CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

22-145

MICROBIOLOGY REVIEW

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

MICROBIOLOGY REVIEW

NDA: 22145 **SN**: 000 **DATE REVIEWED:** 09/28/07

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

NDA #: 22145 **Serial #:** 000

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

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Amendments:

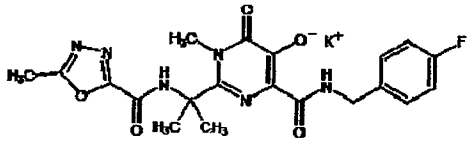
Related/Supporting Documents: IND 69,928 ———

Product Name(s):

Proprietary: ISENTRESS™
Non-Proprietary/USAN: Raltegravir potassium
Code Name/Number: MK-0518

Chemical Name: N-[(4-fluorophenyl)methyl]-1,6-dihydro-5-hydroxy-1-methyl-2-[1-methyl-1-[[(5-methyl-1,3,4-oxadiazol-2-yl)carbonyl]amino]ethyl]-6-oxo-4-pyrimidinecarboxamide monopotassium salt

Structural Formula:



Raltegravir

Molecular Formula: $C_{20}H_{20}FKN_6O_5$
Molecular Weight: 482.51

Dosage Form(s): 400 mg tablet
Route(s) of Administration: Oral
Indication(s): Treatment of HIV-1 infection in combination with other antiretroviral agents in treatment-experience adult patients who have evidence of viral replication and HIV-1 strains resistant to multiple antiretroviral agents
Recommended Dosage: 400 mg twice daily
Dispensed: Rx _X_ **OTC** ___ **(Discipline relevant)**

Abbreviations: AIDS, acquired immunodeficiency syndrome; AZT, zidovudine; BID, *bis in die* (twice a day); bp, base pair; CA, capsid protein; CIC_{95}, 95% cell culture inhibition concentration; EFV, efavirenz; ELISA, enzyme-linked immunosorbent assay; FBS, fetal

bovine serum; FTC, emtricitabine; GSS, genotypic sensitivity scores; HCV, hepatitis C virus; NHS, normal human serum; HIV-1, human immunodeficiency virus type 1; HIV-2, human immunodeficiency virus type 2; IC_{50}, 50% inhibitory concentration; IN, integrase; INSTIs, integrase strand transfer inhibitors; LLOQ, lower limit of quantification; LTR, long terminal repeat; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; OBT, optimized background therapy; PBMC, peripheral blood mononuclear cell; PCR, polymerase chain reaction; PI, protease inhibitor; PIC, pre-integration complex; PSS, phenotypic sensitivity scores; QS, quantitation standard; RAG1/2, recombination activating gene-1 and -2 products; RSS, recombination signal sequence; RT, reverse transcriptase; RT-PCR, reverse transcriptase polymerase chain reaction; SEM, standard error of the mean; TFV, tenofovir; ULOQ, upper limit of quantification

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EXECUTIVE SUMMARY

There are currently 5 classes of antiretroviral drugs approved for the treatment of HIV-1 infection: non-nucleoside reverse transcriptase inhibitors (NNRTIs), nucleoside reverse transcriptase inhibitors (NRTIs), protease inhibitors (PIs), fusion inhibitors (FIs), and CCR5 co-receptor antagonists. Raltegravir is an inhibitor of HIV-1 integrase and is the first drug approved in this new class of antiretroviral drugs. The development of new classes of drugs is important for the treatment of individuals with virus resistant to currently approved drugs or unable to use currently approved drugs because of toxicity concerns.

Raltegravir suppressed HIV-1 replication in MT-4 human T cells infected with HIV-$1_{H9/IIIB}$ with a 95% cell culture inhibitory concentration value (CIC_{95}) of 18.7 ± 14 nM in the presence of 10% fetal bovine serum (FBS). In the presence of 50% normal human serum (NHS), the CIC_{95} value was 31 ± 20 nM, showing a <2-fold reduction in antiviral potency. Raltegravir was active against multiple clinical isolates from HIV-1-infected persons with CIC_{95} values ranging from 6 to 50 nM in human peripheral blood mononuclear cells (PBMCs). Isolates tested included various HIV-1 subtypes, A to F, and both NSI (nonsyncytia inducing M-tropic) and SI (syncytia inducing T-tropic) viruses that use the co-receptors CCR5 and CXCR4, respectively.

Cell-based drug combination studies showed in HIV-$1_{H9/IIIB}$-infected MT-4 cells that the anti-HIV-1 activity of raltegravir was additive to synergistic in combination with each of 18 FDA-approved antiretroviral drugs tested: the NNRTIs delavirdine, efavirenz, or nevirapine; the NRTIs abacavir, didanosine, lamivudine, stavudine, tenofovir, zalcitabine, or zidovudine; the PIs amprenavir, atazanavir, indinavir, lopinavir, nelfinavir, ritonavir, or saquinavir; and the fusion inhibitor enfuvirtide.

Cell-based resistance selection studies in H9 cells infected with HIV-1_{IIIB} identified the Q148K substitution as a primary resistance-associated amino acid substitution. Phenotypic analyses revealed that Q148K conferred 46-fold reduced susceptibility to raltegravir. Further selection in increasing raltegravir concentrations yielded sequential addition of E138A and G140A to the Q148 substitution, which increased overall resistance to 90-fold (E138A/Q148K) and 508-fold (E138A/G140A/Q148K), respectively. The G140A/Q148K double substitutions could also increase resistance to 257-fold. By themselves, however, E138A and G140A conferred undetectable and 3-fold reduced susceptibility, respectively, and the E138A/G140A combination conferred 4-fold reduced susceptibility to raltegravir. Thus, it appeared that the Q148K substitution is a primary contributor to resistance to raltegravir, while the E138A and G140A substitutions play a secondary role in augmenting resistance.

Additional basic amino acid substitutions at Q148, Q148H and Q148R, were observed in the virus populations of 12 patients experiencing virologic failure in the 16-week interim genotypic analyses of Phase 2 protocol 005. Similar to the effect of the Q148K substitution selected in cell culture, Q148H and Q148R also exhibited significant loss of susceptibility to raltegravir: the single substitutions Q148H and Q148R conferred 24-fold and 27-fold reductions, respectively. All 12 patients had a G140S or E138A/K substitution in addition to Q148H/R. Addition of G140S to either the Q148H or Q148R

substitution substantially augmented resistance, conferring 521- and 405-fold resistance, respectively. As with the G140A substitution selected in cell culture, G140S alone exhibited 2-fold reduced susceptibility.

Independently to the Q148H/R substitutions in the 16-week interim genotypic analyses, the N155H substitution emerged in HIV-1 IN from 2 virologic failure isolates. In phenotypic analyses, N155H reduced susceptibility to raltegravir by 13.2-fold. The N155H substitution was observed with the secondary substitution E92Q that increased resistance to 64-fold. E92Q alone conferred 3-fold reduced susceptibility,

Taken together, at least 2 primary pathways, the Q148 pathway or the N155 pathway, appear to be involved independently in the emergence of raltegravir resistance.

The Phase 3 clinical studies, Protocols 018 and 019, examined the antiviral efficacy of raltegravir 400 mg twice daily compared to placebo, both in combination with OBT, in treatment-experienced adult patients who failed antiretroviral therapies with triple-class (NNRTI, NRTI, and PI) antiretroviral drug resistance. FDA analyses indicated that antiviral efficacy of raltegravir (plus OBT) is superior to placebo (plus OBT) in these patient groups, based on Week 16 data from all subjects and Week 24 data from approximately 60% of the subjects. These efficacy results were supported by the 24-week analysis of the Phase 2 study (Protocol 005).

The rates of virologic failure at Weeks 16 and 24 were lower for raltegravir plus OBT recipients, compared to those for placebo plus OBT recipients, 16% versus 50%, and 16% versus 51%, respectively. Treatment failure of the raltegravir recipients was largely due to treatment-emergent virologic rebound (80.6% [58/72] at Week 16; 81.6% [62/76] at Week 24), rather than due to the suboptimal suppression of HIV-1 replication (nonresponse to the treatment). In contrast, in placebo recipients, virologic nonresponse to OBT appears to be a primary cause of the treatment failure, the virologic nonresponse rates (64.2% [77/120] at Week 16; 63.6% [77/121] at Week 24) being higher than the rebound rates. Thus, raltegravir-containing regimens can potently suppress HIV-1 replication but such response may not be durable.

The paired amino acid sequences of HIV-1 IN from the screen and on-treatment samples of 77 evaluable patients with evidence of virologic failure to raltegravir (+ OBT) treatment were analyzed to identify treatment-emergent genotypic changes (mutations) in HIV-1 IN while on raltegravir therapy: 27 from the Phase 3 study (Protocols 018 and 019), out of the 72 virologic failure patients, and 50 from the Phase 2 study (Protocol 005; 35 and 15 during the double-blind phase and the open-label extension phase, respectively).

Amino acid substitutions emerged in the HIV-1 IN from the viruses of 75 patients out of the 77 patients (97.4%) with the average number of genetic changes per patient of 3.9 ± 2.1 (median 4, ranging from 0 to 9). No apparent differences in the average number of changes per patient were observed at different drug concentrations tested in Protocol 5.

A total 94 codons (32.6% of the 288 codons in the HIV-1 IN domain) were found to be mutated in the viruses and most (72 codons) mutated only once or twice. The 3 most

frequently observed changes were at codons 140, 148, and 155 (27, 34, and 29 occurrences each). These codons are highly conserved among HIV-1 isolates and located near the catalytic site (D64, D116, and E152) in the central core domain of HIV-1 IN. In addition, nonpolymorphic substitutions occurred in 4 or more patients at 11 amino acid positions, L74, E92, T97, E138, Y143, V151, G163, H183, Y226, S230, and D232.

Out of the 77 evaluable patients, the Q148H/K/R and N155H substitutions, previously identified as primary substitutions in the 16-week interim analysis of Phase 2 Protocol 005, were detected in 60 patients (77.9%). The other substitutions that were frequently found (≥4 occurrences) in the 77 paired genotypic analyses, with the exception of Y143C/H/P/R, were also found in the same virus population harboring the Q148H/K/R and N155H substitutions; L74M/R, E92Q, T97A, E138A/K, G140A/S, V151I, G163K/R, H183P, Y226C/D/F/H, S230N/R, and D232E/D. Thus, these 11 substitutions may not emerge independently to the primary Q148H/R and N155H substitutions but may emerge as a secondary substitution to augment raltegravir resistance. Phenotypic data supported this implication by showing that some substitutions, such as E92Q, E138A, and G140A/S, were added to the Q148H/K/R and N155H mutations to enhance resistance to raltegravir.

The Y143C/H/P/R substitution was found in 6 patients and 5 of the 6 had HIV-1 population harboring neither Q148H/R nor N155H. Thus, the Y143C/H/P/R substitution is likely to emerge independently as a separate pathway to raltegravir resistance. In the virus population harboring the Y143C/H/R substitutions, the L74M, E92Q, T97A, G163R, and S230R secondary substitutions were also found. Y143 is highly conserved in HIV-1. No phenotypic data of Y143C/H/R against raltegravir are available for this review.

Together, by analyzing 77 evaluable paired genotypic data from patients with virologic failure to raltegravir treatment in Protocols 005, 018, and 019, 3 primary substitutions, Y143C/H/R, Q148H/K/R, and N155H, were identified to emerge independently in HIV-1 IN. Each of the 3 primary substitutions was usually accompanied by one or more of the 11 secondary substitutions, L74M/R, E92Q, T97A, E138A/K, G140A/S, V151I, G163R, H183P, Y226C/D/F/H, S230N/R, and D232N. The primary substitutions developed in 65 patients (84.4%) of the 77 from all 3 Protocols.

The primary substitutions could be detected at early virologic failure isolates, as early as in Day 26 isolates. In Protocols 018 and 019, 5 patients had virologic rebound within 30 days from start of raltegravir (+ OBT) therapy and 4 of the 5 had the primary mutations, Q148H/K/R and N155H, in their rebound virus population. All 4 patients had a PSS ≤1 and had neither enfuvirtide nor darunavir in their OBT. Thus, raltegravir suppression may not be durable in the absence of another fully active agent.

1. Recommendations

1.1. Recommendation and Conclusion on Approvability

This NDA for raltegravir is approvable with respect to microbiology for the treatment of HIV-1 infection in combination with other antiretroviral agents in treatment-experienced adult patients who have evidence of viral replication and HIV-1 strains

resistant to multiple antiretroviral agents.

1.2. Recommendation on Phase 4 (Post-Marketing) Commitments, Agreements, and/or Risk Management Steps, If Approvable.

Post-Marketing Commitments:

(1) Determine the susceptibility in cell culture of HIV-1 harboring a Y143C/H/R amino acid substitution individually and in combination with L74M, E92Q, T97A, G163R, and S230R in a common genetic background.
Protocol Submission Date: December 31, 2007
Final Study Report Submission Date: September 30, 2008
(2) Evaluate the contributions of L74M/R, T97A, V151I, G163R, H183P, Y226C/D/F/H, and S230N/R substitutions to raltegravir resistance by site-directed mutagenesis.
Protocol Submission Date: December 31, 2007
Final Study Report Submission Date: September 30, 2008

Non Post-Marketing Commitments:

(1)

(2)

(3)

2. Summary of OND Microbiology Assessments

2.1. Nonclinical Microbiology

Raltegravir inhibited the HIV-1 IN-catalyzed strand transfer with an IC_{50} value of 2 to 7 nM in a biochemical reaction. No significant inhibitory activity of raltegravir was observed against the DNA polymerase and RNaseH activities of purified HIV-1 RT at concentrations up to 100 μM and 25 μM, respectively, and against purified human DNA polymerases α, β, and γ at concentrations up to 50 μM.

In SupT1 cells infected with VSV-G-pseudotyped HIV-1, raltegravir at 1 μM did not inhibit HIV-1 cDNA synthesis but prevented integration of unintegrated linear HIV-1 cDNA into the host cell genome (50-fold reduction), resulting in a significant increase (approximately 16 fold) of the 2-LTR circular form of unintegrated viral DNA.

Raltegravir exhibited antiviral activity against HIV-1 in MT-4 cells infected with HIV-$1_{H9/IIIB}$ with CIC_{95} values of 18.7 ± 14 nM in the presence of 10% FBS and 31 ± 20 nM

in the presence of 50% NHS.

The CIC_{95} values against clinical isolates (including subtypes A to F, and both NSI and SI viruses) from HIV-1-infected persons in human PBMCs ranged from 6 to 50 nM. Raltegravir was active against 2 HIV-1 isolates resistant to the PI indinavir and to an investigational NNRTI L-697,661 with CIC_{95} values for both of 19 nM, and against a HIV-2 laboratory isolate with the CIC_{95} value of 6.3 nM.

No evidence of cytotoxicity or effects on cell growth by visual inspection of cells was noted when cells were grown in the presence of raltegravir at concentrations up to 1,000-fold greater than those required to inhibit HIV-1 replication.

Raltegravir was bound to highly mouse, rat, dog, and human plasma with average 70, 74, 70, and 83%, respectively.

Raltegravir exerted additive to synergistic antiviral effects when combined with each of 18 FDA-approved antiretroviral drugs: NNRTIs delavirdine, efavirenz, or nevirapine; NRTIs abacavir, didanosine, lamivudine, stavudine, tenofovir, zalcitabine, or zidovudine; PIs amprenavir, atazanavir, indinavir, lopinavir, nelfinavir, ritonavir, or saquinavir; or the entry inhibitor enfuvirtide.

Cell-based resistance selection studies in H9 cells infected with $HIV\text{-}1_{IIIB}$ identified the Q148K substitution as a primary substitution, which persisted until the end of the selection over 18 months and was sequentially supplemented by E138A, G140A, I208M, S230R, Y143C, and D10F. In phenotypic analyses, using a single-cycle HIV-1 infection assay in P4/R5 reporter cells, Q148K was shown to reduce susceptibility to raltegravir by 46-fold. Addition of E138A and G140A to the Q148 substitution increased overall resistance to 90-fold (E138A/Q148K) and 508-fold (E138A/G140A/Q148K), respectively. The G140A/Q148K double substitutions could also increase resistance to 257-fold. By themselves, however, E138A and G140A conferred undetectable and 3-fold reduced susceptibility, respectively, and the E138A/G140A combination conferred 4-fold reduced susceptibility to raltegravir.

In the 16-week interim genotypic analyses of Phase 2 protocol 005, substitutions at Q148 with arginine (Q148R) or histidine (Q148H) were observed in the virus population of 12 patients experiencing virologic failures. Similar to the effect of the Q148K mutation, Q148H and Q148R conferred 24-fold and 27-fold reduced susceptibility, respectively. All 12 patients had G140S or E138A/K in addition to Q148H/R. Addition of G140S to either the Q148H or Q148R substitution increased resistance to 521- and 405-fold, respectively, while G140S alone exhibited 2-fold reduced susceptibility.

The N155H substitution emerged in HIV-1 IN, independently to the Q148H/R substitutions in the 16-week interim genotypic analyses. N155H reduced susceptibility to raltegravir by 13.2-fold. The N155H substitution was observed with the secondary substitution E92Q that increased resistance to 64-fold. E92Q alone conferred 3-fold reduced susceptibility.

None of the HIV-1 variants harboring the IN key substitutions including E138E, G140A, Q148H/K/R, and N155H/S displayed appreciable resistance to zidovudine, efavirenz, emtricitabine, and tenofovir in cell-based cross-resistance studies.

Raltegravir was partially active against HIV-1 variants that conferred reduced susceptibility in cell culture to other investigational HIV-1 IN strand transfer inhibitors such as L-708,906, L-731,988, and L-870,810 that are structurally distinct from raltegravir. The N155S substitution associated with resistance to the diketo acid IN inhibitors, L-708,906 and L-731,988, conferred 19-fold reduced susceptibility to raltegravir. Other substitutions tested, T66I, T125K, V151I and S153Y exhibited no reductions in susceptibility. However, the F121Y substitution alone and in combination with the T125K substitution, conferred 3-fold and 7-fold reduced susceptibility. The triple substitutions T66I/L74M/V151I also conferred 5-fold reduced susceptibility.

2.2. Clinical Microbiology

In the 2 Phase 3 clinical studies (Protocols 018 and 019), antiviral efficacy of raltegravir (400 mg twice daily) was compared to placebo, both in combination with OBT, in treatment-experienced adult patients who failed antiretroviral therapies with triple-class (NNRTI, NRTI, and PI) antiretroviral drug resistance.

Antiviral efficacy of raltegravir (plus OBT) was demonstrated to be superior to placebo (plus OBT) in these patient populations, based on Week 16 data from all subjects and Week 24 data from approximately 60% of the subjects.

The rates of virologic failure at Weeks 16 and 24 were lower for raltegravir plus OBT recipients, compared to those for placebo plus OBT recipients, 16% versus 50%, and 16% versus 51%, respectively.

In treatment failure patients treated with raltegravir plus OBT, the virologic rebound rates were much higher that the virologic nonresponse rates, 80.6% [58/72] versus 19.4% [14/72] at Week 16 and 81.6% [62/76] versus 18.4% [14/76] at Week 24. In contrast, in placebo recipients, the rates of virologic nonresponse to OBT were higher than the rebound rates, 64.2% [77/120] versus 35.8% [43/120] at Week 16 and 63.6% [77/121] versus 36.4% [44/121] at Week 24.

A Total of 77 paired amino acid sequences of HIV-1 IN from the screen and on-treatment samples from patients with evidence of virologic failure to raltegravir(+ OBT) treatment were analyzed to identify treatment-emergent genotypic changes in HIV-1 IN: 27 from the Phase 3 study (Protocols 018 and 019), out of the 72 virologic failure patients, and 50 from the Phase 2 study (Protocol 005; 35 and 15 during the double-blind phase and the open-label extension phase, respectively).

The number of amino acid substitutions emerged in HIV-1 IN while on raltegravir per patient ranged from 0 to 9 (median 4) with the average number of genetic changes per patient of 3.9 ± 2.1. Out of the 77 patients, 75 patients had amino acid substitutions (97.4%). No apparent differences in the average number of changes per patient were

observed at different drug concentrations tested in Protocol 5: 4.2 ± 2.0 (ranging from 0 to 7, median 5) for 200 mg; 4.5 ± 1.7 (ranging from 2 to 7, median 5) for 400 mg; and 3.5 ± 1.8 (ranging from 1 to 7, median 3) for 600 mg.

A total 94 codons (32.6% of the 288 codons in the HIV-1 IN domain) were found to be mutated in the viruses and most (72 codons) mutated only once or twice. The 3 most frequently observed changes were at codons 140, 148, and 155 (27, 34, and 29 occurrences each). In addition, nonpolymorphic substitutions occurred in 4 or more patients at 11 amino acid positions, L74, E92, T97, E138, Y143, V151, G163, H183, Y226, S230, and D232.

The Q148H/K/R and N155H substitutions, identified as primary substitutions in the 16-week interim analysis of Phase 2 Protocol 005, were detected in 60 (77.9%) patients out of the 77 evaluable patients.

The substitutions L74M/R, E92Q, T97A, E138A/K, G140A/S, V151I, G163K/R, H183P, Y226C/D/F/H, S230N/R, and D232E/D were frequently detected in the same virus population harboring the Q148H/K/R and N155H mutations, suggesting that these 11 substitutions may emerge as a secondary mutation: L74M/R (60% of the occurrences), E92Q (88.9%), T97A (87.5%), E138A/K (100%), G140A/S (100%), V151I (100%), G163K/R (83.3%), H183P (40%), Y226C/D/F/H (100%), S230N/R (88.9%), and D232E/D (85.7%).

The Q148H/K/R substitution was most frequently found, occurring in 34 patients (34/77 [44.2%]). The Q148H/K/R substitution was always accompanied by one or more of the 10 secondary substitutions. G163R was not detected. The E138A/K (5 occurrences) and G140A/S substitutions (27 5 occurrences) were exclusively found in the Q148H/K/R mutation-harboring virus population. The G140A/S + Q148H/K/R double mutation was most frequently observed (27/77 [35.1%]).

The N155H substitution was detected in 29 samples (29/77 [37.7%]) and the 10 secondary mutations were also observed in 25 samples: G140A/S was not detected. E138K was detected in 1 patient but is likely to accompany the Q148K mutation, since the virus population harbored the 2 primary mutations, Q148K and N155H. The E92Q (7 patients) and V151I (7 patients) substitutions were most frequently noted in the N155H-containing virus population.

The Y143C/H/P/R substitution was found in 6 patients and 5 of the 6 had HIV-1 population harboring neither Q148H/R nor N155H. In the virus population harboring the Y143C/H/R substitutions, the L74M, E92Q, T97A, G163R, and S230R secondary substitutions were also found. Thus, the Y143C/H/P/R mutation is likely to emerge independently as a separate pathway to raltegravir resistance. No phenotypic data of Y143C/H/R against raltegravir are available for this review.

Three primary substitutions, Y143C/H/R, Q148H/K/R, and N155H, were identified and developed in 65 patients (84.4%) of the 77 evaluable patients from all 3 Protocols. Each of the 3 primary substitutions was usually accompanied by one or more of the 11 secondary substitutions, L74M/R, E92Q, T97A, E138A/K, G140A/S, V151I, G163R,

H183P, Y226C/D/F/H, S230N/R, and D232N.

The primary substitutions were detected at early virologic failure isolates, as early as in Day 26 isolates. In Protocols 018 and 019, 5 patients had virologic rebound within 30 days from start of raltegravir (+ OBT) therapy and 4 of the 5 had the primary mutations, Q148H/K/R and N155H, in their rebound virus population. All 4 patients had a PSS ≤1 and had neither enfuvirtide nor darunavir in their OBT.

In genotypic analyses of the viruses collected at Baseline from 104 patients in Protocols 005, 018, and 019, 56.3% (162/288) of the 288 codons in the IN protein were polymorphic. There was only 1 patient who had virus harboring the Y143H primary substitution at Baseline. In 26 patients, 3 raltegravir secondary substitutions (T97A, V151I, and S230N/R) were detected as naturally occurring polymorphism at Baseline. Out of the 26 patients, 21 were treated with raltegravir (+ OBT), while 5 were treated with placebo (+ OBT). In the raltegravir-treated 21 patients, 12 patients (54.5%) achieved HIV-1 RNA <400 copies/mL at Week 16, comparable to 59.1% (39/66) of the patients without the raltegravir secondary substitutions in the baseline viruses and treated with raltegravir.

3. Administrative

3.1. Reviewer's Signature(s)

_____
Sung S. Rhee, Ph.D.
Microbiologist

3.2. Concurrence

_____Date: .
HFD-530/MicroTL/J. O'Rear

CC:
HFD-530/NDA # 22145
HFD-530/Division File
HFD-530/PM/M. Zeballos

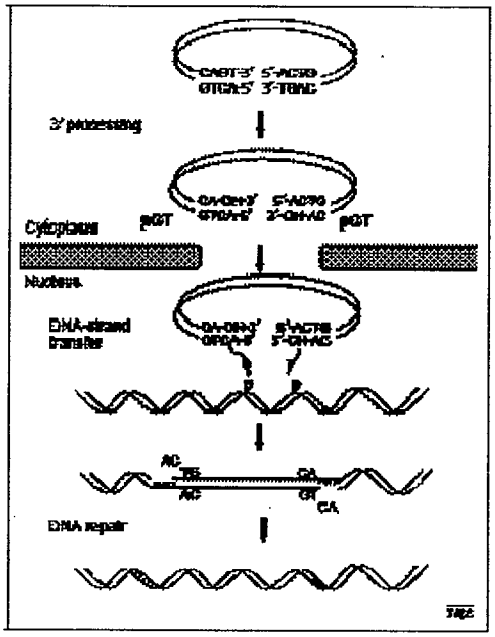
OND MICROBIOLGY REVIEW

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 to form the provirus. 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.

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

Figure 1: Outline of the Integration Reaction



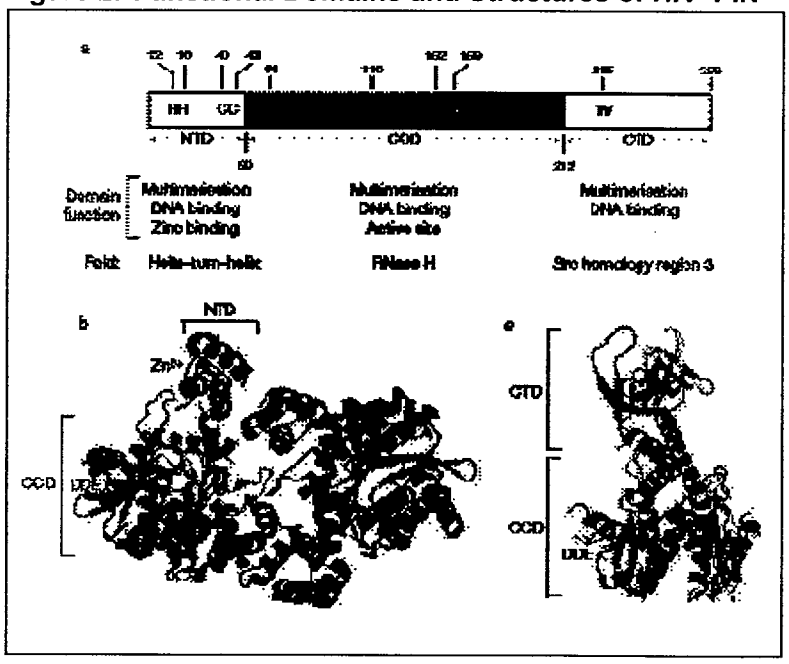
In the first catalytic step, HIV-1 IN recognizes short sequences within the long terminal repeats (LTRs; approximately 20 bp; Sherman and Fyfe, 1990) and removes a terminal GT dinucleotide from the 3'-OH end of each LTR to produce CA-OH-3 that are thus recessed by two nucleotide (Figure 1). This 3'-endonucleolytic processing takes place in the cytoplasm within the pre-integration complex (PIC) where linear viral DNA and several viral proteins including the viral matrix (MA; Bukrinsky *et al.*, 1993) and nucleocapsid (NC; Lapadat-Tapolsky *et al.*, 1993) proteins, 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-OH-3' viral DNA ends to the 5'-phosphate ends of the cellular DNA. 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 is encoded by the *pol* gene of the virus, expressed as part of a large Gag-Pol polyprotein, and cleaved into its mature form of approximately 32 kDa (288 amino acids) by the viral protease. The HIV IN protein 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.

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



The N-terminal domain contains a putative zinc-binding HHCC motif (H12, H16, C40, and C43) and is believed to be involved in multimerization of IN (Pommier *et al.*, 2005; Van Maele and Debyser, 2005). The central core domain contains an absolutely conserved D,D(35)E catalytic motif (D64, D116, and E152) that forms an active site, coordinating a divalent metal ion, either Mg^{++} or Mn^{++} for the IN catalytic activity (Grobler *et al.*, 2002; Pommier *et al.*, 2005; Van Maele

and Debyser, 2005). The less conserved C-terminal domain appears to play a role in binding to viral and host DNA (Pommier *et al.*, 2005; Van Maele and Debyser, 2005). In addition, it was suggested that the C terminus of the IN protein is needed for IN multimerization. Nuclear localization signals, 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 *in vitro* (Drelich *et al.*, 1992; Engelman and Craigie, 1992; Schauer and Billich, 1992; Vink *et al.*,1993).

From 1981 to 2005, more than 900,000 cases of acquired immunodeficiency syndrome (AIDS) were reported in the United States. More than 20 antiretroviral drugs in 5 different mechanistic classes (NNRTIs, NRTIs, PI, fusion inhibitors, and CCR5 co-receptor antagonists) have been approved by FDA for treating HIV infection. Multiple antiretroviral drug therapy introduced in 1996 greatly reduced the morbidity and mortality associated with AIDS, however, the emergence of drug-resistant virus has limited the usefulness of many drugs. Recent studies with 1587 patients in the University of North Carolina HIV Cohort Study by Napravnik *et al.* (2007) showed that the prevalence of HIV-1 triple-class (NNRTI, NRTI, and PI) antiretroviral drug resistance among all antiretroviral-experienced patients was 8% (95% confidence interval [CI] 6%, 9%). Cross-resistance to other members of the class reduces the likelihood of subsequent full suppression of HIV-1 replication with new regimens ultimately leading to failure of the regimen and resistance to the class. Therefore, there is a need for new antiretrovirals with different resistance profiles.

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 responsible for the generation of immunoglobulin and T-cell-receptor diversity in B and T cells, respectively (Melek *et al.*, 2002; see Nonclinical Microbiology for details). Currently, there are no FDA approved drugs targeting HIV-1 IN.

The applicant identified raltegravir to specifically inhibit the strand transfer reaction of HIV-1 IN and consequently suppresses HIV-1 replication in cell culture. The NDA package for raltegravir contains study reports, and datasets primarily from the 2 pivotal Phase 3 studies (Protocols 18 and 19) and the supportive Phase 2 study (Protocol 005), both within the U.S. and at international sites. These studies are randomized, double-blind, superiority trials of raltegravir compared to placebo, both in combination with OBT, in treatment-experienced patients who failed antiretroviral therapies with triple-class (NNRTI, NRTI, and PI) antiretroviral drug resistance. Protocols 018 and 019 were to evaluate raltegravir 400 mg BID plus OBT versus placebo plus OBT, while Protocol 005 was a dose-ranging Phase 2 supportive study evaluating 3 doses of raltegravir (200, 400, and 600 mg BID) plus OBT versus placebo plus OBT. The primary time point was Week 16 in Phase 3 and Week 24 in Phase 2 studies. The overall study hypothesis is that raltegravir treatment would provide superior antiretroviral efficacy compared to placebo, all in combination with OBT.

2. Materials and Methods

2.1. Quantification of Plasma HIV-1 RNA Levels

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

According to the manufacturer, the Amplicor HIV-1 Monitor Test yielded a specificity >99.5%, reducing false positive results when tested in a large seronegative population of over 500 samples. It could distinguish 0.5 $\log_{10}$ copies/mL. In addition, this test utilizes a primer set designed to detect non-B subtypes (HIV-1 Group M), providing reliable viral load measurement of HIV-1 subtypes A-G.

The Roche Amplicor HIV-1 Monitor Test was used with either the standard processing procedure or the ultrasensitive processing procedure. In the standard processing procedure, HIV-1 RNA was isolated directly from plasma by lysis of virus particles with a chaotropic agent followed by precipitation of the RNA with alcohol. HIV-1 RNA can be quantified by this procedure over the range of 400 (lower limit of quantification [LLOQ], 2.60 $\log_{10}$) to 750,000 (upper limit of quantification [ULOQ], 5.88 $\log_{10}$) copies/mL. When HIV-1 RNA levels were <400 copies/mL by the standard processing procedure, the applicant employed the ultrasensitive processing procedure where HIV-1 viral particles are first pelleted from the plasma specimen by high speed ultracentrifugation followed by lysis of the pelleted virus particles with a chaotropic agent and precipitation of the HIV-1 RNA with alcohol. This procedure was reported by the manufacturer to quantify viral loads as low as 50 copies/mL 95% of the time. For specimens to contain high levels of HIV-1 RNA (≥750,000 copies/mL), diluted samples were requantified.

2.2. Nucleotide Sequence Analysis of the HIV-1 IN Coding Region

The applicant submitted on September 08, 2006 (IND 69,928, SN 239) a detailed description of the methodology to analyze the nucleotide sequences of the HIV-1 IN coding domain from plasma-derived viruses.

2.3. HIV-1 Phenotypic/Genotypic Drug Resistance Testing

The HIV-1 phenotypic/genotypic Drug Resistance testing for viruses in plasma of patients was performed by Monogram Biosciences to evaluate genotypic and phenotypic resistance to NNRTIs, NRTIs, and PIs. The phenotypic/genotypic Drug Resistance testing data were used to define phenotypic and genotypic sensitivity scores (PSS and GSS). The PSS was based on a binary algorithm where a drug to which the subject's virus is sensitive is given a score of 1 and a drug to which the subject's virus shows reduced susceptibility is given a score of 0 (IND 69,928 SN 337).

Phenotypic testing using a Phenosense GT assay is to measure directly and quantitatively the ability of the virus to grow in the presence of antiretrovirals evaluated using recombinant technology (Monogram Biosciences). Genotypic testing indirectly measures drug resistance by nucleic acid sequence analysis of the HIV-1 reverse transcriptase and protease genes and by comparing to a reference list of NNRTI, NRTI, and PI resistance-associated amino acid substitutions (Hatano *et al.*, 2006; see Tables 1, 2, and 3 below, from IND 69,928 SN 306). Of note, some drug resistance substitutions published by the International AIDS Society-USA (IAS-USA, 2006) were excluded from the reference list of substitutions with reasons provided in the comments column. Genotypic testing is reliable with over 99% reproducibility with viral loads as low as 500 copies/mL and sensitivity to detect mixtures as low as 10% of the total viral population.

Table 1: Substitutions in the HIV-1 RT Associated with Resistance to NNRTIs

From IAS-USA group (Johnson *et al.*, 2006)	From Hatano *et al.* (2006)	Merck Comments
L100I	L100I	
K103N	K103N	
V106A	V106A	
V106M	V106M	
V108I	V108I	
Y181C	Y181C	
Y181I	Y181I	
Y188C	Y188C	

Y188L	Y188L	
Y188H	Y188H	
G190A	G190A	
G190S	G190S	
P225H	P225H	
	M230L	
P236L	P236L	

Table 2: Substitutions in the HIV-1 RT Associated with Resistance to NRTIs

From IAS-USA group (Johnson *et al.*, 2006)	From Hatano *et al.* (2006)	Merck Comments
M41L	M41L	
	E44D	
A62V		Not counted as an individual mutation in Hatano *et al.* due to clinical significance only in presence of Q151M
K65R	K65R	
D67N	D67N	
T69insertion	T69Sinsertion	
K70E		
K70R	K70R	
L74V	L74V	
V75I		Not counted as an individual mutation in Hatano *et al.* due to clinical significance only in presence of Q151M
F77L		Not counted as an individual mutation in Hatano *et al.* due to clinical significance only in presence of Q151M
Y115F	Y115F	
F116Y		Not counted as an individual mutation in Hatano *et al.* due to clinical significance only in presence of Q151M
	V118I	
Q151M	Q151M	
M184I	M184I	
M184V	M184V	
L210W	L210W	
T215F	T215F	
T215Y	T215Y	
K219E	K219E	
K219Q	K219Q	

Table 3: Substitutions in the HIV-1 Protease Associated with Resistance to PIs

From IAS-USA group (Johnson *et al.*, 2006)	From Hatano *et al.* (2006)	Merck Comments
L10I	L10I	
L10C		
L10F	L10F	
L10R	L10R	
L10V	L10V	
V11L	V11L	
I13V	I13V	
G16E		
K20I	K20I	
K20M	K20M	
K20R	K20R	
K20T		
K20V	K20V	
L24I	L24I	
D30N	D30N	
V32I	V32I	
L33F	L33F	
L33I	L33I	
L33V		
E34Q		
E35G	E35G	
M36I	M36I	
M36L	M36L	
M36V	M36V	
K43T	K43T	
M46I	M46I	
M46L	M46L	
I47A	I47A	
I47V	I47V	

From IAS-USA group (Johnson *et al.*, 2006)	From Hatano *et al.* (2006)	Merck Comments
G48V	G48V	
I50L	I50L	
I50V	I50V	
F53L	F53L	
F53Y		
I54A	I54A	
I54L	I54L	
I54M	I54M	
I54S	I54S	
I54T	I54T	
I54V	I54V	
Q58E	Q58E	
D60E		
I62V		
L63P		Common polymorphism
I64L		
I64M		
I64V		
H69K	H69K	
A71I		
A71L	A71L	
A71T	A71T	
A71V	A71V	
G73A	G73A	
G73C	G73C	
G73S	G73S	
G73T	G73T	
T74P	T74P	
L76V	L76V	
V77I	V77I	
V82A	V82A	
V82F	V82F	
V82I	V82I	
V82L	V82L	
V82S	V82S	
V82T	V82T	
N83D	N83D	
I84V	I84V	
I85V		
N88D	N88D	
N88S	N88S	
L89V	L89V	
L90M	L90M	
I93L		
I93M		

3. Nonclinical Microbiology

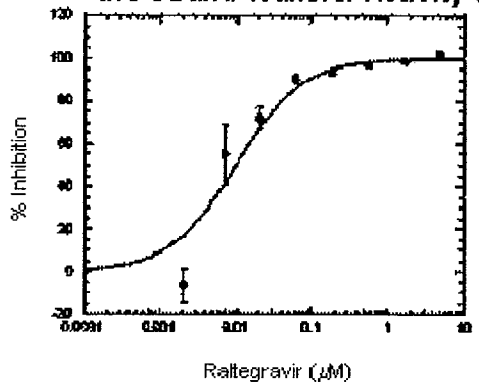
3.1. Mechanism of Action

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

The applicant showed that raltegravir inhibited the HIV-1 IN-catalyzed strand transfer with an IC_{50} value of 2 to 7 nM in a biochemical reaction (Figure 3 from Merck Study Report PD001, page 3). In the strand transfer assay (Hazuda, *et al.*, 1994), purified

recombinant HIV-1_{HXB2} IN proteins that were expressed in *E. coli* and a double-stranded "donor" oligonucleotide substrate mimicking one end of HIV LTR were utilized to measured the inhibitory effect of raltegravir on the strand transfer of the donor DNA into a target DNA. Strand transfer reactions were performed at 37°C for 30 min in 2.5 mM $MgCl_2$ using 0.5 or 5.0 nM target DNA.

Figure 3: Inhibitory Effect of Raltegravir on the Strand Transfer Activity of IN



Raltegravir was evaluated against other mechanistically similar enzymes, viral and cellular, that catalyze a phosphoryltransfer reaction, including the HIV-1 reverse transcriptase (RT) and 3 human DNA polymerases α, β, and γ.

No significant inhibitory activity of raltegravir was observed against the DNA polymerase and RNaseH activities of purified HIV-1 RT at concentrations up to 100 μM and 25 μM, respectively, and against purified human DNA polymerases α, β, and γ at concentrations up to 50 μM (Merck Study Report PD001).

In addition, according to the applicant, no marked off-target inhibitory activities (inhibition of 50% or greater) were observed with raltegravir at ≥10 μM against a panel of 166 human proteins including enzymes, receptors, and transporters Pharmacology Screen, data not shown; Merck Study Report PD001). Thus, raltegravir appears to be selective for HIV-1 IN with the possible exception of RAG1/RAG2 recombinase (Melek *et al.*, 2002).

Consistent with the *in vitro* observations, in the human T cell line SupT1 cells, when infected with VSV-G-pseudotyped HIV-1 in the presence of 1 μM raltegravir, HIV-1 cDNA synthesis was not significantly inhibited, but formation of the provirus (integrated viral DNA) was decreased by approximately 50-fold at 48 hours post-infection (Merck Study Report PD002). In contrast, the presence of raltegravir resulted in a significant increase (approximately 16 fold) of the 2-LTR circular form of unintegrated viral DNA in cells. The 2-LTR circles are the product of the cellular non-homologous DNA end-joining pathway (Li *et al.*, 2001), and were reported to be unable to sustain HIV-1 replication and to increase in abundance when viral IN activity is genetically absent (Wiskerchen and Muesing, 1995) or pharmacologically inhibited (Hazuda *et al.*, 2000). Of note, since HIV-1 cDNA can integrate into random sites in the human genome, integrated HIV DNAs were quantified using the human DNA repetitive element Alu-PCR as previously described by Butler *et al.* (2001). These results strengthened the *in vitro* findings that raltegravir does not inhibit the process of reverse transcription of the HIV-1 RNA genome but specifically inhibits the HIV-1 proviral integration.

3.2. Anti-HIV-1 Activity in Cell Culture

The anti-HIV-1 activity of raltegravir was assessed in MT-4 human T cells infected with the H9/IIIB laboratory isolate of HIV-1. Binding to human serum proteins can limit the efficacy of antiviral agents, thus, the antiviral activity of raltegravir was determined in the presence of fetal bovine serum (FBS) as well as normal human serum (NHS).

Briefly, growing MT4 cells were infected with HIV-$1_{H9/IIIB}$ at a multiplicity of infection of approximately 0.01 for 24 hours and then re-suspended in fresh culture medium supplemented with 10% FBS or with 50% NHS in the presence or absence of raltegravir (in serial 2-fold dilutions at concentrations ranging from 1000 nM to 1.95 nM) to be cultured for 72 hours. Virus production in the culture medium was quantified using an HIV-1 p24 antigen ELISA [redacted]. As a positive control, the protease inhibitor (PI) indinavir was included in this assay. The 95% cell culture inhibitory concentration (CIC_{95}) was calculated as the lowest concentration of test compound that inhibited virus p24 antigen production by at least 95% relative to untreated control cultures. Results are summarized in Table 4 (Merck Study Report PD002, page 3).

The CIC_{95} values for raltegravir were 18.7 ± 14 nM in the presence of 10% FBS and 31 ± 20 nM in the presence of 50% NHS, showing a <2-fold reduction in antiviral potency in the presence of 50% human serum. The antiviral potency of raltegravir was similar to that of indinavir (CIC_{95} vales of 27.1 ± 11 nM when tested in 50% NHS).

Table 4: Antiviral Activity (CIC_{95}, nM) of Raltegravir against HIV-$1_{H9/IIIB}$ in MT4 Cells

	CIC_{95} in 10% FBS	CIC_{95} in 50% NHS
Raltegravir	18.7 ± 14 (n=77)	31 ± 20 (n=90)
Indinavir	35.8 ± 14 (n=94)	27.1 ± 11 (n=94)

Values shown represent the mean ± standard deviation of multiple experiments.
n, the number of independent experiments for each compound and condition

Raltegravir was tested in human peripheral blood mononuclear cells (PBMCs) against multiple clinical isolates from HIV-1-infected persons. Clinical HIV-1 isolates obtained from HIV-1-infected persons by PBMC co-cultivation were used to infect cells at a multiplicity of infection of approximately 0.01. At 24 hours post-infection, cells were washed and cultured in fresh medium with 20% FBS in the presence or absence of raltegravir for 48 hours. Fresh medium, containing the appropriate concentration of compound, added and cells were cultured for an additional 72 hours to quantify virus production using the HIV-1 p24 antigen ELISA [redacted] Results are summarized in Table 5 (Merck Study Report PD002, page 5).

Raltegravir was active against all tested clinical isolates from diverse subtypes (subtypes A to F) with CIC_{95} values ranging from 6 to 50 nM (Table 5). Isolates tested included both NSI (non-syncytia inducing M-tropic) and SI (syncytia inducing T-tropic) viruses that use the co-receptors CCR5 and CXCR4, respectively. In addition, raltegravir was similarly effective against isolates, 1002WK60 and 5024WK16, displaying resistance to the PI indinavir or to an investigational NNRTI L-697,661 (Merck; Goldman *et al.*, 1991), respectively.

Table 5: Antiviral Activity (CIC_{95}, nM) of Raltegravir against Clinical HIV-1 Isolates in PBMCs

Isolate	Clade	Tropism	Raltegravir	Indinavir
RW/92/026	A	NSI	21 (N = 3)	42 (N = 3)
RW/93/022	A	NSI	59 (N = 2)	91 (N = 1)
IN/93/005	C	NSI	8 (N = 3)	39 (N = 3)
MW/93/999	C	NSI	18 (N = 5)	21 (N = 8)
UG/93/067	D	SI	33 (N = 3)	41 (N = 3)
UG/94/E17	D	SI	17 (N = 3)	52 (N = 3)
THA/92/009	E	NSI	6 (N = 3)	<8 (N = 3)
THA/93/072	E	ND	19 (N = 3)	23 (N = 3)
BR/93/020	F	SI	19 (N = 2)	16 (N = 2)
BR/93/029	F	NSI	13 (N = 2)	16 (N = 2)
1002 WK60	B	SI	19 (N = 2)	>1000 (N = 2)
1114 WK76	B	SI	13 (N = 2)	500 (N = 2)
116 WK7	B	SI	13 (N = 2)	23 (N = 2)
5024 WK16	B	SI	19 (N = 2)	91 (N = 3)
1397-0	B	NSI	9 (N = 3)	47 (N = 3)
EPIIIB	B	SI	12 (N = 19)	25 (N = 19)

ND, not determined

The applicant performed HIV-2 replication assays to determine whether raltegravir has the potential to be an effective therapeutic agent for HIV-2. An HIV-2 laboratory isolate was used to infect growing CEMx174 human lymphoid cells at a multiplicity of infection of approximately 0.01. At 24 hours post-infection, cells were cultured in fresh culture medium with 10% FBS in the presence or absence of raltegravir (in serial 2-fold dilutions at concentrations ranging from 1,000 nM to 7.81 nM) for 48 to 72 hours. Cells were then fed by replacing half of the spent culture medium with fresh culture medium containing the appropriate concentration of compound. At 144 hours post infection, virus replication was assessed by measuring the production of viral core antigen p27 in the culture medium using an SIV core antigen ELISA ——————. The CIC_{95} value for raltegravir was 6.3 ± 0 nM, comparable to that of indinavir (21 ± 9 nM), suggesting that raltegravir may be active against HIV-2 replication (Merck Study Report PD002). The sponsor needs to evaluate the activity of raltegravir against additional HIV-2 isolates to confirm this result.

3.3. Cytotoxicity

No formal cytotoxicity reports that describe conventional cell-based assays to assess the cytotoxicity of raltegravir were included in this NDA package.

According to the applicant, no evidence of cytotoxicity or effects on cell growth was observed by visual inspection of cells in cell culture experiments showing anti-HIV-1 activity of raltegravir described above, even at concentrations 1,000-fold greater than those required to inhibit HIV-1 replication (IND 69,928 SN 337). No quantitative surrogate assays were conducted to evaluate cell viability.

In studies to evaluate the potential for raltegravir to cause chromosomal aberrations (Merck Study Reports TT #03-8681 and TT #03-8687), cytotoxicity was assessed in the Chinese hamster ovary (CHO) cell line CHO-WBL cells with a cell-cycle time of approximately 12 to 13 hours. In cells treated with 100 μM raltegravir for 3 hours, cell growth (by viable cell count using ——————————) at 20 hours from the beginning of treatment (about 1.5 normal cell-cycle lengths) was 100% that of control

untreated cells. When treated with 750 μM raltegravir, cell growth was slightly suppressed to 81% of controls. Longer duration of raltegravir treatment resulted in more suppression of cell growth: treatment for 20 hour at 100 μM suppressed cell growth to 86% of controls (CC_{50} value of 400 μM). Similarly, no significant cytotoxicity was noted in primary rat hepatocytes treated with raltegravir for 3 hours at concentrations up to 400 μM with relative cell viability ranging from 100 to 94% (Merck Study Reports TT #03-8381 and TT #03-8394).

Melek *et al.* (2002) noted that there are mechanistic similarities between actions of HIV-1 integrase and human RAG1/2 recombinase responsible for V(D)J site-specific recombination in the rearrangement of immunoglobulin and T-cell receptor (TCR) genes in lymphocytes. These DNA rearrangements are initiated by RAG1/2, a complex of recombination activating gene-1 and -2 products, that catalyzes a single-strand DNA nick between the recombination signal sequence (RSS) and coding DNA to expose a 3'-OH group at the end of the coding DNA, and a subsequent attack on the phosphodiester bond of the opposite DNA strand to produce a hairpin on the coding DNA (McBlane *et al.*, 1995; van Gent *et al.*, 1996a and 1996b). Thus, Melek and coworkers determined whether RAG1/2 activity can also be inhibited *in vitro* by diketo acid HIV-1 IN inhibitors such as 5CITEP (Goldgur *et al.*, 1999) and L-708,906 (Hazuda *et al.*, 2000).

Both of the IN inhibitors tested interfered with DNA cleavage and disintegration activities of RAG1/2 (Melek *et al.* 2002). Especially, L-708,906 (another Merck investigational integrase inhibitor) inhibited many of the biochemical activities of RAG1/2, including nicking between the RSS and coding DNA, hairpin formation by transesterification, and disintegration of transposition intermediates, with an IC_{50} value of 20 μM. Thus, these results implied that HIV-1 IN inhibitors may have the potential to interfere with T-cell receptor and immunoglobulin gene rearrangement in human. These authors, however, were not able to determine whether IN inhibitor are able to interfere with RAG1/2 activity in cells. Of note, raltegravir inhibited the HIV-1 IN-catalyzed strand transfer with an IC_{50} value of 2 to 7 nM *in vitro* and showed similar potency against HIV-1 grown in cell culture. No data are available to evaluate the potential for raltegravir to interfere with RAG1/2 activities and consequently with the development of the specific immune system. Thus, it is recommended that the potential effects of raltegravir on human RAG1/2 activities be determined in a cell-based assay.

3.4. Serum Protein Binding

The *in vitro* plasma protein binding of raltegravir was determined using [^{14}C]-raltegravir by ultrafiltration (Merck Study Report PK003). Binding of raltegravir was high in mouse, rat, dog, and human plasma with average 70, 74, 70, and 83%, respectively, and was independent in all species of raltegravir concentrations evaluated (2, 5, and 10 μM).

3.5. Combination Activity Relationships of Raltegravir with Approved Antiretrovirals

Combinational anti-HIV-1 activities of raltegravir were evaluated with each of 18 FDA-approved antiretroviral drugs in growing MT-4 cells that were infected with HIV-$1_{H9/IIIB}$ at

a multiplicity of infection of ~0.01. At 24 hours post-infection, cells were cultured (10% FBS) for an additional 72 hours in the absence or presence of raltegravir and/or one of the test compounds at 16 different concentrations (highest concentration exceeding the compound's CIC_{95} value; the lowest concentrations tested showed no evidence of inhibiting viral replication). Virus production was quantified by measuring the amounts of HIV-1 p24 antigen in the culture medium using an HIV-1 p24 antigen ELISA [redacted] The determination of whether each compound pair acts synergistically, additively, or antagonistically was made using Independent Joint Action models. Results are summarized in Table 3 (Merck Study Report PD004, pages 3 and 5).

As a positive antagonism combination control, a combination of zidovudine and ribavirin was included in this assay. Ribavirin does not inhibit HIV replication, but has been documented to antagonize the antiviral activity of zidovudine in HIV replication assays (Margot and Miller, 2005). Zidovudine (CIC_{95} value of 50 nM) was tested at concentrations ranging from 0.586 to 100 nM, and ribavirin (CIC_{50}=39 μM) was tested at concentrations ranging from 586 nM to 100 μM. According to the applicant, consistent with published data, the addition of ribavirin to zidovudine-treated HIV-1_{H9IIIB}-infected MT4 cells resulted in antagonism of zidovudine's antiviral activity. This observation established that the HIV-1_{H9IIIB}/MT4 assay system utilized here is capable of detecting an antagonistic relationship between 2 inhibitors.

According to the sponsor, raltegravir displayed additive to synergistic activity, depending on compound concentration, with each of all 18 FDA-approved antiretroviral drugs tested. Raltegravir appeared to act synergistically with each tested drug when both were tested at higher concentrations, beginning at a concentration approaching its CIC_{95} value and extending to higher concentration (Table 6; see columns for synergistic concentration of raltegravir and tested drug when combined). However, these combinations at higher concentrations are in the asymptotic portion of the dose response curve where additivity and synergy cannot be distinguished. Of note, recently FDA approved darunavir (PI, 206), maraviroc (CCR5 co-receptor antagonists, 2007), and tipranavir (PI, 2005) were not included in this study.

Table 6: Effects of Raltegravir in Combination with Other Anti-HIV-1 Drugs

Drug	Drug Class	Concentrations tested (nM)	CIC_{95} (nM)	CIC_{50} (nM)	CIC_{50} of raltegravir (nM)	Synergistic concentration of raltegravir combined with tested drug (nM)	Synergistic concentration of tested drug combined with raltegravir (nM)
Abacavir	NRTI	58.6 - 10000	6760	609.3	3.340	≥ 4.688	≥ 5000
Didanosine	NRTI	293 - 50000	12500	1199.9	3.170	≥ 3.125	≥ 1171.88
Lamivudine	NRTI	586 - 100000	12500	2103.2	1.917	≥ 4.688	≥ 585.94
Stavudine	NRTI	5.86 - 1000	229	30.1	1.364	≥ 4.688	≥ 62.50
Tenofovir	NRTI	0.586 - 100	34	5.6	1.602	≥ 4.688	≥ 12.50
Zalcitabine	NRTI	58.6 - 10000	5243	1138.5	1.882	≥ 9.375	≥ 2500
Zidovudine	NRTI	0.586 - 100	50	2.8	1.977	≥ 6.250	≥ 12.50
Delavirdine	NNRTI	1.17 - 200	69	10.1	1.790	≥ 6.250	≥ 18.75
Efavirenz	NNRTI	0.058 - 10	4.3	0.9	2.568	≥ 6.250	≥ 1.25
Nevirapine	NNRTI	2.93 - 500	125	12.6	0.876	≥ 4.688	≥ 62.50
Amprenavir	PI	0.586 - 100	48.5	6.1	1.179	≥ 9.375	≥ 25.00
Atazanavir	PI	0.146 - 25	9.4	1.5	2.356	≥ 6.250	≥ 6.25

Indinavir	PI	0.146 - 100	37.5	4.0	1.652	≥ 6.250	≥ 18.75
Lopinavir	PI	0.586 - 100	50	9.8	2.676	≥ 3.125	≥ 25.00
Nelfinavir	PI	0.586 - 100	25	3.8	1.119	≥ 3.125	≥ 12.50
Ritonavir	PI	0.586 - 100	60.8	8.2	1.143	≥ 9.375	≥ 50.00
Saquinavir	PI	0.586 - 100	12.5	3.6	2.259	≥ 3.125	≥ 6.25
Enfuvirtide	Entry	0.586 - 100	75	9.4	1.737	≥ 9.375	≥ 50.00
Rategravir	IN	0.145 - 25	18.7	NA	NA	NA	NA
Ribavirin	HCV	586 - 100000	NA	NA	NA	NA	NA

NA: not applicable

3.6. Resistance Development in Cell Culture

HIV-1 variants resistant to raltegravir were selected by serially passaging the laboratory HIV-1 isolate IIIB in H9 cells in the presence of increasing concentrations, from 25 nM to 50 μM, of the inhibitor. The viruses were passaged a total of 18 times over a period of more than 18 months. Viral RNA was prepared using the Viral RNA [redacted] from the viruses that were able to replicate at the end of each passage and subjected to indirect nucleotide sequence analysis of the HIV-1 IN coding region as described in Materials and Methods. Results are summarized in Table 7 (Merck Study Report PD005, page 7).

Table 7: Emergence of Substitution in HIV-1 IN during Resistance Selection in Cell Culture

Passage	Raltegravir (nM)	Days in Passage (Cumulative Days in Selection)	Q148K	E138A/ Q148K	E138A/ G140A/ Q148K	E138A/ G140A/ Q148K/ I208M/ S230R	E138A/ G140A/ Y143C/ Q148K/ I208M/ S230R	D10E/ E138A/ G140A/ Y143C/ Q148K/ I208M/ S230R
1	25	24 (24)	YES(4/9)					
2	50	21 (45)	YES (5/8)[1]					
3	50	18 (63)	YES(6/6)					
4	100	18 (81)	YES(10/10)					
5	100	14 (95)	YES(7/7)					
6	500	28 (123)	YES(9/9)					
7	500	40 (163)	YES(7/7)					
8	1,000	46 (209)		YES (7/7)[2]				
9	1,000	26 (235)		YES (6/6)				
10	5,000	42 (277)			YES (9/9)[3]			
11	5,000	38 (315)			YES (7/7)[4]			
13	10,000	43 (392)				YES (7/9)[5]		
14	10,000	28 (420)				YES (7/7)[6]		
15	20,000	30 (450)					YES (5/9)	YES (4/9)
16	20,000	18 (468)					YES (1/7)	YES (6/7)
17	50,000	21 (489)						YES (8/8)
18	50,000	21 (510)						YES (7/7)[7]

[1]The E138K/G and Y143H substitutions were also found but gone the next passage.
[2]The E138K substitution was also found in 1 clone.
[3]The S230R substitution was also found in 3 clones.
[4]The I208M (2 clones), D279G (2 clones), and S230R (1 clone) substitutions were also found.
[5]The D10E substitution was also found in 2 clones. Remaining 2 clones had the E138A/G140A/Q148K/I208M mutations.
[6]The D10E (1 clone) and Y143C (1 clone) were also found.
[7]The F181L substitution was also found in 2 clones.

The first noted amino acid change in the HIV-1 IN was a glutamine (Q)-to-lysine (K) substitution at codon 148 (Q148K) that emerged at passage 1 while cultured for 24 days

at 25 nM (Table 7), similar to the CIC_{95} value of raltegravir against HIV-1_{H9IIIB} in H9 cells (18.7 ± 14 nM [n=77], Table 4). Importantly, the Q148K substitution persisted until the end of the selection. The glutamine residue at position 148, highly conserved among HIV-1 isolates, is located within the central core domain (CCD) of IN containing the 3 active site residues, aspartic acid (D), aspartic acid (D), and glutamic acid (E) at positions 64 (D62), 116 (D116), and 152 (E152), respectively (Engelman and Craigie, 1992; Kulkosky *et al.*, 1992; Polard and Chandler, 1995). At later passages (Table 7), the Q148K substitution was sequentially supplemented by E138A appearing at passage 8 (1,000 nM), G140A appearing at passage 10 (5,000 nM), I208M appearing at passage 13 (10,000 nM), S230R appearing at passage 13 (10,000 nM), Y143C appearing at passage 15 (20,000 nM), and D10F appearing at passage 15 (20,000 nM). Additional mutations, F181L and D279G, were observed in a small number of clones at passages 18 (50,000 nM) and 11 (5,000 nM), respectively (Table 7).

The phenotypic significance of the key substitution Q148K and some of the additional substitutions (E138A, G140A, and S230R) independently and in conjunction with the Q148K substitution were assessed using a single-cycle HIV-1 infection assay in a reporter cell line P4/R5 (Table 8; Merck Study Report PD005, pages 14 and 15). In addition, the sponsor included in this assay the following substitutions: (1) substitutions E92Q, G140S, Q148H, Q148R, and N155H that were identified from clinical virus samples isolated from patients who were failing raltegravir-containing regimens (Table 9; Study PD005, pages 14 and 15); and (2) substitutions, T66I, F121Y, T125K, V151I, S153Y, and N155S, that were selected in cell culture in the presence of other structurally distinct HIV-1 IN strand transfer inhibitors (INSTIs) such as L-708,906, L-731,988, and L-870,810 (Hazuda *et al.*, 2000 and 2004; see Section 3.7. Cross-Resistance with Other Investigational HIV-1 IN strand transfer inhibitors).

Percent (%) inhibition for each drug dose and the CIC_{50} value for each virus were calculated. The fold change in CIC_{50} values was determined by dividing the CIC_{50} value of each IN mutant virus by the wild-type virus CIC_{50} value. In addition, percent (%) infectivity of each mutant virus was determined in the absence of raltegravir by dividing virus-induced β-galactosidase activity by that of wild-type virus.

Table 8: Sensitivity to Raltegravir of HIV-1 Harboring Raltegravir-Selected IN Substitutions

	E92Q (n=1)	E138A (n=6)	G140A (n=6)	G140S (n=2)	Q148H (n=2)	Q148K (n=9)	Q148R (n=2)	N155H (n=6)
FC[1] (range)	3	[redacted] (1 – 1)	[redacted] (1 – 4)	2 ± 0 (2 – 2)	24 ± 11 (13 - 35)	46 ± 8.6 (16 – 89)	27 ± 10 (17 - 37)	13.2 ± 2.4 (8 – 23)

% Infectivity[2] (range)	76	48 ± 5 (36 – 65)	46 ± 3 (37 – 59)	100 ± 11 (89 – 110)	52 ± 6 (46 - 58)	33 ± 3 (21 – 44)	61 ± 5 (56 – 65)	59 ± 6 (47 – 86)
	S230R (n= 2)	E92Q/ N155H (n=1)	E138A/ G140A (n=5)	E138A/ Q148K (n=3)	G140A/ Q148K (n=2)	G140S/ Q148H (n=2)	G140S/ Q148R (n=2)	E138A/ G140A/ Q148K (n=4)
FC[1] (range)	2 ± 0 (2 - 2)	64	4 ± 0.6 (2 – 5)	90 ± 11.5 (76 – 113)	257 ± 2.5 (254 – 259)	521 ± 35 (486 – 556)	405 ± 158 (247 – 563)	508 ± 152.7 (279 – 958)
% Infectivity[2] (range)	74 ± 2 (72 – 76)	82	35 ± 4 (26 – 49)	31 ± 0 (31 – 31)	37 ± 5 (32 – 42)	93 ± 5 (87 – 98)	46 ± 4 (42 – 49)	35 ± 5 (24 – 45)

[1]FC: average fold change in CIC_{50} values ± standard error of the mean (SEM)
[2]% Infectivity: average % infectivity compared to wild-type virus infectivity ± standard error of the mean (SEM)

The first substitution that emerged during selection, Q148K, conferred 46-fold reduced susceptibility to raltegravir (Table 8, highlighted in yellow). Further selection in increasing raltegravir concentrations yielded sequential addition of E138A and G140A substitutions, which increased overall resistance to 90-fold (E138A/Q148K) and 508-fold (E138A/G140A/Q148K), respectively (highlighted in yellow). The G140A/Q148K double substitutions could also increase resistance to 257-fold. By themselves, however, E138A and G140A conferred undetectable and 3-fold reduced susceptibility, respectively, and the E138A/G140A combination conferred 4-fold reduced susceptibility to raltegravir (highlighted in green). Thus, it appeared that the Q148K substitution is a primary contributor to resistance to raltegravir, while the E138A and G140A substitutions play a secondary role in augmenting resistance. Interestingly, all of the viruses containing the Q148K substitution showed reduced infectivity, <40% infectivity of the wild-type virus (Table 8), indicating that the addition of the secondary substitutions, E138A and G140A, did not improve fitness of the viruses harboring the Q148K substitution in the HIV-1 IN.

In the 16-week interim analysis of Phase 2 protocol 005, the sponsor identified initially 15 subjects as on-treatment Virologic Failures and submitted the HIV IN Genotypic analysis data of those subjects's isolates (IND 69928, SN 254). Table 9 shows substitutions observed in the Virologic Failure samples but not in the subject's Baseline samples. Mutations observed in more than one subject's virus are underlined.

Table 9: Amino Acid Substitutions Observed in Virologic Failure Samples but Not in Baseline Samples of 15 Subjects Failing a Raltegravir Regimen in the Phase 2 Protocol 005

Baseline Number	Integrase Amino Acid Changes from Baseline to Virologic Failure
A	N155H, E92Q,
B	N155H, T97T/A, T112I, I135I/V, V201V/I
C	G140S, Q148H, T124A, E157Q
D	G140S, Q148H (No Baseline Sequence)
E	G140S, Q148H, I84I/V
F	G140S, Q148H
G	G140S, Q148H, K7K/R, M50L
H	G140S, Q148H, H183H/P
I	G140S, Q148H, V31V/G, H183H/P, S255N
J	G140S, Q148H, R20K, I72V,
K	G140S, Q148H, L101I, K111R
L	Q148R, E138E/K,
M	Q148R, E138A, D232N, V31I, K103R
N	G140S, Q148R, V31I
O	G59R/G, K136N, D207D/N, Y226Y/N, R228R/T, D229D/N

Among the IN amino acid sequences in the 15 subject isolates, all but 1 contained a substitution at either position 148 (12 of 15; Q148H or Q148R) or 155 (2 of 15; N155H). These clinically identified substitutions, Q148H, Q148R, and N155H, were frequently associated with additional substitutions, E92Q, G140S, or E138A/K. Genotype-phenotype relationships for raltegravir resistance were also assessed for these substitutions (Table 8).

Similar to the effect of the Q148K mutation selected in cell culture, substitutions of Q148 with other basic amino acid residues, arginine (R) or histidine (H), also exhibited significant loss of sensitivity to raltegravir: the single substitutions Q148H and Q148R conferred 24-fold and 27-fold reduced susceptibility, respectively. Addition of G140S to either the Q148H or Q148R substitution substantially augmented resistance, conferring 521- and 405-fold resistance, respectively. As with the G140A substitution selected in cell culture, G140S alone exhibited 2-fold reduced susceptibility.

The N155H substitution, only observed in clinical virus samples, conferred 13.2-fold reduced susceptibility to raltegravir. Addition of E92Q, which by itself conferred 3-fold reduced susceptibility, that yields the E92Q/N155H double substitutions increased resistance to 64-fold. It appeared that the addition of E92Q also improved the infectivity of the N155H virus from 59% to 82% (Table 8). Interestingly, the N155H substitution was also observed after 28-32 days of L-870,812 (Merck) therapy in viremic rhesus macaques (plasma HIV-1 RNA ≥25 copies/mL by a quantitative real-time RT-PCR assay [Heid *et al.*, 1996]) infected with the simian-human immunodeficiency virus (SHIV) 89.6P (Hazuda *et al.*, 2004). Thus, the N155H substitution may also be selected by structurally distinct INSTIs and cross resistant to other structurally diverse HIV IN strand transfer inhibitors.

As summarized in Table 10 (Merck Study Report PD005, page 16), none of the HIV variants harboring the IN substitutions phenotypically tested above displayed appreciable resistance to antiretroviral drugs directed at RT such as zidovudine (AZT), efavirenz (EFV), emtricitabine (FTC), and tenofovir (TFV). These results confirmed that the reduced susceptibility shown by the viruses containing these IN substitutions is specific to raltegravir.

Table 10: Anti-HIV Activity of NRTIs and NNRTI against HIV-1 Variants Harboring IN Substitutions in P4/R5 Cells

	E138A	G140A	G140S	Q148H	Q148K	Q148R	N155H	N155S
AZT	1	1	1	1	1	1	1.5	1
FTC	1	1	1	1	1	1.5	1.5	1.5
Tenofovir	1	1	1	1	1	1	1	1
Efavirenz	1	1	1	1	1	1	1	1
	E138A/ G140A	E138A/ Q148K	G140A/ Q148K	G140S/ Q148H	G140S/ Q148R	E138A/ G140A/ Q148K	•	•
AZT	1	1	1	1	1	1	•	•
FTC	1	1	1	1.5	1	1	•	•
Tenofovir	1	1	1	1	1	1	•	•
Efavirenz	1	1	2	1	1	1	•	•

Numbers represents fold change in CIC_{50} values with respect to the wild-type virus

Taken together, at least 2 primary pathways, the Q148 pathway or the N155 pathway, appear to be involved independently in emergence of raltegravir resistance. Substitution of Q148 with any of the basic amino acids, H, K, or R (Q148K in cell culture; Q148H or Q148R in clinical samples) conferred significant resistance to raltegravir. The Q148H/K/R substitution was frequently observed with the secondary substitutions, G140A/S (G140A in cell culture; G140S in clinical samples) and/or E138A (E138A both in cell culture and in clinical samples) that augmented resistance to raltegravir.

In the N155 pathway, the N155H substitution alone was observed in clinical virus samples and conferred significant resistance to raltegravir. The N155H substitution was also observed with the secondary substitution E92Q that enhanced raltegravir resistance.

In the phase 3 raltegravir clinical studies, Protocols 018 and 019, HIV-1 IN substitutions at Q148 (Q148H/K/R) and/or N155 (N155H) were found, frequently accompanied by the G140A/S and E92Q substitutions, respectively, in the isolates of patients who had shown evidence of Virologic Failure to raltegravir-containing regimens (see Section 4.2. Clinical Resistance Analyses).

3.7. Cross-Resistance with Other Investigational HIV-1 IN Strand Transfer Inhibitors

There are currently no FDA approved drugs targeting HIV-1 IN. Therefore, cross-resistance studies for raltegravir are limited to determining the activity of the agent against HIV-1 variants that displayed reduced susceptibility in cell culture to other investigational HIV-1 IN strand transfer inhibitors (INSTIs) such as L-708,906, L-731,988, and L-870,810 that are structurally distinct from raltegravir (Hazuda *et al.*, 2000 and 2004). The diketo acid compounds, L-708,906 and L-731,988, selected substitutions T66I, S153Y, M154I, and N155S, whereas naphthyridine carboxamide INSTI L-870,810 selected substitutions V72I, F121Y, and T125K. Phenotypic assays were done with substitutions T66I, F121Y, T125K, V151I, S153Y, and N155S, singly or in combination, as described above (see Section 3.6. Resistance Development in Cell Culture). Results are summarized in Table 11 (Merck Study Report PD005, pages 12 and 13).

The greatest raltegravir resistance among these substitutions was conferred by N155S, a 19-fold reduction in susceptibility (Table 11), similar in magnitude to the resistance this substitution displayed against L-731,988 and L-870,810 (12-fold and 20-fold, respectively). The N155S substitution conferred no appreciable resistance to AZT, FTC, TFV, and EFV (Table 10). When combined with S153Y or T125K/S153Y, susceptibility was, however, decreased to 3- or 2.5-fold, respectively. T125K and S153Y by themselves exhibited no reductions in susceptibility, as observed with the other 2 substitutions, T66I and V151I. The F121Y substitution, alone and in combination with the T125K substitution, conferred 3-fold and 7-fold reduced susceptibility. The triple substitutions T66I/L74M/V151I also conferred 5-fold reduced susceptibility. Therefore, these studies indicate partial cross resistance between raltegravir and other structurally diverse HIV IN strand transfer inhibitors.

Table 11: Sensitivity to Raltegravir of HIV-1 Mutant Viruses Harboring IN Substitutions Selected with Other Structurally Distinct INSTIs

	T66I (n=1)	F121Y (n=3)	T125K (n=5)	V151I (n=2)	S153Y (n=2)	N155S (n=9)	T66I/ V151I (n=2)
FC[1] (range)	1	3 ± 0.6 (2 – 4)	1 ± 0 (1 – 1)	1 ± 0 (1 – 1)	1 ± 0 (1 – 1)	19 ± 4.3 (5 – 35)	1 ± 0 (1 – 1)
% Infectivity[2] (range)	110	46 ± 5 (36 – 51)	95 ± 14 (71 – 140)	95 ± 25 (70 – 120)	123 ± 2 (121 – 124)	39 ± 5 (20 – 62)	68 ± 32 (36 – 99)
	T66I/ S153Y (n=1)	**T66I/ M154I (n=4)**	**F121Y/ T125K (n=4)**	**S153Y/ N155S (n=2)**	**T66I/ L74M/ V151I (n=4)**	**T125K/ S153Y/ N155S (n=2)**	•
FC[1] (range)	1	1 ± 0 (1 – 1)	7 ± 2.3 (4 – 14)	3 ± 0 (3 – 3)	5 ± 0.6 (4 – 6)	2.5 ± 0.5 (2 – 3)	•
% Infectivity[2] (range)	21	77 ± 16 (42 – 107)	46 ± 9 (21 – 63)	15 ± 0 (15 – 15)	66 ± 8 (43 – 78)	14 ± 1 (13 – 14)	•

[1]FC: average fold change in CIC_{50} values ± standard error of the mean (SEM)
[2]% Infectivity: average % infectivity compared to wild-type virus infectivity ± standard error of the mean (SEM)

4. Clinical Microbiology

4.1. Antiviral Efficacy

The antiviral efficacy analyses were conducted to determine whether raltegravir 400 mg BID treatment would provide superior antiretroviral activity compared to placebo, both in combination with OBT, in treatment-experienced adult patients who failed antiretroviral therapies with triple-class (NNRTI, NRTI, and PI) antiretroviral drug resistance.

The primary efficacy endpoint in the 2 Phase 3 studies (Protocols 018 and 109) was the proportion of patients achieving HIV-1 RNA <400 copies/mL at Week 16. Antiviral efficacy of raltegravir (plus OBT) was also assessed compared to placebo (plus OBT) for following secondary endpoints: (1) proportions of patients with HIV-1 RNA levels <400 copies/mL or >1.0 $\log_{10}$ reduction from baseline in HIV RNA at Week 16; (2) proportions of patients with HIV-1 RNA <50 copies/mL at Week 16; (3) change in HIV-1 RNA ($\log_{10}$ copies/mL) from baseline at Week 16; and (4) change in $CD4^+$ T cell count from baseline at Week 16. Protocols 018 and 019 are identically designed international, multi-center, double-blind, randomized, placebo-controlled trials of raltegravir (400 mg BID) with the exception of the location of the study sites: Protocol 018 was conducted in Europe, Asia/Pacific, and South America and Protocol 019 was in North and South America.

Both Week 16 data (Table 12, from Statistical Review and Evaluation by Karen Qi, Ph.D.) from all subjects in the 2 pivotal Phase 3 studies and Week 24 data (Table 13, from Statistical Review and Evaluation by Karen Qi, Ph.D.) from approximately 60% of the subjects provided evidence that the antiviral activity of raltegravir plus OBT is superior to placebo plus OBT in those patient groups. These efficacy results were supported by the 24-week analysis of the Phase 2 study (Protocol 005). Please refer to the reviews by Medical Officer Sarah Connelly, M.D. and Statistician Karen Qi, Ph.D. for a detailed analysis of the efficacy of raltegravir. Protocol 005 was a multicenter, double-blind, randomized, dose-ranging, placebo-controlled Phase 2 trial to evaluate the safety,

pharmacokinetics, and efficacy of raltegravir BID at three different doses (200, 400, and 600 mg) compared to placebo, both in combination with OBT, in HIV-1-infected patients with documented triple-class antiretroviral drug resistance.

Table 12: Key Efficacy Endpoints at Week 16

Outcomes at Week 16	Protocol 018		Protocol 019		Overall	
	Raltegravir (N=232)	Placebo (N=118)	Raltegravir (N=230)	Placebo (N=119)	Raltegravir (N=462)	Placebo (N=237)
Patients with HIV-1 RNA <400 copies/mL, n (%)	179 (77)	49 (42)	180 (78)	51 (43)	356 (77)	100 (42)
Patients with HIV-1 RNA <50 copies/mL, n (%)	146 (63)	40 (34)	143 (62)	43 (36)	289 (63)	83 (35)
Patients with HIV-1 RNA >1 log_{10} drop or <400 copies/mL, n (%)	198 (85)	50 (42)	193 (84)	61 (51)	391 (85)	111 (47)
Mean (SD) change from baseline in HIV-1 RNA (log_{10} copies/mL)	-2.3 (1.1)	-1.0 (1.3)	-2.4 (1.2)	-1.3 (1.3)	-2.3 (1.2)	-1.2 (1.3)
Mean (SD) change from baseline in $CD4^+$ T cell count (cells/mm^3)	81 (94)	32 (73)	84 (96)	39 (74)	82 (95)	36 (74)
Virologic failure (confirmed), n (%) Nonresponse Rebound	34 (15) 5 (2) 29 (13)	63 (53) 44 (37) 19 (16)	38 (17) 9 (4) 29 (13)	57 (48) 33 (28) 24 (20)	72 (16) 14 (3) 58 (13)	120 (50) 77 (32) 43 (18)

Source: Statistical Review and Evaluation by Karen Qi, Ph.D., Table 13

Table 13: Key Efficacy Endpoints at Week 24

Outcomes at Week 24 responders / evaluable (%)	Protocol 018		Protocol 019		Overall	
	Raltegravir (N=232)	Placebo (N=118)	Raltegravir (N=230)	Placebo (N=119)	Raltegravir (N=462)	Placebo (N=237)
Patients who had Week 24 data	158/232 (68)	81/118 (69)	128/230 (56)	69/119 (58)	286/462 (62)	150/237 (63)
Patients with HIV-1 RNA <400 copies/mL, n (%)	120/158 (76)	33/81 (41)	97/128 (76)	27/69 (39)	217/286 (76)	60/150 (40)
Patients with HIV-1 RNA <50 copies/mL, n (%)	95/158 (60)	28/81 (35)	83/128 (65)	23/69 (33)	178/286 (62)	51/150 (34)
Patients with HIV-1 RNA >1 log_{10} drop or <400 copies/mL, n (%)	129/158 (82)	38/81 (47)	104/128 (81)	30/69 (43)	233/286 (81)	68/150 (45)
Mean (SD) change from baseline in HIV-1 RNA (log_{10} copies/mL)	-2.2 (1.2)	-1.1 (1.3)	-2.4 (1.3)	-1.1 (1.4)	-2.3 (1.3)	-1.1 (1.3)
Mean (SD) change from baseline in $CD4^+$ T cell count (cells/mm^3)	83 (98)	33 (71)	92 (98)	39 (71)	87 (98)	36 (71)
Virologic failure (confirmed), n (%) Nonresponse Rebound	36/232 (15) 5/232 (2) 31/232 (13)	63/118 (53) 44/118 (37) 19/118 (16)	40/230 (17) 9/230 (4) 31/230 (13)	58/119 (49) 33/119 (28) 25/119 (21)	76/462 (16) 14/462 (3) 62/462 (13)	121/237 (51) 77/237 (32) 44/237 (19)

Source: Statistical Review and Evaluation by Karen Qi, Ph.D., Table 14

The rates of virologic failure at Weeks 16 and 24 were lower for raltegravir plus OBT recipients, compared to those for placebo plus OBT recipients, 16% versus 50%, and 16% versus 51%, respectively (Tables 12 and 13, respectively). Interestingly, treatment failure of the raltegravir recipients was largely due to treatment-emergent virologic rebound (80.6% [58/72] at Week 16; 81.6% [62/76] at Week 24), rather than due to the suboptimal suppression of HIV-1 replication (nonresponse to the treatment). In contrast, in placebo recipients, virologic nonresponse to OBT appears to be a primary cause of the treatment failure, the virologic nonresponse rates (64.2% [77/120] at Week 16; 63.6% [77/121] at Week 24) being higher than the rebound rates. These results suggest that raltegravir-containing regimens can potently suppress HIV-1 replication but such response may not be durable (see Section 4.2. Clinical Resistance Analyses for details).

Of note, virologic failure was defined as non-response where a <1.0 $\log_{10}$ HIV-1 RNA reduction and >400 copies/mL HIV-1 RNA was achieved by Week 16, or viral rebound at Week 16, which was defined as: (1) HIV-1 RNA >400 copies/mL on 2 consecutive measurements at least 1 week apart after initial response with HIV-1 RNA <400 copies/mL, or (2) >1.0 $\log_{10}$ increase in HIV-1 RNA above nadir level on 2 consecutive measurements at least 1 week apart.

4.2. Clinical Resistance Analyses

Genotypic resistance analyses were performed, in order to identify treatment-emergent genotypic changes (mutations) in HIV-1 IN while on raltegravir therapy, with the paired IN amino acid sequences of the screen and on-treatment virologic failure isolates from Protocols 005, 018, and 019. HIV-1 IN genotypic data were planned to be collected from all patients displaying virologic failure with plasma HIV-1 RNA >500 copies/mL. In this review, paired genotypic data from 77 evaluable patients with evidence of virologic failure to raltegravir (+ OBT) treatment in 3 different protocols were analyzed (Table 14): 27 from Protocols 018 and 019; 35 from Protocol 005 during the double-blind phase; and 15 from Protocol 005 during the open-label extension phase.

For Protocols 018 and 019, paired genotypic data of 27 evaluable patients, out of the 72 patients who showed evidence of virologic failure to raltegravir (+ OBT) treatment (Table 12), were available by genotyping cut-off date November 15, 2006 to be analyzed (additional genotypic data will be submitted in the 48-week clinical study reports).

In Protocol 005 during the double-blind phase, out of the 133 total patients treated with raltegravir plus OBT, virologic failure was observed in 38 patients (28.6%), and paired genotypic data from 35 of those were available for the genotypic resistance analysis. In addition, 15 paired genotypic data were also available from patients who failed to raltegravir treatment during the open-label extension phase of Protocol 005.

Table 14: Number of Evaluable Patients Whose IN Genotypic Data Were Analyzed

	Raltegravir + OBT Treatment			Placebo + OBT Treatment		
Genotypic Data	Paired[1]	Failure only[2]	Screen only[3]	Paired[1]	Failure only[2]	Screen only[3]
018 + 019	**27**	**13**	**7**	**0**	**0**	**0**
018	12	8	3	0	0	0
019	15	5	4	0	0	0
005 (DB[4] and Open-label)	**50**	**0**	**2**	**12**	**2**	**2**
DB phase (subtotal)	35	0	2	12	2	2
200 mg	11	0	0	-	-	-
400 mg	13	0	2	-	-	-
600 mg	11	0	0	-	-	-
Open-label phase (subtotal)	15	0	0	-	-	-
Switched from 200 mg	1	0	0	-	-	-
Switched from 400 mg	3	0	0	-	-	-
Switched from Placebo	11	0	0	-	-	-
Total	**77**	**13**	**9**	**12**	**2**	**2**
Subtype B	74	11	8	12	2	1
Nonsubtype B	3	2	1	0	0	1

Nonresponse	9	2	-	7	1	-
Rebound	68	9	-	5	1	-

[1]Paired, amino acid sequences of the HIV-1 IN protein available from the screen and on-treatment virologic failure isolates
[2]Failure only, amino acid sequences of the HIV-1 IN protein available only from the on-treatment virologic failure isolates
[3]Screen only, amino acid sequences of the HIV-1 IN protein available only from the screen isolates
[4]DB, Double-Blind phase in Protocol 005

4.2.1. Baseline Polymorphism of the HIV-1 IN protein

The HIV-1 IN protein is well conserved across HIV-1 groups and subtypes exhibiting 96% and 94% identity within and between group M (main) subtypes, respectively (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. Further analyses of 2081 HIV-1 group M IN sequences from 1744 IN inhibitor-naïve persons by Zioni *et al.* (2007) showed that 162 amino acids (56.3%) of the 288 codons in HIV-1 IN were polymorphic. The authors reported that all catalytic residues were nonpolymorphic and extended active residues were conserved with low rates of conserved polymorphism at positions I141V (1.6%), V151I (1.7%), N155H (1.5%), and K156N/Q/H (2.3/1.2/1.1%).

The N155H substitution as naturally occurring polymorphism is particularly interesting, since it was shown to be associated with raltegravir resistance. The N155H substitution was frequently observed in viruses isolated from patients experiencing virologic failure to raltegravir treatment (see Section 4.2.2. Raltegravir Treatment-Emergent Amino Acid Substitutions in HIV-1 IN for details) and reduced susceptibility to raltegravir in cell culture (13.2-fold, Table 8).

Recently, Lataillade *et al.* (2007) also 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 IN inhibitors were shown to occur as naturally occurring polymorphism in the HIV-1 IN domain: V72I, L74I, T97A, T112I, A128T, E138K, Q148H, V151I, S153Y/A, M154I, N155H, K156N, E157Q, G163R, V165I, V201I, I203M, T206S, S230N, and R263K. As with the N155H mutation, the Q148H mutation was also frequently observed in viruses isolated from patients with virologic failure to raltegravir treatment (see Section 4.2.2. Raltegravir Treatment-Emergent Amino Acid Substitutions in HIV-1 IN for details) and reduced susceptibility to raltegravir in cell culture (24-fold, Table 8).

These results suggested that IN polymorphism may affect clinical responses to HIV-1 IN inhibitors in patients. Thus, genotypic data of the viruses collected at Baseline (available from 104 patients in Protocols 005, 018, and 019) were analyzed to determine the occurrence of IN polymorphism in these study patient population.

In agreement with previous findings (Lataillade *et al.*, 2007; Zioni *et al.*, 2007), 56.3% (162/288) of the 288 codons in the IN protein were polymorphic, and 15 amino substitutions that have been previously reported to be associated with resistance to HIV-1 IN inhibitors (listed by Lataillade *et al.*, 2007) occurred as naturally occurring polymorphism in these patient population: V72I, T97A, T112I, A128T, Y143C/H/R, V151I, M154I, K156N, E157Q, V165I, V201I, I203M, T206S, S230N/R, and R263K. In contrast, 17 amino acid substitutions also associated with IN inhibitor resistance (H51Y,

T66I, L74M/R, E92Q, F121Y, T125K, E138A/K, G140A/S, Q146K, S147G, Q148H/K/R, S153A/Y, N155H/S, K160D, G163R, V249I, and Ç280Y) were not detected in the viruses collected at Baseline. Of note, 3 primary mutations, Y143C/H/R, Q148H/K/R, and N155H, that were identified to be involved independently in emergence of raltegravir resistance are highlighted in yellow, and secondary substitutions that were frequently detected with the primary mutations in the viruses of patients with virologic failure to raltegravir treatment in Protocol 005, 018, and 019 are underlined (see below Section 4.2.2. Raltegravir Treatment-Emergent Amino Acid Substitutions in HIV-1 IN for details).

Out of 104 patients with baseline genotypic data, there was only 1 patient who had virus harboring one of the primary substitutions at Baseline. Patient 3285 in Protocol 005 had the Y143H substitution in virus collected at Screen and was treated with placebo plus OBT in the double-blind phase. Interestingly, the Y143H substitution became undetectable in the failure isolates at Day 116.

In 26 patients, 3 raltegravir secondary substitutions (T97A, V151I, and S230N/R) were detected as naturally occurring polymorphism at Baseline. Out of the 26 patients, 21 were treated with raltegravir (+ OBT), while 5 were treated with placebo (+ OBT). In the raltegravir-treated 21 patients, 12 patients (54.5%) achieved HIV-1 RNA <400 copies/mL at Week 16, comparable to 59.1% (39/66) of the patients without the raltegravir secondary substitutions in the baseline viruses and treated with raltegravir. Thus, these 3 raltegravir secondary substitutions as naturally occurring polymorphism at Baseline are unlikely to contribute significantly to reduced raltegravir potency. However, it is unclear whether these substitution-harboring viruses may affect durability, i.e. predispose the viruses to more rapidly acquire drug resistance upon longer treatment. The genotypic data currently provided in this NDA are not sufficient to study this possibility.

4.2.2. Raltegravir Treatment-Emergent Amino Acid Substitutions in HIV-1 IN

The viral amino acid sequences present in virologic failure isolates were compared to the sequences present at Screen to identify treatment-emergent genotypic changes (mutations) in the IN-coding region of the HIV-1 polymerase gene. This analysis employed direct nucleotide sequence analysis of the complete HIV-1 IN domain (288 amino acids) in PCR fragments that were amplified from the plasma by RT-PCR.

When a total of 77 paired baseline and on-treatment samples from patients who showed evidence of virologic failure to raltegravir treatment were compared, amino acid substitutions emerged in the HIV-1 IN from the viruses of 75 patients (97.4%). No treatment-emergent genotypic changes were found from samples of 2 patients (Patient IDs 3299 and 15062). Viruses from these 2 patients harbored polymorphic substitution(s) in the HIV-1 IN at Baseline (Table 15, underlined) that were reported to be associated with resistance to HIV-1 IN inhibitors (Lataillade *et al.*, 2007): A128T (associated with S-1360 resistance), V151I (associated with L870,810 resistance), and V201I (associated with S-1360 resistance). Interestingly, both patients experienced virologic nonresponse by Week 16. It is not clear whether these polymorphic substitution(s) in IN at Baseline contributed to virologic failure to raltegravir-containing regimens.

Table 15: Baseline Polymorphism in HIV-1 IN of 2 Patients with No Genetic Changes Developing on Raltegravir

Patient ID	Raltegravir	HIV-1 subtype	GSS/PSS[1]	HIV-1 RNA (log_{10} copies/mL)			Baseline Polymorphism
				Baseline	Week 16 (change)	Day Isolated	
3299	200 mg	B	0/0	4.96	4.88 (-0.1)	5.21 (Day 84)	H16Y, D25E, K42N, L101I, K103R, S119G, T122I, T124A, V151I, V201I
15062	400 mg	B	2/2	5.21	5.53 (0.31)	5.53 (Day 129)	S17N, T125M, A128T, G163E, I208L, K211Q, E212A, I220L

[1]GSS/PSS, genotypic sensitivity score/phenotypic sensitivity score

As summarized in Table 16, the actual number of amino acid substitutions emerged in HIV-1 IN while on raltegravir per patient ranged from 0 to 9 (median 4) with the average number of genetic changes per patient of 3.9 ± 2.1. No apparent differences in the average number of changes per patient were observed at different drug concentrations tested in Protocol 5 (Table 16). It appears that viruses isolated in the double-blind phase of Protocol 005 harbored slightly more genetic changes compared to those in Protocols 018 and 019, 4.1 ± 1.8 (median 4) versus 3.3 ± 2.0 (median 3). It is possible that viruses may accumulate additional substitutions in HIV-1 IN over time. The viruses isolated in Protocols 018 and 019 had been on raltegravir treatment for an average of 79.7 ± 34.7 (ranging from 26 to 129 days) days, whereas the viruses in the double-blind phase of Protocol 5 had been on raltegravir treatment for an average 148.7 ± 51.8 (ranging from 84 to 295 days) study days.

Table 16: Raltegravir Treatment-Emergent Genotypic Changes in HIV-1 IN

Protocol	Number of genetic changes per person (range, median)	Total number of genetic changes	Study days (range)	Codons changed in 3 or More Patients
Overall (n=77)	3.9 ± 2.1 (0 – 9, 4)	94	151.6 ± 87.3 (26 - 356)	See Appendix 1
018 + 019 (n=27)	3.3 ± 2.0 (0 – 8, 3)	48	79.7 ± 34.7 (26 - 129)	92, 140, 143, 148, 151, 155, 230,
018 (n=12)	3.8 ± 2.3 (1 – 8, 3.5)	30	71.7 ± 34.8 (26 - 127)	92, 143, 155
019 (n=15)	2.8 ± 1.7 (0 – 7, 3)	29	86.1 ± 34.5 (29 - 129)	92, 140, 148, 155
005 (DB[1] and Open-label; n=50)	4.3 ± 2.0 (0 – 9, 4)	74	190.5 ± 82.3 (64 - 356)	31, 74, 81, 97, 138, 140, 148, 151, 155, 162, 163, 183, 224, 226, 230, 232, 255
DB phase (subtotal n=35)	4.1 ± 1.8 (0 – 7, 4)	56	148.8 ± 51.8 (84 - 295)	31, 97, 138, 140, 148, 151, 155, 162, 183, 224, 226, 230, 232
200 mg (n=11)	4.2 ± 2.0 (0 – 7, 5)	29	143.6 ± 60.2 (84 - 295)	140, 148, 155, 224
400 mg (n=13)	4.5 ± 1.7 (2 – 7, 5)	34	151.2 ± 46.2 (109 - 249)	140, 148, 155, 232
600 mg (n=11)	3.5 ± 1.8 (1 – 7, 3)	19	151.2 ± 5.8 (112 - 239)	31, 140, 148, 155, 183
Open-label phase (subtotal n=15)	4.7 ± 2.3 (1 – 9, 5)	36	287.7 ± 52.3 (167 - 356)	140, 148, 151, 155, 183, 230, 232

[1]DB, double-blind phase in Protocol 005

A total 94 codons (32.6% of the 288 codons in the HIV-1 IN domain) were found to be mutated in the viruses and most (72 codons) mutated only once or twice (see Appendix 1 for details). Some of these less frequent ones may be clinically significant but the low number of occurrences preclude making definitive conclusions. Without drug selection

pressure, amino acid substitutions in HIV-1 IN appeared to occur less frequently with the average number of changes per patient of 1.4 ± 2.1 (ranging from 0 to 7), when 12 paired data from virologic failure patients treated with placebo plus OBT in Protocol 005 were analyzed (Appendix 1). Only 16 codons (5.6% of the codons in the HIV-1 IN domain) were mutated and all but one (codon 260) were mutated once (see Appendix 1): substitutions at codon 260 were observed in 2 patients.

Table 17 shows the 16 codons, out of the 94 codons mutated in the viruses of patients with virologic failure to raltegravir treatment (Appendix 1), that were changed in 4 or more patients. The 3 most frequently observed changes were at codons 140, 148, and 155 (27, 34, and 29 occurrences each). These codons are highly conserved among HIV-1 isolates and located near the catalytic site (D64, D116, and E152) in the central core domain of HIV-1 IN. According to the IN genotypic analysis results of 243 HIV-1 subtype B, IN inhibitor-naïve clinical isolates by Lataillade *et al.* (2007), no polymorphic substitution was observed at G140, while low rates of polymorphism were observed at Q148H (0.4%) and N155H/D (0.8%). Furthermore, it was noted that these residues were nonpolymorphic in the baseline viruses collected from the 104 patients in Protocols 005, 018, and 019 as described in Section 4.2.1. Baseline Polymorphism of the HIV-1 IN protein. The amino acid substitutions at these codons have already been associated with both genotypic and phenotypic resistance to raltegravir (Tables 8 and 9). It was implied that the mutations at Q148 and N155 codons are involved independently in emergence of raltegravir resistance. It should be noted that the 2 mutations at codons 148 and 155 were observed frequently in all protocols and at different raltegravir concentrations (Tables 16 and 17). Detailed analyses of these mutations are described in Section 4.2.3. Key Mutations Associated with Resistance to Raltegravir.

Table 17: Codons Changed in 4 or More Patients with Virologic Failure to Raltegravir Treatment in Paired Sequences

Protocol	Overall	Phase 3 (Protocols 018 & 019)			Phase 2 (Protocol 005)					
		018 + 019 Total	018	019	005 Total	DB[1]				OL[2]
						DB[1] Total	200 mg	400 mg	600 mg	
Number of Patients	77	27	12	15	50	35	11	13	11	15
Number of genetic changes	94	48	30	29	74	56	29	34	19	36
Codon	Number of Occurrences									
31	6	1	1		5	5		2	3	
74	5	1		1	4	2		2		2
92	9	7	4	3	2	2		1	1	
97	8	2	2		6	4	2	1	1	2
138	5	1		1	4	3		2	1	1
140	27	4	1	3	23	16	4	6	6	7
143	6	4	3	1	2	2	1	1		
148	34	7	2	5	27	19	4	8	7	8
151	9	3	2	1	6	3	2	1		3
155	29	11	5	6	18	13	5	5	3	5
163	6	2	1	1	4	2	2			2
183	10				10	7	2	2	3	3
224	7	1	1		6	4	3	1		2

226	5				5	3	1		2	2
230	9	3	2	1	6	3	1	1	1	3
232	14	2	2		10	7	2	4	1	5

[1]'DB' double-blind phase in Protocol 005
[2]OL, open-label phase in Protocol 005

In addition to the 3 most frequent substitutions at amino acid positions 140, 148, and 155, substitutions at 13 amino acid positions, V31, L74, E92, T97, E138, Y143, V151, G163, H183, R224, Y226, S230, and D232, were observed in 4 or more patients (Table 17). Substitutions of these amino acids with the exception of V31, H183, R224, Y226, and D232 have been reported to be associated with resistance to HIV-1 IN inhibitors (Lataillade *et al.*, 2007; Table 18). The V31I and R224G/W substitutions were frequently found in the viruses at Baseline (39.4% and 8.7%; Table 18), thus these mutations detected in the viruses of patients with virologic failure are unlikely associated with the emergence of viral resistance to raltegravir. In contrast, the H183P substitution was more frequently found in treatment-emergent viruses than in Baseline viruses, 13% versus 7.7% (Table 18). Amino acid residues Y226 and D232 appeared to be polymorphic, Y226C and D232E being detected in 5.8% and 4.8% of the 104 patients with baseline genotypic data. However, Y226D/F/H and D232N substitutions were not present in the baseline viruses, suggesting that these substitutions are nonpolymorphic changes. Based on these observation, in addition to the 3 most frequent substitutions (G140A/S, Q148H/R/R, and N155H), 11 additional substitutions (L74M/R, E92Q, T97A, E138A/K, Y143C/H/P/R, V151I, G163R, H183P, Y226D/F/H, S230R, and D232N) were further analyzed in Section 4.2.3. Key Mutations Associated with Resistance to Raltegravir.

Table 18: Summary of Amino Acid Substitutions Frequently Found in Patients with Virologic Failure to Raltegravir Treatment in Paired Sequences

Codon	Polymorphic substitutions at Baseline		Substitutions developing on raltegravir		Raltegravir resistance in cell culture (FC[1])	Associated with IN inhibitor resistance (Lataillade *et al.*, 2007)
	Occurrence [%]	Substitutions	Occurrence [%]	Substitutions		
V31	41 [39.4%]	V31I	6 [7.8%]	V31I		
L74	2 [1.9%]	L74I	5 [6.5%]	L74M/R		L74M/R
E92	0	-	9 [11.7%]	E92Q	3	E92Q
T97	1 [1.0%]	T97A	8 [10.4%]	T97A		T97A
E138	0	-	5 [6.5%]	E138A/K	1	E138A/K
G140	0	-	27 [35.1%]	G140A/S	2 - 3	G140A/S
Y143	1 [1.0%]	Y143H	6 [7.8%]	Y143C/H/P/R		Y143C/H/R
Q148	0	-	34 [44.2%]	Q148H/K/R	24 - 46	Q148H/K/R
V151	5 [4.8%]	V151I	9 [11.7%]	V151I		V151I
N155	0	-	29 [37.7%]	N155H	13	N155H/S
G163	4 [3.9%]	G163E	6 [7.8%]	G163K/R		G163R
H183	8 [7.7%]	H183P	10 [13%]	H183P/L		
R224	9 [8.7%]	R224G/W	7 [9.1%]	R224G/W		
Y226	6 [5.8%]	Y226C	5 [6.5%]	Y226C/D/F/H		
S230	21 [20.2%]	S230N	9 [11.7%]	S230N/R	2 ± 0	S230N/R
D232	5 [4.8%]	D232E	14 [18.2%]	D232E/N		

[1]FC: average fold change in CIC_{50} values ± standard error of the mean

4.2.3. Key Amino Acid Substitutions Associated with Resistance to Raltegravir

Q148H/R and N155H were previously identified as primary substitutions which emerged in the on-treatment virologic failure viruses of 15 subjects in the 16-week interim analysis of Phase 2 Protocol 005 (IND 69928, SN 254). One or both of these were detected in 60 (77.9%) patients out of the 77 evaluable patients with the paired genotypic data in Protocol 005, 018 and 019. The other 11 substitutions that were frequently found (≥4 occurrences) in the 77 paired genotypic analyses, with the exception of Y143C/H/P/R, were also found in the same virus population harboring the Q148H/K/R and N155H mutations; L74M/R (60% of the occurrences), E92Q (88.9%), T97A (87.5%), E138A/K (100%), G140A/S (100%), V151I (100%), G163K/R (83.3%), H183P (40%), Y226C/D/F/H (100%), S230N/R (88.9%), and D232E/D (85.7%). Thus, these observations suggested that these 11 substitutions may not emerge independently to the primary Q148H/R and N155H mutations. Phenotypic data (Table 8) supported this implication by showing that some substitutions, such as E92Q, E138A, and G140A/S, were added to the Q148H/K/R and N155H mutations to enhance raltegravir resistance. Single-genome nucleotide sequence analyses of these viruses could determine whether these secondary substitutions are developed on raltegravir in combination with the 2 primary mutations in the same viral genome. It is recommended to evaluate the phenotypic significance of these secondary substitutions including L74M/R, T97A, V151I, G163R, H183P, Y226D/F/H, S230R, and D232N and their contributions to raltegravir resistance in cell culture.

Substitutions of Q148 with basic amino acid residues, arginine (R), histidine (H), or lysine (K) were most frequently noted, occurring in 34 patients (34/77 [44.2%]). The Q148H/K/R substitution was always accompanied by one or more of the 10 secondary substitutions. G163R was not detected. Interestingly, the E138A/K and G140A/S substitutions were exclusively found in the Q148H/K/R mutation-harboring virus population. In cell culture (Table 8), the Q148H/K/R conferred 24- to 46-fold reduced susceptibility to raltegravir and the addition of E138A or G140A/S to Q148 variants substantially increased resistance to 90-fold (E138A/Q148K), 257-fold (G140A/Q148K), 521-fold (G140S/Q148H), and 405-fold (G140S/Q148R). E138A, G140A, and G140S alone conferred undetectable, 3-fold reduced, and 2-fold reduced susceptibility, respectively. Thus, it supported clinical observation that the Q148H/K/R substitution is a primary contributor to resistance to raltegravir, while the E138A/K and G140A/S substitutions play a secondary role in augmenting resistance. The G140A/S + Q148H/K/R double mutation was most frequently observed (27/77 [35.1%]).

The other primary mutation, N155H, was detected in 29 samples out of the 77 evaluable paired ones (37.7%) and the 10 secondary mutations were also observed in 25 samples: G140A/S was not detected. Of note, E138K was detected in 1 patient but is likely to accompany the Q148K mutation, since the virus population harbored the 2 primary mutations, Q148K and N155H. The E92Q (7 patients) and V151I (7 patients) substitutions were most frequently noted in the N155H-containing virus population. In cell culture (Table 8), the N155H conferred 13-fold reduced susceptibility to raltegravir. Addition of E92Q, which by itself conferred 3-fold reduced susceptibility, increased resistance to 64-fold. Thus, the N155H mutation alone could confer significant resistance to raltegravir, which could be enhanced with the additional E92Q substitution.

Additional virologic failure genotypic data were submitted in the absence of the baseline

data for 13 patients treated with raltegravir in Protocols 018 and 019. Out of the 13, 8 patients (61.5%) had viruses harboring one or both of the 2 primary substitutions, Q148R and N155H. The Q148R substitution-containing virus population also had the secondary substitutions, E138K, G140S, and D232N, while the secondary substitutions, L74M, E92Q, T97A, and S230N, were found in the N155H mutation-containing virus population. It should be noted that no primary substitutions were detected in 14 placebo (+ OBT)-treated virologic failure isolates.

The Y143C/H/P/R substitution was found in 6 patients, and only 1 patient had HIV-1 population harboring both the Y143H and N155H mutations. Thus, the Y143C/H/P/R mutation is likely to emerge independently as a separate pathway to raltegravir resistance. Y143 is well conserved among mammalian retroviral INs, although nonconservative amino acid substitutions occur naturally in some INs (Engelman and Craigie, 1992; Johnson *et al.*, 1986). However, this residue appears to be nonpolymorpic in HIV-1 (Lataillade *et al.*, 2007; Zioni *et al.*, 2007). No phenotypic data of Y143C/H/R against raltegravir are available for this review. In the virus population harboring the Y143C/H/R substitutions, the L74M, E92Q, T97A, G163R, and S230R secondary substitutions were also found. Current genotypic data obtained by population sequencing could not determine whether these substitutions are developed on raltegravir in combination with Y143C/H/R in the same virus genome. It is recommended to determine the susceptibility in cell culture of HIV-1 harboring Y143C/H/R, individually and in combination with L74M, E92Q, T97A, G163R, and S230R in a common genetic background.

Together, by analyzing 77 evaluable paired genotypic data from patients with virologic failure to raltegravir treatment, 3 primary substitutions, Y143C/H/R, Q148H/K/R, and N155H, were identified to emerge independently in HIV-1 IN. Each of the 3 primary substitutions was usually accompanied by one or more of the secondary substitutions, L74M/R, E92Q, T97A, E138A/K, G140A/S, V151I, G163R, H183P, Y226C/D/F/H, S230N/R, and D232N. The primary substitutions developed in 65 patients (84.4%) of the 77 from all 3 Protocols, although Y143C/H/R was more frequently found in Protocols 018 and 019 than in Protocol 005 (Table 19). This uneven occurrences of the Y143C/H/R mutation may not be statistically significant, since the sample size is rather small (n=6). No apparent relationships exist between development of the 3 primary substitutions and drug concentrations (Table 19).

Table 19: Frequencies of Primary Substitutions in Patients with Virologic Failure to Raltegravir Treatment in Paired Sequences

Protocol	Number of patients[1]	No genetic changes (%)	Genetic Changes			
			Y143C/H/R (%)	Q148H/K/R (%)	N155H (%)	Others (%)
018 and 019	27	1 (3.7)	20 (74.1)			6 (22.2)
			4 (14.8)	7[2] (25.9)	11[2] (40.7)	
005 in DB period	35	1 (2.9)	33 (94.3)			1 (2.9)
			2[3] (5.7)	19 (54.3)	13[3] (37.1)	
200 mg	11	1	1	4	5	0
400 mg	13	0	1[3]	8	5[3]	0
600 mg	11	0	0	7	3	1

005 in open label period[4]	15	0 (0)	0 (0)	8[5] (50.0)	5[5] (30.0)	3 (20.0)
Overall	77	2 (2.6)	65 (84.4)			10 (13.0)
			6 (7.8)	34 (44.2)	29 (37.7)	

[1]Data available in paired sequences
[2]Both Q148K/R and N155H substitutions were observed in viruses from 2 patients.
[3]Both Y143H and N155H substitutions were observed in viruses from 1 patient.
[4]Patients who failed to Raltegravir treatment during the open-label period
[5]Both Q148R and N155H substitutions were observed in viruses from 1 patient.

The primary substitutions could be detected at early virologic failure isolates, as early as in Day 26 isolates. In Protocols 018 and 019, 5 patients had virologic rebound within 30 days from start of raltegravir (+ OBT) therapy (Table 20). All of these patients were infected with HIV-1 subtype B. All patients except 15047 had a PSS ≤1 and had neither enfuvirtide nor darunavir in their OBT. The 2 raltegravir-resistant primary mutations, Q148H/K/R and N155H, were present in the rebound virus population of these 4 patients. Thus, raltegravir suppression may not be durable in the absence of another fully active agent. Patient 15047 had a PSS ≥3 and had both enfuvirtide (prior use) and darunavir (naïve) in his OBT. No primary substitutions were noted in the rebound virus isolates.

Table 20: Genotypic Changes in Patients with Early Virologic Rebound (within 30 Days from Start of Raltegravir plus OBT Therapy)

Patient ID	Day Isolated	GSS/PSS[1]	HIV-1 RNA (log_{10} copies/mL)		Genotypic changes in HIV-1 IN developing on raltegravir
			Baseline	Week 16 (change)	
8236	29	0/1	5.47		G140S/G, Q148Q/H/R, A169A/G, D279N/D
8256	26	0/0	5.8		D41N/D, Q148K/Q/R, F223I/F, R224R/G, S230N/S
8274	28	1/0	4.68		T111I, N155H, V201I
15047	29	1/3[2]	4.66		S24N/S, A179A/T, W243W/C
16279	30	0/1	5.27		G140G/S, Q148H, V260V/G, R269R/M/W/L

[1]GSS/PSS, genotypic sensitivity score/phenotypic sensitivity score
[2]3, PSS ≥3

4.2.4. Raltegravir Treatment-Emergent Amino Acid Substitutions in HIV-1 IN in the Absence of the 3 Primary mutations, Y143C/H/R, Q148H/K/R, and N155H

Of the 77 patients with virologic failure to raltegravir treatment whose paired baseline and on-treatment genotypes were submitted with this application, the primary IN substitutions, Y143C/H/R, Q148H/K/R, and N155H were not detected in the viruses from 12 patients (15.6%; 2 patients with no treatment-emergent genetic changes described above (Table15) and 10 patients with genetic changes). Table 21 summarized IN substitutions found in the virologic failure isolates of those 10 patients. All but 1 (Patient ID 16323) patients experienced virologic rebound: Patient 16323 failed to achieve virologic suppression (nonresponder). A total of 19 codons (6.6% of the 288 codons in the HIV-1 IN domain) were found to be mutated in the viruses of the 10 patients and all were only mutated once or twice, unlikely to be of any statistical significance. Interestingly, the IN secondary mutations (highlighted in yellow) were detected in 3 patients, all of whom were treated with raltegravir in the open-label phase of Protocol

005.

Table 21: Amino Acid Substitutions Found in Patients (n=10) without the 3 Primary IN Mutations, Y143C/H/R, Q148H/K/R, and N155H, in Paired Sequences

Patient ID	Protocol	GSS/PSS[1]	HIV-1 subtype	HIV-1 RNA (log_{10} copies/mL)		Genotypic changes in HIV-1 IN developing on raltegravir
				Baseline	Isolated (DAY)	
2982	005 open-label	0/0	B	5.79		A80T/A, S91A/S, Q95E/Q, H183H/P, Y/F227Y/D
3234	005 open-label	0/0	F1	5.51		L74I/M/L, F121N/I, V151I/V, D232N/D
3292	005 open-label	0/1	B	5.27		H183H/P
3882	005 600 mg	2/1	B	5.75		K14K/R
6404	018	3/3[2]	Complex	3.6		I113I/V
8209	018	1/2	B	5.47		L242L/F
15047	019	1/3	B	4.66		S24N/S, A179A/T, W243W/C
15605	019	3/3	B	3.97		R228R/G
16202	019	0/3	B	5		V72I/V
16323	019	0/-	B	6.63		K7K/R, R20K/R

[1]GSS/PSS, genotypic sensitivity score/phenotypic sensitivity score
[2]3, GSS ≥3; PSS ≥3

4.2.5. Summary

The findings from the raltegravir clinical resistance analyses of Protocols 005, 018, and 019 can be summarized as follows:

- 56.3% (162/288) of the 288 codons in the HIV-1 IN protein were polymorphic in the study patient population (Protocols 005, 018, and 019), based on the baseline genotypic data of 104 patients.
- Four patients, out of the 104 patients, had amino acid substitutions T97A, Y143H, V151I, and S230N/R that were observed in these studies to be associated with resistance to raltegravir.
- In the paired sequence analysis of baseline and on-treatment samples, 97.4% (75/77) of 77 evaluable patients with evidence of virologic failure had genotypic changes in the HIV-1 IN.
- Three amino acid substitutions, Y143C/H/R, Q148H/K/R, and N155H, were identified as primary substitutions which emerged independently in HIV-1 IN from the on-treatment virologic failure isolates.
- Each of the 3 primary substitutions was usually accompanied by one or more of the secondary substitutions, L74M/R, E92Q, T97A, E138A/K, G140A/S, V151I, G163R, H183P, Y226C/D/F/H, S230N/R, and D232N.
- The Q148H/K/R substitution were detected in 34 patients (34/77 [44.2%]).
- The E138A/K and G140A/S substitutions appeared tightly associated with the Q148H/K/R substitution: all patients that carry E138A/K (5 patients) or G140A/S (27 patients) were found to have the Q148H/K/R mutation.
- The N155H substitution were detected in 29 patients (29/77 [37.7%]).

- The E92Q substitution was frequently detected in the N155H-containing virus population (7/9 [77.8%]).
- The Y143C/H/P/R substitution was found in 6 patients (6/77 [7.8%]) and the L74M, E92Q, T97A, G163R, and S230R secondary substitutions were also noted in the virus population harboring the Y143C/H/P/R substitution.
- No apparent relationships were noted between development of the 3 primary substitutions and drug concentrations.
- The primary substitutions could be detected at early virologic failure isolates, as early as in Day 26 isolates.

5. Conclusion

This NDA for raltegravir is approvable with respect to microbiology for the treatment of HIV-1 infection in combination with other antiretroviral agents in treatment-experience adult patients with evidence of HIV-1 replication despite ongoing antiretroviral therapy. Raltegravir plus OBT showed superior antiviral efficacy to placebo plus OBT in studied patient population. Genotypic analysis revealed that 3 amino acid substitutions, Y143C/H/R, Q148H/K/R, and N155H, were primarily associated with virologic failure to raltegravir therapy. Each of the 3 primary substitutions was usually accompanied by one or more of the 11 secondary substitutions, L74M/R, E92Q, T97A, E138A/K, G140A/S, V151I, G163R, H183P, Y226C/D/F/H, S230N/R, and D232N.

6. Recommendations

-
- Determine the susceptibility in cell culture of HIV-1 harboring Y143C/H/R, individually and in combination with L74M, E92Q, T97A, G163R, and S230R in a common genetic background.
- Evaluate the contributions of L74M/R, T97A, V151I, G163R, H183P, Y226C/D/F/H, and S230N/R substitutions to raltegravir resistance by site-directed mutagenesis.
- Characterize phenotypically and genotypically virus selected in cell culture for resistance to raltegravir using distantly related non-clade B HIV-1 isolates.
- Perform genotypic and phenotypic analyses of HIV-1 from patients who experience virologic failure to raltegravir (plus OBT) therapy out to 48 and 96 weeks in ongoing clinical trials.
-

7. Microbiology Package Insert

Mechanism of Action

Raltegravir inhibits the catalytic activity of HIV-1 integrase, an HIV-1-encoded enzyme that is required for viral replication. Inhibition of integrase 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. Raltegravir did not significantly inhibit human phosphoryltransferases including DNA polymerases α, β, and γ.

Antiviral Activity in Cell Culture

Raltegravir at concentrations of 31 ± 20 nM resulted in 95% inhibition (EC_{95}) of viral spread (relative to an untreated virus-infected culture) in human T-lymphoid cell cultures infected with the cell-line adapted HIV-1 variant H9IIIB. In addition, raltegravir at concentrations of 6 to 50 nM resulted in 95% inhibition of viral spread in cultures of mitogen-activated human peripheral blood mononuclear cells infected with diverse, primary clinical isolates of HIV-1, including isolates resistant to reverse transcriptase inhibitors and protease inhibitors. Raltegravir also inhibited replication of an HIV-2 isolate when tested in CEMx174 cells (EC_{95} = 6 nM).

Additive to synergistic antiretroviral activity was observed when human T-lymphoid cells infected with the H9IIIB variant of HIV-1 were incubated with raltegravir in combination with non-nucleoside reverse transcriptase inhibitors (delavirdine, efavirenz, or nevirapine); nucleoside analog reverse transcriptase inhibitors (abacavir, didanosine, lamivudine, stavudine, tenofovir, zalcitabine, or zidovudine); protease inhibitors (amprenavir, atazanavir, indinavir, lopinavir, nelfinavir, ritonavir, or saquinavir); or the entry inhibitor enfuvirtide.

Resistance

The mutations observed in the HIV-1 integrase coding sequence that contributed to raltegravir resistance (evolved either in cell culture or in subjects treated with raltegravir) generally included an amino acid substitution at either Q148 (changed to H, K, or R) or N155 (changed to H) plus one or more additional substitutions (i.e., L74M/R, E92Q, T97A, E138A/K, G140A/S, V151I, G163R, H183P, Y226D/F/H, S230R, and D232N). Amino acid substitution at Y143C/H/R is another pathway to raltegravir resistance.

8. Appendices

Appendix 1

Identity and Frequency of Individual Codon Changes in Paired Sequences of Samples from Patients with Virologic Failure to Raltegravir Treatment

Protocol	Overall	Phase 3 (Protocols 018 & 019)			Phase 2 (Protocol 005)						
		018 + 019 Total	018	019	005 Total	DB[1]				OL[2]	Placebo
						DB[1] Total	200 mg	400 mg	600 mg		
Number of Patients	77	27	12	15	50	35	11	13	11	15	12
Number of genetic changes	94	48	30	29	74	56	29	34	19	36	16
Codon	Number of Occurrences										
7	2	1		1	1	1	1				
8	1	1		1							
14	2				2	2		1	1		

20	2	1		1	1	1	1				
23	1				1	1	1				
24	3	1		1	2	2	1		1		
29	1				1					1	
31	6	1	1		5	5		2	3		
32	1				1	1			1		
33	1				1	1		1			
37	1				1					1	
39	1	1	1								
41	1	1	1								
50	1				1					1	1
54	1				1	1	1				
57	1				1					1	
68	1				1	1		1			
72	2	1		1	1	1		1			
74	5	1		1	4	2		2		2	
75	1	1		1							
78	1				1					1	
80	1				1					1	
81	3				3	1			1	2	1
84	1				1	1		1			
88	2				2	2	1	1			
91	3	2	1	1	1					1	
92	9	7	4	3	2	2		1	1		
95	2				2					2	
97	8	2	2		6	4	2	1	1	2	
103	1				1	1		1			
104	1	1	1								
111	2	1	1		1					1	
112	2	1	1		1	1	1				
113	1	1	1								
118	1				1	1		1			
121	1				1					1	
122	1	1	1								
124	2	1		1	1	1			1		1
125	1				1					1	
128	1				1	1		1			
133	1				1	1	1				
135	1				1	1	1				
136	1	1		1	1	1		1			
138	5	1		1	4	3		2	1	1	
140	27	4	1	3	23	16	4	6	6	7	
142	1				1	1	1				
143	6	4	3	1	2	2	1	1			
148	34	7	2	5	27	19	4	8	7	8	
151	9	3	2	1	6	3	2	1		3	
155	29	11	5	6	18	13	5	5	3	5	
160	1				1					1	
161	2	1		1	1	1	1				
162	3				3	3		2	1		
163	6	2	1	1	4	2	2			2	
169	1	1	1								
171	1	1	1								1
178	1				1	1		1			
179	1	1		1							
181	1				1	1		1			
183	10				10	7	2	2	3	3	1
185	1				1	1		1			
189	1	1	1		1						
201	2	1	1			1	1				1
203	2	2	1	1							
206											1
212											1
213											1
216											1
218	1	1		1							
222	1	1		1							
223	2	1	1		1					1	
224	7	1	1		6	4	3	1		2	1
226	5				5	3	1		2	2	
227	2				2	1	1			1	

228	1	1		1							
229	1				1					1	
230	9	3	2	1	6	3	1	1	1	3	
231											1
232	14	2	2		10	7	2	4	1	5	1
234	2				2	1		1		1	
235	1				1					1	
239	2				2	1		1		1	
242	2	2	2								
243	1	1		1							
247	1				1	1		1			
249	1	1		1							
253	1				1					1	
255	3				3	2			2	1	
259	1				1	1	1				
260	3	2	1	1	1	1			1		2
268											1
269	1	1		1							
270	2				2	2	1	1			
272	1				1					1	
276	1				1	1		1			
279	2	2	2								
282	1				1	1	1				
283	2	1	1		1	1		1			
284											1
285	1				1	1	1				
286	1				1					1	

[1]DB, double-blind phase in Protocol 005
[2]OL, open-label phase in Protocol 005

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