CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

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MICROBIOLOGY / VIROLOGY REVIEW(S)

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)

VIROLOGY REVIEW

NDA: 203-093 **SDN:** 038 **DATE REVIEWED:** 07/25/14

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

Reviewer Name(s): Sung S. Rhee, Ph.D.

Initial Submission Dates:

Correspondence Date: April 04, 2014
CDER Receipt Date: April 04, 2014
Reviewer Receipt Date: April 04, 2014
DAVP Action Date: October 03, 2014
PDUFA Goal Date: October 04, 2014

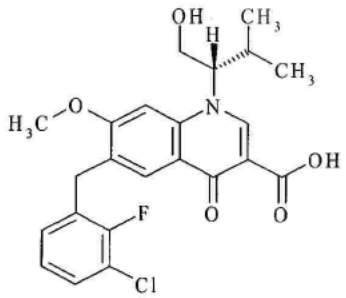
Related/Supporting Documents: IND 72,177, NDA 203-100

Product Name(s):

Proprietary: VITEKTA®
Non-Proprietary/USAN: Elvitegravir (EVG)
Code Name/Number: GS-9137

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

Structural Formula:



Molecular Formula: $C_{23}H_{23}ClFNO_5$
Molecular Weight: 447.9

Indication(s): Treatment of HIV-1 infection in combination with a ritonavir-boosted protease inhibitor and with other antiretroviral agents in antiretroviral treatment-experienced adults

Dosage Form(s): Tablets (85 and 150 mg)
Route(s) of Administration: Oral
Recommended Dosage: One tablet taken once daily with food

Dose of VITEKTA	Dose of Concomitant Ritonavir-Boosted Protease Inhibitor
85 mg once daily	Atazanavir 300 mg/Ritonavir 100 mg once daily
	Lopinavir 400 mg/Ritonavir 100 mg twice daily

150 mg once daily	Darunavir 600 mg/Ritonavir 100 mg twice daily
	Fosamprenavir 700 mg/Ritonavir 100 mg twice daily
	Tipranavir 500 mg/Ritonavir 200 mg twice daily

Dispensed: Rx _X_ OTC ___ (Discipline relevant)

Abbreviations: CMC, Chemistry, Manufacturing, and Controls; EVG, elvitegravir; FTC, emtricitabine; HIV-1, human immunodeficiency virus type 1; NDA, new drug application; NRTI, HIV-1 nucleos(t)ide reverse transcriptase inhibitor; TDF, tenofovir disoproxil fumarate

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EXECUTIVE SUMMARY

VITEKA® (elvitegravir; EVG) is an inhibitor of the HIV-1 integrase-catalyzed strand transfer (INSTI) that prevents the covalent insertion, or integration, of unintegrated linear HIV-1 DNA into the host cell genome preventing the formation of the HIV-1 provirus. The provirus is required to direct the production of progeny virus, so inhibiting integration prevents propagation of the viral infection. An EVG-containing complete regimen STRIBILD™ (Gilead Sciences, Inc.) where EVG is co-formulated as a single tablet with two FDA-approved HIV-1 NRTIs, FTC (200 mg; Emtriva®) and TDF (300 mg; Viread®), and a pharmacokinetic enhancer cobicistat (150 mg) received U.S. marketing approval by FDA for the treatment of HIV-1 infection in antiretroviral treatment-naïve adult patients in August, 2012 (NDA 203-100).

The New Drug Application for VITEKTA® as a single agent was originally submitted in June, 2012 and issued a complete response letter in April, 2013 due to CMC deficiencies: no virology concerns were raised. Non-clinical and clinical virology data of EVG submitted in support of FDA approval were reviewed in Virology reviews N203100.001 and N203093.000, respectively. The applicant resubmitted the NDA for VITEKTA® tablets to provide new or updated information and data that address the deficiencies listed in the Complete Response Letter. In this resubmission, no new virology data were included and no changes were made to the Microbiology section of the label (Section 12.4).

1. Recommendations

1.1. Recommendation and Conclusion on Approvability: Approval of this original NDA for elvitegravir tablets (85 and 150 mg) is recommended with respect to Clinical Virology.

1.2. Recommendation on Phase 4 (Post-Marketing) Commitments, Agreements, and/or Risk Management Steps, If Approvable: None

2. Administrative

2.1. Reviewer's Signatures

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

2.2. Concurrence

_____

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

CC:
HFD-530/NDA # 203093
HFD-530/Division File
HFD-530/PM/P. Hong

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
07/28/2014

JULIAN J O REAR
07/28/2014

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)

VIROLOGY REVIEW

NDA: 203-093 **SDN:** 000 **DATE REVIEWED:** 03/19/13

Clinical Virology Reviewer: Sung S. Rhee, Ph.D.

NDA Number: 203-093 **Supporting Document Numbers:** 000

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

Reviewer's Name: Sung S. Rhee, Ph.D.

Initial Submission Dates:

Correspondence Date: June 27, 2012
CDER Receipt Date: June 27, 2012
Reviewer Receipt Date: June 27, 2012
Review Complete Date: March 19, 2013
DAVP Action Date: April 26, 2013
PDUFA Goal Date: April 27, 2013

Amendments:

Errors corrected in Clinical Study Reports (SDN 002): August 06, 2012

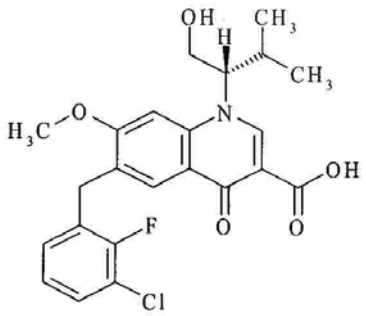
Related/Supporting Documents: IND 72,177, NDA 203-100

Product Name(s):

Proprietary: VITEKTA
Non-Proprietary/USAN: Elvitegravir (EVG)
Code Name/Number: GS-9137

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

Structural Formula:



Elvitegravir (EVG)

Molecular Formula: $C_{23}H_{23}ClFNO_5$
Molecular Weight: 447.9

Dosage Form(s): Tablets (85 and 150 mg)
Route(s) of Administration: Oral
Indication(s): Treatment of HIV-1 infection in combination with a ritonavir-boosted protease inhibitor and with other antiretroviral agents in antiretroviral treatment-experienced adults

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)

VIROLOGY REVIEW

NDA: 203-093 **SDN:** 000 **DATE REVIEWED:** 03/19/13

Clinical Virology Reviewer: Sung S. Rhee, Ph.D.

Recommended Dosage: One tablet taken once daily with food

Dose of VITEKTA	Dose of Concomitant Ritonavir-Boosted Protease Inhibitors
85 mg once daily	Atazanavir/Ritonavir 300/100 mg once daily
	Lopinavir/Ritonavir 400/100 mg twice daily
150 mg once daily	Darunavir/Ritonavir 600/100 mg twice daily
	Fosamprenavir/Ritonavir 700/100 mg twice daily
	Tipranavir/Ritonavir 500/200 mg twice daily

Dispensed: Rx _X_ **OTC** ___ **(Discipline relevant)**

Abbreviations: ABC, abacavir; ART, antiretroviral therapy; ARV, antiretroviral; ATV, atazanavir; ATV/r, ritonavir-boosted atazanavir; BID, twice daily; BR, background regimen; CC_{50}, 50% cytotoxic concentration; COBI, cobicistat; CPI/r, comparator ritonavir-boosted protease inhibitor; DDI, didanosine; DRV, darunavir; DRV/r, ritonavir-boosted darunavir; EC_{50}, effective concentration inhibiting viral replication by 50%; EC_{90}, effective concentration inhibiting viral replication by 90%; ETR, etravirine; EVG, elvitegravir; EVG^{R}, elvitegravir resistance-associated; EVG/r, ritonavir-boosted elvitegravir; FPV, fosamprenavir; FPV/r, ritonavir-boosted fosamprenavir; FTC, emtricitabine; GSS, genotypic sensitivity scores; HIV-1, human immunodeficiency virus type 1; IC_{50}, 50% inhibitory concentration; IL-2, interleukin 2; IN, HIV-1 integrase; INSTI, HIV-1 integrase strand transfer inhibitor; ITT, Intent-to-treat; LAM, lamivudine; LPV, lopinavir; LPV/r, ritonavir-boosted lopinavir; LTR, HIV-1 long terminal repeat; MVC, maraviroc; NDA, new drug application; NNRTI, HIV-1 non-nucleoside reverse transcriptase inhibitor; NRTI, HIV-1 nucleoside/nucleotide reverse transcriptase inhibitor; PBMC, peripheral blood mononuclear cell; PCR, polymerase chain reaction; PHA, phytohemagglutinin; PI, HIV-1 protease inhibitor; PI/r, ritonavir-boosted HIV-1 protease inhibitor; PIC, pre-integration complex; PR, HIV-1 protease; PSS, phenotypic sensitivity scores; QD, once daily; QS, quantitation standard; RAL, raltegravir; RAL^{R}, raltegravir resistance-associates; RT, HIV-1 reverse transcriptase; RTE, resistance testing eligible; RTV, ritonavir; T-20, enfuvirtide; TDF, tenofovir disoproxil fumarate; TPV, tipranavir; TPV/r, ritonavir-boosted tipranavir; TVD, Truvada; VF, virologic failure; VR, virologic rebound; ZDV, zidovudine

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EXECUTIVE SUMMARY

This application was submitted in support of a new drug application (NDA) for VITEKTA tablets (85 and 150 mg). The proposed indication for the VITEKA tablet is for once daily use in combination with a ritonavir-boosted protease inhibitor and with other antiretroviral agents for the treatment of HIV-1 infection in adult patients who are antiretroviral treatment-experienced. VITEKA (elvitegravir; EVG) is an inhibitor of the HIV-1 integrase-catalyzed strand transfer (INSTI) that prevents the covalent insertion, or integration, of unintegrated linear HIV-1 DNA into the host cell genome preventing the formation of the HIV-1 provirus. The provirus is required to direct the production of progeny virus, so inhibiting integration prevents propagation of the viral infection. Recently, an EVG-containing complete regimen STRIBILD™ (Gilead Sciences, Inc.) where EVG is co-formulated as a single tablet with two FDA-approved HIV-1 NRTIs, FTC (200 mg; Emtriva®) and TDF (300 mg; Viread®), and a pharmacokinetic enhancer cobicistat (150 mg) was approved by FDA for the treatment of HIV-1 infection in antiretroviral treatment-naïve adult patients in August, 2012 (NDA 203-100). This original NDA was submitted for U.S. marketing approval of EVG tablets as a single agent. Currently, ISENTRESS® Raltegravir; RAL) is the only FDA-approved drug in the INSTI class (approved in October, 2007).

The NDA package for EVG includes clinical and virology study reports and datasets from the pivotal Phase 3 study (GS-US-183-0145) and two supportive studies, a Phase 2 dose-finding study (GS-US-183-0105) and an open-label roll-over study (GS-US-183-0130). The efficacy of EVG is primarily based on the analyses through 96 weeks from the Phase 3 study that is a randomized, double-blind, active-controlled study to assess the noninferiority of EVG versus RAL, each administered with a background regimen containing a fully active ritonavir-boosted protease inhibitor (PI/r) and a second agent in HIV-1 infected, antiretroviral treatment-experienced adults.

In as-treated antiviral efficacy (potency and durability) analyses, EVG was noninferior to RAL, based on the Week-48 and -96 efficacy data from the Phase 3 study GS-US-183-0145 with virologic success rates of HIV-1 RNA suppression <50 copies/mL comparable between the EVG and RAL treatment groups: 66% versus 66% at Week 48, and 61% versus 65% at Week 96, respectively. Antiviral response to both treatments appeared to be durable as 86% and 87% of EVG and RAL recipients who achieved virologic suppression at Week 48 and continued to receive their assigned treatment in Year 2 remained aviremic at Week 96. Higher rates of virologic success were observed in the subgroup of subjects with lower baseline viral load (negative correlation between baseline HIV-1 RNA levels and antiviral efficacy of EVG). Subjects with baseline viral RNA levels <5 $\log_{10}$ copies/mL achieved and maintained virologic success at lower rates, 72% and 67% at Weeks 48 and 96, respectively, while the virologic success rates for subjects with higher baseline viral RNA levels ≥5 $\log_{10}$ copies/mL were 48% and 45%, respectively.

Using the genotypic and phenotypic data obtained from virus samples (pre- and on-treatment) from subjects receiving EVG-containing regimens in several clinical trials of EVG who remained viremic (HIV-1 RNA >400 copies/mL) by the time of efficacy evaluation (up to 96 weeks), substitutions at 9 amino acid positions in the HIV-1 integrase protein, T66A/I/K, E92A/G/Q, T97A, F121C/Y, P145S, Q146I/L/R, S147G, Q148H/K/R, and N155H/S, were identified to be primarily associated with EVG-treatment virologic failure and resistance to EVG. These primary substitutions appeared to emerge independently as separate pathways to EVG

resistance. Overall, substitutions at E92 (E92A/G/Q, 39% of those evaluated virologic failures) and at N155 (N155H/S, 27% of those evaluated virologic failures) were most frequently observed. Additional substitutions were observed frequently to emerge in the same virus population harboring these primary EVG resistance-associated substitutions: H51Y, L68I/V, G70R, V72A/I/N, I73V, Q95K/R, T112A, S119R, T124A, E138A/K, G140A/C/S, E157Q, K160N, E170A, S230R, and D232N. These substitutions (referred to as secondary EVG resistance-associated substitutions) that may be involved in EVG resistance by being co-selected with primary substitutions were detectable in post-baseline virus samples from 64% of subjects who developed genotypic resistance to EVG with evidence of emerging primary substitutions.

In Study GS-US-183-0145, by Week 96, 24 subjects developed genotypic resistance to EVG with emerging primary EVG resistance-associated substitutions detectable in their post-baseline virus samples: T66A/I (n=8), E92G/Q (n=7), T97A (n=5), Q146R (n=1), S147G (n=4), Q148R (n=4), and N155H (n=5). In 14 of these subjects (58%), emergence of 11 secondary EVG resistance-associated substitutions was also observed in the same virus populations harboring primary substitutions: H51Y, L68V, G70R, V72A/I/N, Q95K, T112A, T124A, E138K, G140A/C/S, E170A, and D232N. The emergence of primary EVG resistance-associated substitutions resulted in median decreases in susceptibility to EVG of 8-fold, ranging from 2- to >158-fold (29 isolates with evaluable data from 21 subjects), compared to wild-type reference HIV-1.

Broad cross-resistance was noted between EVG and RAL. In the pooled resistance analysis, 94% of isolates resistant genotypically and phenotypically to EVG by harboring primary EVG resistance-associated substitutions and displaying >2.5-fold (up to 301-fold) reduced susceptibility to EVG (above the biological cutoff for EVG) were also phenotypically resistant to RAL with >1.5-fold (up to >257-fold) reduced susceptibility (above the biological cutoff for RAL). Similar high level of cross-resistance was observed with isolates collected from RAL recipients in Study GS-US-183-0145, as 96% of those genotypically resistant to RAL with evidence of emerging primary RAL resistance-associated substitutions (E92Q, Y143C/H/R, Q148H, and N155HY observed) displayed phenotypic resistance both to RAL (2- to >170-fold reduction in susceptibility) and to EVG (3- to 301-fold reduction). In Study GS-US-183-0145, among the 24 subjects who developed genotypic resistance to EVG with evidence of emerging primary EVG resistance-associated substitutions, 14 (67%) of the 21 subjects with evaluable data showed phenotypic resistance to EVG, and 11 (79%) of those 14 subjects had HIV-1 variants phenotypically resistant both to EVG and RAL.

1. Recommendations

1.1. Recommendation and Conclusion on Approvability: Approval of this original NDA for elvitegravir tablets (85 and 150 mg) is recommended with respect to Clinical Virology. Elvitegravir, co-administered with a ritonavir-boosted protease inhibitor and with other antiretroviral agents, is indicated for the treatment of HIV-1 infection in antiretroviral treatment-experienced adults.

1.2. Recommendation on Phase 4 (Post-Marketing) Commitments, Agreements, and/or Risk Management Steps, If Approvable: None

2. Summary of OND Clinical Virology Assessments

Elvitegravir (EVG) inhibits the strand transfer activity of HIV-1 integrase and thus 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. Nonclinical virology information on EVG can be found in the Microbiology review of the STRIBILD™ NDA, NDA203100.001, and in the US Prescribing Information.

Pooled resistance analysis of EVG was conducted in order to identify EVG resistance pathways using the genotypic and phenotypic data obtained from virus samples (pre- and on-treatment) of resistance testing eligible subjects in 6 clinical trials sponsored by the applicant (GS-US-183-0105, -0130, and -0145, and GS-US-236-0102, -0103, and -0104). Resistance testing eligible (RTE) subjects are defined by the protocol for each trial but in general, are those who received EVG-containing regimens and remained viremic (HIV-1 RNA >400 copies/mL) at the time of efficacy evaluation (e.g., Weeks 48 and 96) or at the time of study discontinuation. A total of 828 baseline and post-baseline virus isolates of 285 RTE subjects with evaluable IN genotypic and/or phenotypic resistance data were included in the pooled analysis.

Development of substitutions T66A/I/K, E92A/G/Q, T97A, F121C/Y, P145S, Q146I/L/R, S147G, Q148H/K/R, and N155H/S in the HIV-1 integrase protein was primarily associated with resistance to EVG. Of the 266 virologic failures with evaluable IN genotypic data, 72.2% (192/266) developed genotypic resistance to EVG as being infected with HIV-1 variants expressing at least one of the primary EVG resistance-associated (EVG^R) substitutions. Substitutions at the 9 primary resistance-associated amino acid positions appeared to emerge independently as separate pathways to EVG resistance as these were detected singly in treatment-failure isolates from 96 subjects. In the remaining 96 failures with emerging primary EVG^R substitutions, multiple primary substitutions were detected in their genotyped virus populations. Overall, substitutions at E92 (E92A/G/Q, n=103, 38.7% of evaluated virologic failures) and at N155 (N155H/S, n=72, 27.1% of evaluated virologic failures) were mostly frequently observed. Phenotypic resistance to EVG with ≥2.5-fold reductions (up to 301-fold) in EVG susceptibility (above the biological cutoff) was observed in 95.4% (167/175 with evaluable phenotypic data) of subjects who developed EVG genotypic resistance with evidence of emerging primary EVG^R substitutions. When these primary EVG^R substitutions (T66A/I/K, E92G/Q, T97A, F121Y, P145S, Q146I/L/R, S147G, Q148H/K/R, and N155H/S) were introduced individually into a wild-type virus $HIV\text{-}1_{HXB2}$ by site-directed mutagenesis, all but one (T97A) conferred >2.5-fold reduced susceptibility to EVG (up to 133.9-fold). In addition, as expected with a shared mechanism of action against HIV-1 integrase, broad cross-resistance was noted between EVG and RAL, as 94.1% (225/239 with evaluable phenotypic data) of isolates resistant genotypically and phenotypically to EVG by harboring primary EVG^R substitutions and displaying >2.5-fold reduced susceptibility to EVG were also phenotypically resistant to RAL with >1.5-fold (up to >256.8-fold) reduced susceptibility (above the biological cutoff for RAL). A similar high level of cross-resistance was observed with isolates collected from RAL recipients in Study GS-US-183-0145 with 95.8% (23/24) of those genotypically resistant to RAL with evidence of emerging primary RAL^R substitutions (E92Q, Y143C/H/R, Q148H, and N155HY observed) displaying phenotypic resistance both to RAL (1.6- to >169.5-fold reduction in susceptibility) and to EVG (2.8- to 301-fold reduction).

Emergence of 16 substitutions in the HIV-1 integrase protein was frequently observed in the same virus populations harboring primary EVG^R substitutions: H51Y, L68I/V, G70R, V72A/I/N, I73V, Q95K/R, T112A, S119R, T124A, E138A/K, G140A/C/S, E157Q, K160N, E170A, S230R,

and D232N. These substitutions (referred to as secondary EVG^R substitutions) that may be involved in EVG resistance by being co-selected with primary EVG^R substitutions were detectable in post-baseline virus samples from 122 (63.5%) of the 192 subjects with genotypic resistance to EVG. Substitutions G70R, S119R, and E170A were exclusively found in the N155H-harboring virus population, while substitutions I73V and G140A/C/S were also exclusively found in the virus isolates harboring E92Q or Q148H/K/R primary EVG^R substitutions, respectively.

Additional clinical virology analyses were conducted to evaluate the antiviral efficacy (potency and durability) and resistance development of EVG, when administered with a background regimen (BR) including a fully-active PI/r, in HIV-1-infected ART-experienced adult subjects in Study GS-US-183-0145. The proposed indication of EVG is directly supported by this pivotal Phase 3 study.

The antiviral efficacy (potency and durability) analyses were conducted in the censored, as-treated population including subjects who had at least one on-treatment viral load measurement in Study GS-US-183-0145 to determine whether EVG is noninferior to RAL (comparator), each administered with a BR, for the treatment of HIV-1 infection in ARV treatment-experienced adult subjects. Baseline virologic characteristics were similar between the 2 treatment groups with a median baseline HIV-1 RNA level of 4.4 $\log_{10}$ copies/mL and approximately 26% of subjects in each group had HIV-1 RNA >100,000 copies/mL. The majority (93.8%, 648/691) of subjects were infected with HIV-1 subtype B: 93.9% (325/346) and 93.6% (323/345) of EVG and RAL recipients, respectively.

Antiviral efficacy of EVG was noninferior to RAL, based on the Week-48 and -96 efficacy data. The rates of virologic success of HIV-1 RNA suppression <50 copies/mL through Week 96 were comparable between the two EVG and RAL treatment groups in censored, as-treated snapshot analysis: 65.9% (216/328) versus 65.7% (207/315) at Week 48, and 60.9% (190/312) versus 64.5% (191/296) at Week 96, respectively. Furthermore, antiviral response to both treatments appeared to be durable as 86.1% (180/209) and 87.2% (177/203) of EVG and RAL recipients who achieved virologic suppression at Week 48 and continued to receive their assigned treatment in Year 2 remained aviremic at Week 96. In both treatment groups, virologic rebound was the primary cause of the virologic failure (having HIV-1 RNA ≥50 copies/mL at Weeks 48 and 96, or at the time of early study drug discontinuation). Of those EVG-treated virologic failures, 71.6% (73/102) and 78.1% (89/114) experienced virologic rebound by Weeks 48 and 96, respectively, while virologic rebound was observed in 81.7% (76/93) and 88.1% (89/101) of the RAL-treated virologic failures. Negative correlation between baseline HIV-1 RNA levels and antiviral efficacy of EVG (+BR) was observed. Subjects with baseline viral RNA levels ≥5 $\log_{10}$ copies/mL achieved and maintained virologic success at lower rates, 47.6% (40/84) and 45.1% (37/82) at Weeks 48 and 96, respectively, while the virologic success rates for subjects with baseline viral RNA levels <5 $\log_{10}$ copies/mL were 72.1% (176/244) and 66.5% (153/230), respectively.

In Study GS-US-183-0145, by Week 96, among 72 virologic failures receiving EVG-containing regimens with evaluable resistance data, primary EVG^R substitutions were detectable in 22 subjects' failure isolates. In addition, two subjects' viruses also developed primary EVG^R substitutions while they experienced transient virologic rebound. Primary EVG^R substitutions were detectable at 7 of the 9 identified resistance-associated amino acid positions in post-

baseline virus samples of these 24 subjects with EVG genotypic resistance: T66A/I (n=8), E92G/Q (n=7), T97A (n=5), Q146R (n=1), S147G (n=4), Q148R (n=4), and N155H (n=5). In 14 of these subjects (58.3%), emergence of 11 secondary EVG^R substitutions was also observed in the same virus populations harboring primary substitutions: H51Y, L68V, G70R, V72A/I/N, Q95K, T112A, T124A, E138K, G140A/C/S, E170A, and D232N. The emergence of primary EVG^R substitutions resulted in median decreases in susceptibility to EVG of 8.2-fold, ranging from 1.6- to >158.1-fold (29 isolates with evaluable data from 21 subjects), compared to wild-type reference HIV-1. Several of these isolates (20/29) appeared to be cross-resistant to RAL with >1.5-fold reduced susceptibility, ranging from 1.9- to 53-fold. Overall, among the 24 subjects who developed genotypic resistance to EVG with evidence of emerging primary EVG^R substitutions, 14 (66.7%) of the 21 subjects with evaluable data showed phenotypic resistance to EVG. In addition, 12 (57.1%) of those 21 evaluated subjects (11 [78.6%] of the 14 subjects with EVG phenotypic resistance) had HIV-1 variants phenotypically resistant to RAL.

3. Administrative

3.1. Reviewer's Signature(s)

_____
Sung S. Rhee, Ph.D.
Clinical Virology Reviewer

3.2. Concurrence

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

CC:
HFD-530/NDA # 203093
HFD-530/Division File
HFD-530/PM/P. Hong

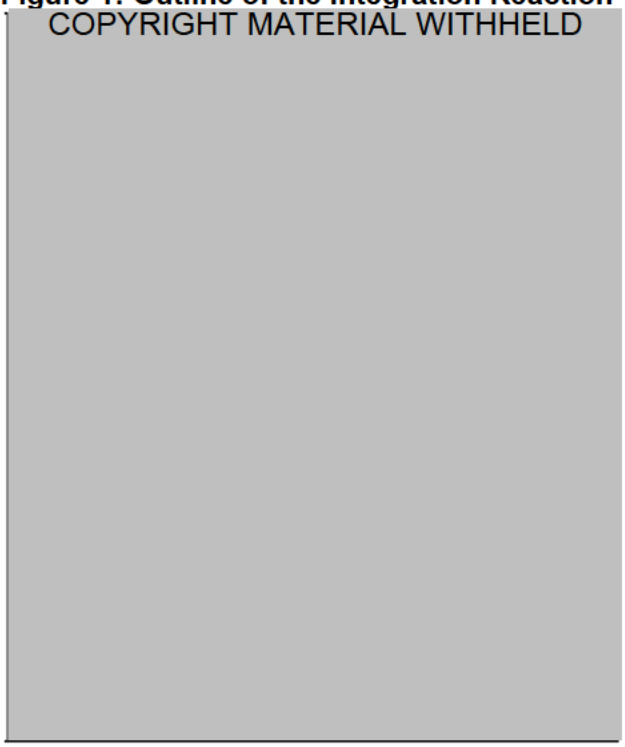
OND VIROLOGY REVIEW

1. Introduction and Background

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). 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 (Figure 1, 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. Thus, proviral integration is one of the essential steps in the HIV-1 life cycle and requires the HIV-1 integrase (IN) present in the virion (reviewed by Freed, 2001).

HIV-1 DNA integration is mediated by a complicated series of reactions that includes two catalytic reactions (Figure 1): 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 reaction*n*s 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 IN 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).

Figure 1: Outline of the Integration Reaction



In the first catalytic step, HIV-1 IN recognizes short sequences within the LTR (long terminal repeat; approximately 20 bp; Sherman and Fyfe, 1990) and removes a terminal GT dinucleotide from the 3'-OH end of each LTR to produce CA-3'-OH that are thus recessed by two nucleotides (Figure 1). 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.

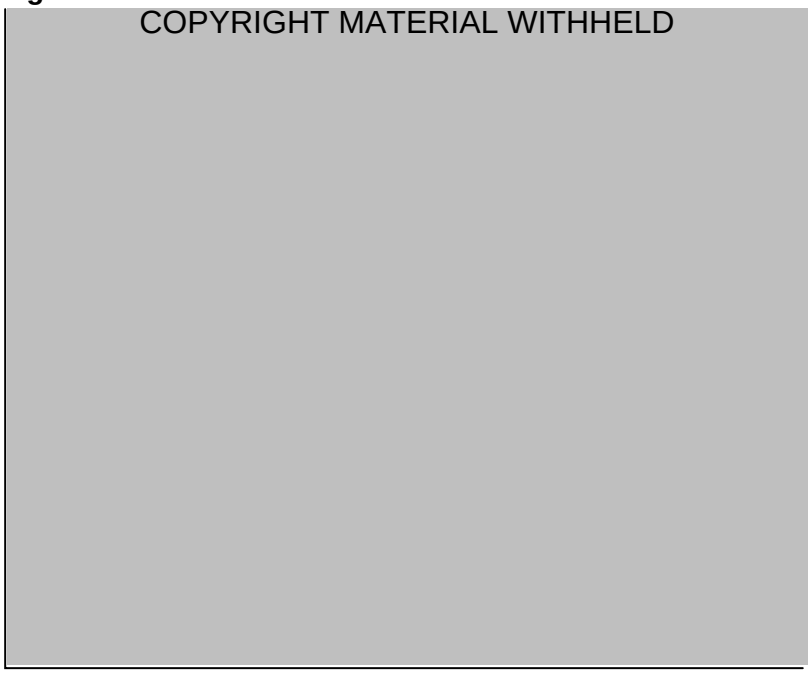
Following the nuclear import of the PIC, the compact structure of the PIC disintegrates and the linear viral DNA is joined to the cellular DNA. This strand-transfer step involves a nucleophilic attack of the phosphodiester bond of the host cellular DNA by the 3'-OH group of the viral DNA to yield a staggered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

The HIV-1 integrase (IN) protein, one of the three virally encoded enzymes, is encoded by the *pol* gene of the virus, expressed as part of a large Gag-Pol polyprotein, and cleaved by the viral protease into its biologically active form of IN consisting of two 32-kDa IN subunits (homodimeric). The HIV IN protein (288 amino acids) is 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.

As shown in Figure 2 (from Vandegraaff and Engelman, 2007), IN consists of 3 distinct functional domains, an N-terminal domain (NTD) of 50 amino acids, a central core domain (CCD) of 160 amino acids, and a C-terminal domain (CTD) of 80 amino acids. The N-terminal domain (NTD) contains a putative zinc-binding HHCC motif (H12, H16, C40, and C43) and is believed to be involved in multimerization of IN (reviewed by Pommier *et al*., 2005; Van Maele and Debyser, 2005). The central core domain (CCD) 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

Figure 2: Functional Domains and Structures of HIV-1 IN



conserved C-terminal domain (CTD) 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. Mutational analyses of HIV-1 IN indicated that all 3 domains are required for 3' endonucleolytic processing and DNA strand transfer (Drelich *et al*., 1992; Engelman and Craigie, 1992; Schauer and Billich, 1992; Vink *et al*.,1993).

The HIV-1 IN catalytic core 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. 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 (INSTIs) 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.

HIV IN has been considered an attractive target for drug development, since integration is absolutely required for productive viral replication in $CD4^+$ T lymphocytes (Engelman *et al*., 1995) and there are no apparent functional equivalents in human cells of HIV-1 IN perhaps with the possible exception of the RAG1/2 recombinase. Raltegravir (RAL; ISENTRESS®, Merck Sharp & Dohme Corp.) is the first and only member of the HIV-1 integrase strand transfer inhibitor (INSTI) class of antiretroviral (ARV) drugs that was approved by FDA for the treatment of HIV-1 infection in combination with other ARVs in October, 2007 (NDA 022-145). Elvitegravir (EVG) also inhibits the strand transfer reaction of HIV-1 IN and thus suppresses HIV-1 replication. Recently, a complete regimen STRIBILD™ (Gilead Sciences, Inc.) where EVG is co-formulated as a single tablet with two FDA-approved HIV-1 NRTIs, FTC (200 mg; Emtriva®) and TDF (300 mg; Viread®), and a pharmacokinetic enhancer cobicistat (150 mg) was approved by FDA for the treatment of HIV-1 infection in adults who are ARV treatment-naïve in August, 2012 (NDA 203-100). This original new drug application (NDA) is submitted for U.S. marketing approval of EVG tablets (85 and 150 mg) as a single agent. The proposed indication for the

EVG tablets is for once daily use in combination with a ritonavir-boosted protease inhibitor and with other ARVs for the treatment of HIV-1 infection in ARV treatment-experienced adults.

The NDA package for EVG includes clinical and virology study reports and datasets from the pivotal Phase 3 study (GS-US-183-0145, Week-96 data included) and two supportive studies, a Phase 2 dose-finding study (GS-US-183-0105, completed; Week-48 data included) and an open-label roll-over study (GS-US-183-0130, on-going; up to Week-192 data included). The Phase 3 study GS-US-183-0145 is a randomized, double-blind, double-dummy, multicenter, active-controlled study to assess the noninferiority of EVG versus RAL, each administered with a background ARV regimen containing a fully active ritonavir-boosted protease inhibitor (PI/r) and a second agent in HIV-1 infected, ARV treatment-experienced adults. The efficacy of EVG is primarily based on the analyses through 96 weeks from this Phase 3 study, and EVG was non-inferior in achieving HIV-1 RNA <50 copies/mL when compared to RAL. The Phase 2 study GS-US-183-0105 is a completed randomized, partially blinded (EVG dose), multicenter, multiple-dose, active-controlled, dose-finding study to assess noninferiority of ritonavir-boosted EVG (EVG/r) relative to PI/r, both in combination with a background ARV regimen, in HIV-1 infected, ARV treatment-experienced adults. Subjects who completed this 48-week study were offered open-label EVG in a rollover study (GS-US-183-0130).

2. Nonclinical Virology

Please refer to the Microbiology review of the STRIBILD™ NDA, NDA203100.001, for the nonclinical virology of EVG, including mechanism of action, antiviral activity in cell culture, combination activity relationships with FDA-approved ARVs**,** resistance development in cell culture, and cross-resistance with RAL. Below nonclinical virology data from previously submitted studies for the approval of STRIBILD™ tablet are briefly summarized.

EVG prevents the HIV-1 integrase (IN)-catalyzed integration of unintegrated linear HIV-1 DNA into the host cell genome by specifically inhibiting the strand transfer activity of HIV-1 IN. In a biochemical reaction, EVG was shown to inhibit the DNA strand transfer step of HIV-1 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 RAL. 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 (reviewed by Champoux, 2001). In HIV-1-infected MT-4 cells, EVG inhibited the integration of HIV-1 DNA into the host chromosomal DNA (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).

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). 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 (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). 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 was not antagonistic when evaluated in pair-wise combination antiviral activity assays with 22 FDA-approved ARV drugs in HIV-1-infected cells: the INSTI raltegravir, 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 (T-20), and a CCR5 co-receptor antagonist maraviroc.

In the cell-based resistance selection experiments, HIV-1 variants that were resistant to EVG were selected, in which substitutions at 13 amino acid positions in HIV-1 IN were consistently 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 amino acid positions, substitutions at 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 amino acid positions conferring varying degrees of reductions in susceptibility 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).

Cross-resistance between EVG and RAL 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 above, HIV-1 variants harboring IN substitution(s) selected by EVG 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 addition, among the 3 primary RAL resistance-associated substitutions examined (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-associated IN substitutions for EVG and RAL (i.e., T66I and Y143H, respectively), a high degree of overlapping resistance between EVG and RAL was observed. In contrast, those IN substitutions selected by EVG in the cell-based resistance selection experiments conferred no significant cross-class resistance to other ARVs 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. Furthermore, 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).

3. Clinical Virology

3.1. Antiviral Efficacy (Potency and Durability) of EVG in Study 145

The antiviral efficacy (potency and durability) analyses were conducted in the censored, as-treated population including subjects who had at least one on-treatment viral load measurement (n=691) in the Phase 3 study GS-US-183-0145 (Study 145) to determine whether EVG is noninferior to RAL, each administered with a background regimen (BR), for the treatment of HIV-1 infection in ARV treatment-experienced adult subjects. A brief description of the trial design of the study is provided in Table 1. Assays for HIV-1 RNA quantification are described in Appendix 1. For the overall efficacy of EVG+BR in the Intent-to-treat (ITT) population, please refer to the reviews by Medical Officer Russell Fleischer, PA-C and Statistical Reviewer Lei Nie, Ph.D. At Week 48 using the FDA-defined snapshot analysis in the ITT population, 59.8% (210/351) of subjects in the EVG+BR treatment group and 57.5% (202/351) of subjects in the RAL+BR treatment group achieved virologic success with HIV-1 RNA <50 copies/mL. Thus, EVG QD+BR was noninferior (pre-specified noninferiority margin of 10%) to RAL BID+BR with the stratum-adjusted difference in the virologic success rate of 2.2% (95% CI: -5.0% to 9.3%). At Week 96, 52.4% (184/351) of subjects in EVG+BR treatment group and 53.0% (186/351) of subjects in the RAL+BR treatment group had virologic success. The stratum-adjusted difference between EVG and RAL treatment groups was -0.5%, and the 95% CI was -7.9% to 6.8%.

Table 1: Summary of Trial Design: Study GS-US-183-0145

Title	A Multicenter, Randomized, Double-Blind, Double-Dummy, Phase 3 Study of the Safety and Efficacy of Ritonavir-Boosted Elvitegravir (EVG/r) versus Raltegravir (RAL) Each Administered with a Background Regimen in HIV-1 Infected, Antiretroviral Treatment-Experienced Adults
Study population	- HIV-1 infected with plasma HIV-1 RNA levels ≥1,000 copies/mL at screening - ARV treatment-experienced - Documented resistance from ≥2 different classes of ARVs - Fully sensitive to the selected PI (ATV, DRV, FPV, LPV, or TPV)
Stratification	- Baseline HIV-1 RNA level ≤100,000 or >100,000 copies/mL - Class of the second agent (NRTI versus other classes)
Treatment group	- Group 1: EVG 150 mg QD (EVG 85 mg QD for subjects taking ATV/r or LPV/r as part of their BR) + RAL placebo BID + BR - Group 2: RAL 400 mg BID + EVG placebo QD + BR
Background regimen (BR)	The BR was constructed by the investigator based on viral resistance testing and was to be composed of (1) a fully-active ritonavir-boosted PI (ATV/r, DRV/r, FPV/r, LPV/r, or TPV/r), defined as being below the clinical or biological cutoff by phenotypic resistance analysis (PhenoSense GT® assay, Monogram Biosciences) AND (2) a second agent that may or may not have been fully active and could have been one NRTI, etravirine, maraviroc, or enfuvirtide.
Duration of treatment:	- Blinded phase: 144 weeks - Optional open-label extension phase: 144 weeks
Primary efficacy point	Proportion of subjects who achieved and maintained confirmed HIV-1 RNA <50 copies/mL through Week 48

A total of 691 subjects received at least one dose of study medication and had at least one HIV-1 RNA measurement after Baseline, who were included in the as-treated antiviral efficacy

analysis (Table 2, 346 and 345 subjects in the EVG and RAL treatment groups, respectively). Baseline virologic characteristics were similar between the 2 treatment groups with a median baseline HIV-1 RNA level of 4.4 $\log_{10}$ copies/mL and approximately 26% of subjects in each group had HIV-1 RNA >100,000 copies/mL (Table 2). The majority (93.8%, 648/691) of subjects were infected with HIV-1 subtype B: 93.9% (325/346) and 93.6% (323/345) of EVG and RAL recipients, respectively. Antiretroviral medications used in the BR were also balanced between the 2 treatment groups. DRV/r was used most frequently as a fully-active PI/r background agent (Table 2): 57.5% (199/346) and 59.1% (204/345) of subjects in the EVG and RAL treatment groups, respectively. For the second background agent, most (80.3% in each treatment group) received NRTIs (ABC, DDI, FTC, LAM, TDF, or ZDV; Table 2). The most frequently used NRTIs were TDF and Truvada (TVD; fixed-dose combination tablet containing FTC 200 mg and TDF 300 mg): 46% (159/346) and 47.8% (165/345) of EVG and RAL recipients received TDF (without FTC), respectively, and 26.0% (90/346) and 19.1% (66/345) received TVD. The NNRTI ETR was used by 12.7% (44/346) and 15.4% (53/345) of subjects in the EVG and RAL treatment groups, respectively, while 6.9% (24/346) and 5.2% (18/345) used MVC. T-20 was used only by 3 subjects (2 EVG recipients and one RAL recipient).

In as-treated snapshot analysis using viral load data measured by the Roche AMPLICOR HIV-1 Monitor™ Test (version 1.5; Appendix A1.1.), the rates of virologic success at Week 48 were comparable between the two treatment groups (Table 2): 65.9% (216/328) of EVG(+BR) recipients and 65.7% (207/315) of RAL(+BR) recipients had HIV-1 RNA <50 copies/mL in the 48-week evaluation window (Treatment Days 309 - 364, inclusive). Antiviral response to both treatments appeared to be durable through Year 2 with 60.9% (190/312) and 64.5% (191/296) of subjects in the EVG and RAL treatment groups, respectively, having HIV-1 RNA <50 copies/mL in the 96-week evaluation window (Treatment Days 645 - 700, inclusive; Table 2). Furthermore, of the 209 and 203 EVG and RAL recipients who achieved virologic suppression (HIV-1 RNA <50 copies/mL) at Week 48 and continued to receive their assigned treatment in Year 2, 86.1% (180/209) and 87.2% (177/203) remained suppressed at Week 96 (or at their last on-treatment evaluation time-point in Year 2). Of note, subjects who discontinued assigned treatment before the end of each study year (e.g., Weeks 48 and 96) while they had a suppressed viral load were excluded from the virologic rate calculation described in Table 2. Virologic responses to EVG(+BR) treatment by baseline HIV-1 RNA levels, HIV-1 subtype, baseline genotypic/ phenotypic sensitivity score, and type of PI or the second agent in the BR are summarized below in Tables 3-6.

In both treatment groups, virologic rebound was the primary cause of the virologic failure (having HIV-1 RNA ≥50 copies/mL in the Week-48 and Week-96 windows or at the time of early study drug discontinuation; Table 2). Of those EVG-treated virologic failures, 71.6% (73/102) and 78.1% (89/114) experienced virologic rebound by Weeks 48 and 96, respectively, while virologic rebound was observed in 81.7% (76/93) and 88.1% (89/101) of the RAL-treated virologic failures. 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. It should be noted that 16.7% (52/312) and 11.8% (35/296) of subjects in the EVG and RAL treatment groups, respectively, never had HIV-1 RNA levels <50 copies/mL through 96 weeks of treatment (Table 2), and the majority (51.9% [27/52] and 65.7% [23/35]) experienced virologic rebound with >1 $\log_{10}$ copies/mL increase in viral load from treatment nadir.

Table 2: Baseline Virologic Characteristics and Virologic Response to Treatment at Weeks 48 and 96 in Study 145

Treatment group	EVG QD + BR (n=346[1])		RAL BID + BR (n=345[1])	
Median baseline HIV-1 RNA, log_{10} copies/mL (range)	4.4 (1.7[2] - 6.6)		4.4 (1.7[2] - 6.1)	
Baseline HIV-1 RNA levels				
≤100,000 copies/mL	257 (74.3%)		256 (74.2%)	
>100,000 copies/mL	89 (25.7%)		89 (25.8%)	
PI/r randomized background agent				
ATV/r	59 (17.1%)		50 (14.5%)	
DRV/r	199 (57.5%)		204[3] (59.1%)	
FPV/r	14 (4%)		19[3] (5.5%)	
LPV/r	68 (19.7%)		66 (19.1%)	
TPV/r	6 (1.7%)		7 (2%)	
Second randomized background agent				
NRTI	278[4] (80.3%)		277[5] (80.3%)	
Other ARV classes	70[4] (20.2%)		72[5] (20.9%)	
Virologic response analysis[6]	**Week 48** (n=328[6])	**Week 96** (n=312[6])	**Week 48** (n=315[6])	**Week 96** (n=296[6])
Virologic success[7]	216 (65.9%)	190 (60.9%)	207 (65.7%)	191 (64.5%)
Virologic failure (VF)[8]	102[8] (31.1%)	114[8] (36.5%)	93[8] (29.5%)	101[8] (34.1%)
Virologic rebound (VR)[9]	73 (22.3%)	89 (28.5%)	76 (24.1%)	89 (30.1%)
Never suppressed[10]	54 (16.5%)	52 (16.7%)	38 (12.1%)	35 (11.8%)

[1] A total of 691 subjects received at least one dose of study medication and had at least one HIV-1 RNA measurement after Baseline (as-treated subject population): 346 subjects in the EVG treatment group and 345 subjects in the RAL treatment group.
[2] Five and 6 subjects in the EVG and RAL treatment groups, respectively, had HIV-1 RNA <50 copies/mL at Baseline.
[3] One subject had two fully active PIs, DRV and FPV, in his background regimen (BR).
[4] Two subjects had one NNRTI (ETR) + one or two NRTIs in their BR.
[5] Four subjects had one NNRTI (ETR, n=3) or T-20 (n=1) + one or two NRTIs in their BR.
[6] Virologic responses to treatment at Weeks 48 and 96 were determined using the FDA-defined snapshot analysis in the censored, as-treated population that includes all randomized subjects who received at least one dose of study medication and had 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). In the EVG treatment group, 328 and 312 subjects were included in the Week-48 and Week-96 analyses, respectively, as 18 and 34 virologic suppressors who discontinued before Week 48 and Week 96 were censored. In the RAL treatment group, 30 and 49 virologic suppressors discontinued before Week 48 and Week 96, respectively, hence 315 and 296 subjects were included in the Week-48 and Week-96 analyses.
[7] Virologic success is defined as having a plasma viral load <50 copies/mL in the 48- and 96-week evaluation window (between Treatment Days 309 and 364, and between Treatment Days 645 and 700, inclusive, respectively).
[8] Virologic failure (VF) is defined as having a plasma viral load ≥50 copies/mL in the 48- and 96-week evaluation window, at the time of early discontinuation, or at the time of change in background regimen. If subjects experienced viral load blips in the evaluation window, they were not considered to have experienced virologic failure or virologic suppression. Viral blips were observed in the 48- and 96-week evaluation window in 10 and 8 EVG recipients, and 15 and 4 RAL recipients, respectively.
[9] Virologic rebound (VR) 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.
[10] Never having a plasma viral load <50 copies/mL during the 48- and 96-week treatment duration.

3.1.1. Virologic Response by Baseline HIV-1 RNA Levels

The correlation between HIV-1 RNA levels at Baseline and antiviral efficacy of EVG QD + BR was analyzed and results are summarized in Table 3. Lower rates of virologic success were observed at Weeks 48 and 96 in the subgroup of subjects with higher baseline viral load

(negative correlation between baseline HIV-1 RNA levels and antiviral efficacy of EVG+BR). Compared to overall virologic success rates of 65.9% (216/328) and 60.9% (190/312) at Weeks 48 and 96 (Table 2), subjects with baseline viral RNA levels ≥5 $\log_{10}$ copies/mL achieved and maintained virologic success at lower rates, 47.6% (40/84) and 45.1% (37/82), respectively. The virologic success rates for subjects with baseline viral RNA levels <5 $\log_{10}$ copies/mL were 72.1% (176/244) and 66.5% (153/230), respectively. In agreement with these observations, EVG+BR-treated subjects with virologic success at Week 96 had slightly lower median baseline viral load than those with virologic failure (4.2 versus 4.7 $\log_{10}$ HIV-1 RNA copies/mL). Similar negative correlation was also observed in subjects who received RAL BID + BR treatment (Table 3).

Table 3: Correlation between Baseline HIV-1 RNA Level and Virologic Response to Treatment in Study 145

Baseline HIV-1 RNA ($\log_{10}$ copies/mL)	Virologic success rate[1]			
	EVG QD + BR		RAL BID + BR	
	Week 48 (n=328)	**Week 96** (n=312)	**Week 48** (n=315)	**Week 96** (n=296)
<4	76% (95/125)	71.7% (81/113)	78.7% (85/108)	77.2% (78/101)
≥4 but <5	68.1% (81/119)	61.5% (72/117)	69.7% (85/122)	67.5% (77/114)
≥5 but <6	50% (39/78)	47.4% (36/76)	43.4% (36/83)	45.6% (36/79)
≥6	16.7% (1/6)	16.7% (1/6)	50% (1/2)	0% (0/2)

[1] Virologic success is defined as having a plasma viral load <50 copies/mL in the 48- and 96-week snapshot evaluation window (between Treatment Days 309 and 364, and between Treatment Days 645 and 700, respectively). This analysis was done using the censored, as-treated population that includes all randomized subjects who received at least one dose of study medication and had 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).

3.1.2. Virologic Response by HIV-1 Subtype

In Study 145, the majority (93.6%, 602/643) of subjects who were included in the censored, as-treated antiviral efficacy analysis were infected with HIV-1 subtype B (Table 4): 93.9% (308/328) and 93.3% (294/315) of those receiving EVG(+BR) and RAL(+BR) treatment, respectively. HIV-1 subtype B is the predominant subtype in North America, Western Europe, and Australia, while subtype B is estimated to contribute to 11% of the worldwide epidemic (Hemelaar *et al*., 2011). Among the 41 subjects (6.4%, 41/643) with non-B subtype infection, various forms of subtype A were most common (n=21, including A, A1, A2, AE, and AG), followed by subtype G (n=8) and subtype C (n=5; Table 4). Other subtypes (D, F, and complex) and recombinant forms of subtype B (B/C, BF, and B/G) were observed in 7 subjects (Table 4).

EVG(+BR)-treated subjects with HIV-1 non-B subtype infection (n=20) had a lower virologic success rate at Week 48, compared to those with subtype B infection (60% [12/20] versus 66.2% [204/308]; Table 4), but their Week-96 virologic success rate became higher (68.4% [13/19] versus 60.4% [177/293]). Baseline HIV-1 RNA levels were comparable between the two subgroups of subjects with median baseline HIV-1 RNA levels of 4.4 $\log_{10}$ copies/mL for subtype B-infected subjects (range, 1.7 - 6.5) and of 4.2 $\log_{10}$ copies/mL for non-B subtype-infected subjects (range, 2.6 - 6.6). Overall, subjects with HIV-1 non-B subtype infection did not appear to have a reduced antiviral response to their EVG-containing regimens. However, due to the small sample size (only 20 subjects infected with non-B subtype), this subgroup analysis

does not provide a definitive conclusion. Of note, in the cell culture nonclinical study using human PHA/IL-2-activated PBMCs, EVG was found to be active against all B and non-B subtype HIV-1 clinical isolates tested (subtypes A, C, D, E, F, G, and Group O) with mean EC_{50} values ranging from 0.1 to 1.26 nM. With limited numbers of subjects with non-B subtype infection (n=21), RAL-containing regimens were also found to be effective against HIV-1 non-B subtypes as well as subtype B (Table 4): 81% [17/21] versus 64.6% [190/294] at Week 48 and 73.7% [14/19] versus 63.9% [177/277] at Week 96, respectively.

Table 4: Virologic Response to Treatment by HIV-1 Subtype in Study 145

HIV-1 Subtype	Virologic success rate[1]			
	EVG QD + BR		RAL BID + BR	
	Week 48 (n=328)	**Week 96** (n=312)	**Week 48** (n=315)	**Week 96** (n=296)
B	66.2% (204/308)	60.4% (177/293)	64.6% (190/294)	63.9% (177/277)
Non-B	60% (12/20)	68.4% (13/19)	81% (17/21)	73.7% (14/19)
A	-	-	100% (1/1)	100% (1/1)
A1	66.7% (2/3)	66.7% (2/3)	100% (3/3)	100% (3/3)
A2	100% (1/1)	100% (1/1)	100% (1/1)	(0/0)
AE	50% (1/2)	50% (1/2)	-	-
AG	60% (3/5)	80% (4/5)	60% (3/5)	50% (2/4)
BF	100% (1/1)	100% (1/1)	-	-
B/C	-	-	0% (0/1)	0% (0/1)
B/G	-	-	100% (1/1)	100% (1/1)
C	100% (1/1)	100% (1/1)	100% (4/4)	75% (3/4)
D	100% (2/2)	100% (2/2)	-	-
F	100% (1/1)	100% (1/1)	-	-
G	0% (0/4)	0% (0/3)	75% (3/4)	75% (3/4)
complex	-	-	100% (1/1)	100% (1/1)

[1] Virologic success is defined as having a plasma viral load <50 copies/mL in the 48- and 96-week snapshot evaluation window (between Treatment Days 309 and 364, and between Treatment Days 645 and 700, respectively). This analysis was done using the censored, as-treated population that includes all randomized subjects who received at least one dose of study medication and had 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).

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 a Mn^{++}-based assay with IC_{50} values of 14 nM and 18 nM, respectively. However, several studies indicated 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).

3.1.3. Virologic Response by Baseline Genotypic/Phenotypic Sensitivity Score

Using the PhenoSense GT assay (Monogram Biosciences; Appendix A1.2.), each subject's

PR/RT genotype and phenotype were assessed at screening. The phenotypic sensitivity score (PSS) that is an output of the assay for each of the drugs tested was used for the construction of the background regimen (BR). In Study 145, the RTV-boosted PI (PI/r) used in the background regimen was required to show full activity in the phenotypic assay and therefore should have a PSS of 1. For the second agent in the BR, full activity in the phenotypic assay was not required. Of note, the applicant reported the phenotypic results of baseline HIV-1 from each subject in this study using the incremental PSS for the drug tested. A PSS of 1 indicates that the value obtained is below the clinical/biological susceptibility cutoff (or lower cutoff). A PSS of 0 indicates that the value obtained is above the cutoff (or upper cutoff). For drugs with two cutoffs (lower and upper cutoffs), a PSS of 0.5 was obtained when the value falls in between the two cutoffs.

As summarized in Table 5, among 643 subjects who were included in the censored, as-treated antiviral efficacy analysis, 638 subjects (99.2% [638/643]; 324 EVG and 314 RAL recipients) received one or two phenotypically fully active PIs as a PI/r background agent (n=637 with PSS=1 and n=1 with PSS=2). The one subject in the RAL treatment group received 2 fully active PIs (PSS=2), DRV and FPV, who was still viremic at the time of early discontinuation (only 11 days of study-drug treatment). Four subjects (3 EVG and 1 RAL recipients) whose screening viruses were not phenotypically fully susceptible to DRV (PSS of <1; n=1 with PSS=0 and n=3 with PSS=0.5) received DRV as their PI/r background agent and all discontinued early their assigned treatment (≤28 days of treatment) without achieving virologic suppression. The PSS for the PI (ATV) in the BR is missing for one EVG(+BR)-treated subject. Virologic responses to EVG(+BR) treatment by type of PI used in the BR are summarized below in Table 6.

Of those 638 subjects with at least one fully active PI in their BR, most (91.8%, 586/638) also had active 2nd background agents (PSS of ≥1, calculated as the sum of the PSS for the individual 2nd agents in the BR): 560 subjects with PSS=1, 4 subjects with PSS=1.5, and 22 subjects with PSS=2. These subjects had one to four 2nd background agents (n=430 with 1 agent, n=151 with 2 agents, n=4 with 3 agents, and n=1 with 4 agents). The remaining 52 subjects (8.2%, 52/638) had one or two 2nd background agents (n=35 with 1 agent and n=17 with 2 agents) that were not fully active against the subjects' baseline HIV-1 (PSS of <1): 7 subjects with PSS=0 and 45 subjects with PSS=0.5. In both the EVG and RAL treatment groups, higher virologic success rates at Weeks 48 and 96 were observed for the subgroup of subjects receiving phenotypically partially active or inactive 2nd agents of the BR (PSS of <1; Table 5), compared to those for subjects receiving active 2nd background agents (PSS of ≥1; Table 5): 78.3% [18/23] versus 65.8% [198/301] at Week 48 and 69.6% [16/23] versus 61.1% [174/285] at Week 96 of EVG recipients, and 79.3% [23/29] versus 64.6% [184/285] at Week 48 and 85.7% [24/28] versus 62.5% [167/267] at Week 96 of RAL recipients. Furthermore, the rates of virologic success were numerically higher in 7 subjects receiving phenotypically inactive 2nd background agents (PSS=0), compared to those in subjects receiving active 2nd background agents (PSS of ≥1): 71.4% (5/7) versus 65.2% (382/586) at Week 48 and 66.7% (4/6) versus 61.8% (341/552) at Week 96 in the overall study population (EVG- and RAL-treated).

Similar observations were made when virologic response was analyzed using the baseline genotypic sensitivity score (GSS; Table 5). The GSS for each of the drugs tested is also an output of the PhenoSense GT assay and reflects the expected activity of each drug, GSS being either 1 (sensitive) or 0 (reduced susceptibility). Together, in this study population, active 2nd

background agents did not appear to contribute significantly to the short-term antiviral efficacy (96 weeks) of treatment regimens containing at least 2 fully active antiretrovirals (one study drug, EVG or RAL, of the INSTI class and one member of the PI class). The results of these subgroup analyses should be interpreted with caution, since the sample size was small. Virologic responses to EVG(+BR) treatment by ARV class of the second agent in the BR are summarized below in Table 6.

Table 5: Virologic Response to Treatment by Genotypic/Phenotypic Sensitivity Score in Study 145

BR	Virologic success rate[1]			
	EVG QD + BR		RAL BID + BR	
	Week 48 (n=328)	**Week 96** (n=312)	**Week 48** (n=315)	**Week 96** (n=296)
PI, PSS of <1	0% (0/3)	0% (0/3)	0% (0/1)	0% (0/1)
PI, PSS of ≥1	66.7% (216/324)	61.7% (190/308)	65.9% (207/314)	64.7% (191/295)
2nd agent(s), PSS of ≥1	65.8% (198/301)	61.1% (174/285)	64.6% (184/285)	62.5% (167/267)
2nd agent(s), PSS of <1	78.3% (18/23)	69.6% (16/23)	79.3% (23/29)	85.7% (24/28)
Data missing	0% (0/1)	0% (0/1)	-	-
PI, GSS of 0	55.6% (5/9)	55.6% (5/9)	77.8% (7/9)	75% (6/8)
PI, GSS of ≥1	66.4% (211/318)	61.3% (185/302)	65.4% (200/306)	64.2% (185/288)
2nd agent(s), GSS of ≥1	63.9% (175/274)	58.7% (152/259)	63.9% (170/266)	62% (155/250)
2nd agent(s), GSS of 0	81.8% (36/44)	76.7% (33/43)	75% (30/40)	78.9% (30/38)
Data missing	0% (0/1)	0% (0/1)	-	-

[1] Virologic success is defined as having a plasma viral load <50 copies/mL in the 48- and 96-week snapshot evaluation window (between Treatment Days 309 and 364, and between Treatment Days 645 and 700, respectively). This analysis was done using the censored, as-treated population that includes all randomized subjects who received at least one dose of study medication and had 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).

The applicant noted the higher proportion of voluntary discontinuations (defined as study drug discontinuation due to withdrawal of consent, non-adherence, or loss to follow-up) in subjects having a GSS of 2 (two active background agents) in comparison to subjects having a GSS of 1 (one active background agent): 16% (92/575) versus 6.8% (7/103) based on the Week-48 data (ITT analysis). These observations indicated subjects with fewer therapeutic options were more likely to continue on study drug.

3.1.4. Virologic Response by Background Regimen

As summarized above in Table 5, among 643 subjects who were included in the censored, as-treated antiviral efficacy analysis, 638 subjects (99.2%; 324 EVG and 314 RAL recipients) received one or two phenotypically fully active PIs as a PI/r background agent: 637 subjects with one PI (PSS=1) and one subject with 2 PIs (PSS=2). Virologic responses to EVG(+BR) treatment by the type of PI used in the BR were analyzed in those subjects having phenotypically fully active PIs (Table 6). Among those receiving a single PI (n=637), DRV/r was used most frequently (58.1%, 370/637): 57.4% (186/324) and 58.8% (184/313) of subjects in

the EVG and RAL treatment groups, respectively. Two other PIs, ATV and LPV, were used by >30 subjects in both treatment groups (Table 6): 16.4% (53/324) and 20.1% (65/324) of EVG recipients, and 14.1% (44/313) and 19.5% (61/313) of RAL recipients, respectively. In both the EVG and RAL treatment groups, no significant differences were observed in virologic response across subgroups of subjects receiving one of these 3 PIs in their BR (>60% achieving virologic success; Table 6), with early virologic success rates (through Week 48) being numerically slightly higher in those using DRV (68.5-69.9%) or ATV (65.9-66%) than those using LPV (60.7-61.5%).

All subjects included in the censored, as-treated antiviral efficacy analysis of Study 145 received at least one second background agent that may or may not have been fully active against subjects' screening HIV-1. These second agents in the BR included NRTIs (ABC, DDI, FTC, LAM, TDF, or ZDV), ETR, MVC, or T-20. Of those 638 subjects who received at least one phenotypically fully active PI as a PI/r background agent, most (91.8%, 586/638) also had active 2nd background agents (PSS of ≥1; Table 5). Out of the 638 subjects, 124 subjects (19.4% [124/638]; 62 subjects in each treatment group) were taking ETR, MVC or T-20 (without NRTIs) as their second background agent (designated as "ARV from the other classes" in Table 6). In both the EVG and RAL treatment groups, these subjects achieved higher rates of virologic success, compared to those taking NRTIs exclusively as the second background agent (Table 6): 74.2% (46/62) versus 64.6% (168/260) and 72.9% (43/59) versus 58.7% (145/247) of EVG recipients at Weeks 48 and 96, respectively, and 72.6% (45/62) versus 64.1% (159/248) and 70.7% (41/58) versus 63.5% (148/233) of RAL recipients. However, when subjects had TVD as the NRTI class background agents in their treatment regimen containing EVG and an active PI/r, their response rates (72.9% [62/85] at Week 48 and 70.5% [55/78] at Week 96; Table 6) were comparable to those (74.2% [46/62] and 72.9% [43/59], respectively) for the subgroup of subjects having an ARV from the other classes. Overall, significant differences were observed in virologic response between the subgroups of subjects who were taking TVD or an ARV from the other classes (with or without NRTIs) and those who were taking ≥2 members of the NRTI class only (except TVD) in addition to EVG and phenotypically fully RTV-boosted PIs. The virologic success rates were 73.8% (110/149) versus 60.6% (106/175) at Week 48, and 71.9% (100/139) versus 53.3% (90/169) at Week 96, respectively (Table 6). This positive contribution of TVD or an ARV from the other class to virologic response observed in the EVG treatment group was also observed but less prominently in the RAL treatment group (Table 6): 69.6% (87/125) versus 63.5% (120/189) at Week 48, and 68.1% (81/119) versus 62.5% (110/176) at Week 96, respectively.

Table 6: Virologic Response to Treatment by Background Regimen in Study 145

BR	Virologic success rate[1]			
	EVG QD + BR		RAL BID + BR	
	Week 48 (n=324)	**Week 96** (n=308)	**Week 48** (n=314)	**Week 96** (n=295)
By type of PI used in the BR				
DRV/r	69.9% (130/186)	60.9% (106/174)	68.5% (126/184)	68.2% (116/170)

Other PI/r[2]	62.3% (86/138)	62.7% (84/134)	62.8% (81/129)	60.5% (75/124)
ATV/r	66% (35/53)	66.7% (34/51)	65.9% (29/44)	57.1% (24/42)
FPV/r	57.1% (8/14)	64.3% (9/14)	58.8% (10/17)	64.7% (11/17)
LPV/r	61.5% (40/65)	60.3% (38/63)	60.7% (37/61)	63.8% (37/58)
TPV/r	50% (3/6)	50% (3/6)	71.4% (5/7)	42.9% (3/7)
2 PIs including DRV/r[3]	-	-	0% (0/1)	0% (0/1)
By class of the second agent in the BR				
NRTI	64.6% (168/260)	58.7% (145/247)	64.1% (159/248)	63.5% (148/233)
ABC	80% (4/5)	25% (1/4)	63.6% (7/11)	50% (5/10)
DDI	0% (0/1)	0% (0/1)	75% (3/4)	75% (3/4)
FTC	100% (1/1)	100% (1/1)	100% (1/1)	-
LAM	55.6% (5/9)	50% (4/8)	37.5% (3/8)	25% (2/8)
TDF	62.1% (90/145)	54.6% (77/141)	61.9% (91/147)	63.8% (88/138)
ZDV	33.3% (1/3)	33.3% (1/3)	100% (6/6)	80% (4/5)
TVD	72.9% (62/85)	70.5% (55/78)	66.1% (39/59)	66.7% (38/57)
2 NRTIs except TVD[4]	45.5% (5/11)	54.5% (6/11)	75% (9/12)	72.7% (8/11)
ARV from the other classes[5]	74.2% (46/62)	72.9% (43/59)	72.6% (45/62)	70.7% (41/58)
ETR	76.3% (29/38)	75% (27/36)	71.1% (32/45)	69.8% (30/43)
MVC	68.2% (15/22)	71.4% (15/21)	76.5% (13/17)	73.3% (11/15)
T-20	100% (2/2)	50% (1/2)	-	-
NRTI + ARV from other classes[6]	100% (2/2)	100% (2/2)	75% (3/4)	50% (2/4)

[1] Virologic success is defined as having a plasma viral load <50 copies/mL in the 48- and 96-week snapshot evaluation window (between Treatment Days 309 and 364, and between Treatment Days 645 and 700, respectively). This analysis was done using the subject population that includes only subjects who had at least one phenotypically fully active PI (PSS of ≥1) in their BR (see Table 5) among those in the censored, as-treated population.
[2] Including RTV-boosted ATV, FPV. LPV, and TPV.
[3] One subject in the RAL treatment group had 2 phenotypically fully active RTV-boosted PIs (DRV and FPV, PSS=2).
[4] A total of 23 subjects (11 EVG- and 12 RAL-treated subjects) had 2 members of the NRTI class except Truvada in their BR, all including LAM: 3, 2, and 6 EVG recipients had LAM+ABC (PSS=0.5, 1, or 2), LAM+TDF (PSS=1), and LAM+ZDV (PSS=1 or 2), respectively; and 6, 1, 2, and 3 RAL recipients had LAM+ABC (PSS=1 or 2), LAM+DDI (PSS=1), LAM+TDF (PSS=0.5 or 1), and LAM+ZDV (PSS=1).
[5] Including an NNRTI ETR, a CCR5 co-receptor antagonist MVC, or a fusion inhibitor T-20.
[6] A total of 6 subjects (2 EVG- and 4 RAL-treated subjects) had one or two members of the NRTI class and one member of the other classes of ARV: 2 EVG recipients had ETR in combination with TDF (n=1, PSS=1.5) or TVD (n=1, PSS=1.5); and 4 RAL recipients had LAM+ETR (n=1, PSS=2), LAM+ZDV+ETR (n=1, PSS=2), TDF+T-20 (n=1, PSS=1.5), or TVD+ETR (n=1, PSS=1).

3.2. Development of Resistance to EVG

To date, as summarized in Table 7, 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 (Microbiology review N203100.001). Some substitutions listed in Table 7 (e.g., T66A/I/K, E92Q, Y143C/H/R, S147G, Q148H/K/R, N155H) were shown to be the primary cause of EVG and/or RAL resistance (Lampiris, 2012). Among the reported primary substitutions associated with INSTI resistance, substitutions T66I, E92Q, Q148R, and N155H were repeatedly detected in virologic failure subjects treated with STRIBILD™ (Gilead Phase 3 Trials GS-US-236-0102 and GS-US-236-0103 in HIV-1-infected, ARV treatment-naïve, adult subjects; STRIBILD™ US Prescribing Information, approved in August, 2012; Microbiology review N203100.001). Those Virologic failure subjects-derived recombinant viruses expressing primary EVG resistance-associated (EVG^R) substitutions had

median decreases in susceptibility to EVG of 44-fold (range: 6- to >198-fold) compared to wild-type reference HIV-1 (NL4-3). In addition, previously reported INSTI resistance-associated IN substitutions H51Y, L68I/V, G140C, S153A, E157Q, V165I, and H183P were also detected in those isolates harboring primary EVG^R substitutions. STRIBILD™ is a recently FDA-approved complete QD regimen of EVG co-formulated with two FDA-approved HIV-1 NRTIs, FTC (200 mg; Emtriva®) and TDF (300 mg; Viread®), and a pharmacokinetic enhancer cobicistat (150 mg). Using site-directed mutagenesis, the applicant showed T66I, E92Q, Q148R, and N155H substitutions conferred individually >14-fold reduced susceptibility to EVG (ranging from 14.9- to >107.6-fold, Table 8; Microbiology review N203100.001).

As shown in Table 7, the genotypic resistance profiles of EVG and RAL were significantly overlapping. Cross-resistance between EVG and RAL 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). Among the 4 primary EVG resistance-associated (EVG^R) substitutions detected in the STRIBILD-treatment virologic failure isolates, E92Q, Q148R, and N155H individually conferred reduced susceptibility both to EVG (>35-fold) and RAL (>5-fold) when introduced into a wild-type virus by site-directed mutagenesis (Table 8; Microbiology review N203100.001). The T66I substitution conferred >14-fold reduced susceptibility to EVG but <3-fold to RAL (Table 8). Of the primary RAL resistance-associated (RAL^R) substitutions, all tested substitutions (Y143H/R, Q148H/K/R, and N155H) conferred >2.5-fold reductions in susceptibility to EVG (above the biological cutoff for EVG; Table 8).

Table 7: 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, E92A/G/Q, 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, E92A/G/Q, 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 (reported 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 (reported primary substitutions are written in red) can be found in the ISENTRESS® US Prescribing Information (revised in August, 2012).

Table 8: Drug Susceptibilities[1] of HIV-1 Variants Harboring IN Substitution(s) Generated by Site-Directed Mutagenesis

Virus	T66A	T66I	T66K	E92G	E92Q	T97A	F121Y	Y143H	Y143R	P145S
EVG	8.4 - 33	14.9 - 30.8	40.8	38.4	39.4 - 78.1	2.1	12.1	1.6	5.4	31.2
RAL	1.2 - 3.8	1.3 - 2.3	19.4	1.7	5.8 - 11.2	2.4	7.3	3.4	37.7	0.6
Virus	**Q146I**	**Q146L**	**Q146P**	**Q146R**	**S147G**	**Q148H**	**Q148K**	**Q148R**	**N155H**	**N155S**

EVG	133.9	20.8	6	4.8	6.8	4.8	49.6	107.6	35.2 - 67.3	64.9
RAL	3.5	1.4	1.2	1.9	1.2	18.8	26.1	34	21 - 25.9	10.9

[1]Fold-reduction in drug susceptibility of infectious HIV-1_{HXB2} recombinant clones harboring IN substitutions using the 5-day cytopathic assay in MT-2 cells (Study PC-183-2025; Microbiology review N203100.001).

3.2.1. Pooled Analysis of EVG Resistance

A pooled analysis of EVG resistance was performed in order to identify (or confirm) EVG resistance pathways using the phenotypic and genotypic data obtained from virus samples (pre- and on-treatment) of resistance testing eligible subjects in 6 clinical trials of EVG:

- Resistance testing eligible subjects in Studies 102[1], 103[1], 104[2], 105[3], and 145[4]:
  - Subjects with suboptimal virologic response, defined as (1) HIV-1 RNA ≥50 copies/mL and <1 $\log_{10}$ copies/mL reduction from baseline at the Week 8 visit, confirmed at the next scheduled or unscheduled visit, or
  - Subjects who experienced virologic rebound, defined as (1) at any visit, after achieving HIV-1 RNA <50 copies/mL, a rebound in HIV-1 RNA to ≥ 400 copies/mL, which is subsequently confirmed at the following scheduled or unscheduled visit or (2) at any time after Week 12 visit, a confirmed increase in HIV-1 RNA ≥1 $\log_{10}$ copies/mL from nadir, or
  - Subjects who failed to achieved confirmed plasma HIV-1 RNA <400 copies/mL by Week 48, Week 96, or by early study discontinuation (after Week 8)
- Resistance testing eligible subjects in Study 130[5]: Subjects HIV-1 RNA >400 copies/mL at the time of study discontinuation, or upon virologic rebound and the investigator requested to reconfigure the subject's antiretroviral regimen without including EVG/r.

[1]Studies 102 (GS-US-236-0102) and 103 (GS-US-236-0103) are similarly designed, on-going Phase 3, double-blind, multicenter, randomized, active-controlled trials to assess the safety and efficacy of STRIBILD™ versus ATRIPLA® (Study 102) or ATV/r+TVD (Study 103) in HIV-1 infected, ARV treatment-naïve adult subjects. Week-48 resistance data were included in this pooled analysis.
[2]Study 104 (GS-US-236-0104) is a completed Phase 2, double-blind, multicenter, randomized, active-controlled trial to assess the safety and efficacy of STRIBILD™ versus ATRIPLA® in HIV-1 infected, ARV treatment-naïve adult subjects. Week-60 resistance data were included in this pooled analysis.
[3]Study 105 (GS-US-183-0105) is a completed Phase 2, randomized, partially blinded (EVG dose), multicenter, multiple-dose, active-controlled, dose-finding study to assess noninferiority of EVG/r relative to PI/r, both in combination with a background ARV regimen, in HIV-1 infected, ARV treatment-experienced adults. Subjects who completed this 48-week study were offered open-label EVG in a rollover study (GS-US-183-0130). Week-48 resistance data were included in this pooled analysis.
[4]Study 145 (GS-US-183-0145) is an on-going Phase 3, double-blind, multicenter, active-controlled trial to assess the noninferiority of EVG versus RAL, each administered with a background ARV regimen containing a fully active PI/r and a second agent in HIV-1 infected, ARV treatment-experienced adult subjects. Week-96 resistance data were included in this pooled analysis.
[5]Study 130 (GS-US-183-0130) is an on-going, open-label, roll-over study to provide continued access to EVG/r for adult and adolescent study subjects who completed a prior EVG/r study without experiencing any treatment-limiting toxicity. Subjects were rolled-over into Study 130 regardless of their baseline HIV-1 RNA level. Eligible subjects may or may not have received EVG in their prior study. Week-192 resistance data were included in this pooled analysis.

3.2.1.1. Primary EVG Resistance-Associated IN Substitutions

First, IN phenotypic results of recombinant viruses derived from 623 baseline and post-baseline virus isolates of 271 subjects who received EVG in their respective studies were analyzed to

identify virus isolates that had reduced susceptibility to EVG (Table 9). Of note, the 271 subjects included 13 subjects from study 102, 11 subjects from Study 103, 2 subjects from Study 104, 162 subjects from Studies 105 and 130, and 83 subjects from Study 145. Virus isolates resistant phenotypically to EVG (see below for definition) without detectable primary EVG^R substitutions (as listed in Table 7) were further analyzed to determine whether additional IN substitutions (other than those previously reported) were associated with phenotypic resistance to EVG and observed repeatedly among the tested virus samples. Results from this initial analysis updated the list of the primary EVG^R substitutions, which was then used for the pooled EVG genotypic resistance analysis of confirmed virologic failure isolates to identify all EVG treatment-emergent genotypic changes and/or confirm the previously observed EVG^R substitutions in HIV-1 IN. Results from the IN phenotypic assays (Appendix A1.2.) were reported as fold-changes (FC) in EC_{50} values compared to that for a wild-type reference laboratory HIV-1 strain (NL4-3). The biological cutoff FC value was determined in the PhenoSense assay (Monogram Biosciences) as the 99th percentile of the distribution of FC values for EVG of viruses from INSTI treatment-naïve subjects. (b) (4) -reported biological cutoff of 2.5 was utilized to define phenotypic resistance to EVG (above the biological cutoff). Rondelez *et al*. (2008) also reported the biological cutoff 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.

As summarized in Table 9, of the 623 virus isolates of EVG recipients with evaluable EVG susceptibility data, previously reported primary EVG^R substitutions (T66A/I/K, E92A/G/Q, S147G, Q148H/K/R, and N155H/S; Table 7) were detected in 241 isolates, and most (95.0%, 229/241) had also phenotypic resistance to EVG displaying ≥2.5-fold reductions in EVG susceptibility (above the biological cutoff).

Table 9: Summary of Pooled EVG Phenotypic Resistance Analysis of Baseline and Post-Baseline Isolates from 271 EVG Recipients with Evaluable IN Phenotypic Data

Sample collection	Number of isolates	Median FC in EVG susceptibility[1]	Number of isolates with FC values above the biological cutoff[2]
overall	623	1.7 (0.7 - 301)	246 (39.5%)
Baseline	223	1.3 (0.7 - 2.7)	5 (2.2%)
Post-baseline	400	28 (0.8 - 301)	241 (60.3%)
With detectable primary EVG^R IN substitution[3]	241	66 (1.1 - 301)	229 (95%)
Baseline	4	1.3 (1.1 - 2.1)	0 (0%)
Post-baseline	237	67 (1.3 - 301)	229 (96.6%)
Without detectable primary EVG^R IN substitution[3]	382	1.3 (0.7 - >165.1)	17 (4.5%)
Baseline	219	1.3 (0.7 - 2.7)	5 (2.3%)
Post-baseline	163	1.3 (0.8 - >165.1)	12 (7.4%)

[1] The applicant-reported FC (fold-change) values indicate fold-reduction in EVG susceptibility of tested virus isolates compared to wild-type reference HIV-1 (NL4-3).
[2] The biological cutoff was determined to be 2.5 for EVG in the PhenoSense assay (Monogram Biosciences). The biological cutoff is defined as the 99th percentile of the distribution of FC values for EVG of viruses from INSTI treatment-naïve subjects.
[3] Primary EVG^R IN substitutions include T66A/I/K, E92A/G/Q, S147G, Q148H/K/R, and N155H/S that were previously reported in EVG-treatment failure subject isolates and/or selected by passage of virus in cell culture in the presence of EVG as listed in Table 7.

There were 17 virus isolates from 13 subjects (4.5% of those tested) that appeared to be phenotypically resistant to EVG with >2.5-fold reduced susceptibility to EVG in the absence of previously reported primary EVG^R substitutions. All post-baseline isolates (n=12 from 9 subjects) had significantly reduced susceptibility to EVG (ranging from 4- to >165.1-fold), while all 5 baseline virus samples showed marginal reductions in EVG susceptibility (ranging from 2.5- to 2.7-fold) close to the biological cutoff used in this analysis. Appendix 2 Table A2-1 listed 17 individual isolates' amino acid changes (or naturally occurring variations) in the HIV-1 IN proteins, relative to the wild-type HIV-1 (NL4-3) reference amino acid sequence. The 12 post-baseline isolates from 9 subjects were further analyzed to identify additional EVG treatment-emergent amino acid substitutions, if any, that may also be the causes of EVG resistance. Results are summarized in Table 10. Among those observed substitutions, F121Y and Q146I substitutions detected in 5 subjects' isolates were reported to individually confer 12.1- and 133.9-fold reduced susceptibility to EVG (Table 8). Amino acid substitutions involving these two conserved-site positions were observed when drug resistant viruses were selected in cell culture in the presence of EVG or RAL (HIV Drug resistance Database, Stanford University; Kobayashi *et al*., 2008; Margot *et al*., 2012; Shimura *et al*., 2008). In addition, a phenylalanine-to-cysteine change at IN position 121 (F121C) was previously observed in virus samples from two RAL-treatment failure subjects and conferred significantly reduced susceptibility to RAL (up to 37-fold). Recent structure-based modeling studies by Krishnan *et al*. (2010) indicated the Q146 residue directly interacts with EVG and RAL In addition to the DDE active-site residues. Leucine or arginine changes at Q146 (Q146L/R) were also observed in subjects treated with EVG-containing regimens included in this pooled study population (found in the same virus population harboring previously-reported primary EVG^R substitutions), and in cell culture conferred 20.8- and 4.8-fold reduced susceptibility to EVG (Table 8). Thus, F121C/Y and Q146I/L/R appeared to be primary contributors to EVG resistance. Of note, conserved 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 conserved 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).

An IN substitution T97A was also frequently found in this virus population (4 of the 9 subjects), and also found in 9% (24/266) of confirmed virologic failure subjects receiving EVG-containing regimens in the absence of other primary EVG^R substitutions (n=6) or as mixtures with others (n=18; see below Table 12). T97A alone showed <2.5-fold loss of susceptibility to EVG (below the biological cutoff for EVG; Table 8), indicating that T97A may require additional substitutions for EVG resistance (such as L68V and T112A; see below Table 13 for details). Previously, the T97A polymorphic-site substitution was considered as a secondary RAL^R substitution, since the substitution alone had no significant effect on RAL susceptibility but was frequently detected in the same virus population harboring primary RAL^R substitutions (Microbiology review N022145.000). T97A alone showed no significant effect on RAL susceptibility but enhanced RAL resistance in combination with Y143C/H/R primary substitutions (Microbiology review N022145.SE7-001; Fransen *et al*., 2009; Reuman *et al*., 2010). Together, substitutions at IN amino acid positions T97 (T97A), F121 (F121C/Y), and Q146 (Q146I/L/R) were additionally identified as primary EVG^R substitutions through the pooled phenotypic resistance analysis of EVG-treated subjects' virus samples displaying phenotypic resistance to EVG (>2.5-fold reductions in EVG susceptibility, above the biological cutoff). These primary substitutions were frequently accompanied by D232G/N, a previously-reported secondary substitution usually

detectable in the Q148R substitution-containing virus population (Microbiology review N022145.000).

Table 10: EVG Treatment-Emergent IN Substitutions Observed in 12 Post-Baseline Isolates Phenotypically Resistant[1] to EVG in the Absence of Previously Reported Primary EVG^R Substitutions[2] in Pooled Analysis of EVG Resistance

Subject ID	Study	Baseline Sample	On-treatment sample		
		EVG susceptibility[3]	Treatment Day	EVG susceptibility[3]	Treatment-emergent substitutions in HIV-1 IN[4]
1603-2193	105	1.1	114	9.8	T97A, T122T/N, V201V/I, D232D/G
1796-2132	105	2.2	117	158	F121C, D232N
2152-2143	105	1	169	163	Q146I, V72V/I, D232D/N
2486-2276	105	1.4	102	73	Q146I, E138E/K, Q216R, D232D/N
0567-2213	130	1.2	59	>92.8	Q146I, T218T/S, D232N
2488-2199	130	1.3	755	>165.1	S39S/R, T97A, S119R, F121Y, S206S/P, I208I/M, K211K/R, L234L/F, V249V/I, D253H, I268I/V, D288D/A/M/N/Q/S
0959-4027	145	2.6	170	4.1	I32I/V, M50M/T, T112T/A
1808-3124	145	1.2	287	5.4	T97A, D279G
			334	4.7	T97A, D279G
			669	6.6	T97A, T112A, L234L/F, D279G
2058-3269	145	1.3	337	4	T97T/A, (V77V/A)[5]
			406[6]	6.8	T97A

[1] Phenotypic resistance to EVG is defined as ≥2.5-fold reductions in EVG susceptibility (above the biological cutoff).
[2] Previously reported primary EVG^R IN substitutions include T66A/I/K, E92A/G/Q, S147G, Q148H/K/R, and N155H/S as listed in Table 7.
[3] Fold-reduction in EVG susceptibility of tested virus isolates compared to wild-type reference HIV-1 (NL4-3).
[4] Treatment-emergent substitutions in the HIV-1 IN protein were detected in post-baseline samples that were present in their respective baseline samples.
[5] Amino acids in parentheses indicate that substitutions were found in samples isolated at earlier time points but became undetectable.
[6] The isolate was collected 2 days after the last dose.

Through the pooled phenotypic resistance analysis of clinical isolates from subjects receiving EVG-containing regimens, primary EVG^R substitutions at 8 amino acid positions were identified: T66A/I/K, E92A/G/Q, T97A, F121C/Y, Q146I/L/R, S147G, Q148H/K/R, and N155H/S. Of the 246 isolates from 169 subjects that had phenotypic resistance to EVG displaying ≥2.5-fold reductions in EVG susceptibility (above the biological cutoff), one or more of the primary EVG^R substitutions were detected in 240 isolates (97.6%).

Pooled genotypic resistance analysis of pre- and on-treatment samples from subject who received EVG-containing regimens and experienced confirmed virologic failure with HIV-1 RNA ≥400 copies/mL (approximate limit of detection of resistance assay) were conducted to (1) identify additional treatment-emergent genotypic changes detected repeatedly in the absence of the primary EVG^R substitutions, and (2) identify secondary substitutions frequently detected with the primary substitutions in the same virus populations. Subjects with confirmed virologic failure with HIV-1 RNA ≥400 copies/mL are those who remained viremic (HIV-1 RNA levels ≥400

copies/mL) by Weeks 48 (Studies 102, 103, and 105), 60 (Study 104), or 96 (Study 145), or at the time of study drug discontinuation (Study 130). In censored as-treated analyses of these studies, evaluable IN genotypic data were obtained from 266 virologic failures: 14, 9, 1, and 74 subjects from Studies 102, 103, 104, and 145; and 168 subjects from Studies 105+130, including 44 subjects who received EVG-containing regimens in the roll-over study 130. Of these evaluable virologic failures, 71.8% (191/266) developed genotypic resistance to EVG as being infected with HIV-1 variants harboring at least one of the primary EVG^R substitutions. The primary EVG^R substitutions were not detectable in post-baseline isolates (n=121) from the remaining 75 subjects. Table 11 summarizes treatment-emergent IN substitutions found in the post-baseline isolates (n=119) from 73 subjects (excluding 2 subjects whose baseline genotypic data were unavailable). A total of 103 amino acid residues (35.8% of the IN 288 residues) were found to be substituted, and 52 substitutions occurred at HIV-1 IN conserved amino acid positions. Only 5 sites (31, 50, 72, 112, and 234) showed development in >5% of those included in this analysis. All these sites are polymorphic and different amino acid substitutions were observed at residues 31, 50, 72, and 112: substitutions of A/I/M for V or V for I at position 31; substitutions of M for I/L or T/V for M at position 50; substitutions of V for I or A/I/T for V at position 72; and substitutions of A/I/M for T or T for A/I or R for S or A/I/T/V for I/V at position 112. A L234F substitution was seen in 4 subjects' virus samples that displayed <1.4-fold reduced susceptibility to EVG compared to wild-type HIV-1 (ranging from 0.8- to 1.6-fold). Of the 73 subjects, no detectable amino acid substitutions were observed in 20 subjects' isolates, compared to their respective baseline isolate. Post-baseline isolates harboring IN substitutions from the remaining 53 subjects had reductions in susceptibility to EVG ranging from 0.8- to 4.1-fold (median of 1.3-fold) compared to that of wild-type reference HIV-1. Among these isolates, only one isolate (from Subject 0959-4027 in Study 145) was phenotypically resistant to EVG with >2.5-fold reduced susceptibility and 3 treatment emergent IN substitutions, I32I/V, M50M/T, T112T/A, were detected. Based on this analysis, no additional IN substitutions were identified that may be associated with virologic failure to EVG therapy and EVG resistance, other than those 8 amino acid positions associated with primary EVG^R. It should be noted that one subject's failure isolates developed a conserved-site substitution at P145S (Subject 0359-2191 in Study 105; no phenotypic data available) along with a polymorphic-site substitution K136N, and an HIV-1 variant harboring a single amino acid substitution P145S exhibited in cell culture a significant reduction in susceptibility to EVG but not to RAL (31.1-fold and 0.6-fold, respectively). Thus, this observation indicated direct contribution of P145S to the subject's virologic failure to EVG treatment. In addition, P145S was observed in 2 additional subjects (Subjects 0698-2164 and 0898-2039 in Study 105) with emerging S147G and Q148R substitutions. Together, P145S is considered as a primary EVG^R substitution, even though the substitution emerged rarely in EVG-treated subjects.

Table 11: Frequency and Position of Individual Amino Acid Changes Selected in 119 Post-Baseline Isolates with No Evidence of Emerging Primary EVG^R Substitutions from 73 Virologic Failures in Pooled Analysis of EVG Resistance

Occurrence (number of subjects)	Treatment-emergent substitutions at amino acid position in HIV-1 IN	
	Polymorphic positions[1]	Conserved positions[1]
1	22, 23, 24, 41, 60, 96, 119, 134, 135, 136, 151, 165, 173, 205, 207, 208, 215, 216, 218, 219, 220, 221, 222, 255, 265, 270, 278, 281, 286 (n=29)	4, 26, 30, 35, 36, 47, 48, 55, 59, 70, 77, 79, 83, 87, 91, 94, 103, 105, 106, 114, 115, 127, 128, 141, 144, 145, 153, 156, 158, 180, 182, 187, 196, 202, 212, 224 230, 232, 239, 240, 248, 253, 261, 263 (n=44)
2	7, 17, 25, 45, 84, 122, 125, 211 (n=8)	38, 90, 198, 233, 241, 251, 262 (n=7)

3	32, 39, 160, 163,188, 201, 269, 283, 284 (n=9)	242 (n=1)
4	50, 234, (n=2)	(n=0)
5	72 (n=1)	(n=0)
6	31 (n=1)	(n=0)
8	112 (n=1)	(n=0)
Observed substitutions in HIV-1 IN	(K7Rx2), (N17S, S17N), M22V, A23V, S24G/N, (D25E, E25D), (V31A, V31I/M, V31M, V31Ix2, I31V), (V32I, I32Vx2), (S39C, S39I, S39N), D41N, (V45A/L/S, L45I), (I50M, L50M, M50T, M50V), I60T, (I72Vx4, V72A/I/T), (I84M, I84V), E96K, (T112A, A112T, I112T, T112Ix2, T112I/M, I/V112I/V/A/T, S112R), P119A, (V122I, T122I), (T125A, V125A), G134S, V135I, K136N, V151I, (K160N, K160R, R160K), (G163Ex2, G163S), V165I, K173R, (R188K, K188x2), (V201Ix2, I201V), S205A, D207N, I208V, (K211R, K211T), K215N, Q216H, T218I, K219R, L220I, H221Q, T222N, (L234Fx4), S255G, V265A, (R269Gx2, R269K), D270E, D278N, V281A, (S283G, G283Sx2), (G284Rx3), D286N	G4E, F26Y, P30L, E35K, I36M, (A38Vx2), G47E, E48G, D55N, G59E, G70E, V77A, V79A, Y83C, E87K, (P90Sx2), A91T, G94R, K103R, A105T, G106A, H114Y, T115A, K127R, T128A, I141T, N144H, P145S, S153F, K156R, F158L, V180I, I182F, R187K, A196V, (E198K, E198D), D202G, E212D, R224Q, S230N, D232N, (P233L, P233S), A239V, K240T, (L241Fx2), (L242Fx3), A248T, (I251L, I251M), E253D, F261P, (F262R, R262G), R263G

[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).

In summary, through the pooled phenotypic and genotypic resistance analysis of clinical isolates from subjects receiving EVG-containing regimens, substitutions at 9 amino acid positions were associated with primary EVG^R: T66A/I/K, E92A/G/Q, T97A, F121C/Y, P145S, Q146I/L/R, S147G, Q148H/K/R, and N155H/S. Of the 266 virologic failures with evaluable IN genotypic (also some with phenotypic) resistance data, 72.2% (192/266) developed genotypic resistance to EVG as being infected with HIV-1 variants harboring at least one of the primary EVG^R substitutions. All primary substitutions appeared to emerge independently as separate pathways to EVG resistance as these were detected singly in treatment-failure isolates from 96 subjects (Table 11). In the remaining 96 failures with emerging primary EVG^R substitutions, multiple primary substitutions were detected in their genotyped virus populations. Overall, substitutions at E92 (E92A/G/Q, n=103, 38.7% of the examined virologic failures) and at N155 (N155H/S, n=72, 27.1% of the examined virologic failures) were most frequently observed (Table 12). Of note, Y143C, a primary RAL^R substitution, was seen in one subject's virus sample (Subject 1536-2205 in Study 105), but was found as a mixture with N155H. A previous report of a tyrosine-to-cysteine change at this highly conserved-site position did not find a large reduction in susceptibility to EVG (0.7-fold decrease in the EC_{50} value; Fenwick *et al.*, 2011). Thus, Y143C was not considered a primary EVG^R substitution in this analysis. The other amino acid substitutions at Y143 (Y143H/R) associated with RAL^R resistance that could confer 1.6- and 5.4-fold reductions in EVG susceptibility were not observed in this pooled study population (Table 8).

Table 12: Frequency of 9 Primary EVG^R Substitutions Detectable in Virus Samples from 266 Virologic Failure Subjects in Pooled Analysis of EVG Resistance

Primary EVG^R substitution	T66A/I/K	E92A/G/Q	T97A	F121C/Y	P145S	Q146I/L/R	S147G	Q148H/K/R	N155H/S
Total	35 (13.2%)	103 (38.7%)	24 (9%)	2 (0.8%)	3 (1.1%)	10 (3.8%)	43 (16.2%)	45 (16.9%)	72 (27.1%)

Single change	3	41	6	1	1	2	5	14	23
Mixture[1]	32	62	18	1	2	8	38	33	49

[1] Ninety-seven subjects had mixtures of ≥2 primary EVG^R substitutions in their failure virus samples.

3.2.1.2. Additional IN Substitutions Emerged in the Same Virus Population Harboring Primary EVG Resistance-Associated Substitutions

Additional substitutions in HIV-1 IN (referred to as secondary EVG^R substitutions) that may be involved in EVG resistance by being co-selected with primary EVG^R substitutions were identified in 385 post-baseline isolates harboring primary EVG^R substitutions collected from 191 virologic failure subjects with paired genotypic data (some subjects with multiple isolates at different time points). Substitutions at 14 amino positions were observed frequently in ≥5% of those evaluated subjects (≥9 subjects; Table 13): H51Y, L68I/V, V72A/I/N, I73V, Q95K/R, T112A, S119R, T124A, E138A/K, G140A/C/S, E157Q, K160N, S230R, and D232N. In addition, two substitutions, G70R and E170A (7 subjects each), were included because they occurred at conserved amino acid positions (no amino acid sequence variations were observed in all baseline isolates with evaluable IN genotypic data, n=505) and were exclusively found in the N155H-harboring virus population. Substitutions, I73V, S119R, and G140A/C/S, were also exclusively found in the virus isolates harboring E92Q, N155H, and Q148H/K/R primary EVG^R substitutions, respectively. Of the 192 subjects with emerging primary EVG^R substitutions, these 16 secondary substitutions were found in 122 subjects (63.5%). Notably, several of these substitutions (Q95K/R, E138A/K, G140A/S, S230R, and D232N) were also frequently found in subjects failing RAL treatment, particularly accompanying the Q148H/K/R and N155H substitutions (ISENTRESS® US Prescribing Information, revised in August, 2012; Fun *et al*., 2010; Microbiology reviews N022145.000 and N203045.001). These observations were expected with shared genetic pathways of resistance between EVG and RAL involving substitutions E92Q, Q148H/K/R, and N155H.

Table 13: Amino Acid Substitutions Frequently Co-Selected in EVG-Treatment Failure Isolates Harboring Primary EVG^R Substitutions

Reference amino acid sequence in HIV-1 IN[1]		Treatment-emergent IN substitutions observed in the 9 primary EVG^{R,2} substitution-harboring virus population			
Amino acid position	Sequence Variation[3]	**Substitution**	EVG susceptibility[4] (fold-reduction)	Number of subjects	Note[5]
H51	conserved	**Y**	H51Y: 2.9	9	E92A/Q observed in 6 (66.7%) subjects
L68	conserved	**I/V**	L68I: 0.8 L68V: 1.5	32	E92A/G/Q observed in 28/32 (87.5%) subjects
G70	conserved	**R**	-	7	N155H observed in all 7 subjects
V72	polymorphic	**A/I/N**	V72I: 1.6	23	E92A/Q observed in 10/23 (43.5%) subjects
I73	conserved	**V**	-	10	E92Q observed in all 10 subjects
Q95	conserved	**K/R**	-	9	N155H observed in 6 (66.7%) subjects
T112	polymorphic	**A**	-	12	T97A and S147G observed each in 5 (41.7%) subjects
S119	polymorphic	**R**	-	14	N155H observed in all 14 subjects
T124	polymorphic	**A**	-	12	E92G/Q observed in 8/12 (75%) subjects

E138	conserved	**A/K**	E138A: 0.8	E138K: 0.6	34	Q148H/K/R observed in 21/34 (61.8%) subjects
G140	conserved	**A/C/S**	G140S: 3.1		12	Q148H/K/R observed in all 12 subjects
E157	polymorphic	**Q**	E157Q: 2.9		14	E92G/Q observed in 10 (71.4%) subjects
K160	polymorphic	**N**	-		11	S147G observed in 6 (54.5%) subjects
E170	conserved	**A**	-		7	N155H observed in all 7 subjects
S230	conserved	**R**	S230R: 1.0		9	N155H observed in 8/9 (88.9%) subjects
D232	conserved	**N**	-		21	T66A/I/K observed in 10 (47.6%) subjects

[1] Predicted amino acid sequence of the IN protein from the wild-type laboratory HIV-1 strain NL4-3.
[2] Primary EVG^R IN substitutions include T66A/I/K, E92A/G/Q, T97A, F121C/Y, P145S, Q146I/L/R, S147G, Q148H/K/R, and N155H/S.
[3] 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).
[4] Fold-reduction in EVG susceptibility of infectious HIV-1_{HXB2} recombinant clones harboring IN substitutions using the 5-day cytopathic assay in MT-2 cells (Study PC-183-2025; Microbiology review N203100.001).
[5] An observed primary EVG^R substitution with witch the secondary substitution was most frequently co-selected is noted.

3.2.1.3. Cross-Resistance with RAL

Cross-resistance between EVG and RAL 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). HIV-1 variants harboring IN substitution(s) selected by EVG (either in cell culture or in subjects treated with EVG) showed varying degrees of cross-resistance to RAL in cell culture depending on the type and number of IN substitutions (Microbiology review N203100.001). As summarized in Table 8, of the substitutions tested at the 9 primary EVG^R-associated amino acid positions (T66A/I/K, E92A/G/Q, T97A, F121Y, P145S, Q146I/L/R, S147G, Q148H/K/R, and N155H/S), all but three (P145S, Q146L, and S147G) conferred >1.5-fold reduced susceptibility to RAL (above the biological cutoff for RAL) 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 introduced by site-directed mutagenesis. The E92Q substitution, most frequently found in the EVG-treatment virologic failure isolates (Table 12), conferred reduced susceptibility to EVG (39.4- to 78.1-fold) and RAL (5.8- to 11.2-fold; Table 8). Among the 3 primary RAL^R substitutions tested in this study (Y143H/R, Q148H/K/R, and N155H), all but one (Y143H) conferred >2.5-fold reductions in susceptibility to EVG (above the biological cutoff for EVG; Table 8). Thus, although there were unique resistance substitutions for EVG and RAL (i.e., P145S, Q146L, and S147G, and Y143H, respectively), this study showed a high degree of overlapping resistance for primary INSTI resistance-associated substitutions. These cell-based findings of broad cross-resistance between EVG and RAL were confirmed in subject-derived post-baseline isolates with or without those primary resistance-associated substitutions. Results are summarized in Table 14.

The phenotypic susceptibility in cell culture of isolates included in this analysis was assessed for EVG and RAL resistance using the PhenoSense® HIV-1 Integrase Assay (Monogram Biosciences). Evaluable IN genotypic and phenotypic data were available for 400 and 137 post-baseline isolates collected from 262 and 82 subjects receiving EVG- and RAL-containing regimens, respectively, in this pooled study population (Table 14). Among those, primary EVR^R

and RAL^R substitutions were detectable in 251 and 26 isolates, respectively. Primary EVR^R substitutions included T66A/I/K, E92A/G/Q, T97A, F121C/Y, P145S, Q146I/L/R, S147G, Q148H/K/R, and N155H/S, while primary RAL^R substitutions included E92Q, Y143C/H/R, Q148H/K/R, and N155H/S. Of note, data from RAL subjects were obtained from those who received RAL as a comparator in Study 145. As summarized in Table 14, 94.1% (225/239) of post-baseline isolates from EVG recipients genotypically resistant to EVG with evidence of emerging primary EVG^R substitutions were phenotypically resistant not only to EVG with >2.5-fold (up to 301-fold) reduced susceptibility (above the biological cutoff for EVG) but also to RAL with >1.5-fold (up to >256.8-fold) reduced susceptibility (above the biological cutoff for RAL; Fransen *et al*., 2008). Furthermore, a similar high level of cross-resistance was observed with isolates collected from RAL recipients, as 95.8% (23/24) of those genotypically resistant to RAL with evidence of emerging primary RAL^R substitutions (E92Q, Y143C/H/R, Q148H, and N155HY observed) displayed phenotypic resistance both to RAL (1.6- to >169.5-fold reduction in susceptibility) and to EVG (2.8- to 301-fold reduction). In agreement with these observations, 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 RAL-containing regimens were collected at the time of virologic failure and analyzed. The cell-based phenotypic results indicated 3 primary EVG^R substitutions (P145S, Q146L, and S147G) alone may not confer phenotypic resistance to RAL (Table 8). Among those with evaluable data, 2 of 7 subject-derived isolates that harbored a single primary substitution S147G showed phenotypic resistance to RAL (≥1 secondary substitutions detectable in both isolates). In P154S and Q146L substitution-containing isolates, other primary substitutions (i.e., T66A, E92Q, S147G, Q148R, and N155H) were present as a mixture. With these clinical and nonclinical findings of broad cross-resistance between EVG and RAL, the sequential use of these INSTIs can not be a valid treatment option for HIV-1 infection.

The majority (98.5%, 256/260) of isolates in the absence of detectable primary resistance-associated substitutions to EVG or to RAL appeared to remain susceptible both to EVG and RAL, respectively (Table 14). Two isolates from RAL recipients (Subjects 0685-4010 and 2493-3389 in Study 145; Table 13, shaded in yellow) phenotypically resistant to EVG (9-9.8 fold-reduction) and RAL (2.2-2.7 fold-reduction) had several substitutions (4 each) in the IN protein including T97A. The T97A substitution was identified to be primarily associated with EVG treatment virologic failure and resistance. However, it was identified as a secondary substitution in RAL treatment failures because (1) T97A alone did not confer reduced susceptibility to RAL (<1.2 fold-reduction; Microbiology review N022145.SE7-001) and (2) T97A is frequently found in the virus population harboring the Y143C/H/R or N155H primary RAL^R substitutions (Fransen *et al*., 2012; Microbiology review N022145.000).

Table 14: Drug Susceptibility to EVG and RAL of Subject-Derived Post-Baseline Isolates

Treatment	Primary substitutions[1]	Number of isolates				Note
		EVG		RAL		
		Resistance[2]	Sensitive[2]	**Resistance**[3]	Sensitive[3]	
EVG (n=400)	Yes (n=251)	**239**	-	**225 (94.1%)**	14	RAL phenotypic resistance was observed with isolates harboring any of all 9 EVG^R substitutions.
		-	12	**2**	10	
	No	**1**	-	**0**	1	One isolate had 4.1- and 1.2-fold reduced

	(n=149)	-	148	0	148	susceptibility to EVG and RAL, respectively.
RAL (n=137)	Yes (n=26)	23 (95.8%)	1	24	-	EVG phenotypic resistance was observed with isolates harboring any of all 4 RAL^R substitutions.
		-	2	-	2	
	No (n=111)	2	-	2	-	T97A was detected in both isolates
		1	108	-	109	-

[1] Primary EVG^R IN substitutions include T66A/I/K, E92A/G/Q, T97A, F121C/Y, P145S, Q146I/L/R, S147G, Q148H/K/R, and N155H/S. Primary RAL^R IN substitutions include E92Q, Y143C/H/R, Q148H/K/R, and N155H.
[2] Phenotypic resistance to EVG is defined as >2.5-fold reductions in EVG susceptibility (above the biological cutoff; Abram *et al.*, 2012).
[3] Phenotypic resistance to RAL is defined as >1.5-fold reductions in RAL susceptibility (above the biological cutoff; Fransen *et al.*, 2008).

3.2.1.4. 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 polymorphisms 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, 104, 105, and 145 were analyzed to determine the occurrence of IN polymorphism in these pooled study population.

In the five pooled studies, when 503 pre-treatment isolates (baseline or screening; regardless of their relation to the treatment in the study) from 503 subjects (29, 19, 1, 276, and 178 subjects in Studies 102, 103, 104, 105, and 145, respectively), primary EVG^R substitutions were rarely detectable as polymorphisms occurring in 6 (1.2%) of these ARV-naïve subjects. Five of the 6 subjects infected with these genotypically EVG-resistant HIV-1 variants (but all phenotypically sensitive with <2.5-fold reductions in EVG susceptibility) received EVG-containing regimens in Studies 105 and 145: one subject with E92G; one subject with T97A; and 3 subjects with Q148H/R. One subject with E92G (Subject 4099-4121 in Study 145) achieved virologic suppression (HIV-1 RNA <50 copies/mL), while the remaining 4 subjects with baseline HIV-1 harboring Q148H/R or T97A failed to achieve virologic suppression. Interestingly, Q148H/R present in the pre-treatment samples became undetectable in all 3 subjects' failure isolates, and 2 of the 3 subjects developed other primary EVG^R substitutions (i.e., E92Q, T66A, and S147G). Thus, in these 3 subjects, Q148H/R appeared to be outgrown by other EVG resistant HIV-1 variants (n=2; Subjects 0567-2125 and 0926-2178 in Study 105) or by wild-type HIV-1 (n=1; Subject 0595-4154 in Study 145). One subject with baseline T97A (Subject 4114-4173 in Study 145) initially achieved virologic suppression but later experienced virologic rebound. The T97A was persistently detected in the subject's rebound isolates.

Among 16 observed secondary EVG^R-associated amino acid positions, substitutions at 5 conserved-sites, H51Y, G70R, G140A/C/S, E170A, S230R, were not detected as naturally occurring polymorphism at Baseline. Substitutions at the remaining 11 positions were present

in the overall study population (n=503) with frequencies of 0.2 - 69% as naturally occurring polymorphisms with substitutions V71I and T124A being highly polymorphic in INSTI-naïve patients with frequencies of 69% and 33.6% (Table 15). Theses substitutions were not observed more frequently in baseline isolates of EVG-treatment failures subjects (n=263) or in those of EVG-treatment failure subjects with emerging primary EVG^R substitutions (n=191; Table 15). Thus, the presence of these secondary substitutions at baseline may not affect clinical responses to EVG and may not have positive impact on development of genotypic resistance to EVG when subjects failed EVG treatment.

Table 15: No Impact on the Emergence of Primary EVG^R Substitutions of Secondary Substitutions at Baseline as Naturally Occurring Polymorphism

Baseline isolates	Number of subjects					
	L68I/V	**V72I**	**I73V**	**Q95K/R**	**T112A**	**S119R**
Overall study population[1] (n=503)	2 (0.4%)	347 (69%)	11 (2.2%)	1 (0.2%)	24 (4.8%)	13 (2.6%)
EVG-treatment failure subjects[2] (n=263)	1 (0.4%)	176 (66.9%)	5 (1.9%)	1 (0.4%)	15 (5.7%)	8 (3%)
Subjects with Primary EVG^R substitutions[3] (n=191)	0	126 (66%)	4 (2.1%)	0	9 (4.7%)	7 (3.7%)
Baseline isolates	**T124A**	**E138A/K**	**E157Q**	**K160N**	**D232N**	
Overall study population[1] (n=503)	169 (33.6%)	2 (0.4%)	13 (2.6%)	6 (1.2%)	1 (0.2%)	-
EVG-treatment failure subjects[2] (n=263)	94 (35.7%)	2 (0.8%)	8 (3%)	1 (0.4%)	1 (0.4%)	
Subjects with Primary EVG^R substitutions[3] (n=191)	69 (36.1%)	2 (1%)	6 (3.1%)	1 (0.5%)	1 (0.5%)	

[1] Including 503 pre-treatment isolates (baseline or screening; regardless of their relation to the treatment in the study) from 503 subjects from Studies 102, 103, 104, 105, and 145.
[2] Including 263 pre-treatment isolates (baseline or screening) from 263 subjects who received EVG-containing regimens and experienced confirmed virologic failure with HIV-1 RNA ≥400 copies/mL in the pooled study population.
[3] Including 191 pre-treatment isolates (baseline or screening) from 191 subjects who developed primary EVG^R substitutions among 263 subjects who received EVG-containing regimens and experienced confirmed virologic failure with HIV-1 RNA ≥400 copies/mL in the pooled study population.

3.2.2. EVG Resistance Analysis of Study 145

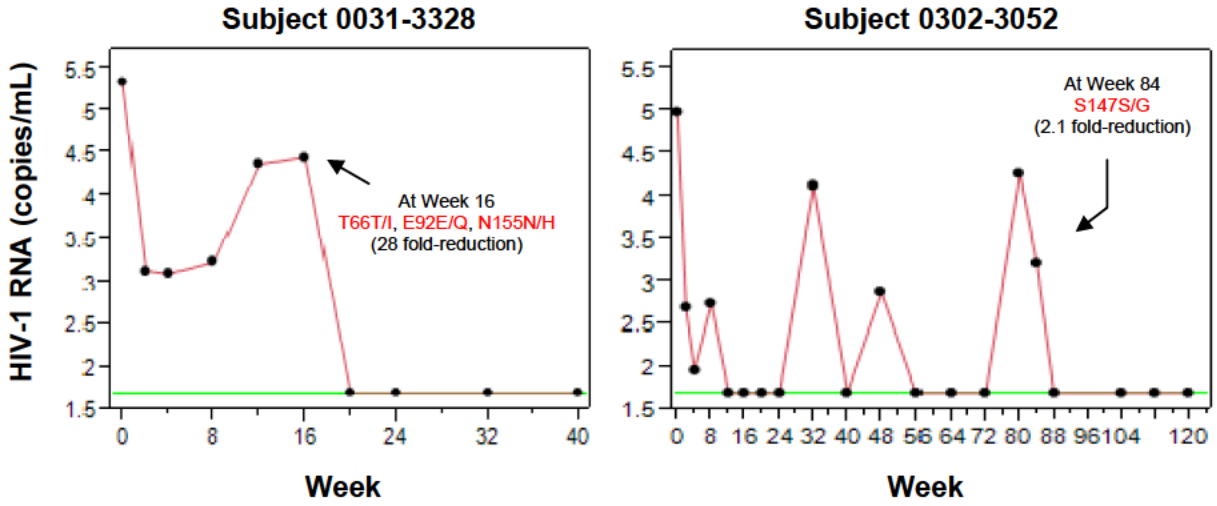
The proposed indication of EVG for the treatment of HIV-1 infection in ARV treatment-experienced adults is directly supported by the pivotal Phase 3 Study GS-US-183-0145 (Study 145), and thus results from the EVG resistance analysis of Study 145 are described in the package insert. In this section, Study 145-specific observations of EVG resistance are summarized.

As described above in Section 3.1, 346 subjects received EVG-containing regimens in the as-treated subject population. By Week 96, 114 subjects experienced virologic failure (HIV-1 RNA >50 copies/mL; Table 2), and 96 of them were eligible for resistance testing with HIV-1 RNA levels ≥400 copies/mL (approximate limit of detection of the resistance assay). Among these resistance testing eligible (RTE) virologic failures, evaluable genotypic resistance data of post-baseline virus isolates were available for 72 subjects (n=68 also with post-baseline drug susceptibility data). Primary EVG^R substitutions were detectable in 22 subjects' failure isolates. In addition, two subjects' viruses also developed primary EVG^R substitutions while they experienced transient virologic rebound (Figure 3). These two subjects eventually achieved virologic suppression and had HIV-1 RNA <50 copies/mL at the time of early discontinuation

(Subject 0031-3328 at Week 40) or at Week 96 (Subject 0302-3052).

Together, 24 subjects developed genotypic resistance to EVG with evidence of emerging primary EVG^R substitutions in Study 145. Substitutions at 7 of the 9 identified primary EVG^R-associated amino acid positions were detectable in these subjects' post-baseline isolates with T66A/I and E92G/Q being most frequently observed (2 substitutions F121C/Y and P145S were undetectable): T66A/I (n=8), E92G/Q (n=7), T97A (n=5), Q146R (n=1), S147G (n=4), Q148R (n=4), and N155H (n=5). In 14 of these subjects (58.3%, 14/24), substitutions at 11 secondary EVG^R-associated amino acid positions were also observed in the same virus populations harboring primary substitutions: H51Y, L68V, G70R, V72A/I/N, Q95K, T112A, T124A, E138K, G140A/C/S, E170A, and D232N (substitutions at 5 positions I73V, S119R, E157Q, K160N, and S230R were not detected). Appendix 2 Table A2-2 listed individual isolates' treatment-emergent substitutions in the HIV-1 IN proteins collected from these 24 subjects.

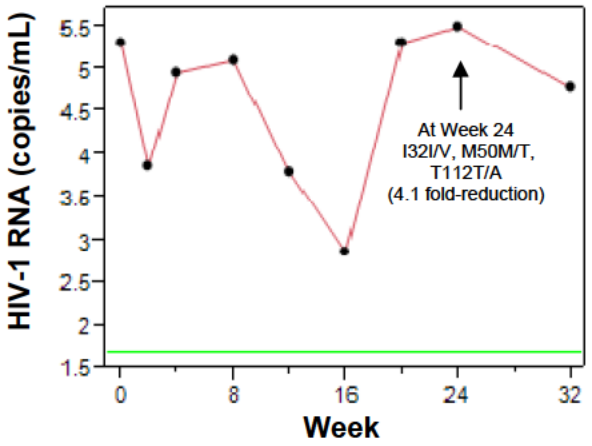
Figure 3: HIV-1 Viral Load in Subjects 0031-3328 and 0302-3052 in Study 145



In phenotypic analysis of 71 subjects' virus isolates, baseline and/or post-baseline, (including 69 confirmed virologic failures) with evaluable drug susceptibility data, these subjects had baseline HIV-1 mostly susceptible to EVG with 0.8- to 2.7- reductions in EVG susceptibility (n=60), compared to wild-type reference HIV-1. Two subjects' isolates displayed 2.6- and 2.7-fold reductions (above the biology cutoff of 2.5 fold for EVG). As noted above, 24 subjects developed genotypic resistance to EVG while on EVG, and the emergence of primary EVG^R substitutions resulted in median decreases in susceptibility to EVG of 8.2-fold, ranging from 1.6- to >158.1-fold (29 isolates with evaluable data from 21 subjects). Some of these isolates (20/29) appeared to be cross-resistant to RAL with >1.5-fold reduced susceptibility, ranging from 1.9- to 53-fold (above the biology cutoff of 1.5 fold for RAL). Overall, among the 24 subjects who developed genotypic resistance to EVG with evidence of emerging primary EVG^R substitutions, 14 (66.7%) of the 21 subjects with evaluable data showed phenotypic resistance to EVG. In addition, 12 (57.1%) of those 21 evaluated subjects (11 [78.6%] of the 14 subjects with EVG phenotypic resistance) had HIV-1 variants phenotypically resistant to RAL.

Of 72 confirmed virologic failures with evaluable genotypic resistance data, 50 subjects did not develop genotypic resistance to EVG. Of these 50 subjects, 49 subjects' post-baseline isolates

Figure 4: HIV-1 Viral Load in Subject 0959-4027 in Study 145



were successfully phenotyped, and all but one had EVG-sensitive failure isolates with <2.5-fold reduced susceptibility (below the biology cutoff for EVG). One subject (0959-4027) who never had a suppressed viral load (HIV-1 RNA <50 copies/mL) through Week 92 experienced virologic rebound at Week 20 (Figure 4). The subject's Week-24 isolate harbored 3 treatment-emergent IN substitutions, I32I/V, M50M/T, and T112T/A, and became phenotypically resistant to EVG with 4.1-fold reduced susceptibility to EVG. All failure isolates (n=76) from those 49 subjects evaluated also remained sensitive to RAL with <1.5-fold reduced susceptibility (below the biology cutoff for RAL).

4. Conclusion

This original NDA for VITEKTA tablets is approvable for the treatment of HIV-1 infection in combination with a RTV-boosted PI and with other ARVs in ART-experienced adult patients with respect to Clinical Virology, based on Week-96 data from a Phase 3 study (Study GS-US-183-0145). VITEKA (elvitegravir; EVG) is an HIV-1 integrase strand transfer inhibitor (INSTI) that prevents the HIV-1 integrase-catalyzed integration of unintegrated linear HIV-1 DNA into the host cell genome. Recently, a EVG-containing complete regimen STRIBILD™ (Gilead Sciences, Inc.) where EVG is co-formulated as a single tablet with two FDA-approved HIV-1 NRTIs, FTC (200 mg; Emtriva®) and TDF (300 mg; Viread®), and a pharmacokinetic enhancer cobicistat (150 mg) was approved by FDA for the treatment of HIV-1 infection in ART-naïve adults in August, 2012 (NDA 203-100). This original NDA was submitted for U.S. marketing approval of EVG tablets (85 and 150 mg) as a single agent.

Antiviral efficacy of EVG was noninferior to raltegravir (RAL as a comparator), each administered with a background regimen including a fully-active PI/r, based on the Week-48 and -96 efficacy data. The rates of virologic success of HIV-1 RNA suppression <50 copies/mL through Week 96 were comparable between the two EVG and RAL treatment groups in censored, as-treated snapshot analysis: 65.9% versus 65.7% at Week 48, and 60.9% versus 64.5% at Week 96, respectively. Furthermore, antiviral response to both treatments appeared to be durable with 86.1% and 87.2% of EVG and RAL recipients who achieved virologic suppression at Week 48 being aviremic at Week 96. Negative correlation between baseline HIV-1 RNA levels and antiviral efficacy of EVG (+background regimen) was observed. Subjects with baseline viral RNA levels ≥5 $\log_{10}$ copies/mL achieved and maintained virologic success at lower rates, 47.6% and 45.1% at Weeks 48 and 96, respectively, while the virologic success rates for subjects with baseline viral RNA levels <5 $\log_{10}$ copies/mL were 72.1% and 66.5%, respectively. Virologic rebound was the primary cause of the EVG-treatment virologic failure with HIV-1 RNA ≥50 copies/mL. Of those EVG-treated virologic failures, 71.6% and 78.1% of subjects experienced virologic rebound by Weeks 48 and 96, respectively.

Through the pooled phenotypic and genotypic resistance analysis of clinical isolates from subjects receiving EVG-containing regimens in 6 clinical trials of EVG, primary EVG resistance-associated (EVG^R) substitutions were identified at 9 IN amino acid positions: T66A/I/K, E92A/G/Q, T97A, F121C/Y, P145S, Q146I/L/R, S147G, Q148H/K/R, and N155H/S. All primary substitutions appeared to emerge independently as separate pathways to EVG resistance as these were detected singly in treatment-failure isolates. Substitutions at E92 (E92A/G/Q, 38.7% of the examined virologic failures) and at N155 (N155H/S, 27.1% of the examined virologic failures) were mostly frequently observed. Additional EVG treatment-emergent substitutions were identified that may be involved in EVG resistance by frequently accompanying the primary EVG^R substitutions: H51Y, L68I/V, V72A/I/N, I73V, G70R, Q95K/R, T112A, S119R, T124A, E138A/K, G140A/C/S, E157Q, K160N, E170A, S230R, and D232N. As expected with a shared mechanism of action against HIV-1 integrase, cross-resistance between EVG and RAL was observed with 94.1% of post-baseline isolates from EVG recipients genotypically resistant to EVG with evidence of emerging primary EVG^R substitutions being phenotypically resistant both to EVG and RAL with above the biological cutoff reductions in drug susceptibility. Similar high level of cross-resistance was observed with isolates collected from RAL recipients, as 95.8% of those genotypically resistant to RAL with evidence of emerging primary RAL^R substitutions displayed phenotypic resistance both to RAL and to EVG.

In Study GS-US-183-0145, by Week 96, 24 subjects receiving EVG-containing regimens developed genotypic resistance with substitutions at 7 of the 9 identified primary EVG^R-associated amino acid positions detectable in their post-baseline virus samples with T66A/I and E92G/Q being most frequently observed: T66A/I (n=8), E92G/Q (n=7), T97A (n=5), Q146R (n=1), S147G (n=4), Q148R (n=4), and N155H (n=5). In 14 of these subjects (58.3%), substitutions at 11 secondary EVG^R-associated amino positions were also observed in the same virus populations harboring primary substitutions: H51Y, L68V, G70R, V72A/I/N, Q95K, T112A, T124A, E138K, G140A/C/S, E170A, and D232N. Among the 24 subjects who developed genotypic resistance to EVG, 14 (66.7%) of the 21 subjects with evaluable data showed phenotypic resistance to EVG. In addition, 12 (57.1%) of those 21 evaluated subjects (11 [78.6%] of the 14 subjects with EVG phenotypic resistance) had HIV-1 variants phenotypically resistant to RAL.

PACKAGE INSERT: Section 12.4 Microbiology

Changes are made to the applicant's draft labeling for VITEKTA, originally included in this NDA package. The words with strikethroughs are the text the applicant was requested to delete and the words written in red are recommended insertions.

Mechanism of Action

Elvitegravir is an HIV-1 integrase strand transfer inhibitor (INSTI). Integrase is 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.

Antiviral Activity in Cell Culture

The antiviral activity of elvitegravir against laboratory and clinical isolates of HIV-1 was assessed in T lymphoblastoid cells, monocyte/macrophage cells, and primary peripheral blood lymphocytes. (b) (4) The 50% effective concentration (EC_{50}) values (b) (4) ranged (b) (4) from 0.02 to 1.7 nM. Elvitegravir displayed antiviral activity in cell culture against HIV-1 clades A, B, C, D, E, F, G, and O (EC_{50} values ranged from 0.1 to 1.3 nM) and activity against HIV-2 (EC_{50} value of 0.53 nM). The antiviral activity of elvitegravir with antiretroviral drugs in two-drug combination studies was not antagonistic when combined with the INSTI 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 (b) (4) cell culture.

Resistance

In Cell Culture

HIV-1 isolates with reduced susceptibility to elvitegravir were selected in cell culture. Reduced susceptibility to elvitegravir was (b) (4) associated with the primary integrase substitutions T66A/I, E92G/Q, F121Y, S147G, and Q148R. Additional integrase substitutions observed in cell culture selection included D10E, S17N, H51Y, (b) (4) S153F/Y, E157Q, D232N (b) (4) R263K, and V281M. (b) (4)

Clinical Studies

Pooled resistance analysis was performed on virus samples from subjects receiving elvitegravir-containing regimens in several clinical trials of elvitegravir who remained viremic (HIV-1 RNA greater than 400 copies/mL) by the time of efficacy evaluation (up to 96 weeks). Development of substitutions T66A/I/K, E92A/G/Q, T97A, F121C/Y, P145S, Q146I/L/R, S147G, Q148H/K/R, and N155H/S in the HIV-1 integrase protein was primarily associated with resistance to elvitegravir. Substitutions E92A/G/Q and N155H/S were mostly frequently observed (39% and

27% of those evaluated subjects). In virus isolates harboring the observed primary elvitegravir resistance-associated substitutions, additional substitutions in integrase were detected including H51Y, L68I/V, G70R, V72A/I/N, I73V, Q95K/R, T112A, S119R, T124A, E138A/K, G140A/C/S, E157Q, K160N, E170A, S230R, and D232N.

(b) (4) *Treatment-Experienced HIV-1-Infected Subjects*: (b) (4) By Week 96, evidence of emerging primary elvitegravir resistance-associated substitutions T66A/I, E92G/Q, T97A, Q146R, S147G, Q148R, or N155H (b) (4) was observed in ~~23~~ 24 of the (b) (4) 74 subjects with evaluable genotypic data in Study 145.- (b) (4)

Post-baseline virus isolates harboring the primary elvitegravir resistance-associated substitutions had median decreases in susceptibility to elvitegravir of 8-fold (N=29, ranging from 2- to greater than 158-fold) and of 5-fold (N=26, ranging from 1- to greater than 58-fold) compared to wild-type reference HIV-1 and to their respective baseline isolates, respectively (b) (4)

(b) (4)

Cross Resistance

Cross-resistance has been observed among INSTIs. Among the 24 subjects who developed genotypic resistance to elvitegravir with evidence of emerging primary elvitegravir resistance-associated substitutions in Study 145, 12/21 (57%) subjects with evaluable drug susceptibility data had HIV-1 with reduced susceptibility to raltegravir (greater than 1.5-fold, above the biological cutoff for raltegravir).

Elvitegravir-resistant viruses showed varying degrees of cross-resistance in cell culture to raltegravir in the (b) (4) INSTI class depending on the type and number of substitutions in HIV-1 integrase. (b) (4)

f the nine primary elvitegravir resistance-associated substitutions tested (T66A/I/K, E92G/Q, T97A, F121Y, P145S, Q146I/L/R, S147G, Q148H/K/R, and N155H/S), all but three (P145S, Q146L, and S147G) conferred greater than 1.5-fold reduced susceptibility to raltegravir when introduced individually into a wild-type virus by site-directed mutagenesis. (b) (4)

f the three primary raltegravir resistance-associated substitutions tested (Y143H/R, Q148H/K/R, and N155H), all but one (Y143H) conferred grater than 2.5-fold reductions in susceptibility to elvitegravir (above the biological

cutoff for elvitegravir).

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APPENDICES

Appendix 1: Virological Assays in Clinical Virology

A1.1. Quantification of Plasma HIV-1 RNA Levels

Phase 3 trial GS-US-183-0145

Plasma HIV-1 RNA levels were quantified using the fully automated Roche COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test (version 1.0; referred to here as the TaqMan assay) and the Roche AMPLICOR HIV-1 Monitor™ Test (version 1.5; referred to here as the Amplicor assay). Both assays received marketing approval from FDA on May 11, 2007 (BP050069) and on March 2, 1999 (BP950005), respectively, as an *in vitro* nucleic acid amplification test for the quantification of HIV-1 RNA in human plasma. These 2 assays correlated significantly (P >0.95) with each other with the correlation coefficient of 98.08% (Oliver *et al*., 2007).

In Study GS-US-183-0145 (Study 145), the TaqMan assay was originally proposed to be utilized for HIV-1 RNA quantification (Microbiology review I072177.182 for the clinical trial protocol) but later the protocol was amended (Amendments 03, submitted on August 07, 2009) so that HIV-1 RNA levels could also be quantified using the Amplicor assay (Microbiology review I103093 037 080709). This change was proposed since the TaqMan assay was reported to be inconsistently sensitive in detecting virus near the detection limit of HIV-1 RNA 50 copies/mL (Lima *et al*., 2009). The authors reported an increased frequency of detectable plasma HIV-1 RNA levels near HIV-1 RNA 50 copies/mL with the TaqMan assay, compared to the Amplicor assay. Thus, a high proportion of individuals who were fully and consistently suppressed on HAART with HIV-1 RNA levels <50 copies/mL using the Amplicor assay experienced unexpected HIV-1 RNA levels >50 copies/mL when the TaqMan assay was introduced. Furthermore, Brumme *et al*. (2012) observed also low but detectable HIV-1 RNA (<250 copies/mL) by the TaqMan assay does not correlate with detectability by the Amplicor assay and is not indicative of impending short-term virological failure or drug resistance. The Division recommended that endpoint assays should not be changed in the middle of a trial, and thus the Amplicor assay (version 1.5) may be added as a additional test to quantify HIV-1 RNA in Study 145 but the TaqMan assay (version 1.0) should be used for the primary endpoint determination (Microbiology review I103093 037 080709). The applicant measured HIV-1 RNA levels using these 2 assays and reported in the efficacy datasets included in this NDA package.

The COBAS AmpliPrep/COBAS TaqMan HIV-1 Test (version 1.0, Roche; TaqMan assay) is based on three major processes: (1) specimen preparation to isolate HIV-1 RNA; (2) reverse transcription of the target RNA to generate complementary DNA (cDNA), and (3) simultaneous PCR amplification of target cDNA and detection of cleaved dual-labeled oligonucleotide probe specific to the target. According to the Package Insert for the TaqMan assay (2007), the assay uses PCR amplification primers that define sequences within the highly conserved region of the HIV-1 *gag* gene and have been optimized to yield comparable amplification of group M

subtypes of HIV-1. The automated processes of reverse transcription, PCR amplification, and detection of HIV-1 target RNA 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. The TaqMan assay gives a linear response from HIV-1 RNA 48 to 10,000,000 copies/mL.

The COBAS AMPLICOR HIV-1 Monitor Test (version 1.5, Roche; Amplicor assay) involves 5 major processes: (1) specimen preparation to isolate HIV-1 RNA; (2) reverse transcription of the target HIV-1 RNA to generate cDNA; (3) PCR amplification of the target sequence in the cDNA using HIV-1 specific complimentary primers (located in a highly conserved region of the HIV-1 *gag* gene); (4) hybridization of the amplified products to oligonucleotide probes specific to the target(s); and (5) detection of the probe-bound amplified products by calorimetric determination. The Amplicor assay utilizes a HIV-1 Quantitation Standard (QS) RNA that is added to the amplification mixture at a known concentration to be carried through the steps of the assay along with the target HIV-1 RNA. The assay can be used with either of 2 specimen processing procedures, the standard procedure and the ultrasensitive procedure. According to the Package Insert for the Amplicor assay (1999), with the standard specimen processing procedure HIV-1 RNA can be quantified over the range of 400 - 750,000 copies/mL. When the ultrasensitive specimen processing procedure is used, the assay can quantify HIV-1 RNA over the range of 50 - 75,000 copies/mL. In Study 145, the ultrasensitive assay was used first, and if the HIV-1 RNA value was >100,000 copies/mL, samples were requantified using the standard assay. If the HIV-1 RNA value based on the standard assay was >750,000 copies/mL, diluted samples were requantified.

Phase 2 trial GS-US-183-0105

Levels of HIV-1 RNA were measured using the Amplicor HIV-1 Monitor Test version 1.5. The ultrasensitive assay was used first, and samples with HIV-1 RNA >75,000 copies/mL were requantified using the standard assay. Samples with HIV-1 RNA >750,000 copies/mL by the standard assay, diluted samples were requantified.

A1.2. HIV-1 Resistance Testing

Phase 3 trial GS-US-183-0145

All resistance analyses were conducted by (b) (4). As required by the enrollment criteria, genotypic and phenotypic analyses of protease (PR) and reverse transcriptase (RT) resistance were assessed for all subjects at screening using the FDA-approved PhenoSense GT® assay (Monogram Biosciences, Inc; Petropoulos *et al*., 2000). These screening data were used for baseline resistance analyses. Post-baseline PR/RT resistance testing was conducted on treatment-failure virus samples from subjects experiencing virologic failure (VF; see below for definition). Genotypic and phenotypic

analyses of integrase (IN) resistance were assessed only for VF subjects (baseline and post-baseline samples) using the Monogram Biosciences GeneSeq/PhenoSense Integrase assays (Fransen *et al*., 2008). HIV-1 tropism was determined for subjects who received maraviroc as part of their background regimen using the Monogram Trofile® assay at both their baseline and post-baseline VF samples (Trinh *et al*., 2008; Whitcomb *et al*., 2007). T-20 resistance was not assessed.

The PhenoSense GT assay combines 3 tests, GeneSeq®, PhenoSense®, and Replication Capacity, to yield PR/RT genotypic and phenotypic data relevant to all currently approved NNRTIs, NRTIs, and PIs, and replication capacity information to evaluate viral fitness. The PR/RT genotype produced by the GeneSeq assay (population-based nucleotide sequence assay) covers the entire PR gene (99 amino acids) and the N-terminal RT sequence spanning amino acids 1 to 305, thus encompassing all clinically relevant PI, NNRTI, and NRTI resistance-associated substitutions (IAS-USA, 2011). The GeneSeq assay also determined the HIV-1 subtype at the time of screening. According to the manufacturer, the GeneSeq assay can be reliably performed on patient plasma samples containing ≥500 HIV RNA copies/mL and capable of detecting minor virus populations when present at roughly 10-30% of the total population. The PhenoSense assay simultaneously determines the drug susceptibility of the virus population from patient plasma samples with HIV-1 RNA ≥500 copies/mL to HIV-1 PR and RT inhibitors using recombinant technology (Petropoulos *et al*., 2000; Qari *et al*., 2002).

The GeneSeq and PhenoSense Integrase assays provide, respectively, the predicted amino acid sequence for the RNase H domain of HIV-1 RT and the entire Integrase (IN) coding region (entire 288 amino acids), and the drug susceptibility of the virus population to HIV-1 IN inhibitors (Fransen *et al*., 2008). The PhenoSense Integrase assay is an adapted version of the original PhenoSense assay to measure inhibition of recombinant viruses containing patient-derived C-terminal *pol* gene sequences containing RNase H and IN coding regions by IN inhibitors. This assay also provides replication capacity data of those recombinant viruses.

*Virologic failure (VF) is defined for resistance analyses as (1) having suboptimal virologic response or virologic rebound or (2) having HIV-1 RNA ≥400 copies/mL at Weeks 48 and 96 (or early study discontinuation after Week 8).

- Suboptimal virologic response is defined as HIV-1 RNA ≥50 copies/mL and <1 $\log_{10}$ copies/mL reduction from baseline at the Week 8 visit, confirmed at the Week 12 visit
- Virologic rebound is defined as (1) at any visit, after achieving HIV-1 RNA <50 copies/mL, a rebound in HIV-1 RNA to ≥400 copies/mL, which is subsequently confirmed at the following scheduled or unscheduled visit or (2) at any time after Week 12 visit, a confirmed increase in HIV-1 RNA ≥1 $\log_{10}$ copies/mL from nadir.

Phase 2 trial GS-US-183-0105

Genotypic and phenotypic analyses of PR and RT resistance were conducted on all subjects' screening samples and on VF subjects'

on-treatment samples in all treatment groups. PR/RT genotypic and phenotypic analyses at screening and at the time of VF (see below for definition) utilized the Monogram Biosciences PhenoSense GT Assay. During the screening program for Study GS-US-183-0105 (Study 105), the protease inhibitor darunavir (Prezista®) had not been incorporated into the PhenoSense GT Assay. Consequently, genotypic and phenotypic data for darunavir were not obtained during the screening program, nor were generated retrospectively on the baseline samples of subjects enrolled in the study. For post-baseline VF sample analyses, darunavir genotypic and phenotypic data were obtained from the updated PhenoSense GT Assay. HIV-1 subtype data were also obtained from the PhenoSense GT Assay.

Genotypic and phenotypic analyses of HIV-1 IN used one of two assays; either the PhenoSense Integrase Assay (which was then an experimental IN genotype and phenotype assay developed at Monogram Biosciences, Inc) or an IN genotyping assay (standard population-based nucleotide sequence assay) developed at Gilead Sciences, Inc. Subjects experiencing VF in each of the three EVG/r dose groups were analyzed using the PhenoSense Integrase Assay (both baseline and confirmed VF time points). Subjects with VF in the comparator arm (CPI/r) were not analyzed using the PhenoSense Integrase Assay. However, subjects who were in the comparator arm, subsequently switched to open-label EVG/r 125 mg, and experienced VF were analyzed for the development of EVG resistance using the PhenoSense Integrase Assay. The Gilead Sciences IN genotyping assay was used for baseline analyses of IN genotype on all other subjects enrolled in the study, including those who did not experience confirmed VF.

Genotypic and phenotypic resistance analyses of HIV-1 envelope glycoproteins were not conducted for subjects who were allowed to use the fusion inhibitor T-20 in the background regimen.

*Virologic failure (VF) is defined for resistance analyses as (1) having HIV-1 RNA >400 copies/mL and <1 $\log_{10}$ copies/mL reduction from Baseline by Week 12 (confirmed subsequently at an unscheduled visit); (2) at any visit on or after Week 16, a rebound in HIV-1 RNA to <1 $\log_{10}$ reduction from Baseline and HIV-1 RNA >400 copies/mL (confirmed at an unscheduled visit); or (3) having HIV-1 RNA ≥400 copies/mL at Weeks 24 or 48 (or early study discontinuation at or after Week 16).

Appendix 2: Individual Subject's Genotypic and Phenotypic Resistance Data

Table A2-1: Amino Acid Changes in HIV-1 IN Observed in 17 Isolates Phenotypically Resistant[1] to EVG in the Absence of Previously Reported Primary EVG^R Substitutions[2] in Pooled Analysis of EVG Resistance

Subject ID	Study	Treatment Day	EVG susceptibility (fold-reduction[3])	Amino acid changes in HIV-1 IN[4]
0407-2040	105	1 (Baseline)	2.6	(S17N), V31V/I, S57S/G, V113I, T124N, T125A, (E157Q), K160Q, G163E, L172I, V234L
1068-2086	105	1 (Baseline)	2.5	V31V/I, S39S/N, (V72I), V79V/A, V113I, S119P, T122I, T124A, V234L, D256E

1603-2193	105	114	9.8	(S17S/N), (V72I), T97A, G106A, V113I, (S119R), T122T/N, S123C, V201V/I, K211R, D232D/G, V234L, D256E
1796-2132	105	117	158	K7Q, E11D, D25E, V31I, (V72I), L101I, V113I, S119P, F121C, T122I, T124A, D232N, V234L
2152-2143	105	169	163	D3E, (S17N), (V72V/I), V113I, T124A, Q146I, K173R, D232D/N, V234L
2191-2138	105	1 (Baseline)	2.7	V72T, T124A, T125A, V176L, V234L
2486-2276	105	102	73	V31I, P90S, K111T, V113I, T124N, T125V, V126M, E138E/K, Q146I, Q216Q/R, D232D/N, V234L, D256E
0567-2213	130	59	>92.8	E11D, S17C, A23V, (V72I), T124A, T125A, Q146I, G163Q, V201I, T218T/S, D232N, V234I
2488-2199	130	755	>165.1	K14K/R, V31I, S39S/R, (V72I), T97A, L101I, T112V, V113I, (S119R), F121Y, T124N, T125V, I135V, V201I, T206P/S, I208I/M, K211K/R, K215N, V234F/L, V249V/I, D253H, D256E, I268I/V, D288D/A/M/N/Q/S
0310-3062	145	1 (Baseline)	2.7	D6E, E11D, (S17N), V31I, L63I, (V72I), L74I, E96D, L101I, T112V, V113I, T124N, T125A, G134N, K136T, (E157Q), K160N, V201I, T206S, K211R, V234I, R269K, S283G, D288N
0959-4027	145	1 (Baseline)	2.6	E11D, V31V/I, V32I, D41D/N, L68V, (V72I), I73V, Q95Q/R, L101L/I, V113I, T125A, I135V, V201I, V234L, R284G
		170	4.1	E11D, V31V/I, V32V/I, D41D/N, M50M/T, L68V, (V72I), I73V, T112T/A, V113I, T125A, I135V, V201I, V234L, R284G
1808-3124	145	287	5.4	K14R, R20R/K, (V72I), A91E, T97A, L101I, V113I, (S119R), T124N, G193D, V201I, T206S, I208I/L, V234L, I268L, D279G
		334	4.7	K14R, R20R/K, (V72I), A91E, T97A, L101I, V113I, (S119R), T124N, G193D, V201I, T206S, I208I/L, V234L, I268L, D279G
		669	6.6	K14R, (V72I), A91E, T97A, L101I, T112A, V113I, (S119R), T124N, G193D, V201I, T206S, I208L, V234F/L, I268L, R269R/K, D279G
2058-3269	145	337	4	S39C, M50I, (V72I), V77V/A, T97T/A, L101I, V113I, S119P, M154L, V201I, T206S, T218S, V234L
		406[5]	6.8	S39C, M50I, (V72I), T97A, L101I, V113I, S119P, M154L, V201I, T206S, T218S, V234L

[1] Phenotypic resistance to EVG is defined as ≥2.5-fold reductions in EVG susceptibility (above the biological cutoff).
[2] Previously reported primary EVG^R IN substitutions include T66A/I/K, E92A/G/Q, S147G, Q148H/K/R, and N155H/S as listed in Table 7.
[3] Fold-reduction in EVG susceptibility of tested virus isolates compared to wild-type reference HIV-1 (NL4-3).
[4] Amino acid changes (or naturally occurring variations) in the HIV-1 integrase (IN) protein, relative to the wild-type HIV-1 (NL4-3) reference amino acid sequence. Previously reported EVG^R substitutions (with exception of primary EVG^R substitutions as listed in Table 7) are written in blue, excluding amino acid changes in parentheses that occurred at the HIV-1 IN polymorphic amino acid positions and were also found in baseline isolates that were phenotypically susceptible to EVG with ≤1.3-fold reduced susceptibility to EVG (below the median fold-reduction for all baseline isolates with evaluable EVG phenotypic data, n=315). 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).
[5] The isolate was collected 2 days after the last dose.

Table A2-2: Amino Acid Changes in HIV-1 IN Observed in 24 Subjects Who Developed Genotypic Resistance to EVG in Study 145

Subject ID	Study Day	Drug susceptibility (fold-reduction[1])		Replication Capacity[2] (%)	Treatment-emergent substitutions in HIV-1 IN[3]
		EVG	RAL		
0031-3075	632	20	1.6	25	A21T, N24S, E92G, V113I, K188K/R, I/V208I/M, E253D
0031-3253	141	1.2	1	ND	(D6D/N)[4], (V31V/G)[4], (E35E/K)[4], D41N, (E48E/K)[4], (V54V/G)[4], (P90P/S)[4], (I203I/T)[4]
	337	1.6	1.6	ND	R/K20K/E, S39S/G, D41N, G70G/R, N155N/H
0031-3283	218	30	3.8	73	E92Q
	274[5]	32	3.2	39	E92Q, A265T
0031-3328	114	28	3	ND	T66T/I, E92E/Q, N155N/H
0302-3052	594	2.1	1.2	86	S147S/G, K156K/R
0302-3222	93	1.4	1.1	ND	(V180V/I)[4]
	274	19	2.6	ND	V72V/I, S147G, D232D/N
	358	24	2.5	ND	V72I, Q95Q/K, S147G, S230S/N, D232D/N
0310-3062	85	108	13	86	E92Q, T210T/I
	176	>158.1	53	99	L68L/V, E92E/Q, G140G/A, Q148Q/R, T210T/I
0433-3021	185	1.1	0.5	133	S261P, L262R
	406	1.1	0.5	ND	135 (Q164E)[4], S261P, L262R
	528	1.9	0.6	ND	V31V/I/M, I72I/N, T97T/A, S261P, L262R
0444-3346	617	47	3.8	25	E92E/Q, E96E/A, N155H
0574-4181	296	ND	ND	ND	(G4G/E)[4], (T66I)[4], (N117N/S)[4]
	338	ND	ND	ND	(T66T/I)[4], Q146R
	394	ND	ND	ND	G82G/D, Q146R

0652-3179	551	2.3	1.4	179	T66T/A, V72V/I, V74V/M
0991-3060	449	9.8	1.3	ND	T66I, I/V101L, T112A, Q148Q/R, M154I
1015-4176	176[6]	1.8	1.4	ND	E91E/A/K/T, V112/V/A/I/T, I122I/V, S123S/C, T124T/N, Q148Q/R, T218T/S
1407-3037	80	1.6	0.8	62	(T97T/A)[4], (K156K/R)[4], (S283S/G)[4]
	170	4.9	1	ND	T66T/A, T124T/A, S147S/G
1534-3056	140	34	4.6	60	(K46K/R)[4], E92Q, (K/R211R/G)[4]
	168[7]	43	3.8	ND	H51H/Y, E92Q
1808-3124	287	5.4	2.2	66	T97A, D279G
	334	4.8	1.9	ND	T97A, D279G
	669	6.6	3	6.3	T97A, T112A, L234L/F, D279G
1960-3172	332	16	1.3	ND	T66T/A, I101I/L, N155N/H, D232D/N
2003-3245	674	ND	ND	ND	E138K, Q148R, R284R/G
2058-3269	142	2.1	0.9	133	(T125T/A)[4], (S255S/G)[4]
	337	4	1.1	ND	T97T/A, (V77V/A)[4]
	406[8]	6.8	1.7	ND	T97A
2058-3282	112	1.7	1.2	136	none
	250	1.6	1.1	ND	(Q164K)[4]
	337	2.5	1.1	108	V88V/I, P90P/S, S147S/G
2152-3093	727	58	7.6	1.6	L101L/F, S153S/F, N155H, E170E/A, H171H/R, L172L/F, L220L/F, D232D/N, L241L/F, L242L/F
2704-3256	84	ND	ND	ND	none
	339	ND	ND	ND	T66T/I, V72A/G, V267V/I
	675	ND	ND	ND	T66T/I, V72A/G, L74L/M, V75V/M, T124T/A, A205A/S, T218T/S, V267V/I
4114-4173	302	2.2	1.3	56	T97A[BL,9], F227F/Y
5007-3471	57	8.2	1.9	115	R20R/K, T66T/I, E92E/Q, R187R/K, V249V/I

ND, not determined
[1] Fold-reduction in drug suscept bility of tested virus isolates compared to wild-type reference HIV-1 (NL4-3).
[2] Replication capacity was determined for HIV-1 with subject-derived integrase (IN) fragments and % replication capacity was calculated compared to wild-type reference HIV-1.
[3] Primary and secondary EVG^R substitutions are written in red and blue, respectively.
[4] Amino acids in parentheses indicate that substitutions were found in samples isolated at earlier time points but became undetectable.
[5] The isolate was collected 3 days after the last dose.
[6] The isolate was collected 56 days after the last dose.
[7] The isolate was collected 1 day after the last dose.
[8] The isolate was collected 2 days after the last dose.
[9] T97A was detected in the subject's baseline isolate with 1.9-fold reduced susceptibility to EVG.

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/s/

SUNG S RHEE
03/19/2013

JULIAN J O REAR
03/20/2013

VIROLOGY FILING CHECKLIST FOR NDA or Supplement

NDA Number: 203-093 **NDA Type:** Original **Stamp Date:** 06/27/2012

Applicant: Gilead sciences, Inc. **Drug Name:** Elvitegravir (GS-9137)

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		Nonclinical virology study reports were previously submitted to NDA 203100 (SDN 001).
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.

Rhee, Sung 08/08/2012

Reviewing Microbiologist Date

Microbiology Team Leader Date

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/s/

SUNG S RHEE
08/13/2012

JULIAN J O REAR
08/13/2012