

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:
21-976

MICROBIOLOGY REVIEW

**DIVISION OF ANTIVIRAL PRODUCTS
OFFICE OF NEW DRUGS
MICROBIOLOGY REVIEW**

NDA: 21-976 SN: 000 DATE REVIEW COMPLETE: 06/19/2006

Microbiology Reviewer: Lisa K. Naeger, Ph.D.

NDA#: 21-976

Serial #: 000

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

Sponsor's Name and Address: Tibotec-Virco, USA
2505 Meridian Parkway
Suite 350
Durham, NC 27713

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Amendments:

Related/Supporting Documents: IND62477

Product Name(s)

Proprietary: PREZISTA/rtv

Non-Proprietary/USAN: Darunavir/rtv; darunavir, TMC114

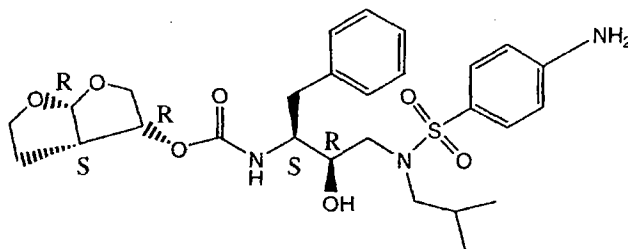
Code Name/Number:

Empirical formula: $C_{27}H_{37}N_3O_7S \cdot C_2H_5OH$

Chemical Name: {3-[(4-amino-benzenesulfonyl)-isobutyl-amino]-1-benzyl-2-hydroxy-propyl} -carbamic acidhexahydro-furo-[2,3-b]furan-3-yl ester.ethanolate

Molecular mass: Relative molecular mass: 547.656 (active moiety) + 46.068 (ethanol, EtOH) = 593.724 (TMC 114-ethanolate)

Structural Formula:



Darunavir

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Drug category: antiviral for HIV infection

Dosage Form(s): Oral; *co-administration of ritonavir as 100-mg soft gelatin capsules*

Route(s) of Administration: Oral

Indication(s): Combination antiretroviral treatment of HIV-1 infected adult subjects with evidence of viral replication who are heavily treatment-experienced or have HIV-1 strains resistant to multiple protease inhibitors.

Dispensed: Rx X **OTC**

Abbreviations: ABC, abacavir; APV, amprenavir; ATV, atazanavir; AZT, zidovudine; Control, comparator PI arm; ddI, didanosine; d4T, stavudine; DLV, delavirdine; EFV, efavirenz; FTC, emtricitabine; HAART, highly active antiretroviral therapy; HIV-1, human immunodeficiency virus-1; IC, inhibitory concentration; IDV, indinavir; LAM, lamivudine; LPV, lopinavir; NFV, nelfinavir; NVP, nevirapine; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; OBT, optimized background therapy; PBMC, peripheral blood mononuclear cells; PCR, polymerase chain reaction; PI, protease inhibitor; /rtv, ritonavir-boosted; RT, reverse transcriptase; SQV, saquinavir; ENF, enfuvirtide; TNF, tenofovir; TPV, tipranavir

EXECUTIVE SUMMARY

Darunavir is an inhibitor of the HIV-1 protease. It selectively inhibits the cleavage of HIV encoded Gag-Pol polyproteins in infected cells, thereby preventing the formation of mature virus particles. Darunavir exhibits activity against laboratory strains and clinical isolates of HIV-1 and laboratory strains of HIV-2 in acutely infected T-cell lines, human peripheral blood mononuclear cells and human monocytes/macrophages with median EC₅₀ values ranging from 1.2 to 8.5 nM (0.7 to 5.0 ng/ml). Darunavir demonstrates antiviral activity in cell culture against a broad panel of HIV-1 group M (A, B, C, D, E, F, G), and group O primary isolates with EC₅₀ values ranging from < 0.1 to 4.3 nM. The EC₅₀ value of darunavir increases by a median factor of 5.4 in the presence of human serum. Darunavir did not show antagonism when studied in combination with the protease inhibitors amprenavir, atazanavir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir, or tipranavir, the N(t)RTIs abacavir, didanosine, emtricitabine,, lamivudine, stavudine, tenofovir, zalcitabine, or zidovudine, the NNRTIs delavirdine, efavirenz , or nevirapine, and the fusion inhibitor enfuvirtide.

Resistance

Cell Culture: HIV-1 isolates with a decreased susceptibility to darunavir have been selected in cell culture and obtained from subjects treated with darunavir/ritonavir. Darunavir-resistant virus derived in cell culture from wild-type HIV had 6- to 21-fold decreased susceptibility to darunavir and harbored 3 to 6 of the following amino acid substitutions S37N/D, R41E/S/T, K55Q, K70E, A71T, T74S, V77I, or I85V in the protease. Selection in cell culture of darunavir resistant HIV-1 from nine HIV-1 strains

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harboring multiple protease inhibitor resistance-associated mutations resulted in the overall emergence of 22 mutations in the protease gene, including L10F, V11I, I13V, I15V, G16E, L23I, V32I, L33F, S37N, M46I, I47V, I50V, F53L, L63P, A71V, G73S, L76V, V82I, I84V, T91A/S, and Q92R, of which L10F, V32I, L33F, S37N, M46I, I47V, I50V, L63P, A71V, and I84V were the most prevalent. These darunavir-resistant viruses had at least eight protease mutations and exhibited 50- to 641-fold decreases in darunavir susceptibility with final EC₅₀ values ranging from 125 nM to 3461 nM

Clinical studies of darunavir/ritonavir in treatment-experienced subjects: In the Phase 2 trials Studies C202, C213 and C215, multiple protease inhibitor-resistant HIV-1 isolates from highly treatment-experienced subjects who received 600/100 mg darunavir/rtv b.i.d. and experienced virologic failure, either by rebound, or by never being suppressed, developed amino acid substitutions that were associated with a decrease in susceptibility to darunavir. The amino acid substitution V32I developed on darunavir/rtv 600/100 mg b.i.d. in greater than 30% of virologic failure isolates and substitutions at amino acid I54 developed in greater than 20% of virologic failure isolates. Other substitutions that developed in 10% to 20% of darunavir/rtv virologic failure isolates occurred at amino acid positions I15, L33, I47, G73 and L89. The median darunavir phenotype (fold change from reference) of the virologic failure isolates was 21-fold at baseline and 94-fold at failure.

Cross-resistance

Darunavir has a <10-fold decreased susceptibility in cell culture against 90% of 3309 clinical isolates resistant to amprenavir, atazanavir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir and/or tipranavir showing that viruses resistant to most protease inhibitors remain susceptible to darunavir. In Studies C202 and C213, 60% (24/40) of subjects with decreased susceptibility to tipranavir (fold change >3) at baseline demonstrated a 1 log₁₀ decrease from baseline at week 24 on darunavir/rtv and 45% (18/40) achieved <50 copies/mL serum HIV RNA levels. In Study C215, 60% (64/107) of subjects with resistance to tipranavir (>3-fold change) at baseline achieved a 1 log₁₀ decrease from baseline at week 24 on darunavir/rtv and 33% (35/107) achieved <50 copies/mL serum HIV RNA levels.

Darunavir-resistant viruses were not susceptible to amprenavir, atazanavir, indinavir, lopinavir, nelfinavir, ritonavir or saquinavir in cell culture. However, six of nine darunavir-resistant viruses selected in cell culture from protease inhibitor-resistant viruses showed a fold change in EC₅₀ values <3 for tipranavir, indicative of limited cross-resistance between darunavir and tipranavir. Of the viruses isolated from subjects experiencing virologic failure on darunavir/ritonavir 600/100 mg b.i.d., greater than 50% were still susceptible to tipranavir while less than 5% were susceptible to other protease inhibitors (amprenavir, atazanavir, indinavir, lopinavir, nelfinavir, ritonavir, or saquinavir).

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Cross-resistance between darunavir and the nucleoside/nucleotide reverse transcriptase inhibitors, the non-nucleoside reverse transcriptase inhibitors or the fusion inhibitor is unlikely because the viral targets are different.

Baseline Genotype/Phenotype and Virologic Outcome Analyses

Genotypic and/or phenotypic analysis of baseline virus may aid in determining darunavir susceptibility before initiation of 600/100 mg b.i.d darunavir/rtv therapy. As-treated analyses were conducted to evaluate the impact of specific baseline protease inhibitor resistance-associated mutations and the number of protease inhibitor resistance-associated mutations at baseline on virologic response. Both specific mutations and the number of baseline mutations as well as susceptible drugs in the optimized background regimen and enfuvirtide use affected darunavir/rtv response rates in Phase 2 Studies C202 and C213.

The presence at baseline of the mutations V32I, I47V, or I54L or M, was associated with a decreased virologic response to darunavir and decreased susceptibility to darunavir. In addition, a diminished virologic response was observed in subjects with ≥ 7 protease inhibitor resistance-associated mutations (any change at amino acid positions 30, 32, 36, 46, 47, 48, 50, 53, 54, 73, 82, 84, 88, or 90) at baseline. The response rate in all subgroups (by type and number of mutations at baseline) was generally higher in the darunavir/rtv group compared to the control group.

Baseline darunavir phenotype (shift in susceptibility relative to reference) was shown to be a predictive factor of virologic outcome. Analyses showed that response rates at Week 24 decreased when the baseline darunavir phenotype was >7 -fold. Phenotypic subgroups of 0-2, >2 -7, >7 -30 and >30 described responses rates in four tiers of 88%, 73%, 52% and 43% with a 1 \log_{10} decrease from baseline, respectively, and 60%, 47%, 24% and 19% with <50 copies/mL, respectively.

The number of susceptible drugs in the optimized background regimen and enfuvirtide use affected darunavir/rtv response rates. In Studies C202 and C213, subjects with no susceptible NRTIs at baseline had lower response rates (38% with 1 \log_{10} decrease and 13% with <50 copies/mL) than those with at least one susceptible NRTI. In addition, for subjects with baseline darunavir phenotypes of >10 in studies C202, C213 and C215, response rates were 81% (13/16) when ENF was used for the first time concomitantly with darunavir while response rates were 36% (27/74) for those who did not use ENF concomitantly.

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1. Recommendations

1.1. Recommendation and Conclusion on Approvability

This NDA for is approvable with respect to microbiology for combination antiretroviral treatment of HIV-1 infected treatment-experienced adult subjects.

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

We request the following to be submitted with traditional approval (included in the approval letter of this NDA):

- 1) Determine response rates based upon presence of specific cleavage site mutations at baseline and submit this analysis with the PREZISTA traditional approval application.
- 2) Determine the protease cleavage site mutations that occur most frequently (>10%) in virologic failure isolates and submit this analysis with the PREZISTA traditional approval application.
- 3) Determine if the most frequently occurring protease cleavage site mutations contributed to decreases in darunavir susceptibility through site-directed mutagenesis and submit this analysis with the PREZISTA traditional approval application.

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2. Summary of Microbiology Assessments

2.1 Non-clinical Microbiology

Complete study reports describing the mechanism of action, antiviral activity, in vitro combination activity, in vitro resistance selection and cross-resistance of darunavir were submitted and reviewed in this NDA. Enzymatic assays and time of addition studies confirmed that darunavir acts as a protease inhibitor. Darunavir had a K_i value of <0.09 nM when tested in enzymatic assays showing that it has comparable inhibition against HIV-1 protease as the currently approved PIs. Crystal structures for darunavir bound to WT or mutant HIV-1 protease showed that darunavir formed van der Waals interactions with protease residues L23, G49, I50, P81, V82 and I84 and interacted with residues A28, D29, D30, V32, I47 and I50.

The median EC_{50} values of darunavir against laboratory strains HIV-1^{IIIB} and HIV-1^{NL4-3} ranged from 2.3 to 6.3 nM in MTT and cell-based assays. The median EC_{50} values were 1.2 nM, 1.7 nM, and 5 nM against HIV-1^{IIIB}, HIV-1^{SF2}, and HIV^{BAL}, respectively, in PBMCs and macrophages/monocytes using a p24 assay. Darunavir demonstrated antiviral activity against HIV-2 and SIV with EC_{50} values less than 10 nM and demonstrated activity against all of the HIV-1 subtype isolates evaluated with EC_{50} values ranging from <0.1 nM to 4.28 nM. These results indicate that darunavir had antiviral activity against a broad range of virus subtypes. The CC_{50} value in MT4 cells in a 5-day MTT assay was >100 μ M giving a therapeutic index of $>26,000$. The 50% toxic concentration of darunavir on cell viability was greater than 200 μ M for the cell types tested.

The antiviral activity of darunavir was decreased by a median 52-fold and 5.4-fold by the presence of 1 mg/mL AAG and 50% human serum, respectively. No loss of activity was observed for darunavir in the presence of 45 mg/mL human serum albumin with a 1.6-fold median change in antiviral activity. The data from in vitro antiviral activity drug combination assays showed that darunavir was not antagonistic with any of the currently approved antiretroviral drugs and that synergy was observed in vitro with three PIs: amprenavir, nelfinavir and ritonavir

In vitro selection experiments were performed starting from wild-type. Replicating viruses could not be selected in the presence of darunavir at concentrations above 220 nM out to 738 days. Selection of viruses was slower with darunavir in comparison with other PIs tested at micromolar concentrations. Darunavir-resistant virus derived in cell culture from wild-type HIV had 6- to 21-fold decreased susceptibility to darunavir and harbored 3 to 6 of the following amino acid substitutions S37N/D, R41E/S/T, K55Q, K70E, A71T, T74S, V77I, or I85V in the protease. Selection in cell culture of darunavir resistant HIV-1 from nine HIV-1 strains harboring multiple protease inhibitor resistance-associated mutations resulted in the overall emergence of 22 mutations in the protease gene, including L10F, V11I, I13V, I15V, G16E, L23I, V32I, L33F, S37N, M46I, I47V, I50V,

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F53L, L63P, A71V, G73S, L76V, V82I, I84V, T91A/S, and Q92R, of which L10F, V32I, L33F, S37N, M46I, I47V, I50V, L63P, A71V, and I84V were the most prevalent. These darunavir-resistant viruses had at least eight protease mutations and exhibited 50- to 641-fold decreases in darunavir susceptibility with final EC₅₀ values ranging from 125 nM to 3461 nM.

2.2 Clinical Microbiology

For the clinical microbiology assessment, genotypes and phenotypes from 319 subjects (1584 total isolates) from study C202 (POWER 2) and 318 subjects (1767 total isolates) from study C213 (POWER 1) were submitted and reviewed for this NDA. In addition, genotypes and phenotypes from 460 subjects (1935 total isolates from study C215 (POWER 3) were submitted and reviewed. Subjects in studies C202, C213, and C215 who rebounded or were never suppressed were analyzed for mutations developing on treatment in the FDA analysis.

The FDA analysis of resistance focused on the comparator PI control group and 600 mg darunavir/rtv bid group from studies C202 and C213 since 600 mg darunavir/rtv bid is the registrational dosage. Baseline genotypic response analyses were performed only on the controlled phase 2 studies C202 and C213 using Week 24 data. Baseline phenotypic response analyses were performed on the phenotypic data from studies C202, C213 and C215 in order to have a larger pool of data to describe baseline darunavir phenotypic subgroups. These baseline response analyses were performed on a censored as-treated dataset. Subjects who discontinued while suppressed or discontinued at week 2 were censored from the analyses. In addition, the virologic failures from studies C202, C213 and C215 were analyzed for mutations developing on darunavir/rtv treatment.

Resistance

In the Phase 2 trials Studies C202, C213 and C215, multiple protease inhibitor-resistant HIV-1 isolates from highly treatment-experienced subjects who received 600/100 mg darunavir/rtv b.i.d. and experienced virologic failure (n=164), either by rebound, or by never being suppressed, developed amino acid substitutions that were associated with a decrease in susceptibility to darunavir. The amino acid substitution V32I developed on darunavir/rtv 600/100 mg b.i.d. in 35% of virologic failure isolates and substitutions at amino acid I54 developed in 24% of virologic failure isolates. Other substitutions that developed in 10% to 20% of darunavir/rtv virologic failure isolates occurred at amino acid positions I15, L33, I47, G73 and L89. The median darunavir phenotype (fold change from reference) of the virologic failure isolates was 21-fold at baseline and 94-fold at failure.

Cross-resistance

Darunavir has a <10-fold decreased susceptibility in cell culture against 90% of 3309 clinical isolates resistant to amprenavir, atazanavir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir and/or tipranavir showing that viruses resistant to most protease

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inhibitors remain susceptible to darunavir. In studies C202, C213 and C215, 60% (88/147) of subjects with decreased susceptibility to tipranavir (fold change >3) at baseline demonstrated a 1 log₁₀ decrease from baseline at week 24 on darunavir/rtv and 36% (53/147) achieved <50 copies/mL serum HIV RNA levels.

Darunavir-resistant viruses were not susceptible to amprenavir, atazanavir, indinavir, lopinavir, nelfinavir, ritonavir or saquinavir in cell culture. However, six of nine darunavir-resistant viruses selected in cell culture from protease inhibitor-resistant viruses showed a fold change in EC₅₀ values <3 for tipranavir, indicative of limited cross-resistance between darunavir and tipranavir. Of the viruses isolated from subjects experiencing virologic failure on darunavir/ritonavir 600/100 mg b.i.d., greater than 50% were still susceptible to tipranavir while less than 5% were susceptible to other protease inhibitors (amprenavir, atazanavir, indinavir, lopinavir, nelfinavir, ritonavir, or saquinavir).

Cross-resistance between darunavir and the nucleoside/nucleotide reverse transcriptase inhibitors, the non-nucleoside reverse transcriptase inhibitors or the fusion inhibitor is unlikely because the viral targets are different.

Baseline Genotype/Phenotype and Virologic Outcome Analyses

Genotypic and/or phenotypic analysis of baseline virus may aid in determining darunavir susceptibility before initiation of 600/100 mg b.i.d darunavir/rtv therapy. Analyses were conducted to evaluate the impact of specific baseline protease inhibitor resistance-associated mutations and the number of protease inhibitor resistance-associated mutations at baseline on virologic response. Both specific mutations and the number of baseline mutations as well as susceptible drugs in the optimized background regimen and enfuvirtide use affected darunavir/rtv response rates in Phase 2 Studies C202 and C213.

The presence at baseline of the mutations V32I, I47V, or I54L or M, was associated with a decreased virologic response to darunavir and decreased susceptibility to darunavir. In addition, a diminished virologic response was observed in subjects with ≥7 protease inhibitor resistance-associated mutations (any change at amino acid positions 30, 32, 36, 46, 47, 48, 50, 53, 54, 73, 82, 84, 88, or 90) at baseline. However, the response rate in all subgroups (by type and number of mutations at baseline) was generally higher in the darunavir/rtv group compared to the control group.

Baseline darunavir phenotype (shift in susceptibility relative to reference) was shown to be a predictive factor of virologic outcome. Response rates of 340 subjects in studies C202, C213, and C215 who received 600/100 mg b.i.d darunavir/rtv therapy assessed by the baseline darunavir phenotype showed that a baseline darunavir phenotype of greater than 7-fold change from reference resulted in decreased response rates at Week 24. The subgroup of subjects with baseline darunavir phenotypes of 0-2 had response rates of 88% (1 log₁₀ decrease from baseline) and 60% (<50 copies/mL). The subgroup of subjects with baseline darunavir phenotypes of >2-7 had response rates of 73% (1 log₁₀

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decrease from baseline) and 47% (<50 copies/mL). The subgroup of subjects with baseline darunavir phenotypes >7-30 had response rates of 52% (1 log₁₀ decrease from baseline) and 24% (<50 copies/mL). The subgroup of subjects with baseline darunavir phenotypes >30 had response rates of 43% (1 log₁₀ decrease from baseline) and 18% (<50 copies/mL).

The number of susceptible drugs in the optimized background regimen and enfuvirtide use affected darunavir/rtv response rates. In Studies C202 and C213, subjects with no susceptible NRTIs at baseline had lower response rates (38% with 1 log₁₀ decrease and 13% with <50 copies/mL) than those with at least one susceptible NRTI. In addition, for subjects with baseline darunavir phenotypes of >10 in studies C202, C213 and C215, response rates were 81% (13/16) when ENF was used for the first time concomitantly with darunavir while response rates were 36% (27/74) for those who did not use ENF concomitantly.

3. Administrative

3.1. Reviewer's Signature(s)

Lisa K. Naeger, Ph.D.
Sr. Microbiologist, HFD-530

3.2. Concurrence

HFD-530/Signatory Authority _____ Signature _____ Date _____

HFD-530/Micro TL _____ Signature _____ Date _____

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4 Microbiology Review

4.1 Important Milestones in Product Development

January 2005: Submission of Phase II resistance data in the HIV resistance template format (SAS datasets) was discussed with the sponsor and agreed upon.

The NDA was proposed for rolling review submission (IND62477 Serial No. 223) and accepted by the Division (FDA facsimile correspondence dated April 5, 2005 and email correspondence dated April 7, 2005).

June 2005: Discussions were held regarding NDA submission of virology reports. The sponsor stated that the initial Virology Summary would be provided in the September 2005 submission and include the currently available in vitro virology data and clinical virology data from trials TMC114-C201 and TMC114-C207. The in vitro virology part contains Mechanism of Action, In vitro Drug Resistance, and Combinations with current HIV-1 inhibitors. The clinical virology part contains exploratory clinical virology data supporting the efficacy in the controlled clinical studies. The updated Virology Summary would be provided in the December 2005 submission to include the available exploratory clinical virology data from trials TMC114-C202 and TMC114-C213 to constitute a comprehensive overview of the virology of darunavir.

June 2005: In an effort to get as much resistance data as possible, the Division requested that the sponsor provide genotypes and phenotypes from study C215. In addition, we requested that sponsor to provide the C_{min} and IQ data in the template datasets.

4.2 Methodology

Genotypes and phenotypes were determined by default on plasma samples taken at predefined timepoints (i.e. screening, Week -2, baseline, Week 2, Week 24, Week 48, Week 96 and final/withdrawal visit) when the viral load was > 1000 copies/mL. Furthermore, samples at other timepoints, such as confirmed virologic failure, were also analyzed upon request of the Protocol Virologist. Isolates from subjects from Studies C202, C213, and C215 who started from Day 1 on the recommended dose (darunavir/rtv 600 mg b.i.d.), and who were rebounders (i.e.: experienced virologic failure by rebound) were analyzed for genotypic and phenotypic changes at virologic failure compared to baseline.

Note: In the exploratory resistance analysis, virologic failure by rebound was defined as viral load > 0.5 log₁₀ HIV-1 RNA copies/mL above nadir at 2 consecutive visits, following a confirmed virologic response of 2 consecutive viral load measurements > 1 log₁₀ HIV-1 RNA copies/mL below baseline.

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Genotypes and phenotypes from 319 subjects (1584 total isolates) from study C202 (POWER 2) were submitted for review [63 subjects in 400 mg bid group, 65 subjects in 400 mg qd group, 64 subjects from 800 mg qd group 66 subjects from 600 mg bid darunavir dosage group and 61 subjects from the control group]. Note: subjects 6603 and 6604 were mixed up in the treatment arm column, so I assigned 6603 to 4Q and 6604 to 8Q. Genotypes and phenotypes from 318 subjects (1767 total isolates) from study C213 (POWER 1) were submitted for review [63 subjects in 400 mg bid group, 64 subjects in 400 mg qd group, 63 subjects from 800 mg qd group, 65 subjects from 600 mg bid darunavir dosage group and 63 subjects from the control group]. In addition, genotypes and phenotypes from 460 subjects (1935 total isolates) from study C215 (POWER 3) were submitted for review. Subjects in study C215 who rebounded (n=46) or were never suppressed (n=78) were analyzed in the FDA analysis.

The FDA analysis of resistance focused on the control group and 600 mg darunavir bid group from studies C202 and C213 since 600 mg darunavir bid is the registration dosage. Baseline genotypic response analyses were only done on the controlled phase 2 studies C202 and C213, but baseline phenotypic response analyses were done on studies C202, C213 and C215. A censored as-treated dataset was used for the baseline response analyses. Subjects who discontinued while suppressed or discontinued at week 2 were censored. In addition, the virologic failures from studies C202, C213 and C215 were analyzed for mutations developing on darunavir treatment.

Genotypic Methods

Genotypic analyses were performed at Virco by automated population sequencing. Individual data were reported as amino acid changes along the PR as compared to the HIV-1/HXB2 wild-type reference. Genotypic mixtures (a combination of different amino acids at 1 position) were reported. In addition, single genome sequencing (SGS) was used to detect mutations that could be missed by population sequencing. SGS was performed at Virco on the baseline samples and used to determine whether the emerging mutations were selected during the treatment period or were already present, at low prevalence, in the baseline species.

Briefly, the HIV-1 complementary DNA (cDNA) region encompassing the PR and the first 400 codons of the RT was derived from plasma RNA by reverse transcription and amplified by polymerase chain reaction (PCR). The corresponding 2.2 kilobases PR-RT fragments were run and extracted from a 1% agarose gel. Ligations and transformations of these fragments were performed using the _____

_____. Sixty-four single colonies were picked and resuspended individually in the PCR reaction mix. The cloned HIV-1 PR-RT segments were then reamplified by PCR and sequenced. For analysis all clones mutation profiles were recorded per unique sequences, and a composite profile of the overall detected mutations was generated.

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Phenotypic Methods

Phenotypic analyses were performed at Virco. Recombinant clinical isolates were constructed according to the Antivirogram® method. Briefly, protease (PR) and reverse transcriptase (RT) coding sequences were amplified from patient-derived viral RNA with HIV-1 specific primers. After homologous recombination of amplicons into a PR-RT deleted proviral clone, the resulting recombinant viruses were harvested and used for in vitro susceptibility testing. Cut-offs used based on the Antivirogram assay to determine resistance are shown in Table 1.

Table 1. Resistance Call Determination Using Biological or Clinical Cut-off (FC) based on the Antivirogram

	Biological Cut-off (FC)	Clinical Cut-off (FC)
NRTI		
3TC ^a	4.5	
ABC ^b	3.2	
AZT ^c	4.0	
d4T ^d	3.0	
ddI ^e	3.5	
FTC ^f	4.5	
TDF ^g		
NNRTI		
DLV ^h	10.0	
EFV ⁱ	6.0	
NVP ^j	8.0	
PI		
APV	2.5	10.0
ATV	2.4	
IDV	3.0	
LPV		
NVF	4.0	
RTV	3.5	
SQV	2.5	
TPV ^k		3.0

4.3 State of antimicrobials used for the indication (s) sought:

An estimated 40 million people worldwide were infected with HIV in 2001 and 3 million died from AIDS. Since HAART regimens have been introduced, the number of AIDS cases has decreased dramatically. HAART does not eradicate HIV from subjects completely and even though the number of HIV RNA copies is reduced to undetectable levels, HIV re-emerges quickly after discontinuation of HAART. Therefore, with the currently available regimens, it is likely that most HIV-infected subjects will require antiretroviral therapy throughout their lives.

There are currently over 20 FDA-approved anti-HIV drugs including seven PIs (amprenavir/fosamprenavir, atazanavir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir, tipranavir), eight NRTIs (abacavir, didanosine, emtricitabine, lamivudine,

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stavudine, tenofovir, zalcitabine, zidovudine), three NNRTIs (delavirdine, efavirenz, nevirapine) and the fusion inhibitor enfuvirtide. PIs work at the late stage of viral replication to prevent virus production from infected cells. They block the HIV protease enzyme, which is necessary for the production of mature virions, resulting in defective particles that are unable to infect new cells. NRTIs mimic nucleosides and target HIV-1 RT by competing with natural deoxynucleoside triphosphates for binding to RT and by incorporating into newly synthesized viral DNA resulting in chain-termination. NNRTIs inhibit HIV-1 RT by binding near the catalytic site of RT and acting as noncompetitive inhibitors. Enfuvirtide (T-20) is a gp41 fusion inhibitor preventing the joining of the viral and cellular membranes necessary for virus entry.

Unfortunately, HIV develops resistance to antiretroviral drugs over time usually from the accumulation of multiple mutations. HAART regimens are also associated with acute toxicities such as diarrhea, kidney stones, rash, CNS toxicities and hepatotoxicity. Long-term toxicities from antiretroviral therapies include mitochondrial toxicities associated with NRTIs (lactic acidosis, myopathy, neuropathy, pancreatitis), and disorders of lipid metabolism (dyslipidemia) and glucose metabolism (lipodystrophy, hypercholesterolemia, hypertriglyceridemia) associated with PIs. These tolerability issues make compliance to therapy more challenging. Compliance is an important determinant of successful virologic suppression for subjects on HAART. Regimens that are well-tolerated and easy to administer with a few pills once daily are likely to aid in patient compliance and improve clinical outcomes. There is a need for new anti-HIV drugs that are well-tolerated and easy to use with new modes of action and low likelihood of viral resistance development. Additionally, drugs that are effective against viruses resistant to all currently approved drugs are needed for the heavily treatment-experienced population.

4.4 Non-clinical Microbiology

Mechanism of Action

A time of addition assay was performed to determine which step in the HIV-1 replication cycle darunavir acts (Report TMC114-20050009-VRR). Cell cultures were maintained at 4° C during the first 30 minutes of infection to allow virus binding, but not fusion or uncoating. Thereafter, cells were incubated at 37° C. Inhibitors were added every 30 minutes until 4 hours postinfection, and hourly thereafter up to 22 hours postinfection. Dextran sulfate (binding inhibitor), AMD3100 (CXCR4 coreceptor inhibitor), C34 (fusion inhibitor), AZT (NRTI), EFV (NNRTI), saquinavir (PI) and darunavir were added at concentrations at least 100-fold higher than their EC₅₀ values. The results indicated the following:

- Dextran sulfate (binding inhibitor): no inhibition of viral infection if drug was added later than 1 hour postinfection.

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- AMD3100 (CXCR4 coreceptor inhibitor): no inhibition of viral infection if drug was added later than 1.5 hour postinfection.
- C34 (fusion inhibitor): no inhibition of viral infection if drug was added later than 2 hours postinfection.
- AZT (NRTI): no inhibition of viral infection if drug was added later than 4 hours postinfection.
- EFV (NNRTI): no inhibition of viral infection if drug was added later than 6 hours postinfection.
- saquinavir (PI): no inhibition of viral infection if drug was added later than 19 hours postinfection.
- darunavir: no inhibition of viral infection if drug was added later than 19 hours postinfection.

In this study, darunavir lost its inhibitory activity when added later than 19 hours postinfection, similar to SQV. This is consistent with the activity of a PI, which inhibits the maturation of the viral proteins, a late event in HIV replication.

The HIV aspartic protease is essential for the cleavage of viral precursor polyproteins needed for maturation of the viral particles. The catalytic activity of darunavir against HIV-1 protease activity was measured using a synthetic quenched fluorescent HIV-1 protease peptide substrate. HIV-1 specific cleavage of this substrate results in a product emitting fluorescence at 520 nm quantitatively. Enzymatic activity was determined by measuring the increase in emission of fluorescence at 520 nm as a function of time and determining the inhibition constants (K_i) using steady state kinetics. The PIs tested (IDV, RTV, NFV, SQV, LPV and APV) all demonstrated inhibitory activity against HIV-1 protease with K_i values ranging from <0.10 to 0.24 nM, consistent with values reported in literature. darunavir had a K_i value of <0.09 nM when tested in this assay showing that darunavir has comparable inhibition against HIV-1 protease as the currently approved PIs.

The time of addition data and inhibitory activity against HIV-1 protease confirm that darunavir acts as a PI.

The cleavage of a chromogenic substrate by WT protease and mutant proteases containing the V82A or I84V mutations were assessed and the K_i values were determined to be 1 nM, 1.3 nM, and 3.2 nM, respectively. For the V82A protease, the 1.3-fold increase in K_i for darunavir was lower than the published increases for APV, IDV, NFV, RTV and SQV. For the I84V protease, the increase in K_i for darunavir of 3.2-fold was lower than the published data for NFV, RTV, and SQV.

darunavir is a close analog of APV, in which the _____
_____ Isothermal titration calorimetry was used to compare the thermodynamics of binding of APV and darunavir to WT protease and multi drug-resistant protease (L63P, V82T, and I84V). Both inhibitors bound with favorable

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changes in enthalpy and entropy resulting in tight binding to the enzyme. darunavir bound to the mutant protease 13-fold less tightly than to WT protease. Binding of darunavir to WT and mutant protease was 87-fold and 33-fold greater, respectively, than that of APV.

Biosensor or surface plasmon resonance (SPR) technology enables detailed kinetic analyses of the direct binding of low molecular weight substrates to their target enzyme, providing an affinity constant (KD) and individual association rate (k_{on}) and dissociation rate (k_{off}) constants. Using the SPR technology, the kinetic parameters for the binding of darunavir to the HIV-1 protease were determined using a instrument darunavir showed a fast association but a very slow dissociation from the HIV-1 protease. darunavir bound to the HIV-1 protease with a high affinity of 4.11×10^{-13} M, which is 3 to 4 \log_{10} higher than that of the other tested PIs. This high affinity was almost completely attributed to the low darunavir dissociation rate of $7.82 \times 10^{-7} \text{ s}^{-1}$ (data from Report TMC114-20050007-VRR).

Co-Crystallization of darunavir with HIV-1 Proteases

Crystal structures for darunavir bound to WT or mutant HIV-1 protease have been described at resolutions between 1.2 and 1.53 Å (R factors 0.117 to 0.168) (data from Report TMC114-20050003-VRR). These structures showed that darunavir formed van der Waals interactions with protease residues L23, G49, I50, P81, V82 and I84 and interacted with residues A28, D29, D30, V32, I47 and I50. Examination of crystal structures identified several features that increased the ability of darunavir to maintain its inhibitory activity against mutant proteases:

- Formation of several hydrogen bonds, mostly with main chain atoms of HIV-1 protease;
- Close fit within the substrate consensus volume;
- Flexibility of darunavir, which appears to compensate for any potential reduction in interaction resulting from mutations in the HIV-1 protease.

Antiviral Activity In Vitro

The antiviral activity of darunavir against HIV^{IIIB}, HIV-1^{NL4-3} and HIV-1^{ROD} was assessed using the MTT assay and a cell-based assay that measures HIV replication using the interaction of HIV tat with the HIV-1 LTR coupled to green fluorescence protein (GFP) as a reporter (Virco/Tibotec) in MT4 cells. The median EC₅₀ values against HIV-1^{IIIB} ranged from 2.3 to 6.3 nM depending on the assay (Table 2). The median EC₅₀ value against HIV-1^{NL4-3} was 3 nM using the GFP cell based assay. The activity of darunavir was also tested against HIV-1 in PBMCs and macrophages/monocytes using a p24 assay, and the median EC₅₀ values were 1.2 nM, 1.7 nM, and 5 nM against HIV-1^{IIIB}, HIV-1^{SF2}, and HIV^{BAL}, respectively (Table 2; Report TMC114-20050014-VRR). Darunavir demonstrated antiviral activity against HIV-2 and SIV with EC₅₀ values less than 10 nM (Table 2).

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Table 2. Antiviral Activity of darunavir against Wild-type HIV and SIV

Virus Strain	Cell Type	Assay	Median EC ₅₀ (nM)	Median EC ₉₀ (nM)
HIV-1/HIB	MT4	MTT	3.80	9.95
HIV-1/HIB	MT4	GFP Type 1	2.29	6.07
HIV-1/HIB	MT4	GFP Type 2	6.26	16.31
HIV-1/HIB	PBMC	p24	1.20	2.80
HIV-1/NL4-3 ^a	MT4	GFP Type 1	3.10	ND ^b
HIV-1/NL4-3	MT4	GFP Type 2	3.29	16.14
HIV-1/SF2	PBMC	p24	1.70	3.20
HIV-1/BaL	M/Ms	p24	5.00	ND
HIV-2/ROD	MT4	MTT	4.70	9.50
HIV-2/ROD	MT4	GFP Type 2	8.49	78.12
SIV/Mac-251	MT4	GFP Type 2	9.28	66.36

^a Reported in TMC114-200500012-VRR

^b ND: Not done

darunavir was tested against three isolates of each of the HIV-1 group M subtypes (A, B, C, D, E, F, and G) and HIV-1 group O in PBMCs. Control compounds LPV and AZT were used in the PBMC assays in parallel with darunavir and the EC₅₀ values fell within acceptable ranges for these compounds. Darunavir demonstrated activity against all of the HIV-1 subtype isolates evaluated with EC₅₀ values ranging from <0.1 nM to 4.28 nM (Table 3; data from Report TMC114-20050011-VRR). The average EC₅₀ value of darunavir for all isolates tested was 1.04 nM with no detectable cytotoxicity at concentrations up to 1000 nM. These results indicate that darunavir had antiviral activity against a broad range of virus subtypes.

Table 3. Average EC₅₀ values for HIV-1 Group M Subtypes and HIV-1 Group O Isolates

	Darunavir (nM) mean EC ₅₀ (range)	LPV (nM) mean EC ₅₀ (range)	AZT (nM) mean EC ₅₀ (range)
Subtype A	0.80 (0.2-1.8)	3.1 (1.19-4.38)	7.5 (1.58-14.9)
Subtype B	1.9 (0.39-4.28)	5.69 (0.42-2.96)	2.11 (1.07-3.39)
Subtype C	0.41 (0.38-0.47)	0.96 (0.43-1.31)	2.00 (0.33-3.29)
Subtype D	0.64 (0.27-1.14)	2.50 (1.13-4.13)	24.03 (1.29-54.6)
Subtype E	0.58 (0.21-0.83)	1.39 (0.48-2.6)	2.75 (1.42-4.75)
Subtype F	0.53 (0.13-1.21)	1.77 (0.48-3.63)	4.28 (0.20-12.4)
Subtype G	0.40 (<0.10-0.65)	1.23 (0.76-2.13)	2.17 (1.49-2.53)
Group O	2.18 (1.59-2.54)	10.89 (6.67-14)	6.57 (0.44-15.4)

50% reduction in cell viability >1000 nM

Three isolates for each subtype were analyzed

The in vitro antiviral activity of darunavir was tested at three multiplicities of infection (MOIs), representing a low, intermediate and high viral input. An increase in the EC₅₀ value (25 nM) was observed for darunavir at an MOI of 1.0 compared to an MOI of 0.01 (6.2 nM) (data from Report TMC114-20050008-VRR). This result is consistent with the mechanism of action of PIs, which act in the late stages of viral replication cycle and cannot prevent the first replication cycle of the virus. Therefore, the EC₅₀ values increase with the MOI.

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Serum Binding

Binding to human serum and plasma proteins can decrease the antiviral activity of some drugs. The in vitro antiviral activity of darunavir against HIV-1^{IIIB} was assessed in the presence of physiological concentrations of serum drug-binding proteins (50% human serum, 45 mg/mL human serum albumin, or 1 mg/mL alpha-1-acid glycoprotein (AAG)) (Report TMC114-20050010-VRR). The antiviral activity of darunavir was decreased by a median 52-fold (interquartile range [IQR] 30 to 97) and 5-fold (IQR 3.6 to 14.4) by the presence of 1 mg/mL AAG and 50% human serum, respectively. No loss of activity was observed for darunavir in the presence of 45 mg/mL human serum albumin with a 1.6-fold median change (IQR 0.9 to 2.8) in antiviral activity compared to activity in its absence.

Using three different batches of human serum gave results varying from 4-fold and 26-fold median changes in antiviral activity. The protein composition in these different human serum batches may explain these differences. The interaction of PIs with AAG is saturable, so the free fraction of darunavir should increase with its concentration. Therefore, the applicant argues that the experiment performed at nanomolar concentrations may not be reflective of the in vivo situation where serum concentration of darunavir is in the micromolar range. They analyzed this and in fact, the median fold change in antiviral activity was ≤ 5 -fold at micromolar darunavir concentrations as compared to >70 -fold change for darunavir concentrations <5 nM.

Cytotoxicity

The in vitro effect of darunavir on cell proliferation and cell viability was analyzed on several human cell types (HepG2, HeLa, MT4, MRC5, A549 and PBMCs). The 50% cytotoxic concentration (CC₅₀) value of darunavir was greater than or equal to 87 μ M for the cell lines tested in a 3-day MTT assay (Table 4; data from Report TMC114-20050016-VRR). The CC₅₀ value in MT4 cells in a 5-day MTT assay was >100 μ M giving a therapeutic index of >26000 . The 50% toxic concentration of darunavir on cell viability was greater than 200 μ M for the cell types tested.

Table 4. In Vitro Cytotoxicity Assessments of darunavir on Human Cell lines

Cells	Characteristics	CC ₅₀ value (μ M)	TC ₅₀ value (μ M)
HepG2	Epithelial liver	140	>200
HeLa	Epithelial cervix	130	>200
MT4	CD4+ lymphocyte from acute T-cell leukemia	>200	>200
MRC5	Normal fetal lung fibroblast	87	>200
A549	Epithelial lung carcinoma	200	>200
PBMCs	Peripheral blood mononuclear cells		>1.0

Results from 3-day MTT assay

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In vitro Anti-HIV Activity of Drug Combinations

The anti-HIV-1 activity of the combination of darunavir with NRTIs (abacavir, didanosine, emtricitabine, lamivudine, stavudine, tenofovir, zalcitabine and zidovudine), the NNRTIs (delavirdine, efavirenz, and nevirapine), the PIs (amprenavir, atazanavir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir, and tipranavir), and the fusion inhibitor (enfuvirtide) were studied in vitro in a MTT cell-based assay. EC₅₀ values of the combined drugs at three different molar ratios were compared to the estimated additive EC₅₀ values. Data were analyzed using the combination index and isobologram approach.

The data showed that darunavir was not antagonistic with any of the currently approved antiretroviral drugs and that synergy was observed in vitro with three PIs: amprenavir, nelfinavir and ritonavir (Table 5; Report TMC114-20050006-VRR).

Table 5. The Effect of the Combination of darunavir with Currently Approved Antiretrovirals on Anti-HIV-1 Activity

TMC114 in Combination	Activity Ratio ^a	CI		Score
		Median	IQR	
With NRTIs / NRTI				
AZT	0.38	0.91	0.91 - 0.96	Additive
	1.2	0.89	0.84 - 0.89	Additive
	3.8	0.93	0.93 - 0.98	Additive
d4T	0.21	0.93	0.91 - 0.97	Additive
	0.61	0.90	0.88 - 0.94	Additive
	1.9	0.88	0.88 - 0.90	Additive
ddI	0.26	0.92	0.85 - 0.96	Additive
	0.78	0.94	0.92 - 0.97	Additive
	2.0	0.94	0.92 - 0.95	Additive
ddC	0.15	0.80	0.79 - 0.86	Additive
	0.40	1.04	0.99 - 1.04	Additive
	1.3	1.01	1.00 - 1.07	Additive
ABC	0.14	0.85	0.83 - 0.89	Additive
	0.39	0.90	0.88 - 0.92	Additive
	1.2	0.93	0.92 - 0.97	Additive
3TC	0.20	0.99	0.86 - 1.38	Additive
	0.59	1.00	0.98 - 1.00	Additive
	1.9	0.91	0.89 - 0.93	Additive
FTC	0.43 ^b	0.94	NA	Additive
	1.2 ^c	0.89	0.84 - 0.94	Additive
	3.5 ^c	0.94	0.93 - 0.94	Additive
TFV	0.36	1.00	0.98 - 1.00	Additive
	1.3	0.95	0.95 - 0.96	Additive
	3.6	0.95	0.95 - 0.96	Additive
With NNRTIs				
NVP	0.16	0.77	0.75 - 0.90	Synergistic
	0.44	0.96	0.90 - 0.98	Additive
	1.4	0.88	0.85 - 0.88	Additive
DLV	0.44	0.83	0.82 - 0.83	Additive
	1.2	0.82	0.78 - 0.84	Additive
	4.2	0.81	0.78 - 0.84	Additive
EFV	0.14	0.86	0.84 - 0.88	Additive
	0.43	0.93	0.87 - 0.94	Additive
	1.3	0.94	0.94 - 0.97	Additive

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TMC114 in Combination	Activity Ratio ^a	CI		Score
		Median	IQR	
With PIs				
IDV	0.25	0.92	0.91 - 0.93	Additive
	0.77	0.87	0.87 - 0.88	Additive
	2.4	0.87	0.86 - 0.87	Additive
RTV	0.27	0.76	0.76 - 0.78	Synergistic
	0.87	0.68	0.66 - 0.68	Synergistic
	2.5	0.69	0.69 - 0.70	Synergistic
SQV	0.20	1.00	0.99 - 1.04	Additive
	0.59	0.99	0.98 - 1.03	Additive
	1.8	0.94	0.94 - 0.95	Additive
NFV	0.15	0.80	0.80 - 0.81	Additive
	0.47	0.66	0.66 - 0.67	Synergistic
	1.3	0.61	0.61 - 0.62	Synergistic
APV	0.33	0.77	0.76 - 0.78	Synergistic
	0.99	0.69	0.69 - 0.70	Synergistic
	3.1	0.65	0.65 - 0.68	Synergistic
LPV	0.34	0.86	0.86 - 0.88	Additive
	1.2	0.85	0.85 - 0.86	Additive
	3.2	0.95	0.93 - 0.95	Additive
ATV	0.38	0.96	0.93 - 0.98	Additive
	1.2	0.94	0.94 - 0.95	Additive
	3.6	0.95	0.95 - 0.98	Additive
TPV	0.45	0.89	0.89 - 0.9	Additive
	1.3	0.95	0.94 - 0.98	Additive
	4.0	0.94	0.94 - 0.90	Additive
With Fusion Inhibitor				
ENF	0.72	0.95	0.90 - 0.95	Additive
	1.9	0.95	0.95 - 0.95	Additive
	6.1	0.94	0.94 - 0.95	Additive
	1.00	0.94	0.89 - 0.95	Additive
	3.0	0.95	0.93 - 0.96	Additive
	7.7 ^c	0.95	0.95 - 0.96	Additive

Combinations were scored synergistic for CI < 0.8, antagonistic for CI > 1.2 or additive for 1.2 > CI ≥ 0.8.

^a Activity ratio = Molar ratio / Ratio of EC₅₀ values

^b Experiment done once

^c Experiment done twice

Development of Resistance In Vitro

In vitro selection experiments were performed starting from wild-type. Replicating viruses could not be selected in the presence of darunavir at concentrations above 220 nM out to 738 days. Selection of viruses was slower with darunavir in comparison with other PIs tested at micromolar concentrations (Fig. 1; Report TMC114-20050012-VRR).

Genotypic analyses of the darunavir-selected laboratory strains and recombinant clinical isolates from naïve subjects showed the emergent of S37N/D, R41E/S/T, K70E, A71T, V77I, and I85V mutations and R41I, K55Q and T74S mutations, respectively (Table 6; Report TMC114-20050012-VRR).

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Figure 1. In Vitro Selection of Resistance HIV-1 in the Presence of darunavir and Currently Approved PIs

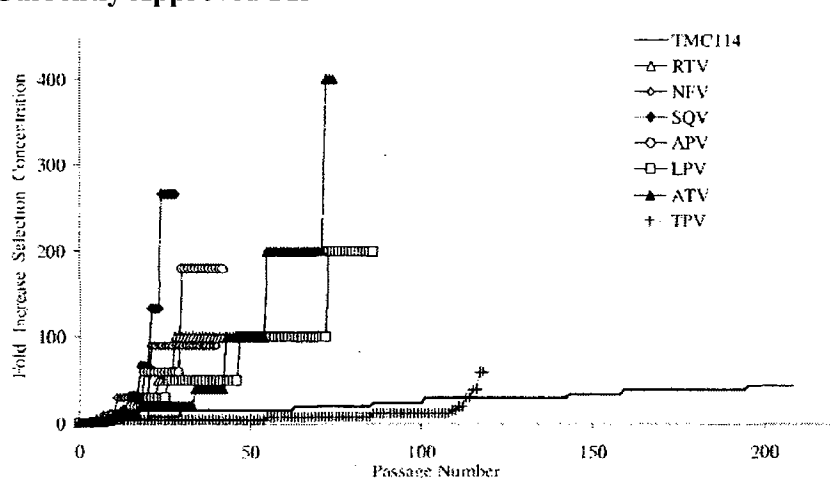


Table 6. Genotypic Analysis of darunavir-In Vitro Selected HIV-1

Initial HIV-1 Strain	Exp #	Original Protease Genotype ^a	PI					Final HIV-1 Strain	Mutations Emerging in the Protease at the end of the Selection Experiment ^a	
			Initial Conc. (nM)	Initial EC ₅₀ ^b	Day (Passage)	Final Conc. (nM)	Final EC ₅₀			
TMCI14										
III B	367	V03I	5	2.36	738 (208)	220	27.74	T21396	S37D, K70E, I85V	
III B	369	V03I	1	2.36	422 (121)	170	0.21	T21159	R41E, K70E	
III B	697	V03I	10	2.36	328 (94)	200	0.44	T14505	S37N, R41S, K70E	
III B	717	V03I	10	2.36	718 (202)	220	49.92	T21273	R41T, K70E, A71T, V77I	
NL4.3	738	V03L S37N	10	3.10	205 (59)	40	6.26	T14493	R41S	
r14054	361	V03I, E35D, S37D/N, I64V	10	0.68	376 (167)	50	14.56	T15585	K55Q, T74S	
r14055	960	V03L I63S, K70R	10	1.60	408 (117)	50	2.71	T15620	R41I	

Phenotypic analysis determined that three selected viruses had a >4-fold change in EC₅₀ values. Mutant viruses harboring S37D, K70E and I85V had a fold change of 12 (Table). Mutant viruses harboring R41T, K70E, A71T and V77I had a fold change of 21. Recombinant clinical isolate r14054 harboring mutations E35D, S37D/N, K55Q, I65V, and T74S had 6-fold change in susceptibility to darunavir. The viruses with reduced susceptibility to darunavir also displayed cross-resistance to other PIs (Table 7; Report TMC114-20050012-VRR).

Table 7. Phenotypic Profile of In Vitro Selected darunavir-Resistant Viruses

HIV-1/	Phenotype ^a (FC)							
	TMC114	RTV	NFV	SQV	APV	LPV	ATV	TPV
T21396	11.8	12.7	2.8	ND	2.9	12.4	9.9	6.2
T21273	21.2	2.8	1.6	27.0	1.9	6.4	12.6	5.9

^a Experiments performed at VIRCO

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Analysis of single recombinant mutants R41K and K70E and the double mutant R41T/K70E show that these mutations alone did not confer significant resistance to darunavir or other PIs (Table 8; Report TMC114-20050012-VRR).

Table 8. Phenotypic Profile of Site-Directed Mutations

HIV-1/HXB2 Containing PI Mutations	Phenotype ^{a,b} (FC)						
	TMC114	RTV	NFV	SQV	APV	LPV	ATV
R41T	0.3	0.2	0.3	0.5	0.3	0.5	0.3
K70E	0.7	0.8	0.9	0.7	0.9	1.0	0.4
R41T and K70E	0.2	0.3	0.3	0.4	0.3	0.4	0.2

^a Experiments performed at VIRCO

^b Data taken from TMC114-20050019-VRR⁶

In vitro selection experiments were also done starting with nine isolates containing PI-resistant mutations. Viruses resistant to darunavir were selected which were able to grow in concentrations of 1.2 to 12 μ M. The nine baseline viruses had 4 to 14 protease mutations with EC₅₀ values for darunavir ranging from <0.15 to 110 nM. The time periods for selection ranged from 162 to 251 days. In these nine selection experiments, 22 protease mutations were selected by darunavir. These included L10F, V11I, I13V, I15V, G16E, L23I, V32I, L33F, S37N, M46I, I47V, I50V, F53L, L63P, A71V, G73S, L76V, V82I, I84V, T91A/S, and Q92R. Mutations L10F, V32I, L33F, S37N, M46I, I47V, I50V, L63P, A71V, and I84V were the most prevalent in the darunavir-selected viruses and were present in more than 50% of the variants analyzed. The nine darunavir-selected viruses had at least eight protease mutations and had 50- to 641-fold decreases in darunavir susceptibility with final EC₅₀ values ranging from 125 nM to 3461 nM (Table 9; Report TMC114-20050013-VRR).

In Vitro Cross-Resistance

The antiviral activity of darunavir was tested on a panel of 20 recombinant clinical isolates resistant to currently approved PIs (amprenavir, indinavir, lopinavir, nelfinavir, ritonavir, and saquinavir) (Table 10; Report TMC114-20050017-VRR). Nineteen of the viruses were selected on the basis of their phenotype (fold change >10 for at least one of the approved PIs) and genotype (PI resistance-associated profile). One virus, r13363, was added on the basis of its resistance to darunavir

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Table 9. In Vitro Selection with darunavir Starting with HIV-1 PI Resistant Viruses

Initial HIV-1 Strain	Exp #	TMC114					Final HIV-1 Strain	Final Genotype	
		Initial EC ₅₀ (nM)	Final EC ₅₀ (nM)	Final EC	Final Conc. (µM)	Days		Mutations Already Present at Baseline ¹	Mutations Emerging in the Protease at the end of the TMC114 Selection Experiment ²
T13610 ^a	802	1.21	124.85	52.9	1.20	183	T14511	L10F, D30N, R41K, K45I, M46I, V77I, I84V, N88D	I13V, V32I, L33F, S37N, L63P
T13708 ^b	819	3.02	259.13	109.8	1.20	251	T14517	L10F, M46I, I54V, L63P, V82A, T91A	I15V, V32I, A71V, I84V
T13527 ^c	381	42.67	303.26	128.5	3.00	162	T20397	L10F, M46I, I47V, I50V	V32I, L33F, S37N, G73S
r20619 ^d	591	< 0.15	370.91	157.2	1.00	225	T21337	L10I, I13V, E35D, M36I, S37I, I62V, L63P, A71T, L90M	L10F, L33F, M46I, I47V, I50V, G73S, V82I, Q92R
r13025 ^e	920	3.60	669.17	282.3	12.00	231	T13717	L10I, V32I, L33M, E35D, S37Y, M46I, Q58E, L63P, K70T, A71V, I72V, I84V, I89V	V32I, I50V, G73S, L76V, V82I
T13548 ^f	371	3.58	750.28	317.9	10.00	233	T20306	L10I, M46I	V11I, L24I, V32I, L33F, I47V, I50V, F53L, A71V, T91S
T14002 ^g	385	0.81	1040.58	440.9	10.00	238	T20472	L10F, V32I, M46I	G16E, S37N, I47V, A71V, L76V, I84V, T91A
T13607 ^h	779	110.31	1120.65	474.9	10.00	203	T14428	L10F, V32I, L33F, M46I, I47V, I50V	I13V, S37N, F53L, G73S
r13080 ⁱ	1100	20.65	3461.30	641.0	10.00	177	T13275	L10F, I13V, V32I, S37N, M46I, L63P, A71V, I84V, I89V, T91A	I47V, I50V, Q92R

^a Selected with NFV from HIV-1/IIIB (NFV EC₅₀ = 1235.56 nM)³

^b Selected with LPV from HIV-1/IIIB (LPV EC₅₀ = 250.96 nM)³

^c Selected with APV from HIV-1/IIIB (APV EC₅₀ = 1701.36 nM)³

^d Recombinant clinical isolate from study TMC114: C213 (Baseline Sample)

^e PI cross-resistant recombinant clinical isolate³

^f Selected with LPV from HIV-1/IIIB (LPV EC₅₀ = 305.37 nM)³

^g Selected with ATV from HIV-1/IIIB (ATV EC₅₀ = 14.21 nM)³

^h Selected with APV from HIV-1/IIIB (APV EC₅₀ = 1532.52 nM)³

ⁱ See Appendices

¹ Also called: TMC114 in Vitro Selection Mutations

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Table 10. Genotypic Profile of Panel of Recombinant PI-Resistant Clinical Isolates

HIV-1	PI-Resistance-Associated Mutations ^a
r13020	L10I, K20R, M36I, F53L, I54V, A71V, V82T, I84V
r13021	L10I, K20R, L24I, M36I, I54V, A71V, V82T, I84V
r13022	L10R/C, K20R, M36I, M46I, F53I, I54V, A71V/I, V82T, L90M
r13023	L10I, M36I, I54V, A71V, G73S, I84V, L90M
r13024	L10I, M36I, A71V, G73S, I84V, L90M
r13025	L10I, M46I, A71V, I84V
r13026	L10I, L24I, L33I, M36V, M46I, I54V, A71V, V82T, I84V
r13027	L10M/I, K20R, M36I, A71V, G73S, V77I, I84V, L90M
r13028	L10I, K20M, I54V, A71V, I84V, L90M
r13029	L10I, L33I, M36I, M46I, A71V, I84V, N88D, L90M
r13030	I10V/I, L33V/I, M36I, I54V, A71V, V82I, L90M
r13031	L24I, G48V, I54V, V77I, V82A, L90M
r13032	L10I, L24I, L33F, M36I, F53L, I54V, V82A, L90M
r13033	L10I, M46I, I54V, A71V, V82A, L90M
r13034	L10I, L24I, M36I, I54V, A71V, I84V
r13035	D30N, V77I, N88D
r13036	L10I, I54L, A71V, G73S, L90M
r13037	L10I, M46I, I54V, A71T, V77I, V82A, L90M
r13080	L10F, M46I, A71V, I84V
r13363	V32I, L33F, M36I, M46I, I47V, I50V, L90M

^a IAS-USA guidelines³

Eighteen of the 20 isolates showed less than 4-fold changes in EC₅₀ values for darunavir with EC₅₀ values ranging from 1.0 to 6.5 nM using a MTT assay in MT4 cells. Isolate r13080 [L10F, M46I, A71V, I84V] had a median EC₅₀ value of 15.4 nM and fold change

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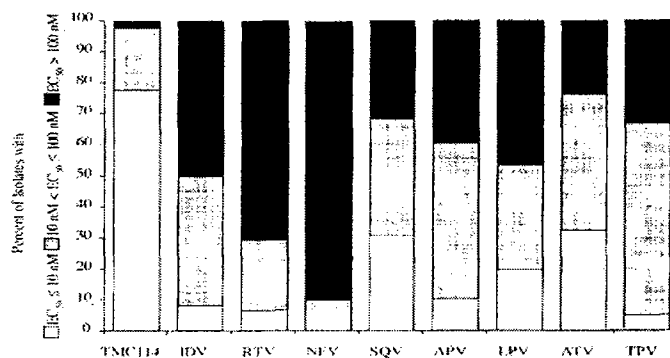
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Table 12. Antiviral Activity against Drug Resistant HIV-1 Primary Isolates in Human PBMCs

Isolate	RT Resistance Mutations	PI Resistance Mutations	EC ₅₀ value (nM)	Fold change from WEJO
WEJO	wild-type	wild-type	3.29	1
MDR769	M41L A62V K65R D67N V75I F116Y Q151M Y181I L210W T215Y	L10I M36M/V M46I I54V L63P A71V V82A I84V L90M	5	1.5
MDR1385	Sequence not available, but resistant to AZT, ddI, 3TC, d4T	L10I V13I M36I M46I I54I/V L63P A71V V82T L90M I93L	1.12	0.34
MDR3761	Sequence not available, but resistant to AZT, ddI, 3TC, d4T	L10I V13I I15V K20I M46I L63P A71I G73T I84V L90M I93L	1.41	0.43
1064-52		L10I I54V L63P A71T V82F L90M	0.78	0.24
1002-60		L10I M46I I54V L63P V82F L90M	4	1.22
052-52		L10R M46I L63P A71V V82T I84V	4.18	1.27
144-44		V32I M46I L63P L90M	5	1.52

Phenotypic analyses were performed on 9181 HIV-1 clinical isolates with darunavir and currently approved PIs using the Virco assay. Of these clinical isolates, 36% (3309/9181) displayed a fold change in EC₅₀ value >4 compared to wild-type to at least one of the currently approved PIs. Darunavir had a median EC₅₀ value of 3.1 nM against the 3309 PI-resistant clinical isolates. Darunavir had EC₅₀ values <10 nM for 78% of the PI-resistant clinical isolates, and EC₅₀ values >100 nM for 3% of the PI-resistant clinical isolates (Fig.2; Report TMC114-20050005-VRR).

Figure 2. Percentage of 3309 HIV-1 PI-Resistant Clinical Isolates Clustered by EC₅₀ Values



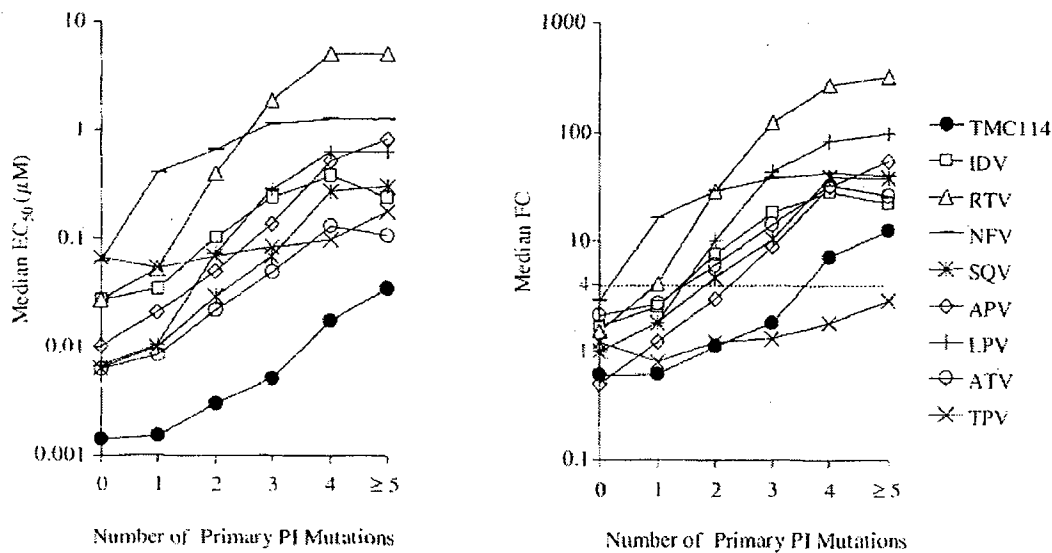
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Genotypes were available for 1113 of the 3309 isolates resistant (fold change >4) for at least one PI. These isolates were grouped by median EC₅₀ values and median fold change on the basis of the number of primary PI mutations (1, 2, 3, 4 or ≥5) and number of PI resistance-associated mutations (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, ≥11) identified in their genotype (Fig. 3 and Fig. 4; Report TMC114-20050005-VRR). Darunavir median EC₅₀ values were found to be below the median EC₅₀ values of the other PIs, but with a fold change >4 as the criteria for resistance, TPV had a lower fold change for isolates with more than three primary PI mutations and eight PI resistance-associated mutations.

Figure 3. Antiviral Activity against 1113 HIV-1 PI-Resistant Clinical Isolates with 0, 1, 2, 3, 4 and ≥5 Primary PI Mutations: Median EC₅₀ Values (Left) and Median Fold Change (Right)



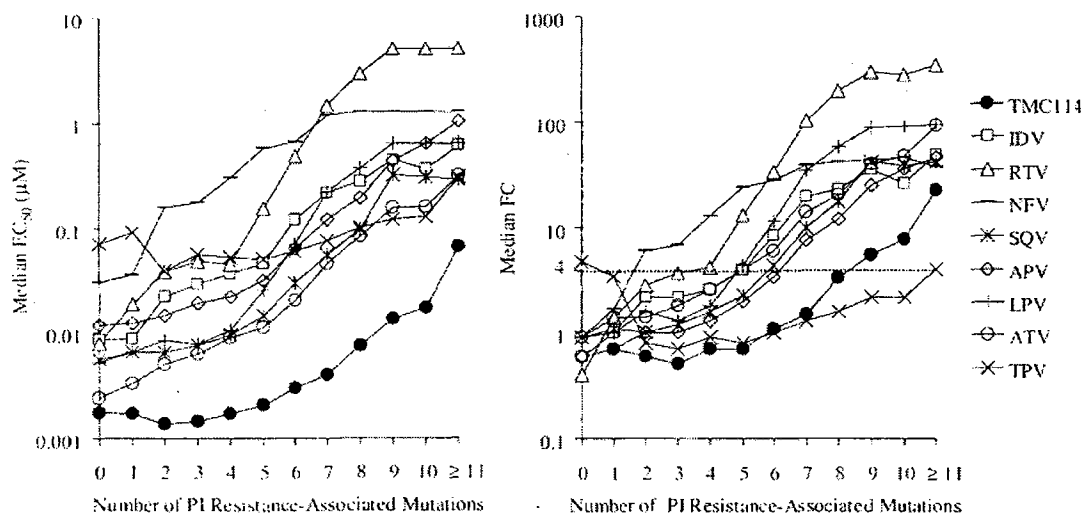
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Figure 4. Antiviral Activity against 1113 HIV-1 PI-Resistant Clinical Isolates with 1 to ≥ 11 Primary PI Mutations: Median EC₅₀ Values (Left) and Median Fold Change (Right)



Darunavir Antiviral Activity against Site-Directed Protease Mutations

Phenotypic profile of a panel of HIV-1^{HXB2} mutants containing single, double or triple point mutations in the protease gene was analyzed. The insertion of single mutations L10I, L10S, L33V, R41T, M46I, M46L, I47V, I50L, I50V, I54L, I54M, I54V, L63P, K70E, A71V, V82A, V82F, V82I, V82L, V82T, I84A, or L90M into HIV-1^{HXB2} did not change the susceptibility to darunavir with fold changes from HIB wild-type <1.5. Most of the combinations of two mutations did not lead to loss of susceptibility to PIs. Nine viruses had a fold change >4 in susceptibility to at least one PI, but none had a >4 fold change in susceptibility to darunavir (Table 13; Report TMC114-20050019-VRR).

Table 13. HIV-1^{HXB2} Containing Two Mutations Associated with Loss of Susceptibility to at Least One PI

Protease Mutations Inserted in the HIV-1/HXB2 SDMs	FC From HIV-1/HXB2							
	TMC114	IDV	RTV	NFV	SQV	APV	LPV	ATV
L33F I50V	0.8	0.6	1.9	0.3	0.8	10.1	0.9	0.2
L33F I84V	2.1	1.2	21.9	1.2	0.9	14.1	25.7	1.3
M46I I50V	0.8	0.4	4.3	0.8	0.4	14.6	2.1	0.3
I47V I50V	0.9	0.3	1.9	0.4	0.2	15.3	4.8	ND
I47V I54M	0.5	ND	2.8	0.3	ND	24.1	2.8	0.4
V32I I54M	0.8	ND	1.7	1.8	ND	6.8	1.1	1.4
V82F I84V	1.1	0.7	10.9	1.3	0.7	4.6	3.4	1.7
V82F L90M	0.2	1.0	5.8	6.8	0.2	2.2	0.6	0.3
I84V L90M	1.4	0.3	4.5	2.1	1.2	9.5	6.6	0.6

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Seven of twelve viruses tested with three or more site-directed PI-resistance associated mutations had resistance >4-fold to at least one PI (Table 14; Report TMC114-20050019-VRR). One of the viruses with mutations M46I, I47V and I50V had a 5.1 fold change in susceptibility to darunavir. These results were concordant with results above where mutations I47V and I50V were included in the R13363 clinical isolate which had 162-fold decreased susceptibility to darunavir.

Table 14. HIV-1^{HXB2} Containing Three or More Mutations Associated with Loss of Susceptibility to at Least One PI

Protease Mutations Inserted in the HIV-1/HXB2 SDMs	FC From HIV-1/HXB2							
	TMC114	IDV	RTV	NFV	SQV	APV	LPV	ATV
L10I V32I I47V	0.7	1.0	3.0	0.4	0.8	4.3	3.9	1.1
L10I L33F I54L	1.0	1.0	5.1	1.2	1.7	4.9	1.1	1.5
V32I I47V I54M	1.2	ND	2.9	0.8	ND	24.1	10.2	0.6
M46I I47V I50V	5.1	0.4	4.3	1.9	0.2	13.8	20.4	0.3
A71V V82F I84V	0.9	3.6	116.1	1.6	0.8	7.8	6.8	4.9
M46I L63P V82F I84V	1.8	3.7	28.8	3.3	0.2	10.9	31.5	1.8
M46I L63P A71V V82F I84V	0.6	0.8	9.6	1.2	0.4	2.3	4.2	1.3

4.5 Clinical Studies

The clinical development program evaluating the efficacy of darunavir/rtv for the treatment of treatment-experienced HIV-1 infected subjects included data from four controlled trials: two completed phase IIa proof of principle trials (C201 and C207) and two phase IIb controlled trials (C202 (POWER 2) and C213 (POWER 1)). In addition, data were available from two ongoing open-label, non-randomized trials, C215 and C208 (POWER 3), designed to provide darunavir/rtv to subjects who had previously received darunavir, but also allowed additional subjects who had not participated in darunavir/rtv trials (de novo subjects) to increase the size of the safety database. Analyses of clinical data revealed that the optimum dose of darunavir/rtv was 600 mg/100 mg bid.

Studies TMC114-C202 and TMC114-C213

Studies C202 and C213 consisted of 2 parts: a partially blinded dose-finding controlled part followed by an open-label part with the recommended dose of darunavir/rtv. The dose-finding part of this trial consisted of a randomized, controlled (standard of care), partially blinded, Phase IIb trial to determine the antiviral activity, safety and tolerability of darunavir, formulated as an oral tablet, and administered with a low dose of ritonavir (RTV). Subjects who were three-class-experienced and who were on a stable protease inhibitor (PI)-containing regimen, that did not include a non-nucleoside analogue reverse transcriptase inhibitor (NNRTI) at screening for at least 8 weeks, and who had > 1000 HIV-1 RNA copies/mL were eligible. Three-class-experience was defined as prior treatment with ≥ 2 nucleoside analogue reverse transcriptase inhibitors (NRTIs) for at least 3 months in total and ≥ 1 NNRTI as part of a failing regimen. Prior enfuvirtide use

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was allowed. In addition, subjects had to have received at least 1 PI for at least 3 months in the past and had to have at least 1 primary PI mutation as defined in the IAS-USA March 2003 list at screening. The study design of the dose-finding part of the trials was a 2-part hybrid. Subjects were randomized to 1 of the 4 darunavir/rtv treatment groups (darunavir/rtv 400/100 mg q.d., 800/100 mg once a day [q.d.], 400/100 mg b.i.d. or 600/100 mg b.i.d.) or to a control group (comparator PI). At baseline, subjects randomized to a darunavir/rtv treatment group substituted their PI(s) with darunavir/rtv and continued the same background NRTIs (with or without enfuvirtide [ENF]), as at screening, for 2 weeks (functional monotherapy). After the 2-week period, subjects continued on their randomized darunavir/rtv dose and changed their background regimen to an optimized background regimen (OBR) consisting of NRTIs with or without ENF. Subjects randomized to the control group changed their therapy at baseline to an investigator selected PI regimen plus OBR (NRTIs with or without ENF). Study C202 was conducted in the United States and Argentina and Study C213 was conducted in Europe, Canada, Brazil and Australia. There were 319 subjects in study C202 and 318 subjects in study C213.

Study TMC114-C215

TMC114-C215 conducted in same sites as C202 and C213 was an open-label, non-randomized trial designed to provide darunavir treatment at the recommended dose of 600/100 mg bid for subjects who previously participated in darunavir trials, but also included 303 additional subjects who had never participated in a darunavir trial in order to expand the evaluation of safety and efficacy of the 600/100 mg bid dose of darunavir.

The roll-over subjects had failed treatment in studies C202 or C213 and were judged by the investigator to potentially benefit from darunavir therapy. Subjects had to have participated in the treatment phase of the original trial for at least 12 weeks and had virologic failure defined as less than 0.5 log₁₀ reduction in plasma HIV-1 RNA level from baseline beyond Week 12. For subjects randomized to the control group in C202 and C213, an additional definition of virology failure that allowed a switch to C215 was applied: subjects not achieving plasma HIV-1 RNA levels below the assay limit of 50 copies/mL following a 12-week treatment period. The 303 newly recruited subjects were at least three-class experienced and who were on a stable PI-containing regimen at screening for at least 8 weeks and who had a plasma HIV-1 RNA > 1000 copies/mL. Three class experience was defined as prior treatment with one or more NRTIs for at least 3 months and one or more NNRTIs as part of a failing regimen. All subjects had to have received at least one PI for a least 3 months and had a least one primary PI mutation

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4.6 Clinical Microbiology

BASELINE ANALYSIS

Studies C202 and C213

Subjects in Studies C202 and C213 were highly treatment-experienced. Baseline phenotypic analysis of 315 and 308 patient isolates at baseline from studies C202 and C213, respectively, showed that 98% of the patient isolates were resistant to at least one PI, 74% were resistant to at least one NNRTI, and 93% were resistant to at least one NRTI. The proportion of patient isolates in studies C202 and C213 resistant to the anti-HIV drugs is shown in Table 15. Only 33% of the subjects in the studies were resistant to TPV. However, most subjects (76%-98%) were resistant to the other approved PIs. Approximately 80% of the subjects were resistant to lamivudine (LAM) and emtricitabine (FTC). Overall, the subjects in Study C202 were more antiretroviral-experienced than the subjects in study C213. Twenty-four percent (75/319) of subjects in Study C202 and 11% (36/318) of subjects in Study C213 had previous ENF use. Using 10-fold as a cutoff for darunavir susceptibility, 59% (35/59) of the subjects receiving 600 mg darunavir/rtv in Study C202 and 70% (45/64) in Study C213 were susceptible to darunavir at baseline.

Table 15. Percent Isolates Resistant to PIs, NNRTIs, and NRTIs at Baseline in Studies C202 and C213

	Study C202	Study C213
Drug (FC)	% Resistant (n) > FC	% Resistant (n) > FC
APV (2.5)	87% (273/315)	79% (244/307)
ATV (2.4)	92% (290/315)	87% (268/307)
IDV (3.0)	91% (286/314)	88% (271/308)
LPV (10.0)	89% (281/315)	85% (262/308)
NFV (4.0)	96% (303/315)	94% (291/308)
RTV (3.5)	98% (306/313)	92% (283/308)
SQV (2.5)	85% (268/314)	76% (233/308)
TPV (3.0)	32% (101/314)	33% (102/307)
DLV (10)	61% (191/314)	51% (158/308)

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EFV (6.0)	56% (177/315)	60% (184/308)
NVP (8.0)	70% (222/315)	68% (208/308)
ABC (3.2)	47% (148/315)	45% (139/308)
ddI (3.5)	24% (75/315)	19% (60/308)
FTC (4.5)	83% (263/315)	76% (234/308)
LAM (4.5)	83% (263/315)	76% (234/308)
d4T (3.0)	14% (43/315)	9.1% (28/308)
ddC (4.0)	33% (105/315)	22% (68/308)
AZT (4.0)	52% (163/309)	48% (146/305)
TDF (1.4)	63% (199/315)	61% (189/308)

Resistance to PIs, NNRTIs and NRTIs were comparable between the 600 mg darunavir arm and the comparator PI arm (Control) in both studies (Tables 16 and 17).

Table 16. Proportion Resistant Isolates at Baseline by Treatment Arm in Study C202

	%PI-R	%NNRTI-R	%NRTI-R
600 mg Darunavir/rtv	65/66 (98%)	49/66 (74%)	63/66 (95%)
Control	58/60 (97%)	41/60 (68%)	53/60 (88%)

Table 17. Proportion Resistant Isolates at Baseline by Treatment Arm in Study C213

	%PI-R	%NNRTI-R	%NRTI-R
600 mg Darunavir/rtv	62/64 (97%)	49/63 (78%)	60/64 (94%)
Control	60/61 (98%)	45/61 (74%)	56/61 (92%)

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An analysis looking at the number of susceptible NRTIs and PIs at baseline showed that the 600 mg darunavir arm and control arm were comparable in both studies. Greater than 60% of subjects had no PIs that their virus was susceptible to at baseline and $\leq 20\%$ had only one susceptible PI at baseline. Interestingly, most subjects (80%-97%) had isolates with >1 susceptible NRTI at baseline in both studies (Table 18).

Table 18. Number of Susceptible Drugs at Baseline by Treatment Arm in Studies C202 and C213

	Study C202		Study C213	
	600 mg Darunavir/rtv	Control	600 mg Darunavir/rtv	Control
0 Susceptible PIs	68% (45/66)	67% (40/60)	62% (40/64)	61% (37/61)
1 Susceptible PI	20% (13/66)	15% (9/60)	19% (12/64)	18% (11/61)
≥ 1 Susceptible PIs	12% (8/88)	18% (11/60)	19% (12/64)	21% (13/61)
0 Susceptible NRTI	14% (9/66)	5% (3/60)	1.5% (1/64)	6.5% (4/61)
1 Susceptible NRTI	6% (4/66)	5% (3/60)	1.5% (1/64)	6.5% (4/61)
≥ 1 Susceptible NRTIs	80% (53/66)	90% (54/60)	97% (62/64)	87% (53/61)

Examining the baseline genotypic data showed a comparable median number of resistance associated mutations in the active and control arm (Table 19) and a similar proportion of subjects with primary PI mutations in both arms (Table 20).

Table 19. Median Number of Resistance Associated Mutations at Baseline in Studies C202 and C213

	PRIM MUT	PIMUT ASS	ALLPR	NRTI MUT	NNRTI MUT	EXT NNRTI	ALL RT	MUT FDA
ALL	3	8	18	6	1	2	29	5
600 mg darunavir/rtv	3	8	18	6	1	2	29	5
Control	3	8	17	5	1	1	29	5

Table 20. Percentage of Subjects with Primary PI Resistance Associated Mutations in Studies C202 and C213 by Arm

	Study C202		Study C213	
	600 mg Darunavir/rtv	Control	600 mg Darunavir/rtv	Control
Primary Mutations ¹ ≥ 3	62% (41/66) ³	69% (42/61) ³	46% (30/65) ⁴	56% (35/63) ⁴
Primary Mutations ¹ >3	22% (18/66) ⁵	36% (22/61) ⁵	22% (14/65)	19% (12/63)
FDA Mutations ² ≥ 5	59% (39/66)	59% (36/61)	52% (34/65)	56% (35/63)
FDA Mutations ² >5	33% (22/66)	34% (21/61)	29% (19/65)	30% (19/63)

¹ IAS-USA 2005

² D30, V32, M36, M46, I47, G48, I50, F53, I54, G73, V82, I84, N88, L90

³ p value = 0.43

⁴ p value = 0.29

⁵ p value = 0.29

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Study C215

The baseline characteristics of 225 subjects who received 600 mg darunavir/rtv de novo and reached week 24 of treatment in study C215 were analyzed. Baseline phenotypic analysis showed that 98% (214/218) of the patient isolates were resistant to at least one PI, 73% (160/218) were resistant to at least one NNRTI, and 96% (209/218) were resistant to at least one NRTI.

The proportion of subjects in study C215 with isolates that were resistant (>3-fold) to tipranavir at baseline was 48% (107/225), higher than that seen in studies C202 and C213. Thirty-one percent (69/225) had prior ENF use, also higher than the prior ENF use in studies C202 and C213. Using a 10-fold cutoff for darunavir susceptibility, 74% (161/217) in Study C215 were susceptible to darunavir at baseline.

The median number of baseline mutations in study C215 was comparable to the other two studies (Table 21).

Table 21. Median Number of Resistance Associated Mutations at Baseline in Study C215

	PRIM MUT	PIMUT ASS	ALLPR	NRTI MUT	NNRTI MUT	EXT NNRTI	ALL RT	MUT FDA
C215	3	8	20	6	1	2	31	5

BASELINE GENOTYPE AND VIROLOGIC OUTCOME ANALYSES

Virologic response analyses of Week 24 data based on baseline genotype and phenotype were performed on a censored as-treated dataset. Subjects who discontinued while suppressed or discontinued at week 2 were censored. The response rates (proportion <50 copies/mL at week 24, proportion with 1 log₁₀ decrease from baseline at week 24, and median DAVG at week 24) of 125 subjects receiving 600 mg darunavir/rtv in Studies C202 and C213 combined were analyzed by the number of baseline PI resistance associated mutations (FDA list of mutations: any change at protease amino acid positions 30, 32, 36, 46, 47, 48, 50, 53, 54, 73, 82, 84, 88, or 90) and compared to the response rates of the 120 subjects in the control arm. Response rates to darunavir were greater or equal to the overall response rates if subjects had less than seven baseline PI resistance associated mutations (Table 22). A diminished virologic response of 22% with <50 copies/mL to darunavir/rtv was observed in subjects with seven PI resistance-associated mutations. None of the five subjects with eight baseline PI mutations achieved <50 copies/mL or a 1 log₁₀ decrease from baseline to darunavir.

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Table 22. Response by Number of Protease Inhibitor Resistance Associated Mutations in Studies C202 and C213

	Confirmed 1 log₁₀ decrease at Week 24		Proportion of Responders with <50 copies/mL at Week 24	
	600 mg Darunavir/r tv Arm (n=125)	Control Arm (n=120)	600 mg Darunavir/rt v Arm (n=125)	Control Arm (n=120)
Overall Response	68% (85/125)	21% (25/120)	45% (56/125)	13% (15/120)
# FDA Mutations*				
0	1/1	0/1	0/1	0/1
1	2/2	1/1	0/2	0/1
2	1/2	38% (3/8)	0/2	2/8
3	86% (19/22)	24% (4/17)	59% (13/22)	12% (2/17)
4	77% (23/30)	16% (4/25)	43% (13/30)	12% (3/25)
5	67% (18/27)	28% (8/29)	52% (14/27)	21% (6/29)
6	67% (18/27)	18% (4/22)	52% (14/27)	9% (2/22)
7	33% (3/9)	8% (1/12)	22% (2/9)	0% (0/12)
8	0% (0/5)	0% (0/5)	0% (0/5)	0% (0/5)

* Any change at protease amino acid positions 30, 32, 36, 46, 47, 48, 50, 53, 54, 73, 82, 84, 88 and 90

Three subgroups of baseline PI mutations 0-4, 5-6 and ≥ 7 showed a better than, an equivalent to, and a poor response to darunavir/rtv, respectively, compared to the overall response rate in studies C202 and C213 of 68% with a 1 log₁₀ decrease from baseline and 45% with <50 copies/mL at Week 24 (Table 23). Subjects with 0-4 baseline PI mutations had response rates of 81% with a 1 log₁₀ decrease from baseline and 46% with <50% copies/mL. Subjects with 5-6 baseline PI mutations had response rates of 67% with a 1 log₁₀ decrease from baseline and 52% with <50% copies/mL. Subjects with ≥ 7 baseline PI mutations had response rates of 21% with a 1 log₁₀ decrease from baseline and 14% with <50% copies/mL. The response rates for subjects in each number subgroup of baseline PI mutations were higher in the darunavir/rtv group compared to the control group.

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Table 23. Response by Baseline Number of Protease Inhibitor Resistance-Associated Mutations: Studies C202 and C213 As-Treated

#PI Mutations*	Darunavir/rtv 600/100 mg (n=125)				Control (n=120)			
	n	1 log ₁₀ decrease	<50 copies/mL	Median DAVG ₂₄	n	1 log ₁₀ decrease	<50 copies/mL	Median DAVG ₂₄
0 - 4	57	81%	46%	-2.16	52	23%	13%	-0.57
5 - 6	54	67%	52%	-2.13	51	24%	16%	-0.43
≥ 7	14	21%	14%	-0.87	17	6%	0%	-0.13

* Any change at protease amino acid positions 30, 32, 36, 46, 47, 48, 50, 53, 54, 73, 82, 84, 88 and 90

In addition, response rates were analyzed by the presence of specific PI resistance associated mutations at baseline (See Appendix A and B). This analysis showed that the presence at baseline of the mutations resulting in amino acid substitutions V32I, I47V, or I54L or M, was associated with a decreased virologic response to darunavir (Table 24). The presence at baseline of PI mutations V32I or I47V or A resulted in decreased response rates of approximately 20% with 1 log₁₀ decrease from baseline, 10% with <50 copies/mL at week 24 and a DAVG₂₄ of 0.6 log₁₀. The presence of I54L or M at baseline resulted in a decreased response rates of 23% with <50 copies/mL at week 24 and a DAVG₂₄ of 1.03 log₁₀.

Table 24. Studies C202 and C213: Week 24 Response by Specific Baseline Protease Inhibitor Mutation

Baseline PI Mutation	600 mg Darunavir/rtv Arm ¹ % of Responders (n)				Control Arm % of Responders (n)			
	n	1 log ₁₀ decrease from BL	<50 copies/mL	DAVG ₂₄	n	1 log ₁₀ decrease from BL	<50 copies/mL	DAVG ₂₄
Overall	125	68% (85)	45% (56)	-2.06	120	21% (25)	13% (15)	-0.46
V11I/L	13	54% (7)	46% (6)	-2.34	12	33% (4)	25% (3)	-0.69
G16A/E	19	47% (9)	42% (8)	-1.83	16	13% (2)	6% (1)	-0.38
V32I	12	17% (2)	8% (1)	-0.6	22	14% (3)	9% (2)	-0.33
I47V/A	19	21% (4)	11% (2)	-0.67	21	14% (3)	10% (2)	-0.33
F53L	18	50% (9)	33% (6)	-1.65	21	29% (6)	14% (3)	-0.39
I54L or M	22	41% (9)	23% (5)	-1.03	30	17% (5)	17% (5)	-0.27
G73	35	54% (19)	40% (14)	-1.66	39	18% (7)	10% (4)	-0.38
T74	21	52% (11)	29% (6)	-1.86	28	18% (5)	7% (2)	-0.42
I84V	50	58% (29)	42% (21)	-1.86	37	5% (2)	5% (2)	-0.34
L89M/V/S/I	32	53% (17)	22% (7)	-1.84	28	7% (2)	4% (1)	-0.094
T91S	10	50% (5)	20% (2)	-1.97	7	0%	0%	-0.597
Q92K/R	16	63% (10)	25% (4)	-1.61	10	30% (3)	20% (2)	-0.502

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The presence of mutations at I82 with the I84V mutation is a combination detrimental to the antiviral activity of most PIs. Of the subjects receiving darunavir/rtv in Studies C202 and C213 with mutations at I82 and I84V, 81% (17/21) had a 1 log₁₀ decrease from baseline and 67% (14/21) achieved <50 copies/mL HIV RNA. This is compared to 6% (1/16) that achieved a 1 log₁₀ decrease and <50 copies/mL in the comparator PI arm.

BASELINE PHENOTYPE AND VIROLOGIC OUTCOME ANALYSES

An analysis of response by 5-fold increments of baseline darunavir phenotype (fold change from reference) using a censored as-treated dataset showed that response rates <50 copies/mL at week 24 decreased when baseline phenotype was ≥20-fold (Table 25). Baseline darunavir phenotype (shift in susceptibility relative to reference) was shown to be a predictive factor of virologic outcome. Response rates were 54%, 43% and 14% when baseline darunavir phenotype was 0-10, >10-20, and >20, respectively (Table 26). In Studies C202 and C213, the median baseline phenotype of responders was 2.1 (n=85) and the median baseline phenotype of virologic failures was 17 (n=40).

Table 25. Proportion of Responders in Studies C202 and C213 by Baseline Phenotype; As-Treated Analysis

Baseline Phenotype n=123	N	1 log ₁₀ decrease at Week 24	<50 copies/mL at Week 25	DAVG ₂₄
0-5	62	82%	58%	-2.28
>5-10	19	79%	42%	-2.06
>10-15	3	67%	0%	-2.68
>15-20	11	55%	55%	-2.34
>20-25	7	29%	14%	-0.64
>25-30	4	50%	25%	-1.81
>30-35	2	50%	50%	-2.35
>35-40	5	40%	20%	-1.77
>40	10	20%	0%	-0.796

Table 26 . Response to 600/100 mg Darunavir/rtv by Baseline Darunavir Phenotype: Studies C202 and C213 As-Treated

Baseline Darunavir Phenotype n=123	1 log ₁₀ decrease at Week 24	<50 copies/mL at Week 24	DAVG ₂₄
0-10	81% 66/81	54% 44/81	-2.20
>10-20	57% 8/14	43% 6/14	-2.34
>20	32% 9/28	14% 4/28	-1.03

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Analyzing an additional 225 subjects (217 with phenotypic data) from POWER 3 which had reached week 24 in an as-treated analysis determined that a 10-fold darunavir phenotypic cutoff resulted in a reduced response of 41% with a 1 log₁₀ decrease from baseline and 16% with <50 copies/mL (Table 27).

Table 27. Proportion of Responders in Study C215 by Baseline Phenotype

Baseline Phenotype n=217	N	1 log ₁₀ decrease at Week 24	<50 copies/mL at Week 24
0-5	137	86% (118)	55% (76)
>5-10	24	58% (14)	33% (8)
>10-15	7	29% (2)	0% (0)
>15-20	6	33% (2)	17% (1)
>20-25	0	0% (0)	0
>25-30	4	0% (0)	0
>30-35	7	71% (5)	29% (2)
>35-40	2	0% (0)	0
>40	30	47% (14)	20% (6)

Adding the phenotypic data from POWER 3 to POWER 1 and 2 resulted in 340 subjects with a median baseline darunavir phenotype of 3. In these three studies, the median baseline phenotype of responders was 2.1 (n=238) and the median baseline phenotype of virologic failures was 15 (n=102). Phenotypic subgroups of 0-3, >3-10 and >10 described responses rates in three tiers of 87%, 69% and 41% with a 1 log₁₀ decrease from baseline, respectively, and 59%, 38%, and 19% with <50 copies/mL, respectively. Subjects who had darunavir phenotypes >10 at baseline had a median DAVG of -1.06 at week 24. After darunavir labeling negotiations with the sponsor, the sponsor preferred response rates for four phenotypic ranges. Therefore, the response rates for the quartile ranges of the virologic failures (0-2, >2-7, >7-30 and >30) were displayed in the package insert (Table 28). See Appendix C and D for more phenotypic subgroup data.

Table 28. Response to 600/100 mg Darunavir/rtv by Baseline Darunavir Phenotype: Studies C202, C213 and C215 As-Treated Analysis

Baseline Darunavir Phenotype n=340	Proportion with ≥1 log ₁₀ decrease at Week 24	Proportion with <50 copies/ml at Week 24	DAVG ₂₄
0 - 2	88% 119/136	60% 82/136	-2.28
>2 - 7	73% 62/85	47% 40/85	-2.14
>7 - 30	52% 33/63	24% 15/63	-1.56
>30	43% 24/56	18% 10/56	-1.19

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RESPONSE BY SUSCEPTIBLE NRTIS AND ENFUVIRTIDE USE

The number of susceptible drugs in the optimized background regimen and enfuvirtide use affected darunavir/rtv response rates in Studies C202 and C213. Subjects with no susceptible NRTIs at baseline had lower response rates (38% with 1 log₁₀ decrease and 13% with <50 copies/mL) than those with at least one susceptible NRTI (Table 29).

Note: 81% (102/125) of subjects in Studies C202 and C213 taking 600 mg darunavir/rtv were also taking tenofovir disoproxil fumarate in their current ARV and 65% (81/125) had previous tenofovir use.

Table 29. Response to 600 mg Darunavir/rtv by Number of Susceptible NRTIs

# Susceptible NRTIs	1 log ₁₀ decrease at Week 24	<50 copies/mL at Week 24	DAVG ₂₄
0	38% (3/8)	13% (1/8)	-1.51
1	75% (3/4)	50% (2/4)	-2.16
2	79% (11/14)	64% (9/14)	-1.85
3	41% (9/22)	32% (7/22)	-1.65
>3	76% (58/76)	47% (36/76)	-2.20

Twenty-four percent (75/319) of subjects in Study C202 and 11% (36/318) of subjects in Study C213 had previous ENF use. Fifty percent of subjects in Study C202 and 43% of subjects in Study C213 used ENF concomitantly with darunavir in the POWER 1 and 2 studies. Enfuvirtide use with darunavir/rtv in subjects that had never received ENF before resulted in better than the overall response rates (54% achieved <50 copies/mL) (Table 30). Those who had prior ENF use and received ENF with darunavir/rtv had low response rates (18% achieved <50 copies/mL). Response rates for those who received darunavir/rtv without concomitant ENF responded similarly to the overall response (46% achieved <50 copies/mL).

Table 30. Response (1 log₁₀ decrease and <50 copies/mL) by ENF Use in Studies C202 and C213

Studies C202 and C213	Darunavir/rtv ¹		Control ²	
	1 log ₁₀ decrease	<50 copies/mL	1 log ₁₀ decrease	<50 copies/mL
ALL	68% (85/125)	45% (56/125)	21% (25/120)	13% (15/120)
+ENF* use	88% (36/41)	54% (22/41)	32% (6/19)	21% (4/19)
No ENF use	66% (44/67)	46% (31/67)	20% (19/95)	12% (11/95)
+ENF with prior ENF use	29% (5/17)	18% (3/17)	0% (0/6)	0% (0/6)

* no prior ENF use

¹ Of those on ENF: 71% (41/58) no previous use; 29% (17/58) previous use

² Of those on ENF: 76% (19/25) no previous use; 24% (6/25) previous use

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The significance of concomitant ENF use was seen when response was analyzed by baseline phenotype and ENF use in Studies C202 and C213 (Table 31). For subjects with baseline darunavir phenotypes of >10, response rates were 81% (13/16) when ENF was used for the first time concomitantly with darunavir/rtv while response rates were 17% (3/18) for those who did not use ENF concomitantly. For subjects with baseline darunavir phenotypes of >10 in Studies C202, C213 and C215, response rates were 81% (13/16) when ENF was used for the first time concomitantly with darunavir/rtv while response rates were 36% (27/74) for those who did not use ENF concomitantly (Table 32).

Table 31. Proportion of Responders by Baseline Phenotype and ENF Use in Studies C202 and C213

Baseline Darunavir Phenotype n=123	1 log₁₀ decrease Week 24 <50 copies/mL at Week 24	+ENF Use 1 log₁₀ decrease Week 24 <50 copies/mL at Week 24	No ENF Use 1 log₁₀ decrease Week 24 <50 copies/mL at Week 24
0-10	81% (66/81) 54% (44/81)	92% (23/25) 52% (13/25)	81% (38/47) 60% (28/47)
>10-20	57% (8/14) 43% (6/14)	100% (7/7) 86% (6/7)	17% (1/6) 0% (0/6)
>20	32% (9/28) 14% (4/28)	67% (6/9) 33% (3/9)	25% (2/12) 8% (1/12)

Table 32. Proportion of Responders by Baseline Phenotype and ENF Use in Studies C202, C213 and C215

Baseline Darunavir Phenotype n=123	1 log₁₀ decrease Week 24 <50 copies/mL at Week 24	+ENF Use 1 log₁₀ decrease Week 24 <50 copies/mL at Week 24	No ENF Use 1 log₁₀ decrease Week 24 <50 copies/mL at Week 24
0 - 5	85% (169/199) 56% (112/199)	94% (15/16) 56% (9/16)	85% (149/176) 57% (100/176)
>5 - 10	67% (29/43) 37% (16/43)	89% (8/9) 44% (4/9)	66% (21/32) 38% (12/32)
>10	41% (40/98) 19% (19/98)	81% (13/16) 56% (9/16)	36% (27/74) 14% (10/74)

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**PROTEASE MUTATIONS DEVELOPING ON DARUNAVIR/RTV
TREATMENT**

In the Phase 2 studies C213 (POWER 1), C202 (POWER 2), and C215 (POWER 3), multiple protease inhibitor-resistant HIV-1 isolates from highly treatment-experienced subjects who received 600/100 mg Darunavir/rtv b.i.d. and experienced virologic failure, either by rebound, or by never being suppressed, developed amino acid substitutions that were associated with a decrease in susceptibility to darunavir. The mutations that developed on darunavir in the virologic failures in these studies were determined by comparing the genotype of the failure isolate with the genotype of the baseline isolate (See Appendix E, F and G). The number of changes at each amino acid position were totaled from each of the studies (Table 33) and summarized in Table 34. Amino acid substitution V32I developed on Darunavir/rtv 600/100 mg b.i.d. in greater than 30% of virologic failure isolates and the substitutions at amino acid position I54 developed in greater than 20% of virologic failure isolates (Table 34). In 80% of cases, the change at amino acid position was I54V to L. Other substitutions that developed in 10% to 20% of Darunavir/rtv virologic failure isolates occurred at amino acid positions I15, L33, I47, G73 and L89.

Table 33. Mutations Developing on 600 mg Darunavir/rtv Treatment

Mutations Developing	Study C202 Power 2 (N=23)	Study C213 Power 1 (N=17)	Study C215 Rebounds (N=46)	Study C215 Never Suppressed (N=78)
T4A/T	0	1 (5.9%)	1 (2%)	
L10F/I/V ²	2 (8.7%)	2 (11.8%)	7 (15%)	4 (5%)
V11I/L ²	3 (13%)	3 (17.6%)	1 (2%)	
T12K/P	1 (4%)	0	3 (6.5%)	
I13V/L ²	1 (4%)	1 (5.9%)	4 (8.7%)	
K14R	0	1 (5.9%)	1 (2%)	
I15V ²	3 (13%)	4 (23.5%)	5 (11%)	10 (13%)
G16A ²	0	0	2 (4.3%)	
K20R/V/T/I	4 (17%)	3 (17.6%)	4 (8.7%)	2 (3%)
L24M	1 (4%)	0	0	
V32I ²	12 (52%)	8 (47%)	16 (35%)	22 (28%)
L33F/M ²	6 (27%)	6 (35%)	4 (8.7%)	6 (8%)
E34Q/T/K	5 (22%)	0	2 (4.3%)	
E35D/N	2 (8.7%)	1 (5.9%)	2 (4.3%)	
M36L/I	3 (13%)	2 (11.8%)	2 (4.3%)	
S37N/T/D ^{1, 2}	3 (13%)	2 (11.8%)	4 (8.7%)	
R41K/Q ¹	0	0	2 (4.3%)	
K43T/R	1 (4%)	2 (11.8%)	3 (6.5%)	
M46I/L ²	4 (17%)	1 (5.9%)	4 (8.7%)	
I47V ²	4 (17%)	2 (11.8%)	5 (11%)	8 (10%)
G48V/M/E	0	0	2 (4.3%)	
I50V ²	2 (8.7%)	2 (11.8%)	2 (4.3%)	
F53L ²	4 (17%)	0	7 (15%)	2 (3%)
I54L/M/V	4 (17%)	5 (29%)	16 (35%)	15 (19%)
K55R/N ¹	3 (13%)	1 (5.9%)	1 (2%)	

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R57K/R	0	1 (5.9%)	0	
Q58E	0	1 (5.9%)	3 (6.5%)	
D60E	1 (4%)	0	0	
Q61E/N/H	0	1 (5.9%)	3 (6.5%)	
L63P ²	0	1 (5.9%)	0	
I66F/V	1 (4%)	0	3 (6.5%)	
G68E/G	1 (4%)	0	0	
K70E ¹	0	1 (5.9%)	1 (2%)	
A71I/V/M ^{1,2}	1 (4%)	2 (11.8%)	3 (6.5%)	
I72L	0	1 (5.9%)	2 (4.3%)	
G73S/D/N/T/A/C/I ²	5 (22%)	2 (11.8%)	9 (19.6%)	5 (6%)
T74S/A/P ¹	2 (8.7%)	1 (5.9%)	6 (13%)	
V77I/T/L ¹	1 (4%)	1 (5.9%)	2 (4.3%)	
P79S/T/D	2 (8.7%)	2 (11.8%)	1 (2%)	
V82I/A ²	2 (8.7%)	2 (11.8%)	2 (4.3%)	
I84V ²	1 (4%)	1 (5.9%)	6 (13%)	
I85V ¹	0	1 (5.9%)	4 (8.7%)	
L89V/I/M/F	4 (17%)	5 (29%)	8 (17%)	11 (14%)
T91S ²	0	1 (5.9%)	1 (2%)	
Q92R ²	0	0	2 (4.3%)	
C95F	0	0	3 (6.5%)	
T96S/T	1 (4%)	0	0	

¹ mutation developed in vitro in naïve strains

² mutation developed in vitro in resistant strains

Table 34. Summary of Mutations Developing on 600 mg Darunavir/rtv Treatment

Mutations Developing	Studies C202, C213, and C215 N=164
L10F/I/V	15 (9%)
I15V	22 (13%)
K20R/V/T/I	13 (8%)
V32I	58 (35%)
L33F/M	22 (13%)
I47V	19 (12%)
F53L	13 (8%)
I54L/M/V	40 (24%)
G73S/D/N/T/A/C/I	21 (13%)
L89V/I/M/F	28 (17%)

The median darunavir phenotype (fold change from reference) of the virologic failure isolates in Studies C202, C213 and C215 was 21-fold at baseline and 94-fold at failure. Amino acid substitutions were also observed in the protease cleavage sites of some darunavir virologic failure isolates.

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IN VIVO CROSS-RESISTANCE

In the C202 and C213 studies, where the subjects were highly treatment-experienced and 98% of subjects were resistant to at least one PIs with 68% having no susceptible PI, 58% (35/60) in Study C202 and 69% (45/65) in Study C213 were susceptible to darunavir at baseline using a 10-fold as a cutoff for darunavir susceptibility. Sixty percent (24/40) of subjects with decreased susceptibility to tipranavir (fold change >3) at baseline demonstrated a 1 log₁₀ decrease from baseline at week 24 on darunavir/rtv and 45% (18/40) achieved <50 copies/mL serum HIV RNA levels. In Study C215, 60% (64/107) of subjects with resistance to tipranavir (>3-fold change) at baseline achieved a 1 log₁₀ decrease from baseline at week 24 on darunavir/rtv and 33% (35/107) achieved <50 copies/mL serum HIV RNA levels.

Of the viruses isolated from subjects experiencing virologic failure on darunavir/ritonavir 600/100 mg b.i.d., greater than 50% were still susceptible to tipranavir while less than 5% were susceptible to other protease inhibitors (amprenavir, atazanavir, indinavir, lopinavir, nelfinavir, ritonavir, or saquinavir) (Table 35).

Table 35. Cross-Resistance of Virologic Failures (N=74)

PI	Median Fold change	% Susceptible (n)
APV	74.9	3% (2)
ATV	82.1	4.1% (3)
IDV	44.9	5% (4)
LPV	99.3	3% (2)
NFV	41.2	3% (2)
RTV	145.3	3% (2)
SQV	32.2	14% (10)
TPV	2.3	57% (42)

5 CONCLUSION

This NDA for is approvable with respect to microbiology for combination antiretroviral treatment of HIV-1 infected treatment-experienced adult subjects.

We request the following to be submitted with traditional approval (included in the approval letter of this NDA):

- Determine response rates based upon presence of specific cleavage site mutations at baseline and submit this analysis with the PREZISTA traditional approval application.
- Determine the protease cleavage site mutations that occur most frequently (>10%) in virologic failure isolates and submit this analysis with the PREZISTA traditional approval application.
- Determine if the most frequently occurring protease cleavage site mutations contributed to decreases in darunavir susceptibility through site-directed

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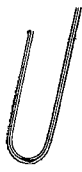
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mutagenesis and submit this analysis with the PREZISTA traditional approval application.

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 § 552(b)(4) Trade Secret / Confidential

 § 552(b)(5) Deliberative Process

✓ § 552(b)(5) Draft Labeling

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7 APPENDICES

**Appendix A. Type of Baseline PI Mutation and Virologic Response
Study C202 and C213: Primary Endpoint - Proportion of Responders with
confirmed $\geq 1 \log_{10}$ decrease at Week 24**

Mutation	C202		C213	
	600 mg DRV n=60	Control N=58	600 mg DRV n=65	Control N=62
Overall	65% 39/60	16% 9/58	71% 46/65	26% 16/62
L10I/F/V/Y	63% 36/57	16% 9/55	70% 43/61	25% 14/57
V11I/L	33% 2/6	43% 3/7	71% 5/7	20% 1/5
I13V/M/A	46% 12/26	13% 4/31	62% 16/26	11% 3/27
I15V	47% 7/15	16% 3/19	69% 18/26	33% 7/21
G16A/Q/E	57% 4/7	10% 1/10	42% 5/12	17% 1/6
K20R/I/M/T	56% 18/32	10% 3/29	70% 31/44	26% 9/35
D30N	2/2	0	75% 3/4	25% 1/4
V32I	25% 2/8	0% 0/10	0% 0/4	25% 3/12
L33F/V/I	61% 14/23	15% 5/34	71% 20/28	14% 4/28
E35D/N/G	57% 16/28	15% 3/20	74% 25/34	24% 7/29
M36I/L/V	54% 19/35	6% 2/31	70% 32/46	19% 8/42
S37N/D/E/H/K/T/C	65% 36/55	16% 9/55	71% 44/62	22% 12/55
R41K/N/T	80% 8/10	14% 3/22	72% 18/25	21% 4/19
K43T/S/I	69% 9/13	15% 2/13	64% 7/11	27% 3/11
M46I/L/V	64% 28/44	15% 6/41	63% 26/41	27% 12/45
I47V/A	11% 1/9	9% 1/11	30% 3/10	20% 2/10
G48V/A/M/Q	80% 8/10	0/5	83% 5/6	25% 1/4
I50V	57% 4/7	0/2	1/2	0/1
F53L	13%	11%	80%	42%

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	1/8	1/9	8/10	5/12
I54V/L/M/A/T	63% 31/49	17% 8/47	72% 38/53	24% 12/51
K55R	69% 11/16	30% 3/10	91% 10/11	21% 3/14
A71V/T/L/I	57% 27/47	17% 7/42	74% 35/47	21% 9/42
G73S/T/A/C	53% 10/19	21% 4/19	56% 9/16	15% 3/20
T74P/S/A/K/R/E	57% 4/7	14% 2/14	50% 7/14	21% 3/14
V77I	73% 11/15	29% 6/21	69% 9/13	36% 5/14
P79R/A/D/S	2/4	0/4	25% 1/4	0/2
V82A/S/T/C/M/L/I/F	73% 29/40	21% 8/39	81% 35/43	26% 11/42
I84V/A	56% 14/25	4% 1/24	62% 16/26	14% 2/14
I85V	67% 4/6	13% 1/8	57% 4/7	80% 4/5
N88D	3/3	1/1	80% 4/5	33% 2/6
L89M/V/S/I	38% 6/16	0% 0/12	69% 11/16	13% 2/16
L90M	48% 16/33	15% 5/34	70% 23/33	21% 7/33
T91S	33% 2/6	0/5	75% 3/4	0/2
Q92K/R	17% 1/6	1/3	90% 9/10	29% 2/7
I93L/M/V	68% 17/25	19% 6/31	67% 18/27	
C95F/V	2/4	0/2	1/1	0/4

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**Appendix B. Type of Baseline PI Mutation and Virologic Response
Study C202 and C213: Primary Endpoint - Proportion of Responders with <50
copies/mL at Week 24**

Mutation	C202		C213	
	600 mg DRV N=60	Control N=58	600 mg DRV N=65	Control N=62
Overall	38% 23/60	9% 5/58	51% 33/65	16% 10/62
L10I/F/V/Y	39% 22/57	9% 5/55	52% 32/61	14% 8/57
V11	33% 2/6	29% 2/7	57% 4/7	20% 1/5
I13V/M/A	27% 7/26	3% 1/31	35% 9/26	7.4% 2/27
I15V	27% 4/15	5% 1/19	50% 13/26	19% 4/21
G16A/Q/E	43% 3/7	10% 1/10	42% 5/12	0/6
K20R/I/M/T	38% 12/32	3% 1/29	45% 20/44	14% 5/35
D30N	2/2	0	2/4	25% 1/4
V32I	13% 1/8	0% 0/10	0% 0/4	17% 2/12
L33F/V/I	43% 10/23	12% 4/34	50% 14/28	7% 2/28
E35D/N/G	39% 11/28	5% 1/20	50% 17/34	10% 3/29
M36I/L/V	37% 13/35	3% 1/31	46% 21/46	9.5% 4/42
S37N/D/E/H/K/T/C	36% 20/55	9% 5/55	50% 31/62	13% 7/55
R41K/N/T	50% 5/10	9% 2/22	40% 10/25	21% 4/19
K43T/S/I	46% 6/13	0% 0/13	36% 4/11	9% 1/11
M46I/L/V	39% 17/44	10% 4/41	49% 20/41	16% 7/45
I47V/A	0% 0/9	9% 1/11	20% 2/10	10% 1/10
G48V/A/M/Q	40% 4/10	0/5	50% 3/6	25% 1/4
I50V	29% 2/7	0/2	1/2	0/1
F53L	0% 0/8	0/9	60% 6/10	25% 3/12
I54V/L/M/A/T	41% 20/49	11% 5/47	55% 29/53	16% 8/51
K55R	25% 4/16	10% 1/10	73% 8/11	7% 1/14

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A71V/T/L/I	38% 18/47	10% 4/42	5% 26/47	12% 5/42
G73S/T/A/C	32% 6/19	11% 2/19	50% 8/16	10% 2/20
T74P/S/A/K/R/E	29% 2/7	0/14	29% 4/14	14% 2/14
V77I	40% 6/15	14% 3/21	54% 7/13	29% 4/14
P79R/A/D/S	2/4	0/4	25% 1/4	0/2
V82A/S/T/C/M/L/I/F	43% 17/40	13% 5/39	60% 26/43	14% 6/42
I84V/A	36% 9/25	4% 1/24	46% 12/26	14% 2/14
I85V	67% 4/6	13% 1/8	43% 3/7	80% 4/5
N88D	3/3	1/1	80% 4/5	17% 1/6
L89M/V/S/I	13% 2/16	0/12	31% 5/16	6% 1/16
L90M	36% 12/33	6% 2/34	55% 18/33	12% 4/33
T91S	17% 1/6	0/5	25% 1/4	0/2
Q92K/R	0/6	0/3	40% 4/10	29% 2/7
I93L/M/V	48% 12/25	13% 4/31	44% 12/27	
C95F/V	2/4	0/2	1/1	0/4

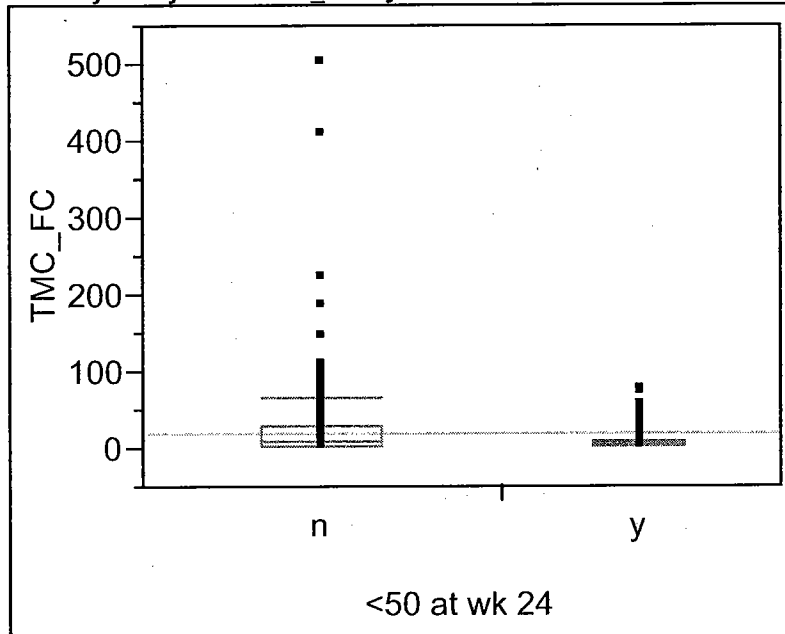
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Appendix C.

Oneway Analysis of TMC_FC By <50 at wk 24



Missing Rows
10

Quantiles

Level	Minimum	10%	25%	Median	75%	90%	Maximum
n	0.1	0.6	1.75	6.8	28.85	69.26	5
y	0.1	0.48	0.8	1.6	4.8	17.46	

n= virologic failures

y= responders

Baseline Darunavir Phenotype n=340	1 log decrease at Week 24	<50 copies/ml at Week 24	DAVG ₂₄
0 - 2	88% 119/136	60% 82/136	-2.28
>2 - 7	73% 62/85	47% 40/85	-2.14
>7 - 30	52% 33/63	24% 15/63	-1.56
>30	43% 24/56	18% 10/56	-1.19

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**Appendix D. Response to 600/100 mg Darunavir/rtv by Different Baseline
Darunavir Phenotype Subgroups: Studies C202, C213 and C215**

Baseline Darunavir Phenotype n=340	1 log decrease at Week 24	<50 copies/ml at Week 24	DAVG₂₄
0 - 2	88% 119/136	60% 82/136	-2.28
>2 - 5	79% 50/63	48% 30/63	-2.24
0 - 5	85% 169/199	56% 112/199	-2.25
>5 - 10	67% 29/43	37% 16/43	-1.92
0-10	82% 198/242	53% 128/242	-2.18
0-3	87% 149/171	59% 101/171	-2.28
>3-10	69% 49/71	38% 27/71	-2.00
>3-6	71% 24/34	41% 14/34	-2.17
>10	41% 40/98	19% 19/98	-1.06
0-6	84% 173/205	56% 115/205	-2.25
6-10	68% 25/37	35% 13/37	-1.87
0-7	82% 181/221	55% 122/221	-2.21
>7	48% 57/119	21% 25/119	-1.44
7-15	68% 21/31	19% 6/31	-1.77
>15	41% 36/88	22% 19/88	-1.06

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APPENDIX E. Mutations Developing on Darunavir/rtv Treatment in Study C202

	Study	PID	TRT GRP	WK	OUTCOME	Baseline PI Mutations	Mutations Developing on Trt	BL FC	EP FC	Change from Baseline
1	C202	202-0405	6B-REC	64	DISCONTINUED BEFORE ACHIEVING SUPPRESSION	V3I L10I I13V K14R I15V/I V32I L33F S37D M46I I47I/V L63P A71V I72I/L/M G73N/S V77I I84V L90M Q92K	V11I/V I15V I47V I54M I72L G73N L89V	3.9	329	84
2	C202	202-0411	6B-REC	16	VF-REBOUND ER	V3I L10I I13V L19V L33F E35D M36I S37N M46I K55R R57K I62V L63P A71T I72V L76V V82F L89I L90M	K20R E34K I54L I84V	27	75	2.8
3	C202	202-0603	6B-REC	29	DISCONTINUED: SUBJECT REACHED A VIROLOGIC ENDPOINT	V3I L10I V32I L33F E35D M36L S37D/N R41K I47V I54M R57K Q58E D60N L63P C67Y A71V G73S V77I N83D L89V L90M	E35N S37N M46I/M V82I/V	74	64	0.87
4	C202	202-1406	6B-REC	60	VF-REBOUND ER	V3I L10F/I/V I15I/V K20R E34Q M36I S37N K43T M46I G51A I54V K55R L63P A71V I72V L76V V82A I93L	L10F T12K I15V V32I T74S	15	149	9.8
5	C202	202-1501	6B-REC	48	VF-REBOUND ER	V3I L10I L33F E34Q M46I/M G48V I54M I62V L63P A71V P79S V82A I84V I93L	V32I M46I V77I	6.7	22.5	3.4

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6	C202	202-1526	6B-REC	3	DISCONTINUED BEFORE ACHIEVING SUPPRESSION	V3I L10F G16A L33F E34Q S37N R41K M46L G51A I54M Q61E/G L63P A71V G73T P79D I84V L89V L90M Q92E I93L		42.5	76.9	1.8
7	C202	202-1537	6B-REC	32	VF-REBOUND	V3I L10V I15V K20R V32I E34K E35D M36I S37D K43T M46L I47V I50V I54V K55R R57K L63P I66F A71V V82A L90M	L33F E34T I54L	40	240	6
8	C202	202-1803	6B-REC	24	DISCONTINUED BEFORE ACHIEVING SUPPRESSION	V3I L10I I13V L33F S37N M46I I47V F53L I54L I62V L63P H69Q A71V I72V G73T V77I V82C L90M	V32I K55K/R	20	16.5	0.8
9	C202	202-2105	6B-REC	32	VF-NEVER SUPPRESSED	V3I L10I I13M K20I L23I E35G M36I S37S/N M46I I50V F53F/L I54V R57K I62V L63P I64V I72V G73I V82I I85V L90M	S37N F53L	0.5*	94	188
10	C202	202-2603	6B-REC	48	VF-REBOUND	V3I L10I G16A L33F S37N M46I G48M F53Y I54S K55R R57K Q58E I62I/V L63P A71V V82A I84V I93L	V32I M36L	6.8	56.5	8.3

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11	C202	202-2632	6B-REC	36	DISCONTINUED BEFORE ACHIEVING SUPPRESSION	V3I L10I I15V G16E L19I K20R E35D M36I S37N M46I I47A I50V F53L Q61H L63T E65D A71V I72T T74S V82I L89M		409	162	0.4
12	C202	202-2636	6B-REC	24	VF-REBOUND ER	V3I L10I V11L I13V Q18H K20M E35D M36I S37N M46L I54L I62V L63P I64V A71V T74S I84V L89I L90M T91S I93L	L10F K20V V32I M46I I47V F53L G73D	21	558	26
13	C202	202-2913	6B-REC	48	VF-REBOUND ER	V3I T4A L10F I13V K20R L33F E35D M36I S37N I54L Q58E I62V L63P A71V I84V L90M Q92K	V11L V32I E34Q K55R/K L89V	26	330	13
14	C202	202-3121	6B-REC	24	VF-NEVER SUPPRESSED	V3I L10I I13V K20R V32I L33F E35D M36I R41K K43T M46I F53F/L I54V L63P A71V I72V G73C V82A L90M I93L C95F	E34E/K I47I/V I50V F53L K55N D60E	0.6	53	89
15	C202	202-3202	6B-REC	48	VF-REBOUND ER	V3I T4S L10F I15V K20V M36I S37D M46I I54V I62V L63P A71T I72K G73T I84V I85V L90M	V32I I54L A71I L89V	16	242	15
16	C202	202-3309	6B-REC	48	VF-NEVER SUPPRESSED	V3I L10F V11I I13V K20V S37N I54V L63P A71I I72L P79A I84V L89V L90M T91S Q92K	L24M V32I L33F I54L G73D/G P79A/S	23	1039	45

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17	C202	202-3803	6B-REC	18	DISCONTINUED AFTER REBOUND	V3I L10I I13V G16A L33F E34Q G48I I54M Q58E L63P H69Y A71V V77I P79S V82S I84V L89I L90M I93L	V32I S37S/T M46I/M I47I/V G68E/G V82A	16.4	300	18
18	TMC1 14- C202	202-4404	6B-REC	48	VF-REBOUND ER	V3I L10F I13V I15V K20I M36I S37D M46I I54V L63P A71V T74P I84V L90M T91S I93L	V11I K20T V32I L33F G73S P79P/T L89V	33	412	13
19	TMC1 14- C202	202-4408	6B-REC	24	VF-REBOUND ER	V3I L10I I15V K20R V32I E35D M36I S37N/S K43T M46I I47V F53L I54L R57K I62V L63P A71V V82A L90M	I13I/V L33F E34Q I50V I66F/I	7.6	331	44
20	C202	202-4913	6B-REC	48	VF-NEVER SUPPRES SED	V3I L10Y I13V Q18H L19V K20V L33F E35D M36I S37D I54V R57K D60E I62V L63P C67F A71V I72F G73S L76V I84V L89V L90M C95L	I15I/V V32I E35D/N M36I/L	17.6	158	9
21	C202	202-4915	6B-REC	24	DISCONTINUED BEFORE ACHIEVING SUPPRESSION	V3I L10F I13V Q18H A22V M36I S37E M46I I47V I54V L63P C67C/Y H69K A71V G73D N83D I84V L89V T91S Q92K I93L	M36L	13.7	99	7
22	C202	202-5508	6B-REC	24	DISCONTINUED BEFORE ACHIEVING SUPPRESSION	V3I L10F V11I I13V I15V Q18H K20I E21K E35D M36I S37D M46I I54S K55R D60E I62V L63P A71V I72V G73D V82A	K20T V32I L33F K43T G73D/N T74A/T T96S/T	4.3	265	62

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						L89V L90M Q92K				
23	C202	202- 6008	6B- REC	24	DISCONTI NUED BEFORE ACHIEVIN G SUPPRESI ON	V3I L10F I13V K20R V32I E35D M36I S37N M46I I47V F53F/L I54L L63P A71T I72V I84V L89V L90M/L	L33F F53L	37.4	198	5.3

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APPENDIX F. Mutations Developing on Darunavir/rtv Treatment in Study C213

	Study	PID	TRT GRP	Wk	OUTCOME	Baseline PI Mutations	Mutations Developing on Trt	BL FC	EP FC	Change from Baseline
1	TMC114-C213	213-0019	6B-REC	54	DISCONTINUED BEFORE ACHIEVING SUPPRESSION	V31 L10I V11I I15V G16A K20I/V V32I L33I/L/M E34Q S37N M46I I47V I54M D60E L63P A71V I72I/L G73S T74I/T V77I P79A/D I84V I85I/V L90M	K20I L33M I72L G73T P79D I85V	146	757	5
2	TMC114-C213	213-0027	6B-REC	48	DISCONTINUED BEFORE ACHIEVING SUPPRESSION	V31 L10I I13V G16E/G K20R/K E34Q S37N M46I I47V I54M Q61N I62I/V L63P I64V T74S L76V I84V	V32I/V L33F/L	503	340	0.68
3	TMC114-C213	213-0030	6B-REC	48	VF-NEVER SUPPRESSED	V31 L10F T12E G16A L33F E34Q S37N M46I I54M L63P A71I I72L G73S V77I P79S I84V L90M I93L	I13V K14R V32I I47I/V A71M	36	492	14
4	TMC114-C213	213-0056	6B-REC	87	DISCONTINUED AFTER REBOUND	V31 L10F K14R I15V L19P K20I E35N M36I S37D R41T K45V I50V F53L I54V I62V I64V A71V V82A I85V L89I	K20T L33F E35D S37N K70E T91S	144	353	2.5
5	TMC114-C213	213-0124	6B-REC	>48	VF-NEVER SUPPRESSED	V31 T4A L10F V11I I13V K20R L33F E35D M36I R41K M46L I54V I62V L63P K70E A71V I72I/V T74P I84V L90M I93L	I15V K43T I54L	23	227	9.8
6	TMC114-C213	213-0135	6B-REC	68	VF-REBOUNDER	V31 T4P L10Y I13V K20R L33F E35D M36I S37D R41K M46L I54V Q58E D60E L63P A71V I72T G73G/S V82A I84V L89V L90M Q92K I93L	I15V V32I G73S	35	132	3.8
7	TMC114-C213	213-0182	6B-REC	8	VF-REBOUNDER	V31 L10I/V I13V K20R V32I L33F E35D M36I S37N P39P/S K43T M46I I47V I54I/L K55R I62V L63P A71V T74P P79P/S I84V L90M I93L	L10I V11I I54L Q61E P79S	64	521	8

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8	TMC114-C213	213-0238	6B-REC	69	DISCONTINUED AFTER REBOUND	V3I L10F I15I/V G16A S37D M46I I47A L63P I84V L89M I93L	V32I L33F M36L R57K/R T74S/T L89M/I	1.6	93	58
9	TMC114-C213	213-0247	6B-REC	20	VF-REBOUNDER	V3I L10I L24I E35D M36I S37N R41K M46L I54V I62V L63P I64V A71V T74S V77I V82A I93L		0.4	2	5
10	TMC114-C213	213-0361	6B-REC	40	VF-REBOUNDER	V3I L10I I15V K20K/R E35D M36I S37D R57K I62V L63P A71A/I/T/V I84V L90M Q92R	L10F K20R V32I L33F I54L Q58E A71V V82I/V L89V	1.4	205	146
11	TMC114-C213	213-0463	6B-REC	8	VF-REBOUNDER	V3I L10I I13V G16A K24F L33F E34Q S37N K43K/P/Q/T I54L R57K Q61E I62V L63P A71T I72T G73T V82A L90M I93L	V32I M36I/M K43T M46L I84V L89V	20	200	10
12	TMC114-C213	213-0584	6B-REC	>48	VF-REBOUNDER	V3I L10F I13V I15V K20T D30N V32I/V M36I S37N M46I I47V F53L L63P V77I N88D	V32I L33F S37N/T I54I/L/M	1.8		
13	TMC114-C213	213-0588	6B-REC	16	VF-REBOUNDER	V3I L10F I13V K20T L33F E35D M36I S37N K43T M46L I54V D60E I62V L63P T74P V82A/V I85V L89M	I15I/V I50I/V I54I/V V77I/V	6.3	392	62
14	TMC114-C213	213-0608	6B-REC	48	VF-REBOUNDER	V3I L10I I13A K20R A22V E35D M36I S37N R41K M46I R57K Q61N I62V L63P I64V A71V L76V N83D I84V T91S	V11I/V I47V K55K/R	7.4	101	13.7
15	TMC114-C213	213-0617	6B-REC	32	DISCONTINUED BEFORE ACHIEVING SUPPRESION	V3I L10I I15I/V K20R V32I E35D M36I S37N K43T M46I I47V I54M I62V L63P I66F A71V I72M G73S L90M	I15V I50V V82I L89V	63	380	6
16	TMC114-C213	213-0632	6B-REC	40	DISCONTINUED AFTER REBOUND	V3I L10I I15V G15A K20T S37N M46I I54V Q58E I62V L63P A71V I72L G73S L76V V82M L90M	T4A/T V11I V32I L89V	3.6	244	68

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17	TMC114- C213	213- 0698	6B- REC	48	VF- REBOUNDER	V3I I15V M36I S37N L63P/L I64V H69Q L90M	L63P	0.4	0.4	1
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APPENDIX G. Mutations Developing on Darunavir/rtv Treatment in Study C215

	Study	PID	TRT GRP	W K	OUT- COME	Baseline PI Mutations	Mutations Developing on Trt	BL FC	EP FC	Change from Baseline
1	TMC11 4-C215	215- 0002	CONTR OL / TMC114	48	VF- REBOUN DER	V3I L10F K14K/R I15V K20I M36I S37D M46I I54M I62V L63P A71V G73S I84V I85V I93L	K20T L33F L89V	7.1	75.8	10.7
2	TMC11 4-C215	215- 0004	CONTR OL / TMC114	8	DISCON TINUED AFTER REBOUN D	V3I L10I I13V K20T L33F E34Q E35D M36V S37E G48M/V I54M I62V L63P I64M H69Q A71I I72V V77I V82A L90M I93L	V32I I47V G48M G73S I84V L89V	37.6	256.7	6.8
3	TMC11 4-C215	215- 0006	CONTR OL / TMC114	32	VF- REBOUN DER	V3I T4P L10F I13V K20T L33F E35D M36I S37E R41K K43T M46L F53F/L I54V K55R R57K/R D60E I62V L63P A71V V82A L90M Q92Q/R I93L C95F	F53L G73S I84V L89V Q92R	6.7	42.9	6.4
4	TMC11 4-C215	215- 0009	CONTR OL / TMC114	44	VF- REBOUN DER	V3I L10F I15I/V L24I L33F E35D M36I S37D/N R41K/R M46L I54V I62V L63P V82A	I15V V32I M36L S37N G73I	0.03	11.6	386.7

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5	TMC11 4-C215	215- 0012	CONTR OL / TMC114	40	VF- REBOUN DER	V3I L5F/L L10V V11I I13V I15I/V G16A/G V32I L33F E34Q S37N K43T M46L I54I/V Q58E/Q L63P A71V G73S T74P/T V77I V82V/A I84V L89V L89V L90M I93L	L5F G16A I54L T74P	55.7	681	12
6	TMC11 4-C215	215- 0013	CONTR OL / TMC114	16	VF- REBOUN DER	L10I G16A/G L33F E34Q S37N M46I I47V F53L I54V D60E I62V L63P I66V H69Y A71T I72E G73A/T V77I I84V L90M I93L C95C/F	V3I G16A I54L G73A C95F	4.8	112	23
7	TMC11 4-C215	215- 0014	CONTR OL / TMC114	32	VF- REBOUN DER	V3I L10V I13V I15V K20R V32I L33F M36I S37N M46I I47V I54MG58E D60E Q61E L63P I64V H69Y A71V V82A L90M I93L	I84V	25.8	405.6	15.7

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8	TMC11 4-C215	215- 0015	CONTR OL / TMC114	42	VF- REBOUN DER	V3I L10V I13V LL33F E35D M36I S37N M46I I54S L63P A71I/V I72V V82T L90M Q92K I93L C95F	L10I T12P V32I I54A/V I62I/V A71V T74P P79P/T I84V	2.5	43.1	17.2
9	TMC11 4-C215	215- 0027	CONTR OL / TMC114	16	DISCON TINUED AFTER REBOUN D	V3I L10I L19VL24I L33F E35D M36I S37T K43T M46L I54V D60E I62V L63P A71V I72M G73S/T V82A	K20R S37D I50V G73C	41.9	74.7	1.8
10	TMC11 4-C215	215- 0032	CONTR OL / TMC114	16	VF- REBOUN DER	V3I L10I V11L/V K14K/R I15I/V L19L/V K20R L33F E35D M36I S37D M46I I54V I62V L63P H69R A71V I72L G73S V82A/V I84V I85V L90M	L10F K14R I15V Q18H V32I G48E/G I54L I66F G73N	6.7	132	20
11	TMC11 4-C215	215- 0033	CONTR OL / TMC114	16	VF- REBOUN DER	V3I L10F V32I L33F S37N K43T M46I I47V F53F/L I62V L63P I66L I72T G73C V77I V82C L90M I93L	F53L I54L C67C/I/V A71T	62	45	0.7

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12	TMC11 4-C215	215- 0037	CONTR OL / TMC114	36	VF- REBOUN DER	V3I L10I I13V K20R L33F M36L S37D R41K I54V Q58E I62V L63P H69K G73S T74K V82A N83D L89V L90M	F53F/L	0.4	0.7	1.8
13	TMC11 4-C215	215- 0039	CONTR OL / TMC114	33	VF- REBOUN DER	V3I L10F V11I I13V K14R I15V K20I M36I/M S37N R41E/K M46I I54V I62V L63P A71V I72M/V G73S P79R I84V L90M T91S	K20V V32I L33F M36I R41K I72M T74A L89V	71	13.8	0.19
14	TMC11 4-C215	215- 0045	CONTR OL / TMC114	8	DISCON TINUED AFTER REBOUN D	V3I L10F I13V K20T V32I L33F E35N M36I S37N K43T M46I I47V I54L R57K I62I/V L63P I66F A71V V82A L89I/L/M L90M	F53L/F G73A/G/S/T	10.4	98.9	9.5
15	TMC11 4-C215	215- 0047	CONTR OL / TMC114	14	VF- REBOUN DER	V3I L10F I13V K20M L24I L33F E35N M36I S37N K43T M46I I54S/T I62V L63P I64I/V T74A/S V82A L89L/M	V32I I54S T74S L89M	16.9	20	1.2

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16	TMC11 4-C215	215- 0064	TMC114 / TMC114	10	VF- REBOUN DER	V3I L10FI/I T12P G16E/G L19V K20R L24I V32I L33F E35D M36I S37N M46I I47V F53F/L I54M K55R I62V L63A I64I/V H69Q A71V V77I V82A I84I/V	I15I/V	18	88	4.9
17	TMC11 4-C215	215- 0065	CONTR OL / TMC114	8	VF- REBOUN DER	V3I K14R I15V S37E P39P/S L63P		0.3	2	6.7
18	TMC11 4-C215	215- 0075	600/100 mg bid DE NOVO	12	VF- REBOUN DER	V3I L10I I13V I15V K20R V32I L33F E35D M36I S37N R41K I47V F53L K55R R57K Q61D L63P H69Y A71V I72T T74A V82L N88D L89M L90M I93L	M46I I50V Q61N	3.6	142	39.4
19	TMC11 4-C215	215- 0076	600/100 mg bid DE NOVO	16	VF- REBOUN DER	V3I L10V I15V K20R E35D M36I S37N I54V G58E D60E I62V L63P A71I V82T I84I/V L90M Q92K	V32I L33F E34Q M46I I47V F53L I54L/V K55R A71V I84V I85V	1.1	4.8	4.4

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20	TMC11 4-C215	215- 0088	600/100 mg bid DE NOVO	8	VF- REBOUN DER	V3I L10I/V V11L/V I13V I15L/V K20R L33F E35D M36I S37D/E/K/N R41K M46I G48A/G/S/W F53Y I54S Q58E/Q Q61H I62V L63T E65D/E A71V I72T T74A V82F L89M L90M I93L	L10V V11L I15V S37T Q58E	9.5	42.5	4.5
21	TMC11 4-C215	215- 0118	600/100 mg bid DE NOVO	16	VF- REBOUN DER	V3I L10V V11I I13I/L D14R I15V Q18E/Q L33F M36L S37D/N R41K K43T M46L I54V K55R I62V L63P C67F H69R A71V I72F G73S V82A I85V L90M	I13L K45R T80I	2.6	44	16.7
22	TMC11 4-C215	215- 0136	600/100 mg bid DE NOVO	12	VF- REBOUN DER	V3I L10F I13M L19T K20I E35T M36V S37N M46I I47V I54V K55R R57K I62V L63P I66I/V A71V G73T V77A/I/T/V P79A I84V L90M I93L	V32I I54L I66V V77T	0.8	x	x

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23	TMC11 4-C215	215- 0138	600/100 mg bid DE NOVO	12	VF- REBOUN DER	V3I L10I T12K/T I13I/M/V L19I/V K20R L33F/L E34K/Q/R E35D/E M36I S37D G48M I50V I54S I62V L63P H69K A71M V82A L90M I93L/M C95F/V	T12K I13V L19I V32I L33F E34Q E35D K43K/T I93L C95F	7.3	X	X
24	TMC11 4-C215	215- 0144	600/100 mg bid DE NOVO	12	VF- REBOUN DER	V3I L10F I13V K20R L23I L33I E35D M36I S37N R41K M46I I50V I54V K55R R57K D60E Q61N L63P A71V I85V N88D L89I L90M	K43K/T I47V F53L Q58E/Q L89I/V T91S/T	6.1	160.5	26
25	TMC11 4-C215	215- 0153	600/100 mg bid DE NOVO	20	VF- REBOUN DER	V3I L10F K20I L33I/L M36V S37N M46I I54V K55R I62V L63P A71V I84V I85V N88D L90M		3	X	X
26	TMC11 4-C215	215- 0155	600/100 mg bid DE NOVO	20	VF- REBOUN DER	V3I L10I L24I E35D/E M36I/M S37N/S R41K M46I I54V L63P I64V H69Q A71V V82A I93L		0.5	X	X

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27	TMC11 4-C215	215- 0205	600/100 mg bid DE NOVO	8	VF- REBOUN DER	V3I L10I I13V L33F S37N M46I G48L I50V F53C/F I54T L63S I64M A71V I72E T74A/T V77I V82A L89M I93L	I47I/V F53L V56A/V Q61N I64L T74A I85I/V	4.3	X	X
28	TMC11 4-C215	215- 0218	600/100 mg bid DE NOVO	12	VF- REBOUN DER	X	X	13.8	X	X
29	TMC11 4-C215	215- 0264	600/100 mg bid DE NOVO	8	VF- REBOUN DER	V3I L10V V11I I13I/V G16A L33F S37D/N R41K/R M46I I54V K55R Q58E/Q I62V L63P I64L I66V A71T I72T V82C I84V L90M I93L C95C/P/G/V	L10I/V I13V V32I S37N I54L Q58E G73S/G C95F	4.6	91	19.8
30	TMC11 4-C215	215- 0299	600/100 mg bid DE NOVO	16	VF- REBOUN DER	V3I L10V I15I/V L19I S37N R41K K43K/R M46I I54V K55R I62V L63P A71V I72V L76V V82T L90M I93L	I15V V32I K43R V82I I84I/V	12.9	28.4	2.2

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31	TMC11 4-C215	215- 0305	600/100 mg bid DE NOVO	19	VF- REBOUN DER	V3I L10F T12P/T I13V K14R K20T L33F E34Q S37N M46L Q58E I62V L63P I66I/V A71V V82A I84V L90M I93L C95F	T12P I54L I66V Q92Q/R	52.4	14.5	0.28
32	TMC11 4-C215	215- 0308	600/100 mg bid DE NOVO	16	VF- REBOUN DER	V3I L10V I15I/V V32I L33F E35D M36L S37N P39S R41K M46I I47V F53L Q58E I62V L63P A71V V77I I84V I93L	L10F/V I54L	100.4	24.8	0.25
33	TMC11 4-C215	215- 0310	600/100 mg bid DE NOVO	16	VF- REBOUN DER	V3I L10F V11I I13V K14R I15V K20I L33I S37N I54L Q58E D60E L63P H69Q A71V I72F G73S I84V L89V L90M	V32I M46I I47V	0.5	68	136

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34	TMC11 4-C215	215- 0311	600/100 mg bid DE NOVO	8	VF- REBOUN DER	V3I L10I I15V L19T K20R L24I V32I L33F E34Q E35D M36L S37N M46L F53L I54V K55R Q58E L63P A71A/V V77I V82A I84V	I13V V77I/L	X	72	X
35	TMC11 4-C215	215- 0336	600/100 mg bid DE NOVO	16	VF- REBOUN DER	V3I L10I V11I L33F M36L S37N M46L I54V K55R Q58E D60E I62V L63P I64V A71V I72K G73T V82A I84V L90M	V32I I54L I85V	32.7	71.1	2.2
36	TMC11 4-C215	215- 0355	TMC114 / TMC114	8	DISCON TINUED AFTER REBOUN D	V3I T4A/T L10I V11I I15V G16A K20T V32I S37N M46I I54V Q58E I62V L63P A71V I72L G73S L76V V82M L89V L90M	T4A	244	48.7	0.2
37	TMC11 4-C215	215- 0358	600/100 mg bid DE NOVO	16	VF- REBOUN DER		X	5.6	29.7	5.3

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38	TMC11 4-C215	215- 0382	600/100 mg bid DE NOVO	16	VF- REBOUN DER	V3I L10I I13V L33F S37N M46L I54L K55R D60E I62V L63P A71V G73S T74P I84V L89V L90M Q92L/Q I93L G94R	V32I G73S/T	48.8	118.6	2.43
39	TMC11 4-C215	215- 0404	600/100 mg bid DE NOVO	13	VF- REBOUN DER	V3I L10I I13V G16A L33F S37N R41K/R M46I I54V K55R I62I/V L63P I66V T74P V82C I84V L90M I93L C95F	L10I/V V32I I54L/V V82C/F/G/V L89F/L	1.1	X	X
40	TMC11 4-C215	215- 0409	600/100 mg bid DE NOVO	16	VF- REBOUN DER	V3I L10I I15V G16E K20R E35D M36I S37K R41K K43T I54V I62V L63C L90M C93L	S37K/N P39/S	0.7	X	X
41	TMC11 4-C215	215- 0416	600/100 mg bid DE NOVO	12	VF- REBOUN DER	V3I L10I V11I I13V K20I L33V E35E/G M36I S37N I54A I62V L63P C67F H69Q A71V G73G/S T74E I84V L89V L90M T91S Q92K I93L C95G	I54I/V I85I/V	15.6	X	X

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42	TMC11 4-C215	215- 0430	600/100 mg bid DE NOVO	12	VF- REBOUN DER	V3I L10F I13V L19V K20T L33F E35S M36I S37E P39P/S M46I/L I54V R57K Q61H/Q I62V L63P I66F A71T T72T G73T I84V L90M C95C/F/IS	E35N/S M46L I54L Q61H E65A/E	80.5	52.8	0.66
43	TMC11 4-C215	215- 0443	600/100 mg bid DE NOVO	8	VF- REBOUN DER	V3I L10I T12Q I13V K14R G16E L33F S37N M46I L63H A71V I72V L76V V82G I93L	V32I K70E/K T74S/T L89L/M	12.6	21.2	1.7
44	TMC11 4-C215	215- 0446	600/100 mg bid DE NOVO	19	VF- REBOUN DER	V3I L10I V11I I13V I15V L19I/L K20V V32I L33F E35D M36I R41K M46I I54A K55R R57K Q58E D60E I62V L63P A71I I72F/L G73S I84V L89V L90M	L10F K20T R41Q I54A/V I72L	3.6	X	X

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45	TMC11 4-C215	215- 0463	600/100 mg bid DE NOVO	16	VF- REBOUN DER	V3I L10I K14R/K I15V G17D K20R L23I V32I E35D M36I M46I I47V F53L I54M K55R R57K D60E I62V L63P A71V I72V V82T I85V L89I L90M		21.8	11.5	0.53
46	TMC11 4-C215	215- 0501	TMC114 / TMC114	13	VF- REBOUN DER	V3I L10F I15I/V K20M V32I L33I M36V S37N M46I I54L I62I/V L63C A71V I72V L76V I84V I93L	X	145.9	X	X
47	TMC11 4-C215	215- 0003	CONTR OL / TMC114		NEVER SUPPRE SSED		L10F I47V	2.7	x	
48	TMC11 4-C215	215- 0005	CONTR OL / TMC114		NEVER SUPPRE SSED		V11I K14K/R I15I/V M46I	33	95.2	2.8589
49	TMC11 4-C215	215- 0011	CONTR OL / TMC114		NEVER SUPPRE SSED		V11I S37N/S Q92L	118	192.3	1.6269
50	TMC11 4-C215	215- 0018	CONTR OL / TMC114		NEVER SUPPRE SSED		I15I/V E35G M36T G73D/G/N/S	50	219.9	4.3892
51	TMC11 4-C215	215- 0022	CONTR OL / TMC114		NEVER SUPPRE SSED		T4A I13I/V L33F I66I/V V82I	9.5		
52	TMC11 4-C215	215- 0026	CONTR OL / TMC114		NEVER SUPPRE SSED		K20R/K Q58E V77L	35	570.6	16.5391
53	TMC11 4-C215	215- 0028	CONTR OL / TMC114		NEVER SUPPRE SSED	V32I/V	V32I	82	291.9	3.5641
54	TMC11 4-C215	215- 0034	CONTR OL / TMC114		NEVER SUPPRE SSED		V32I I54L	27	118.5	4.3889

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55	TMC11 4-C215	215- 0038	CONTR OL / TMC114	NEVER SUPPRE SSED	L10V I13V V32I L33F K43E K45R I47I/V I54M D60D/N C67C/F A71I/V G73S V82A I84I/V L89V L90M	1.2	77.1	64.25
56	TMC11 4-C215	215- 0041	CONTR OL / TMC114	NEVER SUPPRE SSED	V11I/V L24M V32I K43K/R L89V	34	193.7	5.6637
57	TMC11 4-C215	215- 0043	CONTR OL / TMC114	NEVER SUPPRE SSED	V11L/V V32I	16	134	8.535
58	TMC11 4-C215	215- 0055	TMC114 / TMC114	NEVER SUPPRE SSED	V32I L33F L89V	229	462.3	2.0197
59	TMC11 4-C215	215- 0056	TMC114 / TMC114	NEVER SUPPRE SSED	I72I/T I84I/V L89V	75	251	3.3422
60	TMC11 4-C215	215- 0058	TMC114 / TMC114	NEVER SUPPRE SSED	T4S/T H61H/Q C67C/Y V77I/T	182	97.3	0.5352
61	TMC11 4-C215	215- 0059	TMC114 / TMC114	NEVER SUPPRE SSED	NONE	265	77.2	0.2917
62	TMC11 4-C215	215- 0062	TMC114 / TMC114	NEVER SUPPRE SSED	X	74	633	8.6122
63	TMC11 4-C215	215- 0066	TMC114 / TMC114	NEVER SUPPRE SSED	X	215		
64	TMC11 4-C215	215- 0069	TMC114 / TMC114	NEVER SUPPRE SSED	V11I/V I47I/V	211	159	0.7528
65	TMC11 4-C215	215- 0070	TMC114 / TMC114	NEVER SUPPRE SSED	M46I	690	X	
66	TMC11 4-C215	215- 0074	600/100 mg bid DE NOVO	NEVER SUPPRE SSED	E34D/E I47I/V I54M D60E/D T74P/T	46		
67	TMC11 4-C215	215- 0081	600/100 mg bid DE NOVO	NEVER SUPPRE SSED	X		X	

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68	TMC11 4-C215	215- 0085	600/100 mg bid DE NOVO	NEVER SUPPRE SSED		V11I/V E21K V32I K70K/T	2.7	98.4	36.4444
69	TMC11 4-C215	215- 0089	TMC114 / TMC114	NEVER SUPPRE SSED		A22V E34Q K43T V82I	189	534.5	2.8221
70	TMC11 4-C215	215- 0090	600/100 mg bid DE NOVO	NEVER SUPPRE SSED		I54L/V K55K/R Q58R/Q P79A V82I	28	41.6	1.47
71	TMC11 4-C215	215- 0095	600/100 mg bid DE NOVO	NEVER SUPPRE SSED		I15I/V V32I P39P/Q/S V82I L89V	5.6	73.7	13.1607
72	TMC11 4-C215	215- 0110	600/100 mg bid DE NOVO	NEVER SUPPRE SSED		V11L I54L	11.7	121.3	10.3675
73	TMC11 4-C215	215- 0115	600/100 mg bid DE NOVO	NEVER SUPPRE SSED		V32I I47I/V R57K/R Q58E/Q A71A/T	6.5	X	
74	TMC11 4-C215	215- 0123	600/100 mg bid DE NOVO	NEVER SUPPRE SSED		I54I/L F53L	41	19.3	0.4754
75	TMC11 4-C215	215- 0129	600/100 mg bid DE NOVO	NEVER SUPPRE SSED		X	130.7	X	
76	TMC11 4-C215	215- 0131	600/100 mg bid DE NOVO	NEVER SUPPRE SSED		L10F I15V Q61Q/R I85I/V	17	X	
77	TMC11 4-C215	215- 0137	600/100 mg bid DE NOVO	NEVER SUPPRE SSED					
78	TMC11 4-C215	215- 0152	600/100 mg bid DE NOVO	NEVER SUPPRE SSED		E34E/K	38	20.5	0.5467
79	TMC11 4-C215	215- 0174	600/100 mg bid DE NOVO	NEVER SUPPRE SSED		I15V V32I I54L/V K55R/K R57K/R	223	214.2	0.9601

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80	TMC11 4-C215	215- 0183	600/100 mg bid DE NOVO	NEVER SUPPRE SSED		I93V	54	38.2	0.7061
81	TMC11 4-C215	215- 0187	600/100 mg bid DE NOVO	NEVER SUPPRE SSED		V32I	59	X	
82	TMC11 4-C215	215- 0189	600/100 mg bid DE NOVO	NEVER SUPPRE SSED		V11I/V C67W T91S	106	X	
83	TMC11 4-C215	215- 0195	600/100 mg bid DE NOVO	NEVER SUPPRE SSED		T91S	2.7	X	
84	TMC11 4-C215	215- 0203	600/100 mg bid DE NOVO	NEVER SUPPRE SSED		I13V V32I/V I47V L89L/M	28	93.7	3.311
85	TMC11 4-C215	215- 0220	600/100 mg bid DE NOVO	NEVER SUPPRE SSED		V32I E34K A71A/V L89F/L T91S/T	35	181.2	5.1624
86	TMC11 4-C215	215- 0223	600/100 mg bid DE NOVO	NEVER SUPPRE SSED		V32I E34E/Q I54L Q58E/Q L89V	30	43	1.4381
87	TMC11 4-C215	215- 0233	600/100 mg bid DE NOVO	NEVER SUPPRE SSED		K14K/R R57K/R I84V	72	29.1	0.4059
88	TMC11 4-C215	215- 0236	600/100 mg bid DE NOVO	NEVER SUPPRE SSED		V32I E34Q M36I/L K43T F53F/L I54V/L I64I/V T74A V77I/V	5.1	X	
89	TMC11 4-C215	215- 0253	600/100 mg bid DE NOVO	NEVER SUPPRE SSED		I47V I64I/V L76V	16	X	
90	TMC11 4-C215	215- 0285	600/100 mg bid DE NOVO	NEVER SUPPRE SSED		I54V/A I84I/V	16	X	

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91	TMC11 4-C215	215- 0294	600/100 mg bid DE NOVO	NEVER SUPPRE SSED		T74P/A I84I/V	64	X	
92	TMC11 4-C215	215- 0319	600/100 mg bid DE NOVO	NEVER SUPPRE SSED		NONE	0.3	0.6	2
93	TMC11 4-C215	215- 0325	600/100 mg bid DE NOVO	NEVER SUPPRE SSED		L33F/L E35E/D M46I/M Q58E/Q V82A/T/I/V I85I/V	6.8	X	
94	TMC11 4-C215	215- 0339	600/100 mg bid DE NOVO	NEVER SUPPRE SSED		V11L/V I15V V32I G48V R57K P79S V82A	X	42	
95	TMC11 4-C215	215- 0345	600/100 mg bid DE NOVO	NEVER SUPPRE SSED		G16E V32I E34V I54L K55R	6.2	34.4	5.5484
96	TMC11 4-C215	215- 0349	TMC114 / TMC114	NEVER SUPPRE SSED		I13V G73A I85V	169	196.4	1.1614
97	TMC11 4-C215	215- 0350	600/100 mg bid DE NOVO	NEVER SUPPRE SSED		I13V K14K/R I15I/V V32I I54L G94D/G	110	48.9	0.4449
98	TMC11 4-C215	215- 0354	600/100 mg bid DE NOVO	NEVER SUPPRE SSED		V32I L33F/L I54L A71I/V	56	110.8	1.9786
99	TMC11 4-C215	215- 0365	600/100 mg bid DE NOVO	NEVER SUPPRE SSED		G16E/G V32I I85I/V	7	2.1	0.3
100	TMC11 4-C215	215- 0378	600/100 mg bid DE NOVO	NEVER SUPPRE SSED		V32IV M46I/M L89L/V	91	129	1.4223
101	TMC11 4-C215	215- 0388	600/100 mg bid DE NOVO	NEVER SUPPRE SSED		L10F I64I/V I66I/V	6.1	10.2	1.6721

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102	TMC11 4-C215	215- 0413	600/100 mg bid DE NOVO	NEVER SUPPRE SSED		L10I V32I L63L/P I84V	2	X	
103	TMC11 4-C215	215- 0421	600/100 mg bid DE NOVO	NEVER SUPPRE SSED		NONE	56		
104	TMC11 4-C215	215- 0427	TMC114 / TMC114	NEVER SUPPRE SSED		I15I/V L89L/V	118	241.5	2.0414
105	TMC11 4-C215	215- 0436	600/100 mg bid DE NOVO	NEVER SUPPRE SSED		I62V	2.3	1.5	0.6522
106	TMC11 4-C215	215- 0440	TMC114 / TMC114	NEVER SUPPRE SSED		NONE	218	72	0.3298
107	TMC11 4-C215	215- 0444	600/100 mg bid DE NOVO	NEVER SUPPRE SSED		I13I/V K20A/V/M/T	187	280.2	1.5016
108	TMC11 4-C215	215- 0454	TMC114 / TMC114	NEVER SUPPRE SSED		L19V K55I/R	83	79.4	0.9578
109	TMC11 4-C215	215- 0456	TMC114 / TMC114	NEVER SUPPRE SSED		L89V	4.1		
110	TMC11 4-C215	215- 0459	TMC114 / TMC114	NEVER SUPPRE SSED		V11I	159	227.6	1.4332
111	TMC11 4-C215	215- 0460	TMC114 / TMC114	NEVER SUPPRE SSED		NONE	150	79.8	0.5327
112	TMC11 4-C215	215- 0466	TMC114 / TMC114	NEVER SUPPRE SSED		T74A/P C95C/F/L/W	X		
113	TMC11 4-C215	215- 0467	600/100 mg bid DE NOVO	NEVER SUPPRE SSED		M36NL I47V G48Q	95		
114	TMC11 4-C215	215- 0468	TMC114 / TMC114	NEVER SUPPRE SSED		NONE	330	91.2	0.2767

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115	TMC11 4-C215	215- 0477	600/100 mg bid DE NOVO	NEVER SUPPRE SSED		NONE	13	56	4.2748
116	TMC11 4-C215	215- 0478	TMC114 / TMC114	NEVER SUPPRE SSED		L90M	24		
117	TMC11 4-C215	215- 0482	TMC114 / TMC114	NEVER SUPPRE SSED		I13I/V G73C/S	5245	580.8	0.1107
118	TMC11 4-C215	215- 0484	CONTR OL / TMC114	NEVER SUPPRE SSED	I54V/L	I15V V32I I54L Q58E	7.3	56.6	7.7534
119	TMC11 4-C215	215- 0492	600/100 mg bid DE NOVO	NEVER SUPPRE SSED		V32I I54L/V L89L/V	27	50.2	1.8524
120	TMC11 4-C215	215- 0493	TMC114 / TMC114	NEVER SUPPRE SSED		I13I/V L33F S37D/N I54I/V/L	4.8		
121	TMC11 4-C215	215- 0494	TMC114 / TMC114	NEVER SUPPRE SSED		G73A/G/S/T	88	32.2	0.3676
122	TMC11 4-C215	215- 0496	TMC114 / TMC114	NEVER SUPPRE SSED		NONE	413	248.6	0.6025
123	TMC11 4-C215	215- 0498	TMC114 / TMC114	NEVER SUPPRE SSED		I15I/V	6.9	15.1	2.1884
124	TMC11 4-C215	215- 0504	600/100 mg bid DE NOVO	NEVER SUPPRE SSED		NONE	109	74.2	0.6789

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/s/

Lisa Naeger
6/19/2006 03:49:23 PM
MICROBIOLOGIST
microbiology review

Julian O Rear
6/21/2006 12:08:25 PM
MICROBIOLOGIST

James Farrelly
6/22/2006 08:05:04 AM
PHARMACOLOGIST