones at passages 9 and 12 harbored the T66I+D232N double substitutions, and >80% of them at passage 12 acquired D10E on top of T66I+D232N. F121Y and S153Y were rarely found on the same genome (one clone) as being detected in 53.6% (15/28) and 50% (14/28) of the examine clones at passage 12. More than additive effects were shown on the phenotypic resistance to EVG by the interaction of T66I with F121Y or S153Y (Table 44). Single substitutions T66I, F121Y, and S153Y conferred 14.5-, 11.8-, and 4.9-fold reduced susceptibilities to EVG, respectively, whereas 37.2- and 41.3-fold reductions in EVG susceptibility were observed for T66I+F121Y and T66I+S153Y double substitutions (Table 44),

Table 45: Genotypic Results from the Dose-Escalation Selections

Selecting Compound	Final Drug Concentration (nM)	Fold over EC_{50}[a]	Selection Duration (days)	Final Integrase Amino Acid Sequence[b]
EVG #1	1640	2730	171	D10E S17N T66I R263K
EVG #2	3072	5120	121	D10E S17N T66I F121F/Y S153Y/S[c] D232N
RAL	5120	1024	132	Q148R D232N

[a] Wild-Type HIV-1_{IIIB} EC_{50} values for EVG and RAL were 0.6 nM and 3 nM, respectively.
[b] IN substitutions detectable in the selected viruses by population-based nucleotide sequencing, compared to wild-type HIV-1_{LAI}.

[c] The S153Y substitution was not detected by population-based nucleotide sequencing but was present in 50% of the clones in the clonal sequencing analysis.

Overall, 13 IN substitutions were detected in HIV-1 variants resistant to EVG or its metabolites M1 and M4 that were selected in the cell-based resistance selection experiments: D10E, S17N, H51Y, T66A/I, E92G/Q, F121Y, S147G, Q148R, S153F/Y, E157Q, D232N, R263K, and V281M. Of these 13 cell-selected IN substitutions, 5 substitutions H51Y, T66I, E92Q, Q148R, and E157Q were also observed in the E/C/F/T-treatment virologic failure isolates evaluated in Studies 102, 103, and 104 (see below Section 3.2.1. for details and Table 57). No phenotypic resistance data were available for D10E, S17N, D232N, and V281M. Of the remaining 9 substitutions, all but one (E157Q) conferred individually >3-fold reduced susceptibility to EVG (up to 109-fold) with T66A/I, E92G/Q, F121Y, and Q148R resulting in high-level resistance to EVG (>10-fold reduction in susceptibility). Of the 8 substitutions conferring varying degrees of resistance to EVG (H51Y, T66A/I, E92G/Q, F121Y, S147G, Q148R, S153F/Y, and R263K), 4 substitutions (T66A, E92Q, F121Y, and Q148R) also conferred cross-resistance to RAL (3.8- to 37.6-fold reductions in RAL susceptibility).

2.6.2. Development of Resistance to COBI

No cell culture resistance selection studies were conducted as cell culture antiviral activity was not demonstrated.

2.7. Cross-Resistance with Other FDA-Approved Antiretrovirals

2.7.1. Cross-Resistance of EVG

Cross-resistance between EVG and RAL (only approved member of the INSTI class) should be expected with these two inhibitors sharing a common mechanism of action against HIV-1 IN, involving metal chelation of the divalent metal cation Mg^{++} and interaction with the catalytic loop (IN residues 140-149; reviewed by Mouscadet *et al*., 2010). As described in Section 2.6.1., HIV-1 variants harboring IN substitution(s) selected by EVG (or its metabolites M1 and M4) in the cell-based resistance selection experiments showed varying degrees of cross-resistance to RAL depending on the type and number of IN substitutions (Tables 38, 42, and 44). In contrast, these substitution(s), singly and in combination, conferred no significant cross-class resistance to other antiretrovirals from different classes (≤2.5-fold reductions in susceptibility to tested inhibitors including the NRTIs AZT, FTC, and TFV, the NNRTI EFV, and the PI LPV; Tables 38, 42, and 44), as they have different mechanisms of action.

In order to fully assess the phenotypic impact of individual and combinations of IN substitutions on EVG susceptibility and cross-resistance to RAL, drug susceptibility was determined in Study PC-183-2025 using the 5-day cytopathic assay in MT-2 cells of infectious HIV-1_{HXB2} recombinant clones harboring IN substitutions occurring at 21 amino acid positions (Table 46). In addition to the molecular clones with IN substitutions selected by EVG (or its metabolites) in cell culture (Tables 38, 42, and 44), additional IN substitutions were introduced, individually and in combination, by site-directed mutagenesis including those detected in the E/C/F/T-treatment failure isolates evaluated in Studies 102, 103, and 104 (L68I/V and N155H, Table 57) and those known to be associated with RAL resistance (T97A, E138A/K, G140S, Y143H/R, Q148H, N155H, G163R, and S230R). Overall, most viruses containing the examined IN substitution(s)

exhibited significant reductions in susceptibility against EVG and/or RAL (Table 46): only some substitutions H51Y, L68I/V, V72I, T97A, E138A/K, Q146K, E157Q, G163K/R, S230R, and N232D showed <3-fold loss of susceptibility to both EVG and RAL. The E92Q substitution, most frequently found in the E/C/F/T-treatment virologic failure isolates evaluated in Studies 102, 103, and 104 (Table 56), conferred reduced susceptibility to EVG (39.4- to 78.1-fold) and RAL (5.8- to 11.2-fold). Among the 3 primary RAL resistance-associated substitutions tested in this study (Y143H/R, Q148H/K/R, and N155H), all but one (Y143H) conferred measurable reductions in susceptibility to EVG (4.8- to 107.6-fold). All HIV-1 variants expressing multiple IN substitutions in combination with E92Q, Q148H/K/R, or N155H showed high levels of resistance to EVG (42.7- to 868.7-fold) and RAL (6.1- to 755.8-fold). Thus, although there were unique resistance substitutions for EVG and RAL (i.e., T66I and Y143H, respectively), this study showed a high degree of overlapping resistance for key INSTI resistance-associated substitutions.

Table 46: Antiviral Activity of EVG and RAL against HIV-1_{HXB2} Harboring IN Substitution(s) Generated by Site-Directed Mutagenesis

Integrase Genotype	Susceptibility (fold-change in EC_{50} compared to wild-type)						
	EVG	RAL	GS-9200 (M4)	GS-9202 (M1)	TFV	FTC	LPV
H51Y	2.9	1.2	0.8	17.8	1.5	—	1.1
T66A*	8.4	1.2	—	—	1.1	—	—
	33	3.8	14.1	28.8	1.8	—	1.6
T66I	14.9	1.3	10.7	23.1	1.0	0.9	—
	30.8	2.3	9.2	19.9	3.8	—	—
T66K	40.8	19.4	—	—	1.2	—	—
L68I	0.8	0.8	—	—	0.9	—	0.9
L68V	1.5	1.0	—	—	1.1	—	1.0
V72I	1.6	1.6	—	—	1.0	—	1.1
E92G	38.4	1.7	6.5	22.1	1.9	—	1.3
E92Q	39.4	5.8	—	—	0.9	1.1	1.0
	78.1	11.2	22.2	48.5	3.3	—	—
T97A	2.1	2.4	—	—	0.7	—	0.9
F121Y	12.1	7.3	12.5	19.1	1.0	—	—
E138A	0.8	1.1	—	—	0.8	—	0.8
E138K	0.6	0.9	—	—	1.1	—	1.1
G140S	3.1	1.6	—	—	0.9	—	1.0
Y143H	1.6	3.4	—	—	1.0	—	0.8
Y143R	5.4	37.7	—	—	1.0	—	1.0
P145S	31.2	0.6	—	—	0.7	—	0.6
Q146I	133.9	3.5	—	—	0.9	—	1.0
Q146K	2.0	1.0	—	—	0.9	—	0.8
Q146L	20.8	1.4	—	—	0.9	—	0.9
Q146P	6.0	1.2	—	—	1.0	—	0.9
Q146R	4.8	1.9	—	—	0.9	—	1.0
S147G	6.8	1.2	2.7	6.8	1.6	—	1.4
Q148H	4.8	18.8	—	—	0.9	—	0.9

Integrase Genotype	Susceptibility (fold-change in EC_{50} compared to wild-type)						
	EVG	RAL	GS-9200 (M4)	GS-9202 (M1)	TEV	FTC	LPV
Q148K	**49.6**	**26.1**	—	—	0.7	—	0.7
Q148R	**107.6**	**34.0**	—	—	0.7	—	0.9
S153F	5.2	1.9	2.2	3.4	2.4	—	1.3
S153Y	5.0	1.7	1.6	2.1	1.0	—	—
N155H	**35.2**	**21.0**	—	—	1.1	—	0.9
	67.3	**25.9**	**18.8**	**41.4**	2.8	—	—
N155S	**64.9**	**10.9**	—	—	0.7	—	0.6
E157Q	2.9	1.4	—	—	0.9	—	1.1
G163K	1.2	1.4	—	—	0.9	—	0.8
G163R	1.3	1.6	—	—	0.9	—	0.8
S230R	1.0	1.6	—	—	1.0	—	—
N232D	1.8	0.8	—	—	—	—	1.0
R263K	6.4	1.0	3.3	—	0.5	1.2	1.6
H51Y+S147G	**33**	2.1	**12.9**	**144**	2.5	—	1.4
T66A+S153F	**54.1**	1.0	**19.3**	**29.4**	1.7	—	1.6
T66I+F121Y	**38.0**	**10.4**	**22.6**	**41.3**	0.6	—	—
T66I+S147G	**34.4**	1.9	—	—	1.0	—	0.9
T66I+S153Y	**42.2**	0.9	**24.8**	**43.7**	0.6	—	-
T66I+R263K	**107.5**	1.0	**58.3**	**297.4**	0.5	0.8	0.9
E92G+S153F	**30.1**	1.4	8.0	**15.5**	1.8	—	0.9
E92Q+T66I	**107.3**	**25.2**	—	—	0.9	—	0.9
E92Q+L68I	**42.7**	7.4	—	—	0.8	—	0.8
E92Q+L68V	**66.2**	**13.6**	—	—	1.1	—	1.0
E92Q+S147G	**69.5**	6.5	**52.9**	**81.7**	0.8	1.0	0.9
E92Q+N155H	**123.2**	**102.6**	—	—	0.8	—	0.6
Q148H+G140S	**868.7**	**755.8**	—	—	1.0	—	1.1
Q148R+L68V	**137.1**	**71.4**	—	—	0.6	—	0.6
Q148R+N155H	**112.0**	**81.3**	—	—	0.3	—	0.4
N155H+L68V	**50.4**	**23.4**	—	—	0.7	—	0.6
E92Q+H51Y+S147G	**143.9**	6.8	**85.0**	**399.3**	0.9	0.9	1.0
Q148K+E138A+G140A	**372.1**	**354.1**	—	—	0.9	—	0.8
Q148R+E138K+S147G	**152.9**	**33.3**	—	—	0.9	—	0.9
E92Q+H51Y+S147G+E157Q	**171.1**	6.1	**105.8**	**399.3**	0.9	1.1	1.0

"-", not done; Fold-change values >10 are shown in bold.
[a] For some HIV-1 mutants, there were two independent data sets, and both are represented separately. The larger number of the two data sets was used for descriptive purposes in the text.

Study JTK303-PH-010 demonstrated EVG retained full antiviral potency with mean EC_{50} values ranging from 0.02 nM to 1.13 nM against well characterized patient-derived HIV-1 PI-resistant and multidrug-resistant (MDR) isolates (Table 47). Of note, the EC_{50} values of 0.1-1.26 nM were obtained for wild-type clinical isolates of HIV-1 in this assay (see above Table 9 in Section 2.2.1.). The PI-resistant HIV-1 isolates 052-52, 144-44, 1002-60, and 1064-52 were obtained [redacted] (b) (4), and the multidrug-resistant HIV-1 isolates MDR 769,

MDR 807, MDR 1385, and MDR 3761 were obtained [redacted] (b) (4). The genotypic resistance profile for these viruses is provided in Table 47. The virus isolates were originally derived by co-culture of primary PBMCs from each patient with normal human donor PBMCs. The EC_{50} values for these viruses were determined in PHA/IL-2-activated, monocyte-depleted human PBMCs at 7 days post-infection using the HIV-1 p24 ELISA kit (Beckman Coulter).

Table 47: Anti-HIV-1 Activity of EVG against Patient-Derived Drug-Resistant HIV-1 Isolates in PHA/IL-2 Activated PBMCs

Isolate	Resistance Mutations[a]		EC_{50} (nM)			
			EVG	ZDV	EFV	NFV
Protease Inhibitor-Resistant Isolates						
1064–52	Protease[b]	L10I, I54V, L63P, A71T, V82F, L90M	0.63	13.6	0.29	838.5
1002–60	Protease	L10I, M46I, I54V, L63P, V82F, L90M	0.13	6.72	0.21	> 1000
052–52	Protease	L10R, M46I, L63P, A71V, V82T, I84V	0.17	63.9	< 0.10	95.0
144–44	Protease	V32I, M46I, L63P, L90M	0.72	7.24	0.60	165.3
Multidrug-Resistant Isolates						
MDR 769	RT[c]	M41L, K65R, D67N, V75I, F116Y, Q151M, Y181I, L210W, T215Y	0.18	720.7	0.54	> 1000
	Protease	L10I, M36M or V, M46I, I54V, L63P, A71V, V82A, I84V, L90M				
MDR 807	RT	M41L, D67N, M184V, L210W, T215Y, K219N	1.13	159.4	0.71	135.0
	Protease	L10I, G48V, I54T, L63Q, A71V, V82A				
MDR 1385	Protease	L10I, M36I, M46I, I54I or V, L63P, A71V, V82T, L90M	0.02	> 1000	> 1000	> 1000
MDR 3761	Protease	L10I, M46I, I84V, L63P, A71I, L90M	0.73	> 1000	> 1000	> 1000

[a] Amino acid substitution sites are represented as the position number in the RT- or protease-coding region and the amino acid residue in the wild-type and drug-resistant viruses.
[b] Protease gene
[c] RT gene

In Study JTK303-PH-009, the anti-HIV activity of EVG was further evaluated against a large panel of patient-derived drug-resistant HIV-1 recombinant clones (HIV-1_{NL4-3} as a reference clone) using the PhenoSense™ HIV assay (Monogram Biosciences; Petropoulos *et al*., 2000). This study included 120 recombinant clones, 112 with one or more drug resistance-associated substitutions in the protease (PR)- or RT-coding regions and 8 wild-type clones with no amino acid substitutions at crucial positions in these regions listed in Table 48. As summarized in Table 49, EVG retained anti-HIV-1 activity against all of the tested clones resistant to individual and multiple NNRTIs, NRTIs, or PIs with the mean EC_{50} value of 1.06 nM (0.8- to 1.2-fold changes in EC_{50} values, compared to the reference clone of HIV-1_{NL4-3}).

Table 48: Amino Acid Residues in the Protease and RT Regions Crucial to Drug Resistance Development

Phenotype	Amino acid residue and position in RT region in wild type
NRTI*	M41*, K65, D67*, T69, K70*, V118, Q151, M184, L210*, T215* and K219*
NNRTI*	K103, Y181 and Y188
	Amino acid residue and position in protease region in wild type
PI*	D30, M46, V82, I84 and L90

*TAMs: thymidine analogue mutations

Table 49: EVG Susceptibility of Recombinant HIV-1_{NL4-3} with Substitutions Associated with Resistance to NNRTIs, NRTIs, and PIs

RT mutation[a]	N[b]	Fold-increase[c]						
		JTK-303	AZT	3TC	ddI	d4T	ABC	TFV
Wild-type	8	0.9	0.8	1.1	1.1	1.0	0.9	0.8
6 TAMs[d]	5	0.9	531.6	6.7	2.2	6.2	6.9	5.0
6 TAMs, M184	7	1.0	92.1	98.0	2.1	2.7	7.4	1.6
2-5 TAMs[e]	16	1.0	271.3	6.1	2.5	4.8	6.6	3.5
2-5 TAMs, T69ins	4	1.2	506.4	19.5	6.0	17.0	19.3	12.2
2-5 TAMs, K65 and/or Q151	4	1.1	437.8	57.3	17.2	21.0	19.8	8.5
M184	12	0.9	0.4	102.4	1.4	0.8	3.1	0.6
K65 and/or Q151	7	0.9	181.6	40.1	9.6	10.3	13.3	4.0
K65 and/or Q151, M184	12	0.9	262.6	108.0	13.2	9.0	24.1	2.2

RT mutation[a]	N[b]	Fold-increase[c]			
		JTK-303	NVP	DLV	EFV
Wild-type	8	0.9	1.2	1.9	1.3
K103	46	0.9	127.3	86.9	128.6
K103, Y181	14	1.0	187.1	122.1	154.7
K103, Y188	6	0.8	197.0	109.2	218.0

Protease mutation[a]	N[b]	Fold-increase[c]							
		JTK-303	APV	IDV	NFV	RTV	SQV	LPV	ATV
Wild-type	8	0.9	0.6	0.7	0.9	0.8	0.7	0.7	0.7
M46, V82	14	0.9	27.6	31.3	34.6	59.5	42.8	76.8	32.2
M46, V82, I84 or L90	14	1.0	62.3	29.1	41.7	130.6	45.4	112.0	34.7
M46, V82, I84, L90	3	1.0	34.0	32.0	64.0	163.3	108.0	32.3	45.0
D30	8	1.0	1.1	1.6	37.8	1.3	1.3	1.1	3.7
D30, L90	5	1.0	9.6	11.3	209.2	29.2	114.4	11.6	41.4

JTK-303=EVG
[a] Mutation sites are represented as the amino acid position number in the protease- and RT-coding region and the amino acid residue in the wild-type virus.
[b] Number of clones
[c] Fold-increase in the EC_{50} value compared to the wild-type reference clone (HIV-1_{NL4-3})
[d] Six thymidine analogue mutations (at RT positions M41, D67, K70, L210, T215, K219)
[e] Two to 5 thymidine analogue mutations (at RT positions M41, D67, K70, L210, T215, K219)

2.7.2. Cross-resistance of COBI

No cell culture cross-resistance selection studies were conducted as cell culture antiviral activity was not demonstrated.

3. Clinical Virology

Clinical virology analyses were conducted to evaluate the antiviral efficacy (potency and durability) and virological resistance of the single-tablet complete regimen of EVG 150 mg/COBI 150 mg/FTC 200 mg/TDF 300 mg (referred to E/C/F/T through this review) in HIV-1-infected, antiretroviral treatment-naïve (ARV-naïve) adult subjects in 3 clinical trials, two Phase 3 Studies GS-US-236-0102 and GS-US-236-0103 (Studies 102 and 103, respectively) and one Phase 2 Study GS-US-236-0104 (Study 104). In these studies, the comparator groups were treated with a single-tablet complete regimen of EFV 600 mg/FTC 200 mg/TDF 300 mg (Atripla®; ATR; Studies 102 and 104) or 100 mg ritonavir-boosted 300 mg atazanavir (ATV/r) plus TRUVADA® (fixed-dose combination tablet containing FTC 200 mg and TDF 300 mg; TVD) in Study 103. A brief description of the trial design of each study is provided in Table 50. Assays for HIV-1 RNA quantification and resistance testing that were utilized in the clinical virology studies are described in Appendix 1.

Clinical virology analyses were conducted using the censored, as-treated subject population (n=1392, pooled from the three studies) including all randomized subjects who received at least one dose of study medication with at least one virologic observation after Baseline but excludes subjects who discontinued their assigned treatment before the primary efficacy assessment while they had a suppressed viral load (HIV-1 RNA <50 copies/mL). For the analyses, virologic failures were identified as follows:

- In Studies 102 and 103, subjects who failed to achieve HIV-1 RNA <50 copies/mL either at Week 48 (snapshot analysis during the 48-week evaluation window, Treatment Days between 309 and 378) or at the time of early discontinuation
- In Study 104, subjects who failed to achieve HIV-1 RNA <50 copies/mL either at the time of study unblinding (Week 60, Treatment Days 385-429) or at the time of early discontinuation

Table 50: Overview of Study Designs of Studies 102, 103, and 104

Common design features applied all 3 studies			
Study population	- ARV treatment-naïve (no prior use of any approved or experimental ARV for any length of Time) - HIV-1 infected with HIV-1 RNA ≥5,000 copies/mL at screening - Irrespective of $CD4^+$ T cell count		
Stratification	HIV-1 RNA at Screening, ≤100,000 copies/mL or >100,000 copies/mL		
Background treatment	FTC 200 mg and TDF 300 mg		
Study-specific design features			
Study	Study 102	Study 103	Study 104
Phase	3	3	2
Number of study sites	102 (97 in the US; 5 in Puerto Rico)	146 (88 in the US; 58 in other countries)	30 (all in the US)

Active comparator	EFV 600 mg	ATV/r (300/100 mg)	EFV 600 mg
Primary efficacy point (HIV-1 RNA copies/mL)	<50 at Week 48	<50 at Week 48	<50 at Week 24

A total of 105 virologic failures, 7.5% of the 1392 treated-subjects in the three pooled studies, were identified (Table 51). Of subjects receiving E/C/F/T, ATR, and ATV/r+TVD treatment, 7.3% (52/715), 9% (31/346), and 6.6% (22/331) experienced virologic failure, respectively.

Table 51: Virologic Failures in Censored, As-Treated Analyses[1] of Three Studies 102, 103, and 104

Study	**102** (n=657)		**103** (n=668)		**104** (n=67)	
Treatment	E/C/F/T (n=332)	ATR (n=325)	E/C/F/T (n=337)	ATV/r+TVD (n=331)	E/C/F/T (n=46)	ATR (n=21)
VF[2], n=105 (%)	**26** (7.8%)	**28** (8.6%)	**22** (6.5%)	**22** (6.6%)	**4** (8.7%)	**3** (14.3%)
NR[3], n=3	1	1	1	0	0	0
SR[4], n=33	3	10	7	11	1	1
VR[5], n=69 (%)	22 (6.6%)	17 (5.2%)	14 (4.2%)	11 (3.3%)	3 (6.5%)	2 (9.5%)

[1] The censored, as-treated analysis was done using the censored, as-treated population that includes all randomized subjects who received at least one dose of study medication with at least one HIV-1 RNA measurement after Baseline but excludes subjects who discontinued their assigned treatment while they had a suppressed viral load (HIV-1 RNA <50 copies/mL).
[2] VF, virologic failure, is defined as: In Studies 102 and 103, subjects who failed to achieve HIV-1 RNA <50 copies/mL either at Week 48 (snapshot analysis during the 48-week evaluation window [Treatment Days 309 – 378]) or at the time of early discontinuation; and in Study 104, subjects who failed to achieve HIV-1 RNA <50 copies/mL either at the time of study unblinding (Week 60) or at the time of early discontinuation.
[3] NR, virologic non-response, is defined as a <1 $\log_{10}$ copies/mL decrease in HIV-1 RNA from Baseline at the time of early discontinuation without experiencing virologic rebound. All 3 subjects with NR received <10 weeks of treatment.
[4] SR, suboptimal virologic response, is defined as a ≥1 $\log_{10}$ copies/mL decrease in HIV-1 RNA from Baseline but >50 copies/mL at Week 48 (or at the time of early discontinuation) without experiencing virologic rebound. Of the 33 subjects with SR, 31 subjects received <14 weeks of treatment. The remaining 2 subjects (both in Study 103, one treated with E/C/F/T and the other with ATV/r+TVD) showed a >3 $\log_{10}$ copies/mL decline in HIV-1 RNA at Week 48 (both with HIV-1 RNA <200 copies/mL) at Week 48 from Baseline.
[5] VR, virologic rebound, is defined as either confirmed HIV-1 RNA levels >50 copies/mL after HIV-1 RNA levels <50 copies/mL being achieved or confirmed >1 $\log_{10}$ copies/mL increase of HIV-1 RNA from nadir.

3.1. Antiviral Efficacy (Potency and Durability) of E/C/F/T in Studies 102 and 103

The antiviral efficacy (potency and durability) analyses were conducted in the censored, as-treated subject population (n=1325, pooled from the two Phase 3 studies 102 and 103) to determine whether E/C/F/T had potent and durable antiretroviral activity, compared to control treatments (ATR in Study 102 and ATV/r+TVD in Study 103), in treatment-naïve adult subjects with HIV-1 infection. For the overall efficacy of E/C/F/T, please refer to the reviews by Medical Officer Adam Sherwat, M.D. and Statistical Reviewer Wen Zeng, Ph.D. The primary efficacy endpoint was the percentage of subjects with virologic success (HIV-1 RNA <50 copies/mL) at Week 48 using the FDA-defined snapshot analysis. The intent-to-treat population (ITT) was considered the primary analysis population. At Week 48, E/C/F/T was noninferior (noninferiority margin of 12%) in the treatment-naïve, HIV-1-infected subject population both to ATR in Study 102 and to ATV/r+TVD in Study 103 with the differences in the virologic success rate of 3.6% (95% CI: -1.6% to 8.8%) and 3.0% (95% CI: -1.9% to 7.8%), respectively. In Study 102, 87.6% (305/348) of subjects in the E/C/F/T treatment group and 84.1% (296/352) of subjects in the ATR treatment group had virologic success. In Study 103, 89.5% (316/353) of subjects in the E/C/F/T treatment group and 86.8% (308/355) of subjects in the ATV/r+TVD treatment group

had virologic success.

In antiviral efficacy analyses of E/C/F/T in Studies 102 and 103, as summarized in Table 51, the rates of virologic failure (HIV-1 RNA ≥50 copies/mL) at Week 48 were numerically slightly lower or comparable for E/C/F/T recipients, compared to those for ATR and ATV/r+TVD recipients, respectively: 7.8% versus 8.6% and 6.5% versus 6.6%. Of note, plasma HIV-1 RNA levels at Baseline were well matched among the treatment groups (Table 52) with a median baseline HIV-1 RNA level of 4.8 $\log_{10}$ copies/mL for all 3 treatment groups, ranging from 1.7 to 6.6 $\log_{10}$ copies/mL for the subjects in the E/C/F/T treatment arm, from 3 to 6.5 $\log_{10}$ copies/mL for those in the ATR treatment arm, and from 3 to 6.6 $\log_{10}$ copies/mL for those in the ATV/r+TVD treatment arm. Virologic failure of the E/C/F/T recipients was largely due to treatment-emergent virologic rebound (75% of the failures [36/48], pooled from the two studies; Table 51, VR), rather than due to suboptimal response (including nonresponse) to the treatment (25% of the failures [12/48], pooled from the two studies; Table 51, NR+SR). Furthermore, of the 36 E/C/F/T-treated virologic rebounders, 31 subjects achieved virologic suppression initially but failed to maintain suppression as they experiencing virologic breakthrough to HIV-1 RNA >50 copies/mL. Thus, these results indicate that E/C/F/T QD therapy can suppress HIV-1 replication but such antiviral response may not be durable in a small number of virologic failures. When 21 rebounders' resistance data were collected and analyzed, 12 subjects developed amino acid substitutions conferring reduced susceptibility to E/C/F/T (see below Section 3.2. for details). Similarly, in control-drug recipients (ATR or ATV/r+TVD), virologic rebound was also a primary cause of the virologic failure, as 60.7% (17/28) and 50% (11/22) of the virologic failures being virologic rebounders, respectively (Table 51).

Virologic Response to E/C/F/T Treatment by Baseline HIV-1 RNA levels

The correlation between HIV-1 RNA levels at Baseline and antiviral efficacy of E/C/F/T was analyzed. Results are summarized in Table 52. Higher rates of virologic failure and rebound at Week 48 were observed in the subgroup of subjects with higher baseline viral load (negative correlation between baseline HIV-1 RNA levels and antiviral efficacy of E/C/F/T). Compared to overall virologic failure and rebound rates of 7.2% and 5.4%, respectively, in E/C/F/T-treated subjects (pooled from the two studies), subjects with baseline viral RNA levels ≥100,000 copies/mL experienced virologic failure and rebound at higher rates, 12% (31/259) and 9.6% (25/219), respectively. The virologic failure and rebound rates for subjects with baseline viral RNA levels <100,000 copies/mL were 4.1% (17/410) and 2.7% (11/410), respectively. In agreement with these observations, E/C/F/T-treated subjects with virologic failure had slightly higher median baseline viral load than those with virologic suppression (5.2 versus 4.8 $\log_{10}$ HIV-1 RNA copies/mL). Similar negative correlation, however less prominent, was also observed in subjects who received ATR or ATV/r+TVD treatment (Table 52).

Table 52: Correlation between Baseline HIV-1 RNA Level and Virologic Response to Treatment in Studies 102 and 103

Subjects with E/C/F/T (n=669) Median baseline HIV-1 RNA levels of 4.8 $\log_{10}$ copies/mL (range, 1.7-6.6 $\log_{10}$) Overall virologic failure rate of 7.2% and rebound rate of 5.4%		
Baseline HIV-1 RNA ($\log_{10}$ copies/mL)	<5	≥5
Virologic failure rate[1]	4.1% (17/410)	12% (31/259)

Virologic rebound rate[1]	2.7% (11/410)				9.7% (25/259)	
Baseline HIV-1 RNA (log_{10} copies/mL)	<2	≥2 but <3	≥3 but <4	≥4 but <5	≥5 but <6	≥6 (all <7)
Virologic failure rate[1]	0% (0/1)	0% (0/1)	2.9% (2/70)	4.4% (15/338)	10.6% (26/245)	35.7% (5/14)
Virologic rebound rate[1]	0% (0/1)	0% (0/1)	1.4% (1/70)	3% (10/338)	8.6% (21/245)	28.6% (4/14)
Subjects with ATR (n=325) Median baseline HIV-1 RNA levels of 4.8 log_{10} copies/mL (range, 3-6.5 log_{10}) Overall virologic failure rate of 8.6% and rebound rate of 5.2%						
Baseline HIV-1 RNA (log_{10} copies/mL)	<5				≥5	
Virologic failure rate[1]	7.4% (16/217)				11.1% (12/108)	
Virologic rebound rate[1]	4.6% (10/217)				6.5% (7/108)	
Baseline HIV-1 RNA (log_{10} copies/mL)	<2	≥2 but <3	≥3 but <4	≥4 but <5	≥5 but <6	≥6 (all <7)
Virologic failure rate[1]	-	-	3.2% (1/31)	8.1% (15/186)	10.8% (11/102)	16.7% (1/6)
Virologic rebound rate[1]	-	-	3.2% (1/31)	4.8% (9/186)	6.9% (7/102)	0% (0/6)
Subjects with ATV/r+TVD (n=331) Median baseline HIV-1 RNA levels of 4.8 log_{10} copies/mL (range, 3-6.6 log_{10}) Overall virologic failure rate of 6.6% and rebound rate of 3.3%						
Baseline HIV-1 RNA (log_{10} copies/mL)	<5				≥5	
Virologic failure rate[1]	5% (10/201)				9.2% (12/130)	
Virologic rebound rate[1]	2% (4/201)				5.4% (7/130)	
Baseline HIV-1 RNA (log_{10} copies/mL)	<2	≥2 but <3	≥3 but <4	≥4 but <5	≥5 but <6	≥6 (all <7)
Virologic failure rate[1]	-	0% (0/1)	2.9% (1/34)	5.4% (9/166)	9.8% (12/122)	0% (0/8)
Virologic rebound rate[1]	-	0% (0/1)	2.9% (1/34)	1.8% (3/166)	5.7% (7/122)	0% (0/8)

[1] Virologic failure and rebound rates were calculated using the censored, as-treated population that includes all randomized subjects who received at least one dose of study medication with at least one HIV-1 RNA measurement after Baseline but excludes subjects who discontinued their assigned treatment while they had a suppressed viral load (HIV-1 RNA <50 copies/mL).

Virologic Response to E/C/F/T Treatment by HIV-1 Subtype

The majority of subjects in these two Phase 3 studies (Studies 102 and 103) were infected with HIV-1 subtype B, reflecting the US infected population which is over 98% subtype B (Hemelaar *et al.*, 2006). Of note, in 2004 - 2007, subtype B was estimated to contribute to 11% of the worldwide epidemic (Hemelaar *et al.*, 2011). Subtype B was detected in 94.3% of the overall censored, as-treated subject population (1250/1325). In addition, 5 additional pure genetic subtypes (A [n=6], C [n=13], D [n=3], F1 (n=2), and G [n=5]) were found in 2.2% (29/1325) of the studied population and 4 recombinant forms (AE, AG, BF, and complex) in 3.5% (46/1325). In E/C/F/T-treated subjects with HIV-1 non-B subtype infection (n=35), a slightly lower rate of virologic failure was observed compared to those with subtype B infection (5.7% versus 7.3%; Table 53). Baseline HIV-1 RNA levels were comparable between the two subgroups of subjects with median baseline HIV-1 RNA levels of 4.8 log_{10} copies/mL for subtype B-infected subjects (range, 1.7 - 6.6) and of 4.9 log_{10} copies/mL for non-B subtype-infected subjects (range, 3.6 - 6.6). Thus, these limited data indicated that E/C/F/T may be active against non-B subtypes as well as subtype B. However, due to the small sample sizes (≤17 subjects infected with each

detected non-B subtype), it was not conclusively determined whether HIV-1 subtype-specific differences could affect the antiviral efficacy of E/C/F/T.

Table 53: Virologic Response to Treatment by HIV-1 Subtype in Studies 102 and 103

HIV-1 Subtype	Virologic failure rate[1]			
	Overall	Treatment		
		E/C/F/T	ATR	ATV/r
B	7.4% (93/1250[2])	7.3% (46/634)	8.4% (27/320)	6.8% (20/296)
Non-B	6.7% (5/75)	5.7% (2/35)	20% (1/5)	5.7% (2/35)
AE	5.9% (1/17)	0% (0/8)	0% (0/2)	14.3% (1/7)
AG	6.7% (1/15)	12.5% (1/8)	-	0% (0/7)
C	0% (0/13)	0% (0/3)	0% (0/1)	0% (0/9)
Complex	7.7% (1/13)	0% (0/9)	100% (1/1)	0% (0/3)
Others (A, A1, BF, D, F1, G)	11.8% (2/17)	14.3% (1/7)	0% (0/1)	11.1% (1/9)

[1] Virologic rates were calculated using the censored, as-treated population that includes all randomized subjects who received at least one dose of study medication with at least one HIV-1 RNA measurement after Baseline but excludes subjects who discontinued their assigned treatment while they had a suppressed viral load (HIV-1 RNA <50 copies/mL).
[2] In Studies 102 and 103, 98.2% (645/657) and 90.6% (605/668) of the censored, as-treated subjects populations were infected with subtype B, respectively. Of the subjects receiving E/C/F/T, ATR, or ATV/r+TVD, 94.8% (634/669; 97.9% [325/332] in Study 102 and 91.7% [309/337] in Study 103), 98.5% (320/325), and 89.4% (296/331) had HIV-1 subtype B infection, respectively.

The HIV IN protein was reported to be a well conserved protein across HIV-1 groups and subtypes, exhibiting 96% and 94% identity within and between group M (main) subtypes, respectively, when comparing the IN amino acid sequence of 572 individual samples (Hackett *et al*., 2005). IN identity between groups M and O (outlier), O and N (non-M, non-O), and M and N averaged 82%, 80%, and 88%, respectively. Using the purified recombinant subtype-B and -C IN enzymes, Bar-Magen *et al*. (2009) showed similar inhibitory activities of EVG in inhibition of strand transfer using an Mn^{++}-based assay with IC_{50} values of 14 nM and 18 nM, respectively. However, several studies suggested that subtype-dependent IN sequence variability at an amino acid level may affect structure and substrate binding characteristics of IN enzymes, and thus might be important in development of INSTI resistance (Brenner *et al*., 2011; Malet *et al*., 2008; Myers and Pillay, 2008; Wainberg *et al*., 2011). Furthermore, some sequence variations in subtypes A, CRF02_AG, and C might not promote resistance through the Q148H/K/R+G140S pathway (Brenner *et al*., 2011; Maiga *et al*., 2009). Of note, all 24 E/C/F/T-treatment virologic failures with evaluable resistance data for EVG were infected with HIV-1 subtype B.

In addition, the genetic diversity of HIV-1 may also play a role in the emergence of drug-resistance substitutions to NNRTIs, NRTIs, and PIs (reviewed by Santos and Soares, 2010). In cell culture, the K65R substitution in the HIV-1 RT protein was more rapidly selected in subtype C isolates than subtype B, while times to the appearance of M184V under LAM pressure did not vary among subtypes (Brenner *et al*., 2006). Of note, a total of 14 subjects in the three pooled studies (13 from Studies 102 and 103, and 1 from Study 104) were infected with HIV-1 subtype C and none experienced virologic failure.

3.2. Clinical Resistance in Studies 102, 103, and 104

Drug resistance testing, genotypic and phenotypic, was performed for viruses (pre-treatment

and on-treatment) collected from resistance testing eligible (RTE) subjects who experienced virologic failure with HIV-1 RNA ≥400 copies/mL (approximate limit of detection) at Week 48 or at the time of early study drug discontinuation to assess whether the virologic failures developed during therapy amino acid substitutions associated with resistance to the individual antiretroviral components of the regimens.

Of the 1392 treated subjects in the censored, as-treated, pooled population of the three studies (Studies 102, 103, and 104; Table 54), 105 subjects (7.5%) showed evidence of virologic failure (HIV-1 RNA ≥50 copies/mL) by Week 48 (Studies 102 and 103) or at Week 60 (Study 104), and 63 of them were eligible for resistance testing with plasma HIV-1 RNA levels ≥400 copies/mL at the time of virologic failure (or later while still on treatment). Evaluable genotypic and/or phenotypic resistance data were obtained from 45 (71%) of the 63 resistance-testing eligible (RTE) subjects (Table 54): IN data from 24 subjects treated with an EVG-containing regimen for EVG resistance analysis; RT data from 15 subjects treated with an EFV-containing regimen for EFV resistance analysis; and PR data from 6 subjects treated with an ATV/r-containing regimen for ATV/r resistance analysis. In addition, RT data obtained from 43 RTE subjects receiving any of the 3 treatment regimens were utilized for FTC/TDF resistance analysis.

Table 54: Number of Subjects with Evaluable Resistance Data in Studies 102, 103, and 104

Study (n=1392[1])	**102** (n=657)		**103** (n=668)		**104** (n=67)	
Treatment	E/C/F/T (n=332)	ATR (n=325)	E/C/F/T (n=337)	ATV/r+TVD (n=331)	E/C/F/T (n=46)	ATR (n=21)
VF[2], n=105	26	28	22	22	4	3
Resistance testing eligible (RTE[3]), n=63	17	21	14	10	1	0
Genotypic Data (Population-based)						
IN-coding region	14	-	9		1	-
PR-coding region	14	-	7	6	1	-
RT-coding region	14	15	7	6	1	-
Phenotypic data (cell-based drug susceptibility)						
ATV	-	-	-	5	-	-
EFV	-	15	-	-	-	-
EVG	14	-	9	-	1	-
FTC	14	15	7	5	1	-
RTV	-	-	-	5	-	-
TFV	14	15	7	5	1	-

[1] Drug resistance analysis was done using the censored, as-treated population that includes all randomized subjects who received at least one dose of study medication with at least one HIV-1 RNA measurement after Baseline but excludes subjects who discontinued their assigned treatment while they had a suppressed viral load (HIV-1 RNA <50 copies/mL).
[2] VF, virologic failure, is defined as: In Studies 102 and 103, subjects who failed to achieve HIV-1 RNA <50 copies/mL either at Week 48 (snapshot analysis during the 48-week evaluation window [Treatment Days 309 – 378]) or at the time of early discontinuation; and in Study 104, subjects who failed to achieve HIV-1 RNA <50 copies/mL either at the time of study unblinding (Week 60) or at the time of early discontinuation.
[3] RTE, resistance-testing eligible, is defined as subjects who experienced virologic failure and had plasma HIV-1 RNA levels ≥400 copies/mL at the time of virologic failure (or later while still on treatment).

3.2.1. Development of EVG Resistance

To date, as summarized in Table 55, many IN amino acid substitutions have been reported to be detectable in EVG-treatment failure subject isolates (HIV Drug resistance Database, Stanford University; reviewed by Lampiris, 2012; McColl *et al*., 2007; Molina *et al*., 2012) and/or selected by passage of virus in cell culture in the presence of EVG (see above Section 2.6.1.). Some substitutions listed in Table 55 (e.g., T66A/I/K, E92Q, Y143C/H/R, Q148H/K/R, N155H) were shown to be the primary causes of EVG and/or RAL resistance, while others were frequently detected in treatment failure isolates but could confer only small or no decreases in EVG and/or RAL susceptibility. These latter substitutions in general decreased susceptibility further in cell culture when combined with other substitutions including the primary substitutions associated with EVG and RAL resistance. In Study GS-US-183-0105 (McColl *et al*., 2007), the applicant noted 7 IN substitutions, T66A/I/K, L68I/V, E92Q, E138A/D/K, S147G, Q148H/R/K, and N155H, to emerge most frequently in pooled EVG-treatment virologic failures with evaluable paired genotypic data (each of these substitutions were detectable in ≥15% of the tested subjects). Study GS-US-183-0105 was a Phase 2, randomized, active-control, partially blinded (dose of EVG), dose-ranging, 48-week, non-inferiority study of ritonavir-boosted EVG (EVG/r) versus ritonavir-boosted comparator PI (PI/r), each in combination with an optimized background therapy (OBT), in ARV-experienced, HIV-1 infected subjects. Using the PhenoSense INI assay (Monogram Biosciences) and site-directed mutagenesis, the applicant showed T66A/I/K, E92Q, S147G, Q148H/K/R, and N155H substitutions conferred individually ≥4.8-fold reduced susceptibility to EVG (ranging from 4.8- to >107.6-fold; see Table 46 in Section 2.7.1. for details). Thus, these 5 substitutions T66A/I/K, E92Q, S147G, Q148H/R/K, and N155H were identified as primary EVG resistance-associated (EVG^R) substitutions (reviewed by Lampiris, 2012). Of note, 4 of the 5 primary EVG^R substitutions (T66I, E92Q, Q148R, and N155H) were repeatedly detected in E/C/F/T-treatment failures (see below Table 57). The remaining 2 substitutions noted in Study GS-US-183-0105, L68I/V and E138A/D/K, were mostly (100% and 96%, respectively) found in the virus population containing one or more of the 5 primary EVG^R substitutions. Furthermore, both substitutions had little effect individually on EVG susceptibility (≤1.5-fold change; Table 46).

Table 55: INSTI Resistance-Associated IN Substitutions Observed in Cell Culture or in INSTI-treatment Failure Subjects

INSTI	Substitutions in the HIV-1 IN protein
EVG[1] (n=34)	D10E, S17N, H51Y, T66A/I/K, L68I/V, V72A/I/N/T, L74M, E92Q/G/V, Q95K/R, T97A, H114Y, S119G/R, F121C/Y, A128T, E138A/D/K, G140A/C/S, Y143C, P145S, Q146I/K/L/P/R, S147G, Q148H/K/R, V151A/I, S153A/F/Y, N155H/S, E157K/Q, K160N, G163R, V165A/I, R166S, E170A, S230R, D232E/G/H/N, R263K, V281M
RAL[2] (n=15)	L74M, E92Q, Q95K/R, T97A, E138A/K, G140A/S, Y143C/H/R, Q148H/K/R, V151I, N155H, G163R, H183P, Y226D/F/H, S230R, D232N
Cumulative INSTI resistance-associated IN substitutions (n=36)	D10E, S17N, H51Y, T66A/I/K, L68I/V, V72A/I/N/T, L74M, E92Q/G/V, Q95K/R, T97A, H114Y, S119G/R, F121C/Y, A128T, E138A/D/K, G140A/C/S, Y143C/H/R, P145S, Q146I/K/L/P/R, S147G, Q148H/K/R, V151A/I, S153A/F/Y, N155H/S, E157K/Q, K160N, G163R, V165A/I, R166S, E170A, H183P, Y226D/F/H, S230R, D232E/G/H/N, R263K, V281M

[1] Substitutions (primary substitutions are written in blue) were observed in cell culture or in subjects treated with EVG (HIV Drug resistance Database, Stanford University; Lampiris, 2012; McColl *et al*., 2007; Molina *et al*., 2012).
[2] Substitutions (primary substitutions are written in red) can be found in the ISENTRESS® US Prescr bing Information (revised in March, 2012).

3.2.1.1. EVG Treatment-Emergent Amino Acid Substitutions in HIV-1 IN

In order to identify treatment-emergent genotypic changes (amino acid substitutions) and/or confirm the previously observed EVG^R substitutions in HIV-1 IN, EVG resistance analyses were conducted. As summarized in Table 54, 715 subjects (pooled from Studies 102, 103, and 104) received an EVG-containing regimen (EVG/COBI/FTC/TDF) and 52 of them showed evidence of virologic failure (HIV-1 RNA ≥50 copies/mL) by Week 48. Thirty-two subjects were eligible for EVG resistance testing, and evaluable IN genotypic/phenotypic data were obtained from 24 of those. Thirty on-treatment failure sample IN genotypic data from the 24 subjects (6 of them had multiple failure isolates at different time points) were compared to their respective pre-treatment sample genotypic data.

HIV-1 variants harboring EVG-treatment emergent amino acid substitutions in the HIV-1 IN protein were detected in failure virus samples from 20 of the 24 examined subjects (83.3%, Table 56; see Appendix 2 Table A2-1 for individual resistance data). These failure isolates with IN genotypic changes had reductions in susceptibility to EVG ranging from 1- to >198-fold that of wild-type reference HIV-1 (Table 56; when compared to the subject's baseline isolate, 1.2- to 142-fold increases in EC_{50} values). Of note, the applicant stated that the biological cutoff for EVG is 2.5-fold in the PhenoSense assay (Monogram Biosciences). Rondelez *et al*. (2008) also reported the biological cutoff fold-change value for EVG of 2.21 determined as the 97.5th percentile of the normal distribution in the assay (different from the Monogram PhenoSense assay) using INSTI-naïve recombinant viruses. In agreement with these findings, all failure isolates from 4 subjects with no detectable IN genotypic changes (Table 56) had fold-change values in EVG susceptibility below the biological cutoff (ranging from 1.1- to 1.8-fold).

Table 56: Summary of Pooled EVG Resistance Analysis in 24 E/C/F/T-Treated Virologic Failures with Evaluable Resistance Data

Genotypic changes in HIV-1 IN	Number of subjects	EVG Susceptibility (fold- reduction[1])
None	4[2]	1.1 - 1.8
With EVG-treatment emergent substitution(s)	20	1 - >198
• Previously observed EVG^R substitution(s)[3]	11	5.6 - >198
T66I	2	5.6 - 54.3
E92Q	8	5.6 - 166.9
Q148R	3	22.8 - >198
N155H	3	19.7 - 54.3
• without previously observed substitution(s)[4]	9	1 - 2.1

[1] Fold-reduction in EVG susceptibility of virologic failure isolates compared to wild-type reference HIV-1.
[2] One subject (Subject 2475-4507 in Study 104) was included who had genotypic data only from a virologic failure isolate. This subject's Week-12 failure isolate did not harbor detectable IN substitutions associated with resistance to EVG and RAL (see above Table 55 for a cumulative list of INSTI resistance-associated IN substitutions).
[3] EVG^R IN substitutions that were previously observed in EVG-treatment failure subject isolates and/or selected by passage of virus in cell culture in the presence of EVG are listed in Table 55.
[4] Any IN substitutions other than those associated with INSTI resistance listed in Table 55.

A total of 42 amino acid residues (14.6% of the IN 288 residues; Table 57) were found to be substituted in the E/C/F/T treatment-failure viruses collected from the 24 subjects with evaluable IN genotypic data, with the average number of substitutions per isolate of 2.2 ± 2.0 (ranging

from 0 to 8, median of 2). Of those 42 changes, 27 substitutions occurred at HIV-1 IN conserved amino acid positions. Most substitutions (92.9% [39/42]) were not observed commonly, as these were detected only in one (n=33) or two (n=6) failures (Table 57; also see Appendix 2 Table A2-1 for individual resistance data). The remaining 3 substitutions at IN amino acid positions 92 (E92Q), 148 (Q148R), and 155 (N155H) were observed in ≥3 subjects (8, 3, and 3 occurrences each, respectively). Including these 3 frequently observed substitutions, 11 of the 42 substitutions (Table 57, written in blue) were previously identified as INSTI resistance-associated substitutions listed in Table 55: 4 primary EVG^R substitutions (T66I, E92Q, Q148R, and N155H) and 7 additional substitutions (H51Y, I68I/V, G140C, S153A, E157Q, V165I, and H183P).

Table 57: Frequency and Position of Individual Amino Acid Changes Selected in 30 E/C/F/T-Treatment Failure Isolates from 24 Confirmed Virologic Failures with Evaluable Genotypic Data

Occurrence	Treatment-emergent substitutions at amino acid position in HIV-1 IN while on EVG	
	Polymorphic positions[1]	Nonpolymorphic positions
1	6, 31, 45, 50, 57, **157**, 163, **165**, 167, 193, 255, 269, 281 (n=13)	35, 46, 47, **51**, 65, 70, 73, 79, 83, 87, **140**, **153**, 162, **183**, 189, 210, 212, 262, 263, 279 (n=20)
2	14, 284 (n=2)	**66**, **68**, 198, 242 (n=4)
3	-	**148**, **155** (n=2)
8	-	**92** (n=1)
Observed substitutions in HIV-1 IN	D6N, (K14R, 2x), V31A, L45I, M50I, S57G, E157Q, G163S, V165I, D167E, E193G, S255G, R269G, V281A, (G284R, R284G)	E35K, K46R, G47E, H51Y, C65G, (T66I, 2x), (L68I, L68V), G70R, I73V, V79A, Y83C, E87K, (E92Q, 8x), G140C, (Q148R, 3x), S153A, (N155H, 3x), I162V, H183P, G189R, (E198D, E198K), T210I, E212D, (L242F, 2x), R262G, R263G, D279N

[1] Polymorphic residues were identified by levels of amino acid sequence variations occurring in the general population by Heckett *et al*. (2005): frequencies of ≥2% were considered polymorphic in the phylogenetic analysis of 497 HIV-1 group M isolates (76 subtype A, 81 subtype B, 70 subtype C, 43 subtype D, 15 subtype F, 10 subtype G, 1 subtype H, 48 CRF01_AE, 97 CRF02_AG, 56 MOSAIC).

Without drug selective pressure, amino acid substitutions in HIV-1 IN appeared to occur less frequently with the average number of changes per subject of 1 ± 1.6 (ranging from 0 to 6, median of 0), when paired IN genotypic data of pre- and on-treatment virus isolates from 23 subjects who were treated with ATR (n=16) or ATV/r+TVD (n=7) were analyzed. A total of 22 IN amino acid residues (7.6% of the IN 288 residues) were randomly substituted in 9 of the 23 evaluated subjects. The 22 observed substitutions are H12R, K34R, S39N, D41N, L45I, M50I, A86V, T93I, T112I, V113I, T122I, A125V, K188R, T210I, A/D/E/K/N/T212A/D/E/K/N/T/G/R/S, T218S, L234F, L242F, D253G, P261S, K264I, and D286N. All but one (amino acid position 234) were observed once. A substitution at codon 234 (L234F) occurred in 2 ATR-treated subjects in Study 102. It was noted that amino acid substitutions observed in the E/C/F/T-treatment failure isolates were significantly different from those with subjects receiving regimens lacking EVG (ATR or ATV/r+TVD) with only 5 amino acid substitutions, L45I, M50I, T210I, E212D, and L242F (Table 57, written in green), being found in both the examined subject populations. Thus, the limited overlap between the two treatment-emergent IN substitution profiles and greater frequency of substitutions indicate that some substitutions observed in the E/C/F/T treatment-failure viruses, despite their apparent infrequency (once or twice detection),

may be clinically significant in conferring reduced susceptibility to EVG.

Among the 42 observed substitutions in the E/C/F/T treatment-failure viruses, other than those previously observed in INSTI-treatment failures and those selected without EVG selection pressure (Table 57, written in blue and green, respectively), there were only 3 substitutions that were detectable in at least 2 subjects (Table 57, written in red). A lysine-to-arginine change at polymorphic position 14 (K14R) emerged while on EVG in 2 subjects and alone was not sufficient to confer a significant reduction in susceptibility to EVG. One subject isolate harbored K14R with no other detectable IN substitutions displayed a 1.8-fold reduction in EVG susceptibility, while in the other subject isolate showing >198-fold reduced susceptibility to EVG, multiple IN substitutions, in addition to K14R, including Q148R were detected. Substitutions at conserved-site position 198 (E198K and E198D) developed also in 2 subjects with no detectable primary EVG^R substitutions. The virus population harboring E198D (with additional V79A and G163S substitutions) exhibited 1.7-fold reduced susceptibility to EVG. Different amino acid substitutions at polymorphic-site position 284 (G284R and R284G) were seen in 2 failure isolates. G284R conferred 1.3-fold reduced susceptibility to EVG, while the isolate harboring R284G also selected E92Q and displayed a 27.9-fold reduction in EVG susceptibility. Thus, these observations indicated no direct contribution of these amino acid substitutions to reduced susceptibility to EVG but their clinical importance in EVG resistance still remained unclear.

3.2.1.2. Primary EVG Resistance-Associated (EVG^R) IN Substitutions

As summarized in Table 56, the primary EVG^R substitutions were found in 11 of the 24 E/C/F/T-treatment failures with evaluable genotypic data (45.8% of evaluated EVG/COBI/FTC/TDF-treatment failures). These substitutions include T66I (n=2), E92Q (n=8), Q148R (n=3), and N155H (n=3), and isolates harboring these substitutions had reduced susceptibility to EVG (5.6- to >198-fold compared to wild-type reference HIV-1; Table 56). Of the previously identified 5 primary EVG^R substitutions, S147G was not detectable in these studies. E92Q was most frequently found, occurring in 8 subjects (Table 58): 5 subjects had a single E92Q change and 3 subjects had mixtures of E92Q+T66I, E92Q+T66I+N155H, or E92Q+Q148R+N155H in their virus samples (one subject each). The other primary substitutions T66I, Q148R, and N155H were detected in 2, 3, and 3 subjects, respectively (Table 58). Both subjects with T66I had mixtures of T66I+E92Q or T66I+E92Q+N155H in their virus samples. A single Q148R change was detected in 2 subjects and one subject had mixtures of Q148R+E92Q+N155H in his virus samples, while a single N155H change was observed in one subject and mixtures of N155H+ T66I+E92Q or N155H+E92Q+Q148R in 2 subjects. Additional IN substitutions were detected, in addition to the primary EVG^R substitutions, including previously observed IN substitutions associated with INSTI resistance (listed in Table 55), H51Y, L68I/V, G140C, S153A, E157Q, V165I, and H183P.

Table 58: Resistance Data Summary of Virus Isolates Harboring the Primary EVG^R IN Substitutions

Number of subjects	Primary EVG^R IN substitution	Additional IN substitution			EVG Susceptibility (fold-reduction[1])
		Previously observed	Polymorphic[2]	Non-polymorphic	
5	E92Q	H51Y, L68I/V, S153A	M50I, D167E, E193G, R284G	K46R, I73V, I162V, G189R, T210I, L242F	27.9 - 166.9

2	**Q148R**	G140C, V165I, H183P	K14R	none	22.8 - >198
1	**N155H**	none	none	D279N	36.2
1	**T66I+E92Q**	none	D6N, S57G	none	5.6
1	**T66I+E92Q+N155H**	E157Q	none	C65G, G70R	19.7 - 54.3
1	**E92Q+Q148R+N155H**	none	none	none	50.8

[1] Fold-reduction in EVG susceptibility of virologic failure isolates compared to wild-type reference HIV-1.
[2] Polymorphic residues were identified by levels of amino acid sequence variations occurring in the general population by Heckett *et al.* (2005): frequencies of ≥2% were considered polymorphic in the phylogenetic analysis of 497 HIV-1 group M isolates (76 subtype A, 81 subtype B, 70 subtype C, 43 subtype D, 15 subtype F, 10 subtype G, 1 subtype H, 48 CRF01_AE, 97 CRF02_AG, 56 MOSAIC).

3.2.1.3. IN Substitutions Emerged in the Absence of Primary EVG^R Substitutions

Of the 24 E/C/F/T-treatment failures with evaluable genotypic data, failure variants collected from 9 subjects (Table 56; see Appendix 2 Table 2A-1 for individual resistance data) had one or more treatment-emergent IN substitutions in the absence of detectable INSTI^R substitutions (listed in Table 55). These substitutions include K14R, V31A, E35K, L45I, G47E, V79A, Y83C, E87K, G163S, E198D/K, E212D, L242F, S255G, R262G, R263G, R269G, V281A, and G284R (Table 57). The subjects-derived recombinant viruses displayed <2.1-fold reductions in EVG susceptibility (below the biological cutoff for EVG). Thus, these 18 IN substitutions observed with the 9 subjects are unlikely to confer reduced susceptibility to EVG and to exert their effect on virologic response to EVG. Therefore, no new IN substitutions were identified that may be associated with virologic failure to EVG therapy and EVG resistance in these pooled studies, other than those previously identified to be associated with EVG resistance.

3.2.1.4. Cross-Resistance with RAL

The phenotypic susceptibility in cell culture of all isolates that were tested for EVG resistance in the 3 studies, Studies 102, 103, and 104, were also assessed for RAL. With a common mechanism of action against HIV-1 IN (reviewed by Mouscadet *et al.*, 2010) and overlapping genetic pathways of resistance to EVG and RAL, cross-resistance between EVG and RAL should be expected. The cell-based cross-resistance studies showed significant and broad cross-resistance between EVG and RAL (see Section 2.7.1. for details). HIV-1 containing the primary EVG^R substitutions E92Q, Q148H/K/R, or N155H had reductions in susceptibility not only to EVG (4.8- to 107.6-fold) but also to RAL (5.8- to 34-fold), while viruses harboring T66I or S147G EVG^R substitutions showed reduced susceptibility to EVG (6.8- to 30.8-fold) but relatively smaller decreases to RAL (1.2- to 2.3-fold).

Phenotypic sensitivities to EVG and RAL were assessed using the PhenoSense® HIV-1 Integrase Assay (Monogram Biosciences) of failure isolates from 23 of the 24 E/C/F/T-treatment failures with evaluable genotypic data virologic failures in the 3 studies (Table 59). No evaluable data from Subject 2838-6264 in Study 102 who experienced virologic rebound at Week 40 were available for the analysis and the subject's Week-48 isolate had a single E198K IN substitution. As summarized in Table 59, all failure isolates genotypically resistant to EVG with evidence of emerging primary EVG^R substitutions were phenotypically resistant not only to EVG with >2.5-fold reduced susceptibility (above the biological cutoff for EVG) but also to RAL with >1.5-fold reduced susceptibility (above the biological cutoff for RAL; Fransen *et al.*, 2008).

Furthermore, isolates with no genotypic changes or only with substitutions not associated with EVG resistance in the IN protein appeared to remain susceptible to EVG and to RAL with <2.5-fold (ranging from 1- to 2.1-fold) and <1.5-fold (ranging from 0.8- to 1.4-fold) reductions in susceptibility, respectively. These results observed with subject-derived treatment failure isolates confirmed the cell-based cross-resistance findings of broad cross-resistance between EVG and RAL, and thus, the sequential use of these INSTIs can not be a valid treatment option for HIV-1 infection. Recently, Garrido *et al*. (2012) also reported a high degree of cross-resistance between EVG and RAL when virus samples from HIV-infected patients failing on RAL-containing regimens were collected at the time of virologic failure and analyzed.

Table 59: Drug Susceptibility to EVG and RAL of Virologic Failure Isolates

Genotypic changes in HIV-1 IN	Number of subjects	Drug susceptibility (fold-reduction[1])	
		EVG	RAL
None	4	1.1 - 1.8	0.8 - 1.1
With EVG-treatment emergent substitution(s)	19	1 - >198	0.8 - 28.1
• with primary EVG^R substitution(s)[2]	11	5.6 - >198	1.8 - 28.1
• without previously observed substitution(s)[3]	8	1 - 2.1	0.8 - 1.4

[1] Fold-reduction in drug susceptibility of virologic failure isolates compared to wild-type reference HIV-1.
[2] Primary EVG^R IN substitutions observed in these E/C/F/T trials: T66I, E92Q, Q148R, and N155H.
[3] Any IN substitutions other than those associated with INSTI resistance listed in Table 55.

Of the 5 primary EVG^R substitutions, the T66A/I/K and S147G substitutions have never been detected in subjects failing on RAL-containing regimens in Phase 3 clinical trials of RAL, BENCHMRK, STARTMRK, and SWITCHMRK: a total of 149 virologic failures with evaluable genotypic data were analyzed. The cell-based phenotypic studies by site-directed mutagenesis (see Section 2.7.1., Table 46) demonstrated that T66A/I/K substitutions could confer reduced susceptibility to RAL (1.2- to 19.4-fold reduction), while HIV-1 harboring S147G remained susceptible to RAL (1.2-fold reduction). Of note, the S147G substitution did not emerge in the failure isolates examined in the 3 E/C/F/T studies and thus clinical phenotypic resistance to RAL of patient-derived virus samples harboring this substitution was not determined. Of the 3 primary RAL resistance-associated (RAL^R) substitutions, the Y143C/H/R substitution was rarely detected in EVG-treatment failures. No subjects on E/C/F/T developed the Y143C/H/R substitutions in the 3 E/C/F/T trials. One occurrence was noted in a subject failing on a RTV-boosted EVG-containing regimens who developed a Y143C substitution (over 200 failures were examined). A previous report of a tyrosine-to-cysteine change at IN position 143 (Y143C) did not find a large reduction in susceptibility to EVG (0.7-fold decrease in the EC_{50} value; Fenwick *et al*., 2011). The applicant reported 1.6- and 5.4-fold reductions in EVG susceptibility with HIV-1 harboring Y143H and Y143R, respectively (see Section 2.7.1., Table 46). The Y143C substitution does not appear to be a significant resistance pathway for EVG. However, its significance with respect to cross-resistance is unclear at this time.

3.2.1.5. Baseline Polymorphism of the HIV-1 IN Protein

Lataillade *et al*. (2007) reported naturally occurring IN polymorphism (64% of the 288 codons) by analyzing 243 IN-coding domains of HIV-1 subtype B, IN inhibitor-naïve clinical strains. Similar to the Zioni and coworkers' finding (2007), many amino acid substitutions associated

with resistance to HIV-1 INSTIs were shown to occur as naturally occurring polymorphism in the HIV-1 IN domain: V72I, T97A, A128T, E138K, Q148H, V151I, S153A/Y, N155H, E157Q, G163R, V165I, and R263K. Substitutions Q148H and N155H as naturally occurring polymorphisms are particularly interesting, since both are associated with primary resistance to EVG and RAL. These findings indicated that IN polymorphism may affect clinical responses to HIV-1 INSTIs in patients. Thus, IN genotypic data of the viruses collected at Baseline in Studies 102, 103, and 104 were analyzed to determine the occurrence of IN polymorphism in these pooled study population.

In the three pooled studies, when 50 pre-treatment isolates (baseline or screening) from 49 subjects (25, 16, and 8 subjects treated with E/C/F/T, ATR, and ATV/r+TVD, respectively), 37.8% (109/288) of the 288 codons in the IN protein were polymorphic, and 11 polymorphisms were previously reported to be associated with resistance to HIV-1 INSTIs (listed in Table 55): D10E, S17N, V72I, L74M, T97A, S119G/R, A128T, E138D, E157Q, V165I, and D232E. No primary EVG^R substitutions were detectable as naturally occurring polymorphisms in these ARV-naïve subjects. The applicant obtained phenotypic data for EVG on 26 of the 50 baseline isolates. All but one isolates showed <2-fold reductions in EVG susceptibility: one isolate with multiple-site polymorphism including 3 resistance-associated polymorphisms E10, I72, and Q157 showed a 3-fold reduction in EVG susceptibility and was collected from a subject in Study 102 who received ATR and achieved virologic suppression. Of the 26 pre-treatment isolates from 25 subjects who received E/C/F/T, EVG resistance-associated polymorphisms, E10, N17, I72, and/or I165, were detectable in 23 isolates and all 12 of them with evaluable phenotypic data had <2-fold reductions in EVG susceptibility (ranging from 0.9- to 1.6 fold). Since these pre-treatment IN genotypic data were obtained largely from virologic failures (87.8% [43/49]), it is not possible to determine whether the presence of any specific polymorphisms at baseline, EVG resistance-associated polymorphisms in particular, had negative effects on the response to E/C/F/T treatment.

3.2.2. Development of Resistance to FTC/TDF

In Studies 102, 103, and 104, all subjects received a TRUVADA® (TVD) background treatment. At screening, all subjects were tested for genotypic sensitivity to FTC and TDF. Based on a reference list of NRTI resistance-associated amino acid substitutions (IAS-USA, 2011; Table 60), none had baseline HIV-1 harboring RT substitutions associated with primary resistance to FTC and TDF (K65R, K70E, M184I/V), while one or two TAMs (thymidine-associated mutations) were detectable in 39 subjects' baseline isolates (14, 14, and 11 subjects in the E/C/F/T, ATR, and ATV/r+TVD treatment groups, respectively). No phenotypic data for these baseline isolates with TAMs were available for analysis. All but one subjects with baseline TAM(s) achieved virologic suppression by Week 48. One subject (Subject 2475-6378 treated with E/C/F/T in the Study 102) whose baseline isolate harbored 1 TAM, D67N, achieved virologic suppression at Week 4 but experienced transient virologic rebounds of low-level viremia (HIV-1 RNA <400 copies/mL) at Weeks 40 and 48. However, the subject resuppressed at Week 60.

Table 60: Baseline Substitutions in HIV-1 RT Associated with FTC and TDF Resistance

NRTI	RT substitutions associated with resistance (IAS-USA, 2011)	Number of subjects found in the censored, as-treated study population
FTC	K65R, M184I/V	0

TDF	K65R, K70E		0		
Multi-NRTIs	**TAMs**[1]	M41L, D67N, K70R, L210W, T215Y/F, K219E/Q	39	1 TAM	30
				2 TAMs	9
	Q151M complex[2]	Q151M + A62V, V75I, F77L, and F116Y	0		
	T69 insertion complex[3]	T69 insertion + M41L, A62V, K70R, L210W, T215Y/F, and/or K219E/Q	0		

[1] TAMs (thymidine-associated mutations) are selected by thymidine analogues: M41L, D67N, K70R, L210W, T215Y/F, and K219Q/E.
[2] Q151M together with a cluster of four additional substitutions (A62V/V75I/F77L/F116Y)
[3] T69 insertion in combination with multiple TAMs

Of the 105 virologic failures in the virology analyses of these 3 studies, 43 subjects' RT resistance data were obtained. The emergence of RT substitutions associated with FTC and TDF resistance (listed in Table 60) were observed in 32.6% (14/43) of the evaluated failures: 12 (12/22; 54.5%) and 2 (2/15; 13.3%) subjects treated with E/C/F/T and ATR, respectively, and none with ATV/r+TVD (see Appendix 2 Tables A2-1, -2, and -3, respectively, for individual resistance data). As summarized in Table 61, all 14 subjects developed M184I/V, a primary FTC resistance-associated (FTC^R) substitution, and their failure isolates became resistant to FTC with >42-fold reductions in FTC susceptibility. In 6 of these 14 subject isolates (including 4 E/C/F/T recipients; Table 61), K65R associated with both FTC and TDF resistance was also detectable. Three of the 6 isolates with the K65R substitution in addition to M184I/V demonstrated reduced susceptibility both to FTC (>84-fold) and tenofovir (1.5- to 1.8-fold; TFV). Of note, the clinical cutoffs for FTC and tenofovir were determined to be 3.5- and 1.4-fold changes in EC_{50} values, respectively (Monogram Biosciences, 2011). The remaining 29 subjects had failure isolates with no genotypic changes (n=7) in RT or treatment-emergent RT substitutions that were not associated with resistance to any NRTIs with <1.7-fold and <1.3-fold reduced susceptibility to FTC and TFV, respectively. RT substitutions associated with resistance to NRTIs include M41L, A62V, K65R, D67N, T69 insertion, K70E/R, L74V, V75I, F77L, Y115F, F116Y, Q151M, M184I/V, L210W, T215F/Y, K219E/Q (IAS-USA, 2011). None of the examined 43 subjects developed RT substitutions that can confer multi-NRTI resistance (listed in Table 61, "Others").

Table 61: Resistance Data Summary of Treatment-Failure Isolates Harboring RT Substitutions Associated with Resistance to FTC and TDF

RT substitutions associated with FTC and TDF resistance[1]	Number of subjects by treatment				Drug susceptibility (fold-change[2])	
	E/C/F/T	ATR	ATV/r+TVD	Total	FTC	TFV
M184I/V	8	0	0	8	>75	0.4 - 0.7
K65R + M184I/V	4	2	0	6	42.2 - >129	0.8 - 1.8
Others[3]	0	0	0	0	-	-

[1] RT substitutions associated with FTC and TDF resistance include substitutions listed in Table 60.
[2] Fold-change in drug susceptibility of virologic failure isolates compared to wild-type reference HIV-1.
[3] Others include K70E, TAMs, Q151M complex, T69 insertion complex listed in Table 60.

In conclusion, 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. The lower development of M184I/V resistance-associated substitutions in the ATR treatment arm is consistent with other studies of this drug combination (Margot *et al*., 2009; McColl *et al*., 2007). In Study 934, a three-year study comparing a regimen of EFV+FTC+TDF to EFV+COMBIVIR® (fixed-dose combination tablet containing AZT 300 mg and LAM 150 mg) in ART-naïve, HIV-1-infected subjects, the authors noted significantly fewer subjects on EFV+FTC+TDF compared to EFV+COMBIVIR developed the M184V/I RT substitution associated with resistance to FTC and LAM by Week 144: 10.5% (2/19) versus 34.5% (10/29) of virologic failures with evaluable genotypic data on EFV+FTC+TDF and EFV+COMBIVIR, respectively. However, the frequency of development of EFV^R RT substitutions (including A98G, K101E, K103E/N, V179D, Y188H, G190A/S, M230L) between the treatment groups was not significantly different through Week 144: 68.4% (13/19) and 72.4% (21/29).

All 14 isolates harboring the M184I/V RT substitution exhibited cross-resistance to lamivudine with >43.7-fold reduced susceptibility (above the clinical cutoff of 3.5-fold; Monogram Biosciences, 2011), and some to didanosine with 1.4- to 3.7-fold reduced susceptibility (n=11; above the clinical cutoff of 1.3-fold). In addition, 3 of the 6 isolates harboring the K65R RT substitution in addition to M184V/I showed cross-resistance to multiple NRTIs, abacavir, didanosine, emtricitabine, and tenofovir, demonstrating reduced susceptibility to abacavir (4.7- to 6.8-fold, above the clinical cutoff of 4.5-fold), didanosine (2.6- to 3.7-fold), FTC (88.9- to 129.9-fold) and TFV (1.5- to 1.8-fold). No cross-resistance was observed to stavudine and zidovudine (below the clinical and biological cutoffs of 1.7-fold and 1.9-fold for stavudine and zidovudine, respectively).

3.2.3. Concurrent Emergence of IN and RT Substitutions Associated with Resistance to EVG and FTC in Virologic Failures Treated with E/C/F/T

As described in above and summarized in Table 62, a significantly greater proportion of virologic failures on E/C/F/T developed the M184I/V RT substitution by Week 48, compared to those on ATR: 54.6% versus 13.3%. Furthermore, M184I/V was always detectable in the EVG^R substitution-harboring virus populations of all 10 evaluated subjects with E/C/F/T-treatment failure, conferring reduced susceptibility both to EVG and FTC (Table 62). Such concurrent emergence of M184I/V with RT substitutions associated with resistance to EFV (predominantly the K103N substitution), thus conferring reduced susceptibility both to EFV and FTC, were less frequently observed in virologic failures in the ATR treatment group (25% [2/8]; Table 62). Similar observations have also been reported with RAL-treatment virologic failures in clinical studies of RAL in combination with TRUVADA (TVD) of concurrent emergence of M184V/I substitutions in RT and IN substitutions associated with RAL resistance (STARTMRK and QDMRK studies; Eron *et al*., 2011; Lennox *et al*., 2010). In 11 evaluated RAL-treatment failures from the two studies, RAL genotypic resistance (by Y143C/H/R, Q148H, and N155H substitutions) was always noted in association with FTC resistance (by detectable M184I/V RT substitution). In contrast, only 2 of the 5 failures on EFV (as a comparator in the two studies) with evaluable genotypic data developed resistance both to EFV and FTC. Similarly, in Study 934 described in Section 3.2.2., M184I/V was observed only in some of the failure isolates harboring EFV^R substitutions (Margot *et al*., 2009; McColl *et al*., 2007): 15.4% (2/13) and 42.9% (9/21) of the virologic failures who developed the EFV^R substitutions on EFV+FTC+TDF and EFV+COMBIVIR, respectively. Thus, the applicant reasoned concurrent emergence of INSTI resistance-associated IN substitutions with the FTC resistance-associated RT substitution

M184V/I may be an INSTI class-specific effect when used in combination with TVD.

Table 62: Pooled Resistance Analysis by Drug-Resistance Associated Substitutions

E/C/F/T Treatment (24 virologic failures)					
EVG^R-associated substitutions[1] in IN	Number of subjects	FTC^R- and TDF^R-associated substitutions[2] in RT	Drug susceptibility (fold-change[3])		
			EVG	FTC	TFV
YES, n=11	3	K65R, M184V	>111.2	>84	1.1 - 1.6
	7	M184I/V	5.6 - 50.8	>75	0.5 - 0.7
	1	ND	19.7 - 54.3[4]	-	-
NO, n=13	1	K65R, M184I	1.8	42.4	0.8
	1	M184V	1.1	>88	0.4
	10	NONE	1 - 2.1	0.8 - 1.5	0.6 - 1.1
	1	ND	1.3	-	-
ATR Treatment (15 virologic failures)					
EFV^R-associated substitutions[5] in RT	Number of subjects	FTC^R- and TDF^R-associated substitutions[2] in RT	Drug susceptibility (fold-change[3])		
			EFV	FTC	TFV
YES, n=8	2	K65R, M184I/V	>18.7	>42.2	1.4 - 1.8
	6	NONE	1.2 - 68	0.8 - 1.2	0.5 - 1.1
NO, n=7	7	NONE	0.7 - 1.6	0.7 - 1.7	0.7 - 1.3

ND, not determined.
[1] EVG resistance-associated substitutions in HIV-1 IN (Table 55): D10E, S17N, H51Y, T66A/I/K, L68I/V, V72A/I/N/T, L74M, E92Q/G/V, Q95K/R, T97A, H114Y, S119G/R, F121C/Y, A128T, E138A/D/K, G140A/C/S, Y143C/H/R, P145S, Q146I/K/L/P/R, S147G, Q148H/K/R, V151A/I, S153A/F/Y, N155H/S, E157K/Q, K160N, G163R, V165A/I, R166S, E170A, S230R, D232E/G/H/N, R263K, and V281M.
[2] FTC and TDF resistance-associated substitutions in HIV-1 RT (Table 60): K65R, K70E, M184V/I, T69 insertion complex (T69 insertion + M41L, A62V, K70R, L210W, T215F/Y, and/or K219E/Q), Q151M complex (A62V, V75I, F77L, F116Y, and Q151M), and TAMs (M41L, D67N, K70R, L210W, t215F/Y, K219E/Q).
[3] Fold-change in drug susceptibility of virologic failure isolates compared to wild-type reference HIV-1.
[4] Two isolates were evaluated for this subject.
[5] EFV resistance-associated substitutions in HIV-1 RT (IAS-USA, 2011): L100I, K101E/P, K103N/S, V106M, V108I, Y181C/I, Y188L, G190A/S, P225H. Of the 8 subjects with RT substitutions associated with EFV resistance, 7 subjects developed K103N alone or with other known EFV^R-associated RT substitutions V90I, K101E, V108I, Y188F/H/L, and G190A.

As summarized in Table 62, all 10 virologic failures whose isolates harbored EVG^R substitutions (primary ± additional, listed in Table 58) with evaluable RT genotypic data also developed a primary FTC^R substitutions in RT (M184I/V) while on E/C/F/T. Preliminary linkage analysis of failure isolates from 2 of these 10 subjects by sequencing approximately 20 clones per sample revealed that these IN and RT substitutions were present in the same virus genome (Table 63). The presence of the V179I RT substitution in the isolate from Subject 0994-6667 indicates, at least for this subject, that these results are not due to sampling bias. Thus, these two subjects experienced virologic failure with the emergence of HIV-1 variants resistant to at least 2 antiviral components of the single-tablet complete regimen of EVG/COBI/FTC/TDF. Currently, no clones from subjects with K65R+M184I/V RT substitutions were analyzed. Upon completion of the clonal analyses on these virologic failures (targeted for the end of 2012), the applicant plans to submit the data to the FDA.

Table 63: Clonal Analyses of Two Subjects Virologic Failure Isolates Harboring EVG^R and FTC^R Substitutions

Subject ID	Study	Substitutions associated with resistance to EVG and FTC (detected by population-based nucleotide sequencing)		Clonal analysis % (number of clones harboring the substitution / number of clones examined)		
		IN	RT	E92Q	Q148R	M184V
0994-6667[1]	102	E92Q	M184V	100% (25/25)	-	100% (25/25)
2058-7359	103	Q148R	M184V	-	100% (19/19)	100% (19/19)

[1] Subject 0994-6667 developed an additional RT substitution, V179I. Since the subject had a mixture of wild-type and mutant viruses harboring V179I (V179V/I detectable by population-based nucleotide sequencing), V179I was only detectable in 16 (64%) of the 25 examined clones.

3.2.4. COBI Treatment-Emergent Amino Acid Substitutions in the HIV-1 protease

The sponsor proposes to use COBI as a PK enhancer in the absence of a PI in treatment-naïve subjects. Since COBI is structurally similar to RTV, the question was whether the *in vivo* data support COBI not having antiviral activity. The sponsor was asked to evaluate the viral protease (PR) sequences in their clinical studies. There was a disproportionate number of PR amino acid substitutions that developed on-treatment in the E/C/F/T treatment arm (9 substitutions/14 subjects) compared to the ATR arm (4 substitutions/15 subjects; Table 64). Three of the 9 protease substitutions in isolates from the E/C/F/T arm have been associated with resistance to protease inhibitors (M36I, D60E, and V77I) compared to 1 out of 4 in the ATR arm (K20R). The specific amino acid substitutions that developed while on-treatment can be found in Table 65. The only amino acid substitution that developed in more than one subject was the PR R57K/R substitution. This amino acid substitution has not been associated with resistance to any of the approved HIV-1 protease inhibitors. The rate of amino acid substitutions that was observed in the ATR arm is consistent with what was observed in previous studies. In study C209 (RPV/FTC/TDF versus EFV/FTC/TDF in treatment naïve subjects) and C215 (RPV+background regimen containing 2 NRTIs versus EFV+background regimen containing 2 NRTIs), the rates were 8 substitutions/72 subjects and 3 substitutions/34 subjects, respectively (Table 66). Please see the Clinical Virology review of Dr. Lisa K. Naeger, Ph.D. of NDA 202123 (SDN 013) for more details. The clinical relevance of this observation is unclear at this time as the number of subjects was small; however, this issue will require careful follow up.

Table 64: Total Number of Amino Acid Substitutions that Developed on Treatment in the HIV-1 Protease

Study	E/C/F/T	ATR	ATV/r+TVD
102	9 (14 subjects) 0.64 substitution/subject	4 (15 subjects) 0.27 substitution/subject	
103	6 (7 subjects) 0.86 substitution/subject		3 (6 subjects) 0.5 substitution/subject
104	1 (1 subject) 1 substitution/subject		
Total	16 (22 subjects) 0.73 substitution/subject	4 (15 subjects) 0.27 substitution/subject	3 (6 subjects) 0.5 substitution/subject

Table 65: Amino Acid Substitutions that Developed on Treatment in the HIV-1 Protease

Substitution	E/C/F/T	ATR	ATV/r+TVD
V11I/V	1		
I13I/V	1		
I15I/V	1		
I/L19V	1		
M19I/M		1	
K20K/R		1	
E35D			1
I/M36M	1		
M36I/M	1		
V36I/V		1	
S37G/S	1		
N/S37N/S/T		1	
P39S	1		
R57K/R	2		
D60D/E	1		
Q63P/Q	1		
H69H/Y			1
A/V71T	1		
A71A/T			1
I72I/V	1		
I77V	1		
V77I/V	1		

Green: Accessory substitutions for PIs
Red: Secondary substitutions associated with RTV resistance as well as most PIs
M36I: 6 subjects in EVG/COBI/FTC/TDF arm, 2 in ATR arm, 1 in ATV/r arm at BL.
D60E: 3 subjects in EVG/COBI/FTC/TDF arm, 1 in ATV/r arm at BL
L63P: 12 subjects in EVG/COBI/FTC/TDF arm, 8 in ATR arm, 2 in ATV/r arm at BL
A71T: 6 in EVG/COBI/FTC/TDF arm, 2 in ATV/r arm at BL; also A71V: 2 in ATR arm at BL
V77I: 7 subjects in EVG/COBI/FTC/TDF arm, 6 in ATR arm, 4 in ATV/r arm at BL

Table 66: Percent of Virologic Failure Subjects with Emerging PI Substitutions on Treatment in EDURANT Studies C209 and C215

	Rilpivirine Arms	EFV Arms
Any PI Substitution	8 (72 subjects) 0.11 substitutions/subject	3 (34 subjects) 0.09 substitutions/subject
Any IAS-USA PI Substitution	3 (72 subjects) 0.04 substitutions/subject	1 (34 subjects) 0.03 substitutions/subject

The RTV resistance-associated amino acid substitutions as defined by the label are PR I36L, M46I, I54V, A71V/T, I82A/F/T/S, and I84V. The PR I82 substitution is necessary but not

sufficient to confer a reduction in susceptibility to RTV in cell culture assays. There were no amino acid substitutions that developed at amino acid position 82 while on-treatment with E/C/F/T (Appendix 3). Subject 2058-7359 in Study 103 developed the PR A71T amino acid substitution that is an accessory resistance-associated substitution for several HIV-1 protease inhibitors, including RTV (Appendix 3 Table A3-3). Subject 1967-7177 in Study 103 developed the PR A71T amino acid substitution while on treatment with ATV/r+TVD (Appendix 3 Table A3-4). However, there were 6 and 2 subjects whose HIV-1 that had this amino acid substitution at baseline in the E/C/F/T and ATV/r+TVD arms, respectively. Furthermore, the drug susceptibility to HIV-1 PIs (APV, ATV, DRV, IDV, LPV, NFV, RTV, SQV, and TPV) ranged from 0.4- to 2.5 fold shift in susceptibility for all of the isolates tested (Appendix 3). Therefore, it is unclear if this amino acid substitution is associated with COBI exposure. The sponsor needs to follow-up for a longer period. Also, the sponsor might require a clinical study of E/C/F/T-treatment failures treated with a PI-containing regimen against ATR-treatment failures.

4. Conclusion

This original NDA for STRIBILD tablets is approvable for the treatment of HIV-1 infection in antiretroviral treatment-naïve adult patients with respect to Clinical Virology, based on Week-48 data from 2 Phase 3 studies and Week-60 data from a supportive Phase 2 study. STRIBILD tablets contain a fixed-dose combination of two investigational compounds, 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.

Studies evaluating treatment responses and resistance to STRIBILD treatment are continuing to be monitored post 48 weeks. Post 48-week follow-up data of STRIBILD-treated subjects is needed to obtain long term resistance data and to determine the STRIBILD resistance pathway in antiretroviral treatment-naïve subjects. The applicant needs to follow-up with the virologic failure subjects and sequence the protease for a longer period to see if the disproportionate number of amino acid substitutions in the STRIBILD treatment arm compared with the Atripla® continues. Also, the sponsor might require a clinical study of STRIBILD treatment failures treated with a PI-containing regimen against ATR failures.

PACKAGE INSERT: Section 12.4 Microbiology

The words with double strikethroughs are the text the sponsor was requested to delete and the words written in red are recommended insertions.

Mechanism of Action

Elvitegravir: Elvitegravir inhibits the strand transfer activity of HIV-1 integrase (b) (4) (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 polymerase α, β, ε, and mitochondrial DNA polymerase γ.

Tenofovir Disoproxil Fumarate: Tenofovir DF is an acyclic nucleoside phosphonate diester analog of adenosine monophosphate. Tenofovir DF requires initial diester hydrolysis for conversion to tenofovir and subsequent phosphorylations by cellular enzymes to form tenofovir diphosphate. Tenofovir diphosphate inhibits the activity of HIV-1 RT by competing with the natural substrate deoxyadenosine 5′-triphosphate and, after incorporation into DNA, by DNA chain termination. Tenofovir diphosphate is a weak inhibitor of mammalian DNA polymerase α, β, and mitochondrial DNA polymerase γ.

Antiviral Activity in Cell Culture

Elvitegravir, Cobicistat, Emtricitabine, and Tenofovir Disoproxil Fumarate: The triple combination of elvitegravir, emtricitabine, and tenofovir was not antagonistic (b) (4) in cell culture combination antiviral activity assays and was not affected by the addition (b) (4) of cobicistat.

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 (b) (4) 50% effective concentrations (EC_{50}) (b) (4) ranged from (b) (4) 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). The antiviral activity of elvitegravir with antiretroviral drugs in two-drug combination studies was not antagonistic when combined with

the integrase strand transfer inhibitor raltegravir, NNRTIs (efavirenz, etravirine, or nevirapine), NRTIs (abacavir, didanosine, emtricitabine, lamivudine, stavudine, tenofovir, or zidovudine), (b) (4) PIs (amprenavir, atazanavir, darunavir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir, or tipranavir), (b) (4) the fusion inhibitor enfuvirtide, or the CCR5 co-receptor antagonist, maraviroc. Elvitegravir did not show inhibition of replication of HBV or HCV in cell culture. (b) (4)

Cobicistat: (b) (4)

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). No antagonistic effects were observed in (b) (4) two-drug combination studies of emtricitabine with NRTIs (abacavir, lamivudine, stavudine, tenofovir, or zidovudine), NNRTIs (b) (4) (delavirdine, efavirenz, nevirapine, or (b) (4) rilpivirine), PIs (amprenavir, nelfinavir, ritonavir, or saquinavir), or (b) (4) the integrase strand transfer inhibitor elvitegravir (b) (4)

Tenofovir Disoproxil Fumarate: The antiviral activity of tenofovir against laboratory and clinical isolates of HIV-1 was assessed in T lymphoblastoid cell lines, primary monocyte/macrophage cells and peripheral blood lymphocytes. The EC_{50} values for tenofovir were in the range of 0.04-8.5 micromolar. Tenofovir displayed antiviral activity in cell culture against HIV-1 clades A, B, C, D, E, F, G, and O (EC_{50} values ranged from 0.5-2.2 micromolar) and showed strain specific activity against HIV-2 (EC_{50} values ranged from 1.6- (b) (4) -5.5 micromolar). No antagonistic effects were observed in (b) (4) two-drug combination studies of tenofovir with NRTIs (abacavir, didanosine, emtricitabine, lamivudine, stavudine, or (b) (4) zidovudine), NNRTIs (delavirdine, efavirenz, nevirapine, or (b) (4) rilpivirine), PIs (amprenavir, indinavir, nelfinavir, ritonavir, or saquinavir), or (b) (4) the integrase strand transfer inhibitor elvitegravir (b) (4)

Resistance

<u>In Cell Culture</u>

Elvitegravir: HIV-1 isolates with reduced susceptibility to elvitegravir have been selected in cell culture. Reduced susceptibility to elvitegravir was most commonly 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, (b) (4) S153F/Y, E157Q, D232N, (b) (4) R263K, and V281M. (b) (4)

(b) (4)

Emtricitabine and Tenofovir Disoproxil Fumarate: HIV-1 isolates with reduced susceptibility to emtricitabine or tenofovir have been selected in cell culture. Reduced susceptibility to emtricitabine was associated with M184V/I substitutions in HIV-1 RT. HIV-1 isolates selected by tenofovir expressed a K65R substitution in HIV-1 RT and showed a 2-4 fold reduction in susceptibility to tenofovir.

In Treatment-Naïve HIV-1-Infected Subjects

(b) (4)

Cross Resistance

(b) (4)

(b) (4)

Elvitegravir: Cross-resistance has been observed among INSTIs. Elvitegravir-resistant viruses showed varying degrees of cross-resistance in cell culture to (b) (4) raltegravir depending on the type and number of substitutions in HIV-1 integrase. (b) (4) Among the four primary elvitegravir resistance-associated substitutions detected in the STRIBILD-treatment virologic failure isolates, E92Q, Q148R, and N155H individually conferred reduced susceptibility both to elvitegravir (>32-fold) and raltegravir (>5-fold) when introduced into a wild-type virus by site-directed mutagenesis. The T66I substitution conferred >14-fold reduced susceptibility to elvitegravir but <3-fold to raltegravir. Among the three primary raltegravir resistance-associated substitutions (Y143H/R, Q148H/K/R, and N155H), all but one (Y143H) conferred significant reductions in susceptibility to elvitegravir (>5-fold).

Emtricitabine: Cross-resistance has been observed among NRTIs. Emtricitabine-resistant isolates (b) (4) harboring an M184V/I substitution in HIV-1 RT (b) (4) were cross-resistant to lamivudine (b) (4) HIV-1 isolates containing the K65R RT substitution, selected *in vivo* by abacavir, didanosine, and tenofovir, demonstrated reduced susceptibility to inhibition by emtricitabine. (b) (4)

Tenofovir Disoproxil Fumarate: Cross-resistance has been observed among NRTIs. The K65R substitution in HIV-1 RT selected by tenofovir is also selected in some HIV-1-infected patients treated with abacavir or didanosine. HIV-1 isolates with the K65R substitution also showed reduced susceptibility to emtricitabine and lamivudine. Therefore, cross-resistance among these NRTIs may occur in patients whose virus harbors the K65R substitution. HIV-1 isolates from patients (N=20) whose HIV-1 expressed a mean of 3 zidovudine-associated RT amino acid substitutions (M41L, D67N, K70R, L210W, T215Y/F, or K219Q/E/N) showed a 3.1-fold decrease in the susceptibility to tenofovir. Subjects whose virus expressed an L74V RT substitution without zidovudine resistance-associated substitutions (N=8) had reduced response to VIREAD. Limited data are available for patients whose virus expressed a Y115F substitution (N=3), Q151M substitution (N=2), or T69 insertion (N=4) in HIV-1 RT, all of whom had a reduced response in clinical trials. (b) (4)

APPENDICES

Appendix 1: Virological Assays in Clinical Virology

Quantification of Plasma HIV-1 RNA Levels

Plasma HIV-1 RNA levels were quantified using the fully automated COBAS® AMPLICOR HIV-1 Monitor Test (Roche, version 1.5) that received marketing approval from FDA (BP950005) on March 2, 1999 as an *in vitro* nucleic acid amplification test for the quantification of HIV-1 RNA in human plasma. The Test involves (1) reverse transcription of HIV-1 RNA to generate complementary DNA (cDNA), (2) PCR amplification of the target sequence in the cDNA (142 bp, located in a highly conserved region of the HIV-1 *gag* gene) using HIV-1 specific complimentary primers, (3) hybridization of the amplified products to oligonucleotide probes specific to the target(s), and (4) detection of the probe-bound amplified products by calorimetric determination. These processes are carried out simultaneously with the sample HIV-1 RNA to be quantified and a Quantitation Standard (QS) RNA. HIV-1 QS RNA is a non-infectious RNA transcript that contains the identical primer binding sites as the HIV-1 target and a unique probe binding region that allows the QS signal to be distinguished from the target HIV-1 signal.

According to the manufacturer, the AMPLICOR HIV-1 Monitor Test yielded a specificity >99.85%, reducing false positive results when tested in a large seronegative population of over 500 samples. It could distinguish 0.5 $\log_{10}$ copies/mL. In addition, this test utilizes a primer set designed to detect non-B subtypes (HIV-1 Group M), providing reliable viral load measurement of HIV-1 subtypes A-G.

The Test can be used with either the Standard or UltraSensitive specimen processing procedure. When the Standard specimen processing procedure is used, the Test can quantify HIV-1 RNA over the range of 400 - 750,000 copies/mL. For specimens to contain high levels of HIV-1 RNA (≥750,000 copies/mL), diluted samples were requantified. When the UltraSensitive specimen processing procedure is used, the Test can quantify HIV-1 RNA over the range of 50 - 75,000 copies/mL.

HIV-1 Resistance testing

As required by the enrollment criteria, the protease/reverse transcriptase (PR/RT) genotype was assessed for all subjects at screening using the two FDA-approved HIV-1 genotyping assays, either GeneSeq® in the Phase 3 studies 102 and 103 (Monogram Biosciences; performed by (b) (4)) or TRUGENE® in the Phase 2 study 104 (Siemens Healthcare Diagnostics; performed by (b) (4)). The GeneSeq assay also determined the HIV-1 subtype. For samples analyzed by the TruGene genotyping assay, HIV-1 subtype was determined using the Stanford HIV-1 database algorithm (http://hivdb.stanford.edu/). These screening data were used for baseline resistance analyses. No analyses of IN were performed at screening. No PR/RT

phenotypic analysis was conducted at Screening.

For post-baseline resistance analyses of subjects with virologic failure, PR/RT and IN genotyping and phenotyping assays were performed. The Monogram Biosciences PhenoSense GT® assay and GeneSeq®/PhenoSense RH/IN assay (Petropoulos *et al*., 2000) were used for this purpose. The PhenoSense GT assay (combining 3 tests, GeneSeq®, PhenoSense®, and Replication Capacity) yielded PR/RT genotypic and phenotypic data relevant to all currently approved NNRTIs, NRTIs, and PIs, with the exception of the NNRTI rilpivirine (RPV). The PR/RT genotype produced by the GeneSeq assay covers the entire PR gene (99 amino acids) and the N-terminal RT sequence spanning amino acids 1 to 305, encompassing all clinically relevant PI, NNRTI, and NRTI resistance-associated substitutions (IAS-USA, 2011). The GeneSeq/ PhenoSense RH/IN assay yielded IN genotype (entire 288 amino acids) and phenotypic data for EVG and RAL. IN resistance testing were conducted retrospectively at Baseline for those subjects whose post-baseline samples were tested. Samples with successful PhenoSense GT results for PR/RT but with IN assay failure were retested for the IN genotype using the GenoSure® assay (LabCorp CMBP). No analyses of viral tropism were conducted in these studies.

Appendix 2: Virologic Failures with Resistance Data in Studies 102, 103, and 104 by Treatment Arm (IN and RT Genotype and Phenotype)

(b) (4)

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/s/

SUNG S RHEE
07/02/2012

TAKASHI E KOMATSU
07/02/2012

JULIAN J O REAR
07/02/2012

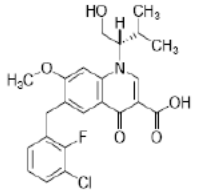
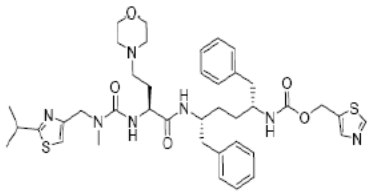
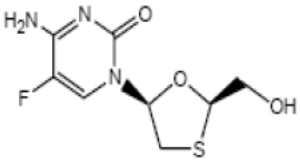
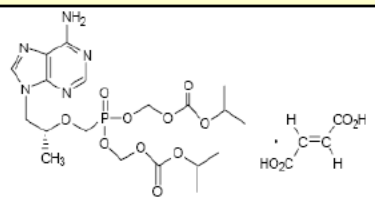
DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW
NDA#: 203-100 **SDN:** 033 **DATE REVIEWED:** 06/26/12

Reviewer: Sung Rhee, Ph.D.
Date Submitted: 04/27/12 **Date Assigned:** 04/30/12
Date Received: 04/27/12

Sponsor: Gilead Sciences, Inc.
333 Lakeside Drive
Foster City, CA 94404
Christophe Beraud, Ph.D.
650-522-5093

Product Name: STRIBILD, a fixed-dose combination tablet containing four active pharmaceutical ingredients, elvitegravir (EVG, 150 mg), cobicistat (COBI, 150 mg), emtricitabine (FTC, 200 mg), and tenofovir disoproxil fumarate (TDF, 300 mg). Chemical characteristics of individual components of STRIBILD are listed below.

Chemical Name, Structure, Molecular Formula, and Other Information:

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 Weight	447.88	776.02
Drug Class	INSTI	Pharmacoenhancer (No anti-HIV-1 activity in cell culture)
Supporting Document	IND (b) (4)	IND (b) (4)
Individual Component	**FTC**	**TDF**
Structure		
Chemical Names	5-fluoro-1-(2R,5S)-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine	9-[(R)-2[[bis[[(isopropoxycarbonyl)oxy]methoxy]phosphinyl]methoxy]propyl]adenine fumarate (1:1)

Molecular Formula	$C_8H_{10}FN_3O_3S$	$C_{23}H_{34}N_5O_{14}P$
Molecular Weight	247.24	635.52
Drug Class	NRTI	NRTI
Supporting Document	IND 53,971 and NDA 21-500	IND52,849 and NDA 21-356

Indication(s): Once-daily complete regimen for the treatment of HIV-1 infection in adults aged 18 years and over who are antiretroviral treatment-naïve or have no known substitutions associated with resistance to the individual components of the regimen

Dosage Form(s): Tablet (EVG 150 mg, COBI 150 mg, FTC 200 mg, and TDF 300 mg)
Route(s) of Administration: Oral
Recommended Dosage: One tablet taken once daily with food
Dispensed: Rx _X_ **OTC** ___ **(Discipline relevant)**

Abbreviations: COBI, cobicistat; EVG, elvitegravir/cobicistat; FTC, emtricitabine; HIV-1, human immunodeficiency virus type 1; INSTI, HIV-1 integrase strand transfer inhibitor; NRTI, HIV-1 nucleoside/nucleotide reverse transcriptase inhibitor; TDF, tenofovir disoproxil fumarate

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BACKGROUND/SUMMARY

This submission contains the sponsor's comments on the FDA Briefing Document provided to the sponsor on April 23, 2012 for the May 11th Antiviral Drug Advisory Committee meeting to discuss NDA 203-100 for the STRIBILD tablet for the treatment of HIV-1 infection in treatment-naïve adult patients. STRIBILD is a fixed-dose combination tablet of elvitegravir/cobicistat/ emtricitabine/tenofovir disoproxil fumarate (EVG 150 mg/COBI 150 mg/FTC 200 mg/TDF 300 mg) and is currently under review for the approval for this indication. The Division's responses to the comments were sent to the sponsor on May 02, 2012.

There was one comment (Comment 2) regarding the Clinical Microbiology Summary in the FDA Briefing Document:

Comment 2: Gilead requests to receive the list of the subjects receiving EVG/COBI/FTC/TDF identified as virologic failures by the Division and included in the resistance analysis population that was used for the analyses of the integrase gene, and, if different, the list of subjects used for the analyses of reverse transcriptase and protease described in the briefing document.

Virology Response: Attached below is the list of 24 virologic failures receiving EVG/COBI/FTC/TDF who were included in the resistance analysis population.

Study 102	Study 103
0031-6257	0123-7332
0310-6332	0598-7203

0637-6041	1925-7108
0652-6556	2058-7359
0698-6012	2058-7461
0698-6182	2493-7299
0994-6667	2838-7562
1598-6101	4140-7130
1950-6503	5124-7476
1978-6091	**Study 104**
2140-6534	2475-4507
2154-6648	-
2838-6264	-
4140-6628	-

Sung S. Rhee, Ph.D.
Clinical Microbiology Reviewer

CONCURRENCE

Date:

HFD-530/MicroTL/J. O'Rear

cc:
HFD-530/Original IND
HFD-530/Division File
HFD-530/RPM/S. Min

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SUNG S RHEE
06/26/2012

JULIAN J O REAR
06/27/2012

VIROLOGY FILING CHECKLIST FOR NDA or Supplement

NDA Number: 203-100 **NDA Type:** Original **Stamp Date:** 10/27/2011

Applicant: Gilead sciences, Inc. **Drug Name:** Elvitegravir/Cobicistat/Emtricitabine/Tenofovir disoproxil fumarate Single Tablet Regimen

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?			NA
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?	X		
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 design of the development package?	X		

VIROLOGY FILING CHECKLIST FOR NDA or Supplement

	Content Parameter	Yes	No	Comments
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	

NA, not applicable

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.

Komatsu, Takashi	12/07/2011
Reviewing Microbiologist	Date
Rhee, Sung	12/07/2011
Reviewing Microbiologist	Date
Microbiology Team Leader	Date

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

SUNG S RHEE
12/16/2011

TAKASHI E KOMATSU
12/16/2011

JULIAN J O REAR
12/16/2011