CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

202022Orig1s000

MICROBIOLOGY REVIEW(S)

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW
NDA: 202022 **SN**: 000 **DATE REVIEWED:** 1/31/11
Microbiology Reviewer: Lisa K. Naeger, Ph.D.

NDA#: 202,022 **Serial #:** 000
Reviewer's Name(s): Lisa K. Naeger, Ph.D.

Sponsor's Name and Address:
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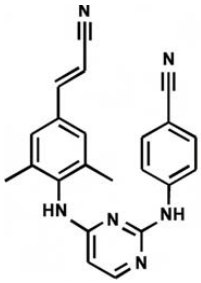
Initial Submission Dates:
Correspondence Date: 07/23/10
CDER Receipt Date: 07/23/10
Assigned Date: 07/23/10
Review Complete Date: 03/25/11
PDUFA Date: May 23, 2011

Amendments: none
Related/Supporting Documents: IND67699
Product Name(s)
Proprietary: *TRADE NAME*
Non-Proprietary/USAN: rilpivirine
Code Name/Number: TMC278
Chemical Name: 4-[[4-[[4-[(1*E*)-2-cyanoethenyl]-2,6-dimethylphenyl]amino]-2-pyrimidinyl]amino]benzonitrile,
Molecular Weight: 366.42 Daltons
Molecular Formula: $C_{22}H_{18}N_6$
Structural Formula:



TMC278

Dosage Form(s):
Route(s) of Administration: Oral
Indication(s): Treatment of HIV-1 infection in ARV treatment-naive adult patients, (b) (4) co-administered with other antiretroviral (ARV) agents.
Dispensed: Rx X OTC_____

Abbreviations: EC_{50}, effective concentration at 50%; EFV, efavirenz; ENF, enfuvirtide; ETR, etravirine; FC, fold change; HSA, human serum albumin; IC_{50}, inhibitory concentration at 50%; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; NVP, nevirapine; PBL, peripheral blood lymphocytes; PI, protease inhibitor; PR, protease; RAMs, resistant-associated mutations; RT, reverse transcriptase; SDM, site-directed mutants;

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EXECUTIVE SUMMARY

Rilpivirine (TMC278) is a non-nucleoside reverse transcriptase inhibitor (NNRTI) of HIV-1. It non-competitively inhibits the viral encoded reverse transcriptase (RT) and thereby disrupts viral replication. Rilpivirine had antiviral activity against wildtype laboratory strain HIV-1_{IIIB} in the MT4 T-cell line with a median EC_{50} value of 0.73 nM and a broad panel of HIV-1 group M (subtype A, B, C, D, F, G, H) primary isolates with EC_{50} values ranging from 0.07 to 1.01 nM. Rilpivirine was less active against group O primary isolates with EC_{50} values ranging from 2.9 to 8.5 nM and demonstrated limited activity in cell culture against HIV-2 with a median EC_{50} value of 5220 nM (range 2510 to 10830 nM).

No antagonism was observed when TMC278 was combined with the NNRTIs efavirenz (EFV), etravirine (ETR), and nevirapine (NVP); NRTIs abacavir, didanosine, emtricitabine, lamivudine, stavudine, tenofovir or zidovudine; the PIs amprenavir, atazanavir, darunavir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir, and tipranavir; the integrase strand transfer inhibitor raltegravir; the fusion inhibitor enfuvirtide; and the CCR5 coreceptor antagonist maraviroc.

Resistance Selection in Cell Culture
Rilpivirine-resistant strains were selected in cell culture starting from wild-type HIV-1 of different origins and subtypes as well as NNRTI-resistant HIV-1. The frequently observed amino acid substitutions that emerged in cell culture selection experiments and conferred decreased susceptibility to rilpivirine included: L100I, K101E and P, V106I and A, V108I, E138K and G, Q, R, V179F and I, Y181C and I, V189I, G190E, H221Y, F227C and M230I and L.

Rilpivirine Susceptibility of Site-Directed NNRTI Mutant Viruses
Rilpivirine had a <2.5 fold reduction in susceptibility against 82% (55/67) of a panel of HIV-1 recombinant site-directed mutant laboratory strains with one NNRTI resistance-associated substitution including the K103N substitution. The single NNRTI substitutions K101P, Y181I and Y181V conferred 52-fold, 15-fold and 12-fold decreased susceptibility to rilpivirine, respectively. Combinations of two NNRTI resistance-associated substitutions in site-directed mutants had varied and complex patterns of rilpivirine resistance with 53% (42/79) of the double NNRTI mutants having ≥2.5-fold decrease in TMC278 susceptibility. The combination of E138K and the emtricitabine/lamivudine resistance-associated substitution M184I showed 6.7-fold reduced susceptibility to rilpivirine compared to 2.8-fold for E138K alone. Greater than 70% of the site-directed mutant viruses tested with combinations of three NNRTI resistance-associated substitutions had decreased susceptibility (≥2.5-fold change) to rilpivirine (fold change range 2.5 - 554).

Treatment-Naïve HIV-1-Infected Subjects
Two 96-week, randomized Phase IIII trials, TMC278-TiDP6-C209 (n=690) and TMC278-TiDP6-C215 (n=678) evaluated the efficacy, safety, and tolerability of rilpivirine (TMC278) 25 mg once daily compared with control (EFV 600 mg once daily) in HIV-1-infected, treatment-naïve adult subjects. There were twice as many subjects who rebounded and were never suppressed in the TMC278 arm of Study C209 14% vs. 7%

in the EFV arm, although this difference was not seen in Study C215. In the combined studies, there were twice as many virologic failures in the >500,000 baseline HIV-1 RNA strata in the TMC278 arm (12%) compared to the EFV arm (6%). Moreover, there were fewer virologic failures with ≤100,000 viral load at baseline in the TMC278 arm than in the EFV arm.

In the pooled resistance analysis from the Phase III Studies C209 and C215, the emergence of resistance was greater in the rilpivirine arms compared to the EFV arms in both studies. In an as-treated analysis of the combined studies, 41% (38/92) of the virologic failures in the rilpivirine arms developed evidence of genotypic and phenotypic rilpivirine resistance compared to 25% (15/60) of the virologic failures in the EFV arms who developed EFV resistance. Moreover, resistance to a background drug occurred in 48% (44/92) of the virologic failures in the rilpivirine arms compared to 15% (9/60) in the EFV arms.

Emerging NNRTI substitutions in the rilpivirine virologic failures were V90I, K101E/P/T, E138K/G, V179I/L Y181I/C, V189I, H221Y, F227C/L and M230L which were associated with a rilpivirine phenotypic fold change range of 2.6 - 621. The E138K substitution emerged most frequently on rilpivirine treatment commonly in combination with the emtricitabine/lamivudine resistance-associated substitution M184I substitution. The emtricitabine/lamivudine resistance-associated substitutions M184I or V and the tenofovir resistance-associated substitutions K65R or N emerged more frequently in rilpivirine virologic failures than in EFV virologic failures.

Cross-resistance
Taking into consideration all of the available cell culture and clinical data, the following amino acid substitutions, when present at baseline, are likely to decrease the antiviral activity of rilpivirine: K101E, K101P, E138A, E138G, E138K, E138R, E138Q, V179L, Y181C, Y181I, Y181V, H221Y, F227C and M230I or M230L.

Cross-resistance to EFV, etravirine and/or nevirapine is likely after virologic failure with a rilpivirine-containing regimen. Of the 38 virologic failures in the rilpivirine arms with evidence of rilpivirine resistance from the pooled analysis of the Phase 3 clinical trials, 89% (n=34) were resistant to etravirine and efavirenz, and 63% (n=24) were resistant to nevirapine. In the efavirenz arm, none of the 15 efavirenz-resistant virologic failures were resistant to etravirine at failure. Subjects experiencing virologic failure on rilpivirine developed more NNRTI resistance-associated substitutions conferring more cross-resistance to the NNRTI class and had a higher likelihood of cross-resistance to all NNRTIs in the class than subjects who failed on efavirenz.

1. RECOMMENDATIONS

1.1. Recommendation and Conclusion on Approvability

This supplemental NDA for TMC278 (rilpivirine) is approvable with respect to virology for the treatment of HIV-1 in NNRTI-treatment-naive subjects. The following statements regarding the indication and usage should be included in the package insert.

Indications and Usage

- More TRADE NAME™ treated subjects with HIV-1 RNA greater than 100,000 copies/mL at the start of therapy experienced virologic failure compared to subjects with HIV-1 RNA less than 100,000 copies/mL at the start of therapy [see Clinical Studies (14)].
- The observed virologic failure rate in TRADE NAME™ treated subjects conferred a higher rate of overall treatment resistance and cross-resistance to the NNRTI class compared to efavirenz [see Microbiology (12.4)].
- More subjects treated with TRADE NAME™ developed lamivudine/emtricitabine associated resistance compared to efavirenz [see Microbiology (12.4)]. (1, 12.4, 14)

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

We have no recommended virology Phase IV commitments for this application.

2. SUMMARY OF OND VIROLOGY ASSESSMENTS

2.1 Non-Clinical Virology

Antiviral Activity in Cell Culture

Rilpivirine (TMC278) is a non-nucleoside reverse transcriptase inhibitor (NNRTI) of HIV-1. It non-competitively inhibits the viral encoded reverse transcriptase (RT) and thereby disrupts viral replication. The median EC_{50} value for rilpivirine against wildtype laboratory strain HIV-1_{IIIB} in the MT4 T-cell line was 0.73 nM (0.27 ng/mL). Rilpivirine demonstrated antiviral activity against a broad panel of HIV-1 group M (subtype A, B, C, D, F, G, H) primary isolates with EC_{50} values ranging from 0.07 to 1.01 nM (0.03 to 0.37 ng/ml) and was less active against group O primary isolates with EC_{50} values ranging from 2.9 to 8.5 nM (1.1 to 3.1 ng/ml). Rilpivirine demonstrated limited activity in cell culture against HIV-2 with a median EC_{50} value of 5220 nM (range 2510 to 10830 nM) (920 to 3970 ng/mL).

The antiviral activity of TMC278 was reduced 19-fold and 39-fold in the presence of 50% human serum and 45 mg/ml human serum albumin, respectively. The CC_{50} value for TMC278 in MT-4 cells was 8.1 μM after 5 days incubation giving a therapeutic index >8,000 (EC_{50} value range 0.39 – 0.98 nM). Rilpivirine does not inhibit the human cellular DNA polymerases α, β and γ.

Combinations of TMC278 with the NNRTIs efavirenz, etravirine, and nevirapine; the NRTIs abacavir, didanosine, emtricitabine, lamivudine, stavudine, tenofovir or zidovudine; the PIs amprenavir, atazanavir, darunavir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir, and tipranavir; the integrase inhibitor raltegravir; the fusion inhibitor enfuvirtide; and the CCR5 coreceptor inhibitor maraviroc were not antagonistic.

Resistance Selection in Cell Culture

Rilpivirine-resistant strains were selected in cell culture starting from wild-type HIV-1 of different origins and subtypes as well as NNRTI-resistant HIV-1. The frequently

observed amino acid substitutions that emerged in the cell culture selection experiments and conferred decreased susceptibility to rilpivirine included: L100I, K101E and P, V106I and A, V108I, E138K and G, Q, R, V179F and I, Y181C and I, V189I, G190E, H221Y, F227C and M230I and L. Changes in rilpivirine susceptibility for the selected resistant variants ranged from 3.8-fold change for a variant with the E138K substitution alone to >10,000–fold change for a variant with multiple NNRTI substitutions L100I, K101E, V179F, Y181C, and F227C. An increased number of NNRTI substitutions were associated with a higher rilpivirine fold change.

Rilpivirine Susceptibility of Site-Directed NNRTI Mutant Viruses
Rilpivirine had a <2.5 fold reduction in susceptibility against 82% (55/67) of a panel of HIV-1 recombinant site-directed mutant laboratory strains with one NNRTI resistance-associated substitution including the K103N substitution. The single NNRTI substitutions K101P, Y181I and Y181V conferred 52-fold, 15-fold and 12-fold decreased susceptibility to rilpivirine, respectively. The combination of E138K and emtricitabine/lamivudine resistance-associated substitution M184I showed 6.7-fold reduced susceptibility to rilpivirine compared to 2.8-fold for E138K alone. Combinations of two NNRTI resistance-associated substitutions had varied and complex patterns of rilpivirine resistance with 53% (42/79) of the double NNRTI site-directed mutants having ≥2.5-fold decrease in TMC278 susceptibility. Greater than 70% of the site-directed mutants tested with combinations of three NNRTI resistance-associated substitutions had decreased susceptibility to rilpivirine (fold change range 2.5 - 554).

2.2 Clinical Virology

Treatment-Naïve HIV-1-Infected Subjects
TMC278-TiDP6-C209 (n=690) and TMC278-TiDP6-C215 (n=678) are 96-week, randomized, double-blind, active-controlled, international Phase III trials in HIV-1-infected, treatment-naïve adult subjects. The trials evaluated the long term efficacy, safety, and tolerability of rilpivirine (TMC278) 25 mg once daily compared with control (efavirenz (EFV) 600 mg once daily). Each NNRTI was given in combination with a background regimen containing emtricitabine and tenofovir disoproxil fumarate in Study C209 and 2 N(t)RTIs (either abacavir/lamivudine, lamivudine/zidovudine, or emtricitabine/tenofovir disoproxil fumarate) in Study C215. In both trials, inclusion criteria included adult subjects with an HIV-1 viral load of ≥5,000 HIV-1 RNA copies/mL, who were treatment-naïve, susceptible to their background regimen at screening, and had no NNRTI resistance-associated substitutions in their genotype at screening.

There were twice as many subjects who rebounded and were never suppressed in the TMC278 arm of Study C209, 14% vs. 7% in the EFV arm, although this was not seen in Study C215. There were twice as many treatment failures in the >500,000 baseline HIV-1 RNA strata in the TMC278 arm (12%) compared to the EFV arm (6%). In an as-treated analysis, 14% of the virologic failures were in the >500,000 baseline HIV-1 RNA strata in the TMC278 arm compared to 10% in the EFV arm. Regardless of baseline phenotype, response rates were higher if baseline viral load was ≤100,000 copies/mL and response rates were lower if baseline viral load was >500,000 copies/mL.

In the pooled resistance analysis from the Phase III Studies C209 and C215, the emergence of resistance was greater in the rilpivirine arms compared to the EFV arms in both studies. In an as-treated analysis of the combined studies, 41% (38/92) of the virologic failures in the rilpivirine arms developed evidence of genotypic and phenotypic rilpivirine resistance compared to 25% (15/60) of the virologic failures in the EFV arms who developed EFV resistance. Moreover, resistance to a background drug occurred in 48% (44/92) of the virologic failures in the rilpivirine arms compared to 15% (9/60) in the EFV arms.

The median fold change in rilpivirine susceptibility of the virologic failures with post-baseline resistance data in the rilpivirine arms (n=75) was 3.3 (range: 0.2 – 621) at failure. The most common emerging NNRTI substitutions in the rilpivirine virologic failures were V90I, K101E/P/T, E138K/G/A, V179I/L, Y181I/C, V189I, and H221Y. The E138K substitution emerged most frequently on rilpivirine treatment commonly in combination with the emtricitabine/lamivudine resistance-associated substitution M184I substitution. The emtricitabine/lamivudine resistance-associated substitutions M184I or V and the tenofovir resistance-associated substitutions K65R or N emerged more often in rilpivirine virologic failures than in EFV virologic failures. Clinical isolates containing the combination of E138K/M184I without other NNRTI substitutions had decreased rilpivirine susceptibility ranging from a 4.9 – 8.8 fold change compared to a 3.6-fold change for one clinical isolate with E138K alone, consistent with the results of site-directed mutant virus grown in cell culture.

At Baseline, the isolates of subjects who responded had a median 1.1 fold change and a mean 1.2 fold-change in TMC278 susceptibility. Comparably, isolates of TMC278 virologic failure subjects (n=38) had a median 1.3-fold change and mean 1.5- fold-change in TMC278 susceptibility at baseline. At virologic failure, the median fold-change in TMC278 susceptibility of the 38 TMC278 virologic failures was 3.5 and the mean fold-change was 18.

The biologic cut-off of 3.7 determined by the sponsor for TMC278 does not appear valid as a clinical cut-off, because multiple TMC278 virologic failure isolates with TMC278 resistance-associated substitutions (i.e., K101E, E138K, M184I, V189I) had fold changes less than 3.7 for TMC278.

Cross-Resistance

Taking into consideration all of the available cell culture and clinical data, the following amino acid substitutions, when present at baseline, are likely to decrease the antiviral activity of rilpivirine: K101E, K101P, E138A, E138G, E138K, E138R, E138Q, V179L, Y181C, Y181I, Y181V, H221Y, F227C and M230I or L.

Cross-resistance to EFV, etravirine and/or nevirapine is likely after virologic failure with a rilpivirine-containing regimen. Of the 38 virologic failures in the rilpivirine arms with evidence of rilpivirine resistance from the pooled analysis of the Phase 3 clinical trials, 89% (n=34) were resistant to etravirine and efavirenz, and 63% (n=24) were resistant to nevirapine.

TaqMan® and Amplicor® HIV Viral Load Assay Discordant Results
The COBAS® TaqMan® HIV-1 assay v1.0 (Roche Diagnostics) was originally used to report viral load results for the Phase III trials C209 and C215. However, the sponsor encountered issues with the TaqMan viral load assay; namely, an increase in blips and persistent low-level viremia (PLLV) with the TaqMan assay while the results were undetectable with the Amplicor viral load assay. Tibotec requested that the COBAS® Amplicor® HIV-1 Monitor (Roche Diagnostics) be used as the primary viral load assay instead of the TaqMan assay. We requested an analysis of the discordant results of undetectable HIV RNA between the TaqMan and Amplicor assay. Tibotec was also asked to analyze whether emergence of resistance occurred in subjects with viral load blips and PLLV seen with the TaqMan assay.

There were 25 and 17 subjects in the TMC278 and EFV arms, respectively, with discordant Amplicor and TaqMan assay results at Week 48; i.e., viral loads were <50 copies/mL with the Amplicor assay and >50 copies/mL with the TaqMan assay in the Week 48 window. The median viral load with the TaqMan assay of these subjects was 91 copies/mL (range of 52 - 6363 copies/mL). Only two had detectable phenotypic resistance at the endpoint; one in each arm had TDF resistance. Substitutions emerged in 7 subject isolates from the TMC278 arm and 7 in the EFV arm. However, only two substitutions were common resistance-associated NRTI substitutions (D67D/G and T69). Review of the data indicates that viral load blips or PLLV detected with the Taqman viral load assay are not always associated with the emergence of resistance. The risk of virologic failure among subjects with viral load blips or PLLV was not different in the TMC278 and control group. Therefore, the difference in virologic failure rate between the two treatment groups does not seem to originate from the subpopulation of patients experiencing viral load blips or PLLV.

3. ADMINISTRATIVE

3.1. Reviewer's Signature

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

3.2. Concurrence

HFD-530/Micro TL _____Date _____

4. OFFICE OF NEW DRUGS VIROLOGY REVIEW

4.1 Important Milestones in Product Development

The COBAS® TaqMan® HIV-1 assay v1.0 (Roche Diagnostics) was originally used to report viral load results for the Phase III trials C209 and C215. This assay is a relatively new viral load assay with more sensitivity at the lower end of viral loads (<200 copies/mL). However, the sponsor encountered issues with the TaqMan viral load assay where they detected an increase in blips and persistent low-level viremia (PLLV) with the TaqMan assay while the results were undetectable with the Amplicor viral load assay. This issue with the TaqMan viral load assay has been widely reported [Oliver, 2007; Manavi, 2008; Gatanaga, 2009; Lima, 2009; Montaner, 2009; Smit, 2009; Pas, 2010; Willig, 2010].

On December 15, 2009, Tibotec provided a briefing package for the Division's review and description of the issues encountered with the viral load assays. Tibotec proposed that the COBAS® Amplicor® HIV-1 Monitor (Roche Diagnostics) should be adopted as the primary viral load assay instead of the TaqMan assay. In the January 13, 2010 correspondence, the Division agreed to the use of the Amplicor assay results to report viral load results for the primary efficacy analyses, but requested an analysis using the TaqMan assay results, outcome, and resistance data in patients with "blips" with the TaqMan assay while undetectable with the Amplicor assay. Tibotec was also asked to analyze whether emergence of resistance is occurring in patients with viral load blips and PLLV seen with the TaqMan assay.

4.2 Methodology

The IAS-USA Drug Resistance Substitution list updated in December 2009 was used for analyses of NNRTI RAMs, NRTI RAMs, and PI RAMs. In addition, for analysis of NNRTI resistance, an extended list of 48 NNRTI RAMs was also used.

IAS-USA NNRTI Resistance-Associated Substitutions (n = 25)
V90I, A98G, L100I, K101E/H/P, K103N, V106A/I/M, V108I, E138A, V179D/F/T, Y181C/I/V,Y188C/H/L, G190A/S, P225H, M230L

Extended NNRTI Resistance-Associated Substitutions (n = 48)
V90I, A98G, L100I, K101E/H/P/Q, K103H/N/S/T, V106A/I/M, V108I, E138A/G/K/Q/R, V179D/E/F/G/I/T, Y181C/I/V, Y188C/H/L, V189I, G190A/C/E/Q/S, H221Y, P225H, F227C/L, M230I/L, P236L, K238N/T, Y318F

IAS-USA NRTI Resistance-Associated Substitutions (n = 20)
M41L, A62V, K65R, D67N, 69ins, K70E/R, L74V, V75I, F77L, Y115F, F116Y, Q151M, M184I/V, L210W, T215F/Y, K219E/Q

IAS-USA Primary PI Resistance Substitutions (n = 22)
D30N, V32I, M46I/L, I47A/V, G48V, I50L/V, I54L/M, Q58E, T74P, L76V, V82A/F/L/S/T, I84V, N88S, L90M

IAS-USA PI Resistance-Associated Substitutions (n = 74)
L10C/F/I/R/V, V11I, I13V, G16E, K20I/M/R/T/V, L24I, D30N, V32I, L33I/F/V, E34Q, E35G, M36I/L/V, K43T, M46I/L, I47A/V, G48V, I50L/V, F53L/Y, I54A/L/M/S/T/V, Q58E, D60E, I62V, L63P, I64L/M/V, H69K, A71I/L/T/V, G73A/C/S/T, T74P, L76V, V77I, V82A/F/I/L/S/T, N83D, I84V, I85V, N88D/S, L89V, L90M, I93L/M

GENOTYPIC METHODS

Genotypic analyses were performed according to the Virco®TYPE methodology based on automated population-based DNA-sequence analysis (ABI Prism BigDye terminator cycle sequencing). Individual sequencing data were reported as amino acid changes as compared to the HIV-1/HXB2 wild-type reference. Genotypic mixtures (a combination of different amino acids at 1 position) were reported as separate amino acids and indicated as "X" if 4 or more amino acids per position were present.

PHENOTYPIC ANALYSES

Phenotypic analyses were performed using the Antivirogram® method. Recombinant clinical isolates were constructed by amplifying PR and RT coding sequences from subject-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 susceptibility testing. Replication of HIV-1 was measured using the specific interaction of HIV-1 Tat protein with the HIV-1 LTR coupled to the green fluorescent protein (GFP) reporter gene. The test compound was serially diluted, MT4 LTR-GFP cells were infected with HIV-1 (multiplicity of infection (MOI) of 0.01) and after 3 days, GFP production was measured. The resistance call for each ARV drug (based on the Antivirogram® Phenotype Assay AVG 2.5.01) was determined based on the biological cut-off (BCO) or the clinical cut-off (CCO) (Table 1). A drug was defined as susceptible (S) if the fold change (FC) value was ≤ BCO or ≤ CCO for the specific drug (indicating that the virus showed susceptibility to the drug). A drug was defined as resistant (R) if the FC value was > BCO or > CCO for the specific drug (indicating that the virus showed decreased susceptibility to the drug).

A biological cut off (BCO) of 3.7 was calculated by the applicant and used to determine susceptibility to TMC278. This preliminary BCO for TMC278 was determined as the 97.5th percentile of fold change measurements, after outlier removal, from 2796 wild-type HIV-1 recombinant clinical isolates from a 3-year period (February 2004 to February 2007). Outliers are the values outside the interval comprising the mean fold change plus 3 standard deviations in the log domain. Wild-type isolates are defined as recombinant clinical isolates containing none of the NNRTI resistance-associated substitutions that are both present in the public domain and identified by Virco's linear modeling algorithm (update from November 2007).

Table 1. Biological or Clinical Cut-Off Values Used for the Interpretation of Phenotypic Susceptibility Based on Antivirogram® Version 2.5.01

Class	Drug	Generic name	Biological / clinical cut-off Antivirogram® (version 2.5.01)
NRTI	AZT	Zidovudine	2.5
	3TC	Lamivudine	2.1
	ddI	Didanosine	2.3
	d4T	Stavudine	2.2
	ABC	Abacavir	3.2*
	FTC	Emtricitabine	3.1
	TDF	Tenofovir	1.4*
NNRTI	NVP	Nevirapine	6.0
	EFV	Efavirenz	3.3
	ETR	Etravirine	3.2
	TMC278	Rilpivirine	3.7
PI	NVF	Nelfinavir	2.2
	IDV	Indinavir	2.3
	SQV	Saquinavir	1.8
	APV	Amprenavir	2.2
	LPV	Lopinavir (a component of Kaletra)	10.0*
	ATV	Atazanavir	2.1
	DRV	Darunavir	10.0*
	TPV	Tipranavir	3.0*

*: Clinical cut-off.

4.3 Prior FDA Virology Reviews

Prior IND67699 reviews were done by Lisa K. Naeger, Sr. Virology Reviewer.

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

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

There are currently twenty-two FDA-approved anti-HIV-1 drugs including including NNRTIs (delavirdine, efavirenz, nevirapine), NRTIs (abacavir, didanosine, emtricitabine, lamivudine, stavudine, tenofovir, zalcitabine, zidovudine), PIs (atazanavir, darunavir, fosamprenavir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir), the fusion inhibitor enfuvirtide, the CCR5 coreceptor antagonist maraviroc and the integrase strand transfer inhibitor raltegravir. Maraviroc inhibits the interaction between the viral envelope glycoprotein gp120 and the human CCR5 receptor membrane protein and inhibits entry of the virus into the cell. Enfuvirtide is a gp41 fusion inhibitor preventing the joining of the viral and cellular membranes necessary for virus entry. NRTIs mimic nucleosides and target HIV-1 RT by competing with natural deoxynucleoside triphosphates for binding to RT and by incorporating into newly synthesized viral DNA resulting in chain-termination. NNRTIs inhibit HIV-1 RT by binding near the catalytic site of RT and acting as noncompetitive inhibitors. Integrase catalyzes the integration of linear viral DNA into

host cell DNA forming the provirus. PIs work at the late stage of viral replication to prevent virus production from infected cells. They block the HIV-1 protease enzyme, which is necessary for the production of mature virions, resulting in defective particles which are unable to infect new cells.

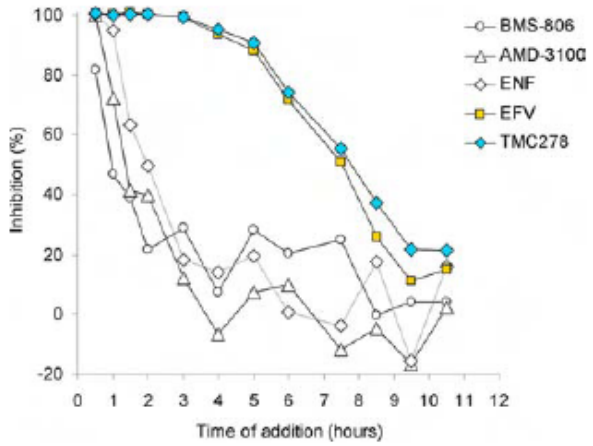
Unfortunately, HIV-1 develops resistance to antiretroviral drugs over time usually from the accumulation of multiple mutations. HAART regimens are also associated with acute toxicities such as diarrhea, kidney stones, rash, CNS toxicities and hepatotoxicity. Long-term toxicities from antiretroviral therapies include mitochondrial toxicities associated with NRTIs (lactic acidosis, myopathy, neuropathy, pancreatitis), and disorders of lipid metabolism (dyslipidemia) and glucose metabolism (lipodystophy, 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 subject 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.

4.5 Non-Clinical Virology

MECHANISM OF ACTION

The antiviral activity of TMC278 was studied at different times of addition of the drug in infected HIV-1_{IIIB} cell culture using BMS-806 (a CD4/HIV-1 gp120 attachment inhibitor), AMD-3100 (a CXCR4 co-receptor antagonist), enfuvirtide (a fusion inhibitor), and efavirenz (EFV) (an NNRTI) as references. The viral replication stage at which TMC278 inhibits the HIV-1 replication cycle was similar to that of the NNRTI EFV (Fig. 1). Using a primer extension-based scintillation proximity assay, the median inhibitory activity (IC_{50}) of HIV-1 RT by TMC278 was 42 nM.

Figure 1. Effect of Time of Addition of TMC278 on Anti-HIV Activity compared with Other ARVs



The crystal structure of the TMC278/HIV-1 reverse transcriptase (RT) complex and freedom-of-motion analyses are consistent with TMC278 adapting to changes in the NNRTI binding pocket which might contribute to a higher genetic barrier to the development of resistance.

ANTIVIRAL ACTIVITY

The median EC_{50} value for TMC278 against HIV-1_{IIIB} in the MT4 T-cell line was 0.73 nM, lower than the median EC_{50} values for EFV, ETR, and NVP by factors of 2, 4, and 47, respectively (Table 1). The antiviral activity of TMC278 was tested in parallel with those of EFV, ETR, and NVP against a panel of nine group M recombinant HIV-1 clinical isolates of subtypes A1, AE, AG, BG, C, D, F1, G, and H (Table 2). All the HIV-1 subtypes were sensitive to TMC278 with EC_{50} values in the subnanomolar range (range 0.06 – 0.52 nM). TMC278 had lower antiviral activity against HIV-2 with a median EC_{50} value of 5.22 μM similar to etravirine but lower than for EFV and NVP (Table 2).
The antiviral activity of TMC278 determined in monocyte-derived macrophages had EC_{50} values of 0.18 and 0.22 nM (0.07 to 0.08 ng/mL) against HIV-1/Ba-L and HIV-1/ADA, respectively.

Table 2. Antiviral Activities of TMC278, EFV, ETR, and NVP against HIV-1_{IIIB}, HIV-2, and a Range of HIV-1 Group M Recombinant Clinical Isolates in Cell Culture (nM)

Viral type (subtype)	Median EC_{50} (Q1–Q3)[a]			
	TMC278	EFV	ETR	NVP
HIV-1 (IIIB)	0.73 (0.39–0.98) (n = 403)	1.73 (1.14–2.42) (n = 889)	2.73 (2.06–3.49) (n = 927)	34.09 (26.23–44.90) (n = 512)
HIV-1 (A1)	0.44 (n = 1)	0.72 (0.60–0.87) (n = 2)	1.39 (n = 1)	9.32 (n = 1)
HIV-1 (AE)	0.21 (0.20–0.21) (n = 2)	0.53 (0.43–0.65) (n = 2)	0.65 (0.63–0.66) (n = 2)	8.94 (n = 1)
HIV-1 (AG)	0.13 (0.10–0.17) (n = 2)	0.53 (0.34–0.81) (n = 2)	0.46 (0.32–0.66) (n = 2)	14.40 (n = 1)
HIV-1 (BG)	0.22 (0.20–0.24) (n = 2)	0.70 (0.46–1.06) (n = 2)	0.67 (0.60–0.75) (n = 2)	25.99 (n = 1)
HIV-1 (C)	0.30 (0.18–0.52) (n = 2)	0.31 (0.31–0.31) (n = 2)	0.52 (0.28–0.97) (n = 2)	<4.88 (n = 1)
HIV-1 (D)	0.30 (0.27–0.33) (n = 2)	1.19 (0.96–1.47) (n = 2)	1.13 (1.01–1.26) (n = 2)	24.17 (n = 1)
HIV-1 (F1)	0.21 (0.18–0.26) (n = 2)	0.66 (0.58–0.76) (n = 2)	0.35 (0.13–0.99) (n = 2)	14.88 (n = 1)
HIV-1 (G)	0.14 (0.06–0.34) (n = 2)	0.31 (0.23–0.42) (n = 2)	0.24 (0.21–0.26) (n = 2)	24.63 (n = 1)
HIV-1 (H)	0.24 (0.22–0.25) (n = 2)	0.49 (0.31–0.77) (n = 2)	0.51 (0.26–0.98) (n = 2)	34.14 (n = 1)
HIV-2 (ROD)	5.22 (2.51–10.83) (n = 2)	24.83 (14.49–32.00) (n = 28)	5.67 (3.10–7.34) (n = 30)	>31.25 (n = 9)

[a] EC_{50}, 50% effective concentration in a cell-based assay; Q1–Q3, first- to third-quartile values. Values are given in nanomolar concentrations for HIV-1 and in micromolar concentrations for HIV-2. n, number of experiments. For MT4 cells, the 50% cytotoxic concentrations in cell-based assays (with first- to third-quartile values in parentheses) were 5.91 μM (5.02 to 8.31 μM) for TMC278 in 381 experiments, 40.02 μM (36.46 to 43.06 μM) for EFV in 805 experiments, >64.00 μM for ETR in 820 experiments, and >32.00 μM for NVP in 457 experiments.

The antiviral activities of TMC278 was determined against HIV-1 primary isolates of group M subtypes A, B, C, D, E, F, and G and group O (Table 3). The median EC_{50} value of TMC278 against all the HIV-1 primary clinical isolates tested in PBMC cultures was 0.26 nM. TMC278 had EC_{50} values ranging from 0.07 to 1.01 nM (0.03 to 0.37 ng/mL) against group M wildtype HIV-1 isolates and 0.73 nM or (0.27 ng/mL) against HIV-1_{IIIB}. No apparent difference in the antiviral activity of TMC278 based on virus tropism was observed. Similarly, except for HIV-1 group O, no apparent difference was found in the antiviral activity of the compound based on HIV-1 subtype. TMC278 was on average approximately 10- to 15-fold less active against the HIV-1 group O isolates than against the HIV-1 group M isolates. TMC278 had EC_{50} values ranging from 2.88 to 8.45 nM (1.06 to 3.10 ng/mL) against HIV-1 group O isolates.

No antiviral activity of TMC278 (EC_{50} values >10 μM) was observed against the human hepatitis B virus, herpes simplex virus 2, human corona virus, influenza A virus, and vaccinia virus. The median EC_{50} value for TMC278 against SIV/mac251 observed in the MT4-LTR-eGFP cell line was 8.55 μM (3.13 μg/mL).

Table 3. Anti-HIV-1 activities of TMC278, EFV, and ETR against primary HIV-1 isolates in Cell Culture

HIV-1 strain	Subtype	Tropism[a]	EC_{50} (nM)[b]		
			TMC278	EFV	ETR
92UG029	A	CXCR4	0.44	0.62	1.45
92UG037	A	CCR5	0.24	1.11	1.42
92RW020	A	CCR5	0.07	0.30	0.48
JR-CSF	B	CCR5	0.51	0.76	1.02
93BR021	B	CCR5	0.23	1.17	1.35
WEJO	B	CXCR4	0.08	0.16	0.43
92BR025	C	CCR5	0.33	2.08	1.70
93IN101	C	CCR5	0.53	1.47	2.72
93MW959	C	CCR5	0.11	0.19	1.01
92UG001	D	DUAL	0.26	0.53	0.90
92UG024	D	CXCR4	0.38	0.72	3.18
92UG035	D	CCR5	0.07	0.34	0.44
92TH006	E	CCR5	0.08	0.48	0.50
93TH073	E	CCR5	0.07	0.39	0.21
CMU08	E	CXCR4	1.01	0.78	3.47
93BR019	F	CXCR4	0.95	0.15	0.46
93BR020	F	DUAL	0.16	0.74	1.08
93BR029	F	CCR5	0.19	0.42	1.06
G3	G	CCR5	0.25	0.12	0.66
JV1083	G	CCR5	0.26	0.70	1.45
RU132	G	CCR5	0.51	0.52	1.70
BCF01	O	CCR5	3.13	72.8	16.4
BCF02	O	CCR5	2.88	57.2	52.8
BCF03	O	CCR5	8.45	194	149

[a] CCR5, chemokine receptor 5; CXCR4, CXC chemokine receptor 4.
[b] EC_{50}, 50% effective concentration in a cell-based assay. The median EC_{50} values for TMC278, EFV, and ETR were 0.26, 0.66, and 1.22 nM, respectively, and the corresponding first- to third-quartile values were 0.15 to 0.52, 0.38 to 1.13, and 0.62 to 1.96 nM, respectively.

Antiviral Activity of TMC278 at Different Multiplicities of Infection

The antiviral activity of TMC278 against HIV-1_{IIIB} was studied at 3 MOIs (0.25, 0.0025, and 0.00025). The median EC_{50} values at each MOI for TMC278 were comparable to those for EFV and ETR (Table 4). The EC_{50} values clearly increased with increasing MOI for NVP and the NRTI control AZT.

Table 4. Influence of the MOI on the EC_{50} Values of TMC278, EFV, ETR, NVP and control (AZT) against HIV-1_{IIIB}

MOI	Median EC50 (IQR) (nM)				
	TMC278	EFV	ETR	NVP	Control (AZT)
0.00025	0.18 (0.17-0.19)	0.22 (0.20-0.24)	0.78 (0.77-0.80)	5.00 (4.85-73.50)	13.00 (11.50-14.50)
0.0025	0.42 (0.40-0.44)	0.79 (0.75-0.83)	0.94 (0.92-1.07)	37.00 (31.55-39.00)	39.30 (36.65-46.15)
0.25	1.40 (1.40-1.78)	4.25 (4.20-4.30)	5.30 (0.51-0.65)	5835.00 (5477.50-6012.50)	890.00 (805.00-922.50)

The results are expressed as the median and interquartile range of 2 independent experiments

ANTIVIRAL ACTIVITY OF TMC278 IN THE PRESENCE OF HUMAN SERUM PROTEINS

The green fluorescent protein (GFP) assay was performed to determine the effects of human serum proteins (50% human serum, 45 mg/ml human serum albumin (HSA), or 1 mg/ml alpha-1-acid glycoprotein (AAG)) on the activities of TMC278, EFV, ETR, and NVP against HIV-1_{IIIB} in MT4 cells. The ratio of the EC_{50} values in the presence of serum protein to that in the absence of serum protein was calculated. The antiviral activity of TMC278 was reduced in the presence of 50% human serum and 45 mg/ml HAS by factors of 18.5 and 39.2, respectively (Table 5). Comparable results were obtained for EFV.

Table 5. Effect of Human Serum Proteins on Antiviral Activity of TMC278 in Cell Culture

NNRTI	Median EC_{50} ratio[a] (Q1–Q3)		
	1 mg/ml AAG	45 mg/ml HSA	50% human serum
TMC278	1.8 (1.5–2.1) (n = 13)	39.2 (25.7–54.1) (n = 12)	18.5 (11.0–40.4) (n = 13)
EFV	3.8 (3.2–4.1) (n = 5)	17.3 (13.2–19.4) (n = 5)	12.3 (10.2–16.2) (n = 5)
ETR	3.4 (2.0–4.4) (n = 17)	4.9 (4.0–5.9.) (n = 17)	5.7 (4.9–12.1) (n = 16)
NVP	2.0 (1.5–2.9) (n = 7)	2.9 (1.5–3.3) (n = 7)	3.8 (1.8–5.1) (n = 7)

[a] Ratio of the 50% effective concentration (EC_{50}) in the presence of serum proteins to that in the absence of serum proteins. Q1–Q3, first- to third-quartile values; n, number of experiments.

CYTOTOXICITY

The CC_{50} value for TMC278 in MT-4 cells was 8.1 µM after 5 days incubation giving a therapeutic index >8,000 (EC_{50} value range 0.39 – 0.98 nM).

The CC_{50} values of TMC278 on A549, HeLa, HEp-2, Hep-G2, and MRC-5 cells were measured at Day 3 and Day 5. The CC_{50} values for TMC278 ranged between 17.34 to 34.51 µM (6.35 to 12.65 µg/mL) and between 16.90 to 35.59 µM (6.19 to 13.04 µg/mL) at Day 3 and Day 5, respectively (Table 6).

Table 6. CC_{50} Values for TMC278 in Cell Culture

Cells	TMC278 CC50 (µM) Median (IQR)	EFV CC50 (µM) Median (IQR)	ETR CC50 (µM) Median (IQR)	NVP CC50 (µM) Median (IQR)
Day 3				
A549	25.48 (25.05 - 25.69)	>40	>40	>40
HeLa	17.34 (17.22 - 17.37)	>40	>40	>40
HEp-2	34.38 (31.88 - 35.43)	>40	>40	>40
Hep G2	33.18 (32.52 - 33.21)	>40	>40	>40
MRC-5	34.51 (32.84 - 34.86)	>40	>40	33.03 (32.02 - 33.74)
Day 5				
A549	26.70 (26.60 - 27.52)	>40	>40	>40
HeLa	16.90 (16.41 - 18.51)	>40	>40	>40
HEp-2	26.27 (25.46 - 28.88)	>40	>40	>40
Hep G2	27.13 (26.99 - 30.83)	>40	>40	>40
MRC-5	35.59 (35.34 - 37.79)	>40	>40	35.96 (35.09 - 36.03)

The results are expressed as the median and interquartile range of 3 independent experiments

The inhibitory activity of TMC278 against three human DNA polymerases α, β, and γ and the Klenow fragment of bacterial *E. coli* DNA polymerase I was tested in biochemical assays. The IC_{50} values for each was >1,000 μM [Report TMC278-1646-0005343].

ANTIVIRAL ACTIVITY OF TMC278 IN COMBINATION WITH OTHER ARVS

Combinations of TMC278 with the NNRTIs EFV, ETR, and NVP; the NRTIs 3TC, ABC, AZT, d4T, FTC, ddI, and TDF; the PIs APV, ATV, DRV, IDV, LPV, NFV, RTV, SQV, and TPV; the integrase strand transfer inhibitor RAL; the fusion inhibitor ENF; and the CCR5 coreceptor antagonist MVC were tested against HIV-1_{IIIB} in MT4 cells at three different molar ratios of their estimated respective EC_{50} values: 3/1, 1/1, and 1/3. Each combination was tested in triplicate in three independent experiments. A combination index (CI) at 50% protection was calculated. A median CI of <0.8 denotes synergy, ≥1.2 denotes antagonism and ≥0.8 and <1.2 denotes additivity between drugs. Combinations of TMC278 with MVC, ETR, and RAL were tested in a checkerboard plate format using five concentrations of MVC, ETR, or RAL in all possible combinations with eight concentrations of TMC278. Combination antiviral activity was evaluated in triplicate, and control wells included cells and virus only. The antiviral activity of TMC278 with MVC was measured as the inhibition of β-galactosidase expression, and the antiviral activity of TMC278 with ETR and RAL was measured with the anti-HIV-1 cytoprotection assay and the MTS assay. The drug combination assay data were then analyzed according to the method of Prichard and Shipman using the MacSynergy II program. On the basis of the methodologies used, all combinations were scored as additive, except for the combinations with 3TC, AZT, and RAL, which showed low levels of synergy.

RESISTANCE DEVELOPMENT IN CELL CULTURE

At high MOIs and fixed drug concentrations, MT4 cells were infected with wild-type HIV-1 or with HIV-1 strains harboring substitutions associated with resistance to RT inhibitors at an MOI of 0.1 to 1.0 in the absence (control) or presence of different concentrations of the inhibitor. Cell cultures were maintained by repeat passages up to a maximum of 32 days in the presence of the initial concentration of each inhibitor and were examined for signs of viral replication. Assessment of cytopathic effect was used to determine viral replication.

At low MOIs and escalating drug concentrations, MT4 cells were infected at an MOI of 0.001 to 0.01 with wild-type HIV-1_{IIIB}, NNRTI-resistant recombinant clinical HIV-1 isolates of various subtypes, or various NNRTI-resistant HIV-1_{HXB2} site-directed substitutions in the presence of TMC278 at initial concentrations ranging from 1 to 10 nM. The cultures were maintained by repeat cell passages, and cells were examined for signs of viral replication. At 100% cytopathic effect, virus supernatants were collected and used to infect fresh cells at 0- to 5-fold-incremented concentrations of TMC278. Susceptibility testing was performed with the Antivirogram Phenotype methodology, using either the MTT or the GFP assay.

After 32 days in culture, no viral replication was detected in cells infected with HIV-1_{IIIB} at a high MOI at any concentration of TMC278 greater than or equal to 40 nM. The same results were also observed for recombinant clinical HIV-1 isolates of group M subtypes

A1, AE, AG, BG, C, D, F1, G, and H and for two HIV-1_{HXB2} site-directed mutants containing either K103N or Y181C. Therefore, the 40 nM concentration of TMC278 appears sufficient to prevent the replication of these HIV-1 strains. Results from the high-MOI selection experiments showed that 10 nM TMC278 was a suboptimal concentration at which new HIV-1 strains emerged from subtypes A1, B, BG, and D and from the two HIV-1 site-directed mutants resistant to TMC278. These emerging HIV-1 strains were subjected to nucleotide sequence analysis and found to harbor combinations of NNRTI substitutions L100I, K101E, V106I, Y181C, Y181I, and/or M230I.

For the low-MOI and escalating drug concentration experiments, mutant strains resistant to TMC278 were isolated from cell cultures containing initial TMC278 concentrations ranging from 5 nM to 16 μM starting from wild-type and NNRTI-resistant strains from various group M subtypes. Twelve selected HIV-1 strains had decreased susceptibility to TMC278 with fold changes of 3.8 to 13,921 (Table 7). These isolates had as many as five NNRTI resistance-associated substitutions and an increased number of NNRTI substitutions were associated with a higher TMC278 fold change. Genotypic analyses of these strains emerging under selective pressure from TMC278 showed the substitutions E138G/K/Q/R as well as L100I, K101E/P, V106A/I, V108I, V179F/I, Y181C/I, V189I, G190E, H221Y, F227C, and M230I/L contribute to decreased susceptibility to TMC278.

CROSS-RESISTANCE

Antiviral Activity of TMC278 against HIV-1 NRTI-Resistant Recombinant Clinical Isolates

To determine if cross-resistance between TMC278 and the NRTI class exists, the antiviral activity of TMC278 was tested against a panel of HIV-1_{HXB2} site-directed mutant viruses harboring NRTI resistance-associated substitutions. TMC278 retained activity against NRTI-resistant site-directed mutant virus (Table 8).

Antiviral Activity of TMC278 against HIV-1 NNRTI-Resistant Recombinant Clinical Isolates

The antiviral activity of TMC278 was evaluated on a panel of 4,786 HIV-1 recombinant clinical isolates resistant to either EFV or NVP or both. The biological cutoff values used were 3.3 for EFV, 3.2 for ETR, and 6.0 for NVP. A biological cutoff of 3.7 was calculated and used to determine resistance for TMC278. Using the biological cutoff values, 62% of this group of HIV-1 recombinant clinical isolates retained sensitivity to TMC278 or etravirine, compared with 11% with sensitivity to EFV and 5% with sensitivity to NVP.

Antiviral Activity of TMC278 against HIV-1_{HXB2} Site-Directed Mutant Viruses Containing NNRTI Resistance-Associated Substitutions

A series of HIV-1 site-directed mutant viruses (n=55) were constructed in an HIV-1_{HXB2} molecular clone backbone in order to study the resistance profile of TMC278 in cell culture in comparison to EFV, ETR, and NVP. Of the 31 site-directed mutants with a single NNRTI resistance-associated substitution, 12 (39%) had ≥2.5-fold decreased susceptibility to TMC278 (Table 9). The highest fold changes in TMC278 susceptibility for single NNRTI substitutions were seen with K101P (52-fold), Y181I (15-fold) and Y181V (12-fold). Isolates with K101E, K101Q, or Y181C had less than 3-fold changes in susceptibility to TMC278. The mutant isolate with K103N was susceptible to TMC278.

Table 7. Resistant Viral Isolates from TMC278 Selection Experiments

Subtype	Strain	Expt	Day[b]	Concn (nM)[c]	FC[d] TMC278	FC[d] EFV	FC[d] ETR	Mutation(s) in HIV-1 reverse transcriptase[e]
Wild types								
AE	r13816[f]		0	0	0.4	1.5	0.9	
		1	69	10	3.8	7.4	5.0	**E138K**
		2	109	40	5.9	13.1	24.3	**E138K**, R358G
	r13817[f]		0	0	0.7	1.6	0.7	
			107	40	66.8	268.3	180.2	**E138G**, **V189I**, **H221Y**, **M230I**
			293	5,000	>1,932.0	4,849.9	>3,787.9	I050T, **E138R**, **V189I**, K219E, **H221Y**, **M230I**, E297K
AG	r13813[f]		0	0	0.7	0.8	0.9	
			108	40	5.4	13.4	26.2	E006A, **E138Q**, I178M, T200V, V245E
B	IIIB[g]		0	0	1.0	1.1	0.9	
		1	69	80	12.0	NA	17.9	**E138K**, **G190E**
			90	600	114.1	>12,345.8	787.4	E006K, **E138K**, **G190E**
			121	5,000	>1,830.2	>15,923.6	>3,906.3	E006K, **L100I**, **E138K**, **G190E**, K219N
		2	66	62	34.6	19.7	45.3	**K101E**, **Y181C**, T386A
			108	4,000	5,490.6	3,895.1	NA	**L100I**, **K101E**, **Y181C**, **F227C**, T386A
			122	16,000	13,920.7	>21,240.8	4,878.0	**L100I**, **K101E**, A158T, **V179F**, **Y181C**, **F227C**, T386A
		3	34	62	100.1	173.5	286.0	**V179F**, **Y181C**, **F227C**, **M230I**
			59	4,000	7,716.3	546.1	7,381.9	**V179F**, **Y181C**, **F227C**, **M230I**
		4	34	5	5.0	5.4	5.7	E040K, **Y181C**
			62	80	315.4	427.8	1,722.2	E040K, **V179F**, **Y181C**, **F227C**, **M230I**
			90	1,500	626.9	783.6	244.8	E040K , V060A, **V090I**, **Y181C**, **F227C**, **M230I**
		5	32	40	23.7	30.9	27.1	E040K, **K101E**, **V108I**, **Y181C**, **H221Y**
			52	200	144.4	402.8	117.1	E040K, **K101E**, **V108I**, **Y181C**, K219E, **H221Y**, R358K
			70	5,000	2,154.6	1,688.0	2,566.5	E040K, **K101E**, **V108I**, **Y181C**, **F227C**, **M230I**
		6	116	2,000	2,022.6	1,025.2	112.0	**L100I**, **K101E**, **Y181C**, P225L, **F227C**
C	v071130[f]		0	0	1.9	0.9	1.6	
			128	80	15.9	174.8	19.9	**E138K**, V241M, E399G
			276	5,000	>1,467.1	6,191.1	>759.9	**V108I**, **E138K**, **M230L**, Y232F, V241 M, G335Y, E399G
D	v071038[f]		0	0	0.4	0.3	0.3	
			93	80	26.0	287.8	224.0	L074V, **L100I**, T139K, **Y181C**, T240I, R284K
			139	3,000	978.9	2,455.1	2,796.7	R072K, L074V, **L100I**, T139K, **Y181C**, T240I, E396K
			163	5,000	>1,984.1	15,027.6	>2,883.5	L074V, **L100I**, **V106A**, **V108I**, T139K, **Y181C**, T240I, V241M
Mutants								
B	SM041[h]		0	0	3.1	1.5	2.0	**E138K**
			90	40	13.2	6.4	3.1	**E138R**, K173Q, **H221Y**, G273E
			160	150	185.6	27.5	60.7	**L100I**, **E138R**, K173Q, **V179I**, **H221Y**, V241I, G273E
			212	5,000	>3,004.8	159.9	>41.3	**L100I**, **V108I**, I135T, **E138R**, K173Q, **V179I**, L214F, **H221Y**, V241I, G273E
	SM051[h]		0	0	0.7	17,097.0	6.1	**L100I**, **K103N**
			93	5,000	>1,830.2	>15,923	333.1	**L100I**, **K103N**, **E138G**, **V179I**, G196R, P225Y
	SM052[h]		0	0	3.2	348	1.7	**K101E**, **K103N**
			118	5,000	>1,947.0	4,238.0	269.4	E028K, **K101E**, **K103N**, **V108I**, **E138G**, **V179I**, **Y181C**, K219R, L228R, T376S
	SDM020001[h]		0	0	3.1	1.8	9.1	**V179I**, **Y181C**
			161	5,000	970.2	130.6	1674.2	K030I, **V106I**, **V108I**, **E138K**, T165A, **V179I**, **Y181C**, **V189I**, L214F, **H221Y**, N348I, E370K
	SDM020059[h]		0	0	8.0	4.5	184.0	**V179F**, **Y181C**
			171	5,000	>2,784.0	>24,630.3	>3,041.4	**L100I**, **V108I**, **V179F**, **Y181C**, L214F, N348I, E370G, V381I
	r9602[f]		0	0	3.2	ND	3.2	K102Q, **K103N**, E122K, **Y181C**, T200I, L214F, S322T, D324E, K366R, T400A
		1	28	40	7.4	102.2	ND	T069I, K102Q, **K103N**, E122K, **E138G**, S163N, **Y181C**, **V189I**, T200I, L214F, S322T, D324E, K366R, T386A, T400A
			52	200	237.7	91.7	409.4	K102Q, **K103N**, E122K, **E138G**, **V179I**, **Y181C**, T200I, L214F, S322T, D324E, N348I, V365I, K366R, T386A, T400A
			63	1,000	156.9	26.1	159.6	K102Q, **K103N**, E122K, **E138G**, **V179I**, **Y181C**, T200I, L214F, S322T, D324E, N348I, K366R, T386A, T400A
		2	74	1,000	142.4	312.8	485.1	L074I, K102Q, **K103N**, E122K, **E138G**, **V179I**, **Y181C**, T200I, L214F, S322T, D324E, N348I, K366R, T386A, T400A
			109	10,000	460.3	332.8	635.2	M041I, L074I, K102Q, **K103N**, **V108I**, E122K, **E138G**, **V179I**, **Y181C**, T200I, E203K, L214F, S322T, D324E, N348I, K366R, T386A, T400A

[a] The experiments for which results are presented are those in which viruses resistant to TMC278 (FC, >3.7) were obtained.
[b] Number of days in cell culture.
[c] TMC278 concentration at which the resistant isolate was selected.
[d] Calculated as the ratio between the 50% effective concentration of the compound for the emerging HIV-1 strain and that obtained in the same experiment with HIV-1/IIIB. Each result is the mean for a single experiment run in duplicate. NA, not applicable; ND, not done.
[e] The first 400 amino acids of the reverse transcriptase were sequenced. Emerging mutations are underscored, and NNRTI resistance-associated mutations are in boldface.
[f] Recombinant HIV-1 clinical isolate.
[g] Laboratory derived HIV-1 strain.
[h] Site-directed mutant derived from HIV-1 HXB2.

Table 8. Antiviral Activity of TMC278 against HIV-1_{HXB2} Site-Directed Mutant Viruses Harboring NRTI Resistance-Associated Substitutions

	In vitro phenotypic susceptibility			
	Median FC			
NRTI RAMs	TMC278	EFV	ETR	NVP
None*	1.0	1.0	1.0	1.0
M041L*	0.7	0.5	0.9	1.3
A062V	0.7	0.9	0.8	2.0
R065R*	1.0	1.4	N.D.	N.D.
L074V	1.0	1.0	1.1	2.0
Y115F	1.0	1.7	1.1	4.3
Q151M	1.8	1.7	1.8	4.5
M184V	0.8	0.9	0.8	1.2
M041L T215Y	0.4	0.7	0.4	1.1
K065R M184V	0.6	0.7	0.6	1.2
Y115F M184V	0.9	1.0	0.9	1.8
M184V T215Y	0.3	0.4	0.3	0.7
M041L L210W T215Y	0.5	0.4	0.3	0.9
T069S-SG M041L T215Y*	0.5	<0.4	0.3	0.9
T069S-SS L210W T215Y*	0.7	0.6	0.5	0.8
M041L D067N K070R T215Y	0.3	0.6	0.2	1.5
M041L V075M L210W T215Y	0.6	0.7	0.5	1.1
M041L D067N L210W T215Y*	0.7	1.0	0.7	1.8
D067N K070R K219Q T215F	0.9	0.7	0.7	0.8
V075I F077L F116Y Q151M	2.2	2.0	2.0	1.9
M041L D067N T069D L210W T215Y*	<0.5	0.5	1.0	1.4
M041L D067N V075M L210W T215Y*	0.6	0.8	0.2	0.4
M041L D067N M184V L210W T215Y	0.3	0.4	0.3	0.7
M041L D067N T069D V118I L210W T215Y	0.4	0.4	0.3	0.7
M041L D067N K070Q M184V L210W T215Y	0.3	0.3	0.3	0.5
M041L M184V L210W R211K L214F T215Y*	<0.5	0.8	0.9	0.6
T069S-SG M041L L210W R211K L214F T215Y*	<0.5	<0.4	0.4	0.4
T069S-SS M041L L210W R211K L214F T215Y*	<0.5	<0.4	<0.2	0.3
M041L D067N T069D L210W R211K L214F T215Y	0.8	0.5	0.6	1.4
A062V T069S M184V L210W R211K L214F T215Y*	0.6	<0.4	0.5	0.7
M041L D067N M184V L210W R211K L214F T215Y*	0.7	0.5	0.6	0.7
M041L E044D D067N L210W R211K L214F T215Y*	0.7	0.9	0.8	1.4
M041L E044D D067N T069D L210W R211K L214F T215Y*	0.7	0.7	0.9	1.6
M041L E044D D067N V118I L210W R211K L214F T215Y*	<0.5	0.9	0.4	1.0
D067N T069D V075M M184V L210W R211K L214F T215Y*	1.0	1.0	1.0	1.3
M041L E044D D067N T069D V118I L210W R211K L214F T215Y*	<0.5	<0.4	0.9	0.9
M041L E044D D067N V118I M184V L210W R211K L214F T215Y*	<0.5	<0.4	0.4	0.8
M041L E044D D067N T069D V118I M184V L210W R211K L214F T215Y*	0.8	0.6	0.5	0.6

Data are presented as the median of 3 independent experiments (unless marked with a* for those done once). All experiments were run in duplicates.

HIV-1 site-directed mutant viruses with two and three NNRTI resistance-associated substitutions were selected based on the current knowledge of the ETR resistance profile. Of the 16 HIV-1_{HXB2} site-directed mutant viruses with two NNRTI resistance-associated substitutions that were investigated, 4 had less than 3-fold changes in susceptibility to TMC278 (i.e., L100I/K101E; K101E/K103N; K103N/F227L; K103N/V108I) (Table 10). High-level cross-resistance between TMC278, EFV, NVP, and ETR was observed for all 8 isolates with three NNRTI resistance-associated substitutions (Table 10).

These observations were extended further by analyzing additional HIV-1_{HXB2} site-directed mutant viruses (n=161) containing one or more NNRTI resistance mutations generated on the basis of data collected from the genotypic profiles of virologic failures identified in the C204 Clinical Trial (Table 9, Appendix A). These results confirm that the 3 HIV-1 site-directed mutant viruses with a single NNRTI resistance-associated substitution K101P, Y181I or Y181V were resistant to TMC278 and confirm that K103N in isolation is not associated with resistance to TMC278.

The combinations of NNRTI resistance-associated substitutions that decrease susceptibility to TMC278 are varied and complex (Appendix A). Overall, 53% (42/79) of the double NNRTI mutant viruses had ≥2.5-fold decrease in TMC278 susceptibility (Table 9). Analysis of the specific NNRTI substitution changes in the double mutants showed that, from those with a combination containing V90I, only those with E138Q or Y181I are resistant to TMC278. From the various combinations with K101E, those with E138K or M184V were sensitive to TMC278. The combination of E138K+M184I substitutions was resistant to TMC278 and ETR, but not to EFV and NVP. In contrast, the combinations of E138A+M184I, E138G+M184I, E138K+M184V and K101E+E138K+M184I were sensitive to TMC278. The addition of M184V to any of the combinations of RT substitutions did not modify their sensitivity to TMC278, EFV, ETR and NVP. Most (>70%; 46/62) of the HIV-1 site-directed mutant viruses with combinations of three NNRTI resistance-associated substitutions showed decreased susceptibility to rilpivirine (≥2.5 FC) (Table 9, Appendix A).

M184I and M184V were not selected in cell culture in the presence of TMC278. However, the E138K with M184I substitutions emerged together frequently in virologic failures in TMC278 arms of the Phase 3 clinical trials (see *Clinical Virology* below). The combination of E138K and M184I confers higher level decreased susceptibility (6.7-fold) to TMC278 than the E138K substitution alone (2.8-fold) indicating a contribution of the M184I substitution to TMC278 resistance (Appendix A). The applicant states that the possible impact of M184I in combination with E138K on HIV-1 resistance to TMC278 is under investigation.

Table 9. Proportion of Site-Directed Mutant Viruses with Decreased Susceptibility to TMC278 (≥2.5 fold change) by Number of NNRTI Substitutions

HIV-1_{HXB2} Site-Directed Mutant Viruses with:	Decreased TMC278 Susceptibility (FC≥2.5) n/N (%)
1 NNRTI Substitution	13/67 (19%)
2 NNRTI Substitutions	42/79 (53%)
3 NNRTI Substitutions	46/62 (74%)

Table 10. Antiviral Activity of TMC278 against Site-Directed Mutant Viruses Harboring NNRTI Resistance-Associated Substitutions (n=55)

NNRTI RAM(s)	Median FC (Q1–Q3)[a] in the antiviral activity of:			
	TMC278	EFV	ETR	NVP
V090I	1.7 (1.4–1.9) (*n* = 4)	1.6 (1.4–1.8) (*n* = 4)	1.5 (1.4–1.6) (*n* = 4)	4.4 (3.4–5.1) (*n* = 4)
L100I	0.9 (0.5–1.1) (*n* = 73)	20.3 (15.5–28.9) (*n* = 106)	1.3 (1.0–1.8) (*n* = 107)	7.3 (4.7–10.1) (*n* = 43)
K101E	2.4 (2.0–4.7) (*n* = 9)	3.8 (2.7–4.2) (*n* = 10)	2.7 (1.5–4.2) (*n* = 10)	ND
K101P	51.7 (34.7–64.1) (*n* = 48)	72.3 (39.0–96.3) (*n* = 45)	5.3 (4.6–6.4) (*n* = 48)	>166.1 (108.9–244.5) (*n* = 3)
K101Q	1.6 (0.9–2.2) (*n* = 8)	2.4 (1.7–3.5) (*n* = 8)	1.4 (1.1–2.5) (*n* = 8)	2.5 (1.9–3.6) (*n* = 5)
K103N	0.9 (0.6–1.2) (*n* = 72)	32.5 (23.9–43.0) (*n* = 113)	0.9 (0.7–1.1) (*n* = 110)	>42.1 (33.5–54) (*n* = 51)
K103S	1.6 (1–2) (*n* = 4)	4.8 (3.8–6.7) (*n* = 3)	0.8 (0.8–1.1) (*n* = 4)	59.4 (35.9–83.0) (*n* = 2)
V106A	0.6 (0.5–0.9) (*n* = 6)	2.0 (1.5–2.8) (*n* = 4)	0.5 (0.5–0.6) (*n* = 6)	49.0 (45.3–81.7) (*n* = 3)
V106M	0.9 (0.7–1.5) (*n* = 7)	2.6 (1.3–3.7) (*n* = 8)	0.9 (0.6–1.0) (*n* = 6)	6.3 (6.2–10.4) (*n* = 5)
V108I	0.7 (0.6–1.0) (*n* = 4)	1.5 (1.2–1.9) (*n* = 5)	0.7 (0.7–0.9) (*n* = 5)	3.1 (2.5–3.9) (*n* = 4)
E138A	2.5 (2.3–2.6) (*n* = 4)	1.6 (1.4–2.2) (*n* = 4)	2.9 (2.6–3.1) (*n* = 4)	2.3 (2.1–3.0) (*n* = 4)
E138G	1.6 (1.4–2.2) (*n* = 4)	0.9 (0.8–1.3) (*n* = 4)	2.4 (1.6–3.3) (*n* = 4)	2.8 (2.5–2.9) (*n* = 3)
E138K	2.8 (1.7–3.9) (*n* = 10)	2.0 (1.4–2.3) (*n* = 10)	2.6 (2.0–4.1) (*n* = 12)	1.1 (1.1–1.3) (*n* = 5)
E138Q	2.7 (2.1–3.9) (*n* = 7)	3.4 (2.8–5.6) (*n* = 8)	3.0 (2.5–3.7) (*n* = 7)	5.1 (3.9–6) (*n* = 6)
E138R	3.3 (2.9–3.6) (*n* = 4)	2.2 (2.0–2.5) (*n* = 4)	3.6 (3.2–4.2) (*n* = 4)	4.4 (4.1–4.7) (*n* = 4)
E138S	2.7 (2.5–2.8) (*n* = 4)	1.4 (1.1–1.7) (*n* = 4)	2.8 (2.1–3.5) (*n* = 4)	3.8 (2.0–5.9) (*n* = 4)
V179D	1.7 (1.1–2.4) (*n* = 6)	2.7 (2.5–2.9) (*n* = 6)	1.9 (1.4–2.5) (*n* = 6)	4.6 (3.7–4.9) (*n* = 3)
V179E	1.2 (0.7–1.9) (*n* = 7)	5.7 (4.0–7.6) (*n* = 9)	1.3 (1.2–3.1) (*n* = 9)	2.1 (1.3–3.2) (*n* = 4)
V179F	<0.1 (0.1–0.1) (*n* = 5)	<0.4 (0.2–0.6) (*n* = 6)	<0.2 (0.1–0.2) (*n* = 5)	0.6 (0.5–1.1) (*n* = 3)
V179T	1.5 (1.5–1.7) (*n* = 3)	1.0 (0.9–1.0) (*n* = 3)	0.7 (0.7–0.8) (*n* = 3)	0.8 (*n* = 1)
Y181C	2.7 (1.9–3.6) (*n* = 151)	2.1 (1.3–3.0) (*n* = 197)	4.0 (3.2–5.4) (*n* = 191)	>43.0 (34.7–76.7) (*n* = 60)
Y181I	15.3 (12.9–16.8) (*n* = 3)	1.6 (1.3–1.9) (*n* = 4)	12.5 (10.7–13.2) (*n* = 3)	>65.7 (60.6–71.5) (*n* = 3)
Y181V	12.2 (10.8–15.2) (*n* = 4)	3.0 (2.3–3.7) (*n* = 4)	15.1 (14.2–20.7) (*n* = 3)	2,155.9 (*n* = 1)
Y188L	2.8 (2.1–4.1) (*n* = 71)	42.6 (31.3–56.5) (*n* = 106)	1.1 (0.9–1.4) (*n* = 110)	>42.1 (33.1–65.4) (*n* = 45)
G190A	1.1 (0.8–1.2) (*n* = 12)	8.1 (6.3–10.1) (*n* = 17)	1.1 (0.8–1.4) (*n* = 14)	>86.6 (49.6–92) (*n* = 11)
G190S	0.2 (0.2–0.4) (*n* = 7)	94.8 (73.4–131) (*n* = 8)	0.2 (0.2–0.3) (*n* = 8)	96.9 (54.2–149.1) (*n* = 4)
H221Y	1.8 (1.3–2.2) (*n* = 4)	1.6 (1.3–1.9) (*n* = 4)	1.6 (1.1–2.2) (*n* = 4)	4.3 (3.7–4.6) (*n* = 4)
M230I	2.7 (2.5–3.7) (*n* = 3)	5.0 (4.6–5.9) (*n* = 10)	3.5 (3–4.1) (*n* = 9)	13.6 (10.8–18.6) (*n* = 8)
M230L	3.4 (2.6–4) (*n* = 4)	5.9 (3.9–11.1) (*n* = 9)	3.6 (2.6–5) (*n* = 8)	20.4 (16.8–21.3) (*n* = 7)
M230V	2.1 (1.5–3.5) (*n* = 4)	1.1 (1–1.3) (*n* = 11)	0.8 (0.8–1) (*n* = 8)	1.4 (1.2–1.7) (*n* = 8)
M236L	1.1 (1–1.3) (*n* = 4)	1.0 (0.6–1.7) (*n* = 4)	1.2 (1.0–1.4) (*n* = 4)	3.4 (*n* = 1)
L100I, K101E	2.1 (1.4–2.7) (*n* = 4)	49.1 (41.3–50.4) (*n* = 3)	0.9 (0.9–1.0) (*n* = 4)	18.1 (12.9–24.5) (*n* = 4)
L100I, K103N	7.0 (4.7–9.7) (*n* = 138)	>575.8 (56–1,172.2) (*n* = 149)	4.0 (2.9–5.3) (*n* = 178)	>42.5 (33.9–66.8) (*n* = 48)
K101E, K103N	2.0 (1.2–3.2) (*n* = 71)	56.4 (39–86.3) (*n* = 149)	1.8 (1.2–2.7) (*n* = 152)	>41.6 (29.9–59.3) (*n* = 85)
K103N, F227L	0.5 (0.4–0.6) (*n* = 3)	8.9 (7.1–16.2) (*n* = 3)	0.5 (0.4–0.6) (*n* = 3)	141.1 (109.0–173.1) (*n* = 2)
K103N, V108I	0.5 (0.5–0.8) (*n* = 3)	96.0 (71.1–107.8) (*n* = 4)	0.7 (0.6–0.7) (*n* = 4)	>205.2 (136.7–237.8) (*n* = 3)
K103N, Y181C	3.5 (2.1–4.8) (*n* = 246)	36.5 (25.2–50.7) (*n* = 420)	4.1 (2.8–5.5) (*n* = 426)	>41.8 (29.6–60.6) (*n* = 128)
K103N, Y181I	94.9 (80.0–117.5) (*n* = 3)	6.4 (5.5–7.5) (*n* = 3)	16.1 (13.5–21.8) (*n* = 3)	>71.5 (68.6–74.4) (*n* = 2)
E138K, M230L	22.6 (20.8–24.0) (*n* = 3)	18.7 (17.9–22.4) (*n* = 3)	19.3 (17.5–26.3) (*n* = 3)	>63.7 (61.9–70.5) (*n* = 6)
V179D, Y181C	6.0 (3.9–6.1) (*n* = 3)	9.3 (9.2–9.7) (*n* = 3)	10.8 (8.6–11.6) (*n* = 3)	>71.5 (68.6–74.4) (*n* = 2)
V179E, Y181C	6.8 (6.0–7.9) (*n* = 3)	13.1 (10.9–14.6) (*n* = 3)	30.6 (24.3–36.9) (*n* = 3)	>71.5 (68.6–74.4) (*n* = 2)
V179F, Y181C	8.7 (6.0–12.2) (*n* = 79)	4.6 (3.6–7.5) (*n* = 76)	158.9 (113.6–211.6) (*n* = 79)	>358.3 (267.4–459.3) (*n* = 7)
V179F, Y181I	11.4 (10.3–27.4) (*n* = 3)	1.0 (1.0–1.1) (*n* = 3)	122.8 (101.4–146.5) (*n* = 3)	>71.5 (68.6–74.4) (*n* = 2)
V179I, Y181C	3.7 (3.2–4.0) (*n* = 3)	1.3 (1.3–2) (*n* = 3)	4.6 (4.3–12.8) (*n* = 3)	139.9 (107.2–172.5) (*n* = 2)
Y181C, F227C	23.6 (17.3–24.7) (*n* = 3)	12.1 (11.5–13.5) (*n* = 4)	25.3 (18.6–31.5) (*n* = 3)	>77.3 (71.5–77.4) (*n* = 3)
Y181C, G190S	3.4 (3.3–4.3) (*n* = 3)	486.0 (363.8–604.7) (*n* = 3)	20.4 (17.0–26.8) (*n* = 3)	>71.5 (68.6–74.4) (*n* = 2)
Y181C, Y188L	30.2 (25.9–37.9) (*n* = 3)	237.5 (178.8–280.2) (*n* = 4)	6.0 (3.7–10.3) (*n* = 4)	>157.7 (134.0–181.5) (*n* = 2)
L100I, K103N, E138G	33.2 (23.6–62.7) (*n* = 6)	1,695.3 (1,453.9–1,796.3) (*n* = 6)	20.8 (10.9–24.3) (*n* = 5)	>58.1 (50.4–65.7) (*n* = 7)
L100I, K103N, T386A	22.8 (16.5–27.5) (*n* = 3)	10,866.5 (8,349.2–12,035.2) (*n* = 3)	12.2 (10.2–15.7) (*n* = 3)	>51.6 (*n* = 1)
L100I, K103N, V179L	46.1 (42.1–63.1) (*n* = 3)	5,660.6 (5,405.2–6,002.4) (*n* = 3)	13.4 (13.1–15.9) (*n* = 3)	>71.5 (68.6–74.4) (*n* = 2)
L100I, K103N, Y181C	80.8 (66.0–109.4) (*n* = 7)	1,812.0 (1,496.9–2,110.8) (*n* = 5)	58.1 (34.8–73.2) (*n* = 7)	468.1 (395.5–540.8) (*n* = 2)
L100I, V179I, Y181C	15.2 (9.1–22.1) (*n* = 4)	16.9 (14.6–17.2) (*n* = 4)	34.1 (27.0–42.6) (*n* = 4)	>205.2 (128.4–267.8) (*n* = 3)
K101P, K103N, V108I	>162.1 (115.8–314.9) (*n* = 3)	12,931.1 (11,343.6–13,462.3) (*n* = 3)	18.4 (17.3–19.7) (*n* = 3)	>51.6 (*n* = 1)
K103N, V179I, Y181C	10.5 (8.2–14.1) (*n* = 9)	16.7 (12.6–27.3) (*n* = 8)	14.5 (10.4–17.2) (*n* = 8)	>429.9 (252.5–445.1) (*n* = 7)
V179F, Y181C, F227C	553.8 (386.7–578.5) (*n* = 3)	25.7 (22.5–57.7) (*n* = 3)	638.6 (559.5–763.1) (*n* = 3)	>71.5 (68.6–74.4) (*n* = 2)

[a] Fold changes (FCs) were calculated as the ratio between the 50% effective concentration of the compound for the HIV-1 SDM and that obtained in the same experiment with HIV-1/IIIB. Q1–Q3, first- to third-quartile values; *n*, number of experiments; ND, not determined.

4.6 Clinical Studies

TMC278-TiDP6-C209

TMC278-TiDP6-C209 is an ongoing, 96-week, randomized, double-blind, double-dummy, active-controlled, international Phase III trial in HIV-1-infected, treatment-naïve adult subjects. The trial was designed to evaluate the long term efficacy, safety, and tolerability of TMC278 25 mg once daily compared with control (efavirenz 600 mg once daily), each in combination with a background regimen containing emtricitabine and tenofovir disoproxil fumarate. Adult subjects with an HIV-1 viral load of ≥ 5,000 HIV-1 RNA copies/mL, who were treatment-naïve, susceptible to their background regimen at screening, and had no NNRTI resistance-associated substitutions in their genotype at screening, were eligible for the trial.

HIV-1 infected subjects (n = 690) were randomized in a 1:1 ratio to TMC278 25 mg q.d. or to EFV 600 mg q.d. for a 96-week treatment period followed by a 4-week follow-up period. The primary objective of this trial was to demonstrate non inferiority of TMC278 versus EFV in regard to the proportion of subjects achieving a confirmed plasma viral load of <50 HIV-1 copies/mL at 48 weeks of treatment, (using time to loss of virologic response [TLOVR] imputation), with a maximum allowable difference of 12%. The lower limit of the 95% confidence interval of the difference between the treatment groups derived from the logistic regression model was >-12% (-5.9; 5.2), thus demonstrating non-inferiority of TMC278 compared with control (at the 12% non-inferiority margin) in regard to the proportion of subjects achieving a confirmed plasma viral load <50 HIV-1 RNA copies/mL at 48 weeks of treatment.

TMC278-TiDP6-C215

TMC278-TiDP6-C215 is an ongoing, 96-week, randomized, double-blind, double-dummy, active-controlled, international Phase III trial in HIV-1-infected, treatment-naïve adult subjects. The trial was designed to evaluate the long-term efficacy, safety and tolerability of TMC278 25 mg once daily compared with control (efavirenz 600 mg once daily). Each of these NNRTIs was given in combination with a background regimen containing 2 nucleoside/nucleotide reverse transcriptase inhibitors [N(t)RTI]. The investigator-selected N(t)RTIs were either abacavir /lamivudine, emtricitabine/tenofovir disoproxil fumarate, or lamivudine/zidovudine. Adult subjects with an HIV-1 viral load of ≥ 5,000 HIV-1 ribonucleic acid copies/mL, who were treatment-naïve, susceptible to their background regimen at screening, and had no NNRTI resistance-associated substitutions in their genotype at screening, were eligible for the trial.

HIV-1 infected subjects (n = 678) were randomized in a 1:1 ratio to TMC278 25 mg q.d. or to EFV 600 mg q.d. after the investigator had selected the N(t)RTI background regimen for 96-week treatment period followed by a 4-week follow-up period. The primary objective of this trial was to demonstrate non-inferiority of TMC278 versus EFV in regard to the proportion of subjects achieving a confirmed plasma viral load of <50 copies/mL at 48 weeks of treatment, with a maximum allowable difference of 12%. The lower limit of the 95% confidence interval of the difference between the treatment groups derived from the logistic regression model was > -12% (-1.7; 8.8), thus demonstrating non-inferiority of TMC278 compared with control (at the 12% non-inferiority margin) in

regard to the proportion of subjects achieving a confirmed plasma viral load <50 HIV-1 RNA copies/mL at 48 weeks of treatment.

4.7 Clinical Virology

PHASE III STUDIES C209 and C215

In Study C209, TMC278 25 mg once daily was compared with EFV 600 mg once daily each in combination with a background regimen containing emtricitabine and tenofovir disoproxil fumarate. In Study C215, TMC278 25 mg once daily was also compared to EFV 600 mg once daily. However, the background regimen could contain combinations ABC/LAM, AZT/LAM or TDF/FTC. The proportions taking each regimen in each arm are shown in Table 11.

Table 11. C215 Background Treatment

	TMC278 Arm	EFV Arm
ABC/LAM	35 (10%)	33 (10%)
AZT/LAM	101 (30%)	103 (30%)
TDF/FTC	204 (60%)	202 (60%)

BASELINE RESISTANCE PARAMETERS

At Baseline, in the pooled analysis, 99.3% and 99.3% of subjects were susceptible to TMC278 and efavirenz, respectively; and 95% and 94% of subjects were sensitive to both their background NRTIs in the TMC278 and the EFV control group, respectively (Table 12). No differences regarding susceptibility to the treatment drugs were observed at baseline between treatments. None of the subjects with baseline resistance to TMC278, EFV, TDF, LAM or AZT were rebounders or virologic failures who never suppressed.

Table 12. Baseline Susceptibility (number above fold-change (FC) cutoff /number with phenotypic data)

	C209 N=690		C215 N=678	
FC cutoff	**TMC278 N=346**	**EFV N=340**	**TMC278 N=340**	**EFV N=338**
TMC278 FC ≥3.7	2/337	3/336	2	2
EFV FC ≥ 3.3	3/337	1/336	1	5
FTC FC ≥3.1	0	0	0	0
TDF FC ≥1.4	25/336	25/335	17 (9 TDF/FTC)	20 (10 TDF/FTC)
ABC FC ≥3.2			0	0
AZT FC ≥ 2.5			4*	6 (4*)
LAM FC ≥ 2.1			1*	1

*drug not in background regimen
Note: No subjects with baseline resistance were rebounders or virologic failures who never suppressed

There were 41 subjects with a ≥2.5 fold change to TMC278 at baseline, of which 32 (78%) were responders. There were 9 subjects with a ≥2.5 fold change to TMC278 at baseline who were virologic failures (7 in Study C209 and 2 in Study C215) (Table 13).

Table 13. Subjects with Baseline TMC278 ≥2.5 FC

PID	Reason for Failure	TMC278 FC at Baseline
TMC278-C209-0066	Virologic failure: Never suppressed	3.4
TMC278-C209-0099	DC	3.2
TMC278-C209-0129	Virologic failure: Never suppressed	3.0
TMC278-C209-0184	DC AE	2.9
TMC278-C209-0405	DC	2.9
TMC278-C209-0495	Virologic failure: Never suppressed	6.1
TMC278-C209-0629	Virologic failure: Rebounder	3.4
TMC278-C215-0208	Virologic failure: Never suppressed	2.5
TMC278-C215-0743	DC AE	3.3

Response by Baseline Genotype

Although presence of baseline NNRTI substitutions was an exclusion criterion, an analysis was undertaken to look at response rates by baseline genotype (Table 14). As expected, there were limited NNRTI substitutions at baseline. The numbers were small, but response rates did not appear to be affected by the baseline substitutions analyzed.

Table 14. Outcome by Baseline Genotype (Snapshot)

Baseline Substitution	TMC278 Response Rate	EFV Response Rate
Overall	83% (566/686)	80% (546/682)
V90I	76% (13/17)	67% (8/12)
V189I/A	9/9	89% (8/9)
K101E/P	3/3	5/5
E138G/K/R/Q or A	0/1	1/1
Y181C/I/V	0	0
H221Y	0	0
L100	0	0
V108	0	0/1
V179	78% (49/63)	80% (43/54)
V179I	79% (45/57)	
F227	0	0
M230	0	0
K103R	89% (17/19)	80% (8/10)

VIROLOGIC FAILURES

Subjects participating in the TMC278-TiDP6-C209 and TMC278-TiDP6-C215 studies were included in this exploratory Week 48 resistance analysis if they experienced virologic failure (rebounder, stopped treatment while not suppressed, or never suppressed).

A subject experiencing a virologic failure (VF) was defined as having either:

- first achieved 2 consecutive viral load values <50 copies/mL, followed by 2 consecutive viral load values of ≥50 copies/mL (also called a 'rebounder' or VF1)
- first achieved 2 consecutive viral load values <50 copies/mL and stopped treatment with a last observed viral load value on treatment of ≥50 copies/mL (also called a 'stopped treatment while not suppressed' or VF2)
- never achieved 2 consecutive viral load values of <50 copies/mL and having an increase in viral load of at least 0.5 $\log_{10}$ copies/mL above the nadir (also called a 'never suppressed' or VF3)

Subjects who rebounded, were never suppressed, or were not suppressed at discontinuation after Week 4 (>Day 30) were included in the FDA resistance analysis. Subjects who discontinued before or on Day 30 were censored from the FDA resistance analysis. More subjects discontinued early before Week 4 in the EFV arms compared to the TMC278 arms and were censored from the analysis. Three subjects were censored from the TMC278 arms of both studies and 30 were censored from the EFV arms of both studies (Appendix B). In addition, subjects who discontinued while suppressed were removed for the as-treated analysis. In the TMC278 arms, 15 and 19 subjects were removed from Study 209 and Study 215, respectively, because they discontinued while suppressed. More subjects discontinued while suppressed in the EFV arms: 42 and 36 subjects were removed from the as-treated analysis from Study 209 and Study 215, respectively.

After censoring and removing subjects who discontinued while suppressed, 93 in the TMC278 and 60 in the control group were included in the FDA virologic failure as-treated analysis (Table 15; PIDs listed in Appendix C). The overall proportion of virologic failures was 14% (93/652) in the TMC278 arm compared to 10% (60/604) in the EFV arm in both Study C209 and Study C215. The proportion of virologic failures was higher in the TMC278 arm (16%) of Study 209 compared to the EFV arm (7%) whereas the virologic failure rates (12%) were more comparable in Study 215. Of note, these numbers differ from the sponsor's number of subjects with virologic failure in the time to loss of virologic response (TLOVR) and snapshot analyses because the sponsor only included virologic failures as defined above and did not use an as-treated analysis.

There were 2 times more virologic failures who rebounded or where never suppressed in the TMC278 arm of Study C209: 14% compared to 6% in the EFV arm (Table 15). However, in Study C215 there were a comparable number of virologic failures who rebounded or where never suppressed – 10% in the TMC278 arm compared to 9% in the EFV arm. In the pooled analysis of the Phase 3 trials, 93 subjects were virologic

failures in an as treated analysis. There were 40 virologic failures with ≥2.5 FC in TMC278 susceptibility in the pooled studies (Appendix E).

Table 15. As-Treated Censored Resistance Analysis

	C209 N=690		C215 N=678	
	TMC278 N=346	EFV Control N=344	TMC278 N=340[1]	EFV Control N=338[1]
As Treated Virologic Failures	54/331 (16%)	24/302 (7%)	39/321 (12%)	36/302 (12%)
Rebounders + Never suppressed	45/331 (14%)	20/302 (7%)	32/321 (10%)	27/302 (9%)
Paired Baseline and Post Baseline Resistance Data[2]	45	17	30	20
Virologic failures with ≥2.5 FC in TMC278 Susceptibility at Failure	26 (58%)	--	14 (47%)	--
Virologic failures with EFV Resistance (>3.3 FC)	--	8 (47%)	--	7 (35%)

[1]Removed responders who switched background NRTIs not permitted by the protocol
[2]Listed in Appendix D

A number of incongruities were found using the snapshot approach for the resistance analysis (Table 16). There were subjects who rebounded during treatment but were <50 copies/mL at Week 48. Since these subjects rebounded and in some cases had evidence of resistance emergence, they were included as virologic failures (rebounders) in the FDA virology resistance analysis. A few subjects who had missing data in the Week 48 window were counted as virologic failures using the snapshot approach. The number of subjects with missing data in the Week 48 was similar between the arms of each study. Additionally, several subjects in each arm who were responding to treatment (<50 copies/mL) but were ≥50 copies/mL at the Week 48 timepoint were counted as rebounders. There were a comparable number of subjects in this category between the arms of each study.

Table 16. Incongruities Using the Snapshot Approach for the Resistance Analysis

ARM/STUDY	Missing data in Week 48 window	Responded but >50 at Week 48 (Rebounders)	Rebounded but <50 copies/mL at Week 48
TMC278 C209	0090 0112 0788	N=7 0383 0538 0555 0683 0750 = 50 week 48 0915 0935	0512 (developed phenoR to all drugs in regimen) 0548 (developed V179I I329I/V no phenoR) 0612 *0679*

			0760
EFV C209	0124 0948	N=6 0054 0076= 50 week 48 0564 0709 0726 0909	0118 (EFV-R) 0583 0908
TMC278 C215	0869	N=9 0095 0130 0227 0387 0426 0446 0646 0656 0793	0494 0565 0914
EFV C215	0066	N=9 0164 0165 0172 = 53 week 48 0228 0266 = 53 week 48 0317 0472 = 55 week 48 0621 0879 = 53 week 48	0251 0319 0490 0591 0702 (EFV-R LAM-R)

Number of Virologic Failures on Different Background Regimens in Study C215
Given that different background regimens were used in Study C215, we determined if the number of virologic failures differed depending on which background regimen was used. The percentage of virologic failures using each background regimen was similar to the proportion of the overall population using the given background regimen (Table 17; compare to Table 11). Therefore, the different background regimens did not appear to contribute to a difference in the virologic failure rate.

Table 17. Background Treatment of Virologic Failures in Study C215

	TMC278 Arm	EFV Arm
ABC/LAM	5/57 (9%)	5/54 (9%)
AZT/LAM	17/57 (30%)	18/54 (33%)
TDF/FTC	35/57 (61%)	31/54 (57%)

Virologic Failures by Baseline Viral Load Strata
The number of virologic failures was assessed by baseline viral load strata (Table 18). There were twice as many treatment failures in the >500,000 baseline HIV-1 RNA strata in the TMC278 arm (12%) compared to the EFV arm (6%). However, in an as-treated analysis, there were 14% TMC278 virologic failures in the >500,000 baseline HIV-1 RNA strata compared to 10% in the EFV arm (Table 19).

Table 18. Treatment Failures and Baseline Viral Load Strata

Treatment Failures	TMC278 N=124	EFV N=108
≤100,000 HIV-1 copies/mL	59 (48%)	66 (61%)
>100,000 and ≤500,000 HIV-1 RNA copies/mL	50 (40%)	35 (32%)
>500,000 HIV-1 RNA copies/mL	15 (12%)	7 (6%)

Table 19. Virologic Failures and Baseline Viral Load Strata

Virologic Failures (Censored As-Treated group)	TMC278 N=92	EFV N=60
≤100,000 HIV-1 copies/mL	39 (42%)	30 (50%)
>100,000 and ≤500,000 HIV-1 RNA copies/mL	40 (43%)	24 (40%)
>500,000 HIV-1 RNA copies/mL	13 (14%)	6 (10%)

GENOTYPIC ANALYSIS OF VIROLOGIC FAILURES

The genotypes of the virologic failures with decrease susceptibility to TMC278 and EFV were assessed for emerging substitutions associated with resistance and virologic failure. For TMC278, a conservative cutoff of 2.5-fold was used in the FDA analysis based on an overall assessment of the virologic failure phenotypic values and assay variation. This conservative approach was used in order not to miss potential emerging substitutions associated with TMC278 resistant and virologic failure. There were 40 virologic failures with ≥2.5 fold change in TMC278 susceptibility in Studies C209 and C215 and they are listed with the emerging RT substitutions and phenotypic data in Table 20.

Table 20. Virologic Failures with Decreased TMC278 Susceptibility (≥2.5 FC) at Failure

PID	Bgrd TRT	Reason for Failure	Baseline RT Substitutions	RT Substitutions Emerging	Baseline Phenotype	Failure Phenotype
C209-0009	FTC TDF	Never suppressed		E28K **V90I/V E138K M184I** M357I		13 TMC278 EFV-R ETR-R FTC-R
C209-0011	FTC TDF	Never suppressed	E138A R211K	E6E/K **V90I Y181I M184I** R211Q		621 TMC278 NVP-R EFV-R ETR-R FTC-R
C209-0066	FTC TDF	Never suppressed		**K65K/R D67D/G K70E/K K101E Y181C M184V H221H/Y**	3.2 TMC278 TDF 2.2	17 TMC278 NVP-R EFV-R, ETR-R FTC-R
C209-0079	FTC TDF	Rebounder	D67D/N A98S Q207E T215I/T K219E/K	A158T **M184I** Q207A K219E P313T		8.6 TMC278 ETR-R FTC-R
C209-0119	FTC TDF	Rebounder	A98S	**E138K M184I** R356K		5.1 TMC278 EFV-R ETR-R FTC-R

C209-0129	FTC TDF	Never suppressed		P4S **L100I** I135I/T **E138K** T139K **M184I** K219E		64 TMC278 NVP-R EFV-R ETR-R FTC-R
C209-0146	FTC TDF	Never suppressed	V90I	**E138K M184I** L214F **H221H/Y**		7 TMC278 ETR-R FTC-R
C209-0161	FTC TDF	DC	A98A/S	**L74I E138K M184V** E358K/R		6.5 TMC278 NVP-R ETR-R FTC-R
C209-0163	FTC TDF	Rebounder		**K101E M184I** I293I/V		4.6 TMC278 NVP-R EFV-R ETR-R FTC-R
C209-0231	FTC TDF	Never suppressed	V179I/V	**E138K V179I M184I/V** V179I K219E/K		32 TMC278 NVP-R EFV-R ETR-R FTC-R
C209-0297	FTC TDF	Never suppressed	A98S V106I	**Y181C M184I H221H/Y**		5.1 TMC278 NVP-R EFV-R ETR-R FTC-R
C209-0361	FTC TDF	Never suppressed		A62V **K65R V90I/V E138E/K Y181C M184I H221H/Y**		14 TMC278 NVP-R EFV-R ETR-R FTC-R
C209-0389	FTC TDF	Rebounder		**K65N** Y115F **E138K** G282G/R		3.6 TMC278 DTR-R FTC-R TDF-R
C209-0495	FTC TDF	Never suppressed		K32E/K K70E/K **V90I/V E138K M184I**	6.1 TDF-R	7.8 TMC278 NVP-R EFV-R ETR-R FTC-R
C209-0512	FTC TDF	Rebounder But <50 copies/mL at Week 48	A98S	E40K **K65R K101E Y181C V189I** K219E		10 TMC278 NVP-R EFV-R ETR-R FTC-R TDF-R
C209-0573	FTC TDF	Never suppressed	V179I	**V108I** E122K **Y181C M184V** K219E		28 TMC278 NVP-R EFV-R ETR-R FTC-R
C209-0594	FTC TDF	Never suppressed		E28E/K D67N K70E/K **V90I E138K M184I** K219R D256D/E Y354H/Y	NVP-R TDF-R	8 TMC278 NVP-R EFV-R ETR-R FTC-R
C209-0629	FTC TDF	Rebounder		L109L/Q I178I/L	3.4 TMC278	3.5 TMC278 NVP-R EFV-R ETR-R
C209-0636	FTC TDF	Rebounder		W88G/W **K101E/K E138K M184I** L214F		5.9 TMC278 NVP-R EFV-R ETR-R FTC-R
C209-0745	FTC TDF	DC		**K65K/R E138K M184I**		8.8 TMC278 EFV-R ETR-R FTC-R
C209-0768	FTC TDF	Never suppressed		**K101E M184I** E204K		4.6 TMC278 EFV-R FTC-R
C209-0779	FTC TDF	Never suppressed		I31I/L **K65K/R E138K M184I** I257L		8.7 TMC278 NVP-R EFV-R ETR-R FTC-R

C209-0787	FTC TDF	DC		**K101E** T165K/T **M184I** T240A/T R277K/R		3.5 TMC278 NVP-R EFV-R ETR-R FTC-R
C209-0807	FTC TDF	Never suppressed		**K101E/K** Y115F **E138K M184V V189I/V** M357L/M T376A/T		7.5 TMC278 NVP-R EFV-R ETR-R FTC-R
C209-0871	FTC TDF	Never suppressed		K64Q **E138K** S162C **M184I** S332T A360T K390R T400A		4.9 TMC278 EFV-R ETR-R FTC-R
C209-0887	FTC TDF	Never suppressed	T215N	**V90I E138K/Q V179I/V M184I V189I/V** L228I/L		7.9 TMC278 NVP-R EFV-R ETR-R FTC-R
C215-0001	FTC TDF	DC	V35I A98S	V35T **K65N D67N** S68G **V106A** E122K **E138K V179I F227L** K281R		29 TMC278 NVP-R EFV-R FTC-R TDF-R
C215-0032	FTC TDF	Never suppressed		E6E/K **K101E E138E/K M184I** K219E E297K		6.9 TMC278 NVP-R EFV-R ETR-R FTC-R
C215-0110	FTC TDF	Never suppressed		**E138K M184I/V/M** T200A/T		8.3 TMC278 EFV-R ETR-R FTC-R
C215-0135	AZT LAM	Rebounder		I31I/L L100I/L **K101E/K E138K M184V** T338S		2.6 TMC278 NVP-R EFV-R ETR-R LAM-R
C215-0181	ABC LAM	Never suppressed	E122K	**L100I K101E/K E138K M184I** K219E/K R356G/R		19.9 TMC278 NVP-R EFV-R ETR-R LAM-R
C215-0208	AZT LAM	Never suppressed	E122K	E28K A62A/V S68G **V90I V108I E138K V179L M184V** G196G/R N348I T377I		64 TMC278 NVP-R EFV-R ETR-R LAM-R
C215-0330	FTC TDF	DC		**V90I/V Y181C M184I** D324D/E		16 TMC278 NVP-R EFV-R ETR-R LAM-R
C215-0339	FTC TDF	Never suppressed	K103R	I135T **M184V F227C M230L**		17 TMC278 NVP-R EFV-R ETR-R FTC-R
C215-0344	FTC TDF	Never suppressed	A98S	**V90I E138K M184I** G285R M357T		6.3 TMC278 EFV-R ETR-R FTC-R
C215-0416	FTC TDF	Never suppressed		**E138K M184I/V/M V189I/V H221H/Y**		9.6 TMC278 NVP-R EFV-R ETR-R FTC-R
C215-0466	AZT LAM	Never suppressed	D123S M184M/V L210F/L	I47L V75I/V **K101P/T** D123N I132L S163T **M184V** L210F		138 TMC278 NVP-R EFV-R ETR-R LAM-R
C215-0515	FTC TDF	Never suppressed	V106I	**K101E/K V118I/V E138E/K M184I/M/V**		5.9 TMC278 NVP-R EFV-R

				E204E/K **H221H/Y**		ETR-R FTC-R
C215-0534	FTC TDF	Never suppressed		**K101E/K** E122K D123N **M184I** **V189I/V** T200A Q207A R307K		**3.1 TMC278** NVP-R EFV-R FTC-R
C215-0783	FTC TDF	DC	E122K	K11K/T		2.6 TMC278

*Removed C209-0629 and C215-0783 from original 40 TMC278 virologic failures with >2.5 FC TMC278 susceptibility and genotypic changes, because C209-0629 had 3.4 FC at baseline and did not develop any of the frequently emerging TMC278 substitutions and C215-0783 did not develop any of the frequently emerging TMC278 substitutions. Therefore, total = 38 rilpivirine virologic failures with evidence of rilpivirine resistance emergence.

- NRTI and NNRTI resistance-associated substitutions are bolded.

The most common emerging NNRTI substitutions in TMC278 virologic failures with ≥2.5 FC in TMC278 susceptibility (N=40) were E138K, K101E/P/T, V90I, V179I/L Y181I/C, V189I, and H221Y (Table 21). These results were confirmed in a second analysis performed by Dr. Julian O' Rear (Virology Team Leader). The emtricitabine/lamividine resistance-associated substitutions M184I or V emerged frequently in association with other NNRTI substitutions. Over half of the virologic failures had a combination of the E138K and M184I substitutions. The fold change in TMC278 susceptibility of the TMC278 virologic failures ranged from 2.6 – 621.

In subject 215-0208, additional substitutions, V90I and V108I, emerged from the failure to the end of treatment timepoints resulting in increased TMC278 resistance (from 21-FC to 64 FC) and cross-resistance to other NNRTIs.

Table 21. Substitutions Emerging on TMC278 in Virologic Failures from Combined Phase 3 Studies (n=93)

Substitutions Emerging on TMC278	Number (%)
E138K/A/G	27 (29%)
E138K M184I	20 (22%)
E138G M184I	1 (1%)
E138A M184I	1 (1%)
E138K M184V	6 (6%)
K101E/P/T	14 (15%)
V179L	1 (1%)
V179I	3 (3%)
V90I	9 (10%)
Y181C/I	7 (8%)
H221Y	6 (6%)
G190	0
V189I	5 (5%)
L100I	3 (3%)
V106A/I	4 (4%)
E28K	4 (4%)
F227C/L	2 (2%)
M230L	1 (1%)

A62V	2 (2%)
K65R/N	7 (8%)
M184I	30 (32%)
M184V	11 (12%)
K219E/R	7 (8%)

For EFV, the 3.3 fold change cutoff for EFV in the Antivirogram assay Version 2.5.01 was used in the FDA resistance analysis. There were 15 EFV virologic failures with EFV resistance in the combined studies and they are listed with emerging RT substitutions and phenotypic data in Table 22.

Table 22. Virologic Failures with EFV Resistance (>3.3 FC)

PID	Bgrd TRT	Reason for Failure	Baseline RT Substitutions	RT Substitutions Emerging	Baseline Phenotype	Failure Phenotype
C209-0118	FTC TDF	Rebounded but <50 copies/mL at Week 48		R83G/R K103N Y181S/Y		EFV-R NVP-R
C209-0007	FTC TDF	Never suppressed		K103N D123D/G		EFV-R NVP-R FTC-R TDF-R
C209-0092	FTC TDF	DC		K103N		EFV-R NVP-R
C209-0176	FTC TDF	Rebounder		K103N V108I/V I178M M184V E248D/E		EFV-R NVP-R FTC-R
C209-0285	FTC TDF	Never suppressed		K103N		EFV-R NVP-R
C209-0333	FTC TDF	Never suppressed		L100I/L K101K/Q K103N V108I/V M184I/V/M K238K/T I257I/L		EFV-R NVP-R FTC-R
C209-0648	FTC TDF	DC	Q207E	V106M Q207K S251N		EFV-R NVP-R
C209-0711	FTC TDF	Never suppressed		K103N M184V P225H		EFV-R NVP-R FTC-R
C215-0109	FTC TDF	Rebounder		K65R V106M Y188C		EFV-R NFV-R FTC-R
C215-0289	ABC LAM	Never suppressed	A98S	K103N V108I/V M184V V189I/V P225P/S N348I		EFV-R NFV-R LAM-R
C215-0594	AZT LAM	Never suppressed	A98S V189I T200I	K103N I135T K173T T200L		EFV-R NFV-R
C215-0596	AZT LAM	DC		K103N		EFV-R NFV-R
C215-0702	AZT LAM	Rebounded but <50 copies/mL at Week 48	V179I H207N	M184V		EFV-R NFV-R LAM-R
C215-	AZT	Never		K101E K103N		EFV-R NFV-R

0773	LAM	suppressed		M184V T200A/T T338S		LAM-R
C215-0860	FTC TDF	Rebounded but <50 copies/mL at Week 48	V179I H207N	V106M V179D/V M184V/M Y188C/Y S251N		EFV-R NFV-R

The common emerging NNRTI substitutions in EFV failures with EFV resistance at failure (N=15) were K103N (n=11/15; 73%) and V106M (n=3; 20%) (Table 23). There was one isolate with the V179I and H207N substitutions at baseline. Other NNRTI substitutions emerging on EFV treatment included L100I, V108I, V179D, Y181S, Y188C and P225H/S. These emerging substitutions are consistent with what has been historically seen in EFV clinical studies.

Table 23. Substitutions Emerging on EFV in Virologic Failures from Combined Phase 3 Studies (n=60)

Substitutions Emerging on TMC278	Number (%)
K103N	12 (20%)
V106M	4 (7%)
V108I/V	3 (5%)
E138K/A/G	0
Y181S/Y	1 (2%)
V179V/D	1 (2%)
L100I/L	1 (2%)
Y188C	2 (3%)
P225H	2 (3%)
V90I	1 (2%)
K101E	1 (2%)
V189I	1 (2%)
G190E	1 (2%)
K65R	2 (3%)
M184I or V	8 (13%)

TMC278 PHENOTYPIC ANALYSIS

The phenotypic data of the virologic failures was analyzed to determine susceptibility to TMC278 or EFV and the background drugs at the failure/endpoint timepoint. Using a conservative cutoff of ≥2.5 for TMC278, 26 and 14 of the virologic failure isolates in the TMC278 arms of Study C209 and C215, respectively, had decreased susceptibility to TMC278 at failure (Table 24). In the EFV arms, EFV resistance emerged in 8 and 7 of the virologic failure isolates in Study C209 and C215, respectively (Table 24). In Study C209, 27 of the virologic failure isolates were resistant to FTC and 3 were resistant to TDF at failure. Emergence of resistance to the background drugs was comparable in Study C215, where 8 virologic failure isolates developed FTC resistance, 8 developed LAM resistance and 2 developed ABC resistance.

Table 24. Decreased Susceptibility at Endpoint (% As-Treated Virologic Failures)

Decreased	**C209**	**C209**	**C215**	**C215**

Susceptibility to:	TMC278 N=54	EFV N=24	TMC278 N=39	EFV N=36
TMC278 ≥2.5	26 (48%)		14 (36%)	
FTC ≥3.1	27 (50%)	4 (17%)	8 (21%)	4 (11%)
TDF ≥1.4	3 (6%)	1 (4%)	0	2 (6%)
EFV ≥3.3	-	8 (33%)	-	7 (19%)
LAM ≥2.1	-		8 (21%)	5 (14%)
ABC ≥3.2	-		2 (5%)	0
AZT ≥2.5	-		0	1 (3%)

Overall, the emergence of resistance was greater in the TMC278 arms compared to the EFV arms in both studies. In the combined studies, 43% of the virologic failures in the TMC278 arms developed TMC278 resistance compared to 25% of the virologic failures in the EFV arms who developed EFV resistance (Table 25). Moreover, resistance to a background drug emerged in 47% of the virologic failures in the TMC278 arms compared to 15% in the EFV arms.

Table 25. Overall Number of Subjects Developing Genotypic Resistance

Decreased Susceptibility to:	TMC278 N=93	EFV N=60
TMC278/EFV	40 (43%)	15 (25%)
Background Drugs	44 (47%)	9 (15%)

Baseline TMC278 Phenotype of Failures vs. Responders

At Baseline, the isolates of subjects who responded had a median 1.1 fold change and a mean 1.2 fold-change in TMC278 susceptibility. Comparably, isolates of TMC278 virologic failure subjects (n=38) had a median 1.3 fold change and mean 1.5 fold-change in TMC278 susceptibility.

TMC278 Phenotype at Virologic Failure

At virologic failure, the median fold-change in TMC278 susceptibility was 3.5 and the mean fold-change was 18 of the pooled virologic failures (n=38) from both studies.

Clinical Phenotypic Cutoff for TMC278

Determining a clinical cutoff for TMC278 is challenging given the closeness of the phenotypic values of failures and responders at baseline (1.1 FC vs. 1.3 FC) and the tightness of the phenotypic values for the virologic failures with and without substitutions associated with decreased susceptibility to TMC278. For example, isolates from Subjects C215-0135 and C215-0783 both had a failure TMC278 phenotypic fold change of 2.6 (Table 26); however, only Subject C215-0135 had substitutions associated with TMC278 resistance (L100I/L, K101E/K, E138K and M184V) while C215-0783 had a K11K/T substitution. Similarly, isolates from Subjects C209-0787 and C209-0629 both had a fold change value of 3.5 for TMC278. Subject C209-0787 had substitutions K101E and M184I, which are associated with decreased susceptibility to TMC278; however, Subject C209-0629 had no TMC278 resistance-associated substitutions (Table 26). Furthermore, the biologic cutoff of 3.7 determined by the sponsor for TMC278 does not appear valid as a clinical cutoff given multiple TMC278 virologic failures with

TMC278 resistance-associated substitutions (i.e., K101E, E138K, M184I, V189I) and fold changes less than 3.7 for TMC278 (e.g., Subject C215-0534 and Subject C209-0389) (Table 26) and the fact that the median fold change for the virologic failures was 3.5.

Table 26. Examples of Phenotypic Values for TMC278 Virologic Failures

PID	RT Substitutions Emerging	Baseline Phenotype	Failure Phenotype
C215-0135	I31I/L **L100I/L K101E/K E138K M184V** T338S		**2.6 TMC278** NVP-R EFV-R ETR-R LAM-R
C215-0783	K11K/T		**2.6 TMC278**
C215-0534	**K101E/K** E122K D123N **M184I V189I/V** T200A Q207A R307K		**3.1 TMC278** NVP-R EFV-R FTC-R
C209-0787	**K101E** T165K/T **M184I** T240A/T R277K/R		**3.5 TMC278** NVP-R EFV-R ETR-R FTC-R
C209-0629	L109L/Q I178I/L	3.4 TMC278	**3.5 TMC278** NVP-R EFV-R ETR-R
C209-0389	K65N Y115F **E138K** G282G/R		**3.6 TMC278** DTR-R FTC-R TDF-R

In cell culture, the combination of E138K and M184I conferred over 2-fold more reduced susceptibility to rilpivirine than the E138K substitution alone. The combination of E138K and M184I emerged in over half of the rilpivirine virologic failures with a ≥2.5-fold FC. It is challenging to determine if the combination of E138K/M184I confers more resistance than E138K alone clinically, because of the confounding with various other NNRTI substitutions that emerged with the E138K and M184I substitutions. There were only two subject isolates (C209-0389 and C215-0001) containing the E138K without a M184 substitution (Table 27). However, isolate C215-0001 also had NNRTI substitutions V106A and V179I. Subject isolate C209-0389 had a phenotypic TMC278 fold change of 3.6. Five subject isolates had E138K and M184I substitutions without other NNRTI substitutions. Isolate C209-0119, C209-745, C209-0779, C209-0871 and C215-0110 had phenotypic TMC278 fold changes of 5.1, 8.8, 8.7, 4.9 and 8.3, respectively (Table 27). These results are consistent with the cell culture data showing that the combination of E138K and M184I confer about 2-fold more TMC278 resistance than the E138K substitution alone. Interestingly, in contrast to cell culture data which showed that the phenotype for site-directed mutant with E138K and M184V substitutions was 3.6, in one clinical isolate, C209-0161, with substitutions M184V and E138K emerging on treatment, the TMC278 fold change was 6.5.

In summary, clinical isolates containing the combination of E138K/M184I without other NNRTI substitutions had decreased rilpivirine susceptibility ranging from a 4.9 – 8.8 fold

change compared to a 3.6-fold change for a clinical isolate with E138K alone, consistent with the results of site-directed mutants in cell culture.

Table 27. Clinical Isolates with E138K and M184I or V Emerging on Treatment

PID	Genotype	TMC278 Phenotype
C209-0389	K65N Y115F **E138K** G282G/R	3.6
C209-0119	**E138K M184I** R356K	5.1
C209-0745	K65K/R **E138K M184I**	8.8
C209-0779	I31I/L K65K/R **E138K M184I** I257L	8.7
C209-0871	K64Q **E138K** S162C **M184I** S332T A360T K390R T400A	4.9
C215-0110	**E138K M184I/V/M** T200A/T	8.3
C209-0161	L74I **E138K M184V** E358K/R	6.5

SUBSET OF PATIENTS BELOW THE MEDIAN LOG_{10} IQ SCALE

In an exposure-response analysis (See Clinical Pharmacodynamics review by Jeff Florian, Ph.D.), a relationship between lower IQ and lower response was seen. In addition, higher baseline viral load is associated with lower response. Therefore, I performed an analysis on the 330 subjects from Studies C209 and C215 with an IQ below the median value to determine the effect of baseline viral load and baseline TMC278 phenotype on response rates.

The median TMC278 fold change of this subset of 330 subjects was 1.6 (quartiles 1.2, 2.0) (range 0.4-6.1). For the responders in this subset, the median was 1.7, which was comparable to the median of 1.65 for the failures in this subject.

Response rates in this subset were lower for subjects with a baseline TMC278 phenotype ≥2.5-fold and even lower for ≥3.0-fold (Table 28). Response rates in the subset were comparable to the response rates for the overall dataset when analyzed by baseline TMC278 phenotype (Table 28).

Table 28. Response Rates by Baseline Phenotype (n=330) n (%)

TMC278 FC	Response Rate for Subset Below Median IQ (n=330)	TMC278 FC	Combined Response Rate All 209 and 215 (n=686)
<1.6 n=159 median	127 (80%)	**<1.1** n=311 median	270 (87%)
≥1.6 n=171	136 (80%)	**≥1.1** n=356	282 (79%)
<2.0 n=237	191 (81%)	**<2.0** n=561	467 (83%)
≥2.0 n=93	72 (77%)	**≥2.0** n=106	85 (80%)
<2.5 n=292	235 (80%)	**<2.5** n=626	520 (83%)
≥2.5 n=38	28 (74%)	**≥2.5** n=41	32 (78%)
<3.0 n=311	251 (81%)	**<3.0** n=648	539 (83%)
≥3.0 n=19	12 (63%)	**≥3.0** n=19	12 (63%)
<3.5 n=324	259 (80%)	**<3.5** n=661	547 (83%)
≥3.5 n=6	4 (67%)	**≥3.5** n=6	4 (67%)

Of the 12 subjects who were responders with ≥3-fold change in baseline TMC278 susceptibility, 9 subjects had a baseline viral load ≤100,000 HIV-1 copies/mL and no subjects with >500,000 copies/mL at baseline. Of the 6 subjects with ≥3.5-fold change in baseline TMC278 susceptibility, 4 were responders and had less than 500,000 HIV-1 copies/mL [3 with ≤100,000 copies/mL]. One of the failures had ≥500,000 copies/mL at baseline and the other had ≥100,000 copies/mL.

An analysis of response rates by both baseline TMC278 phenotype and baseline viral load showed that baseline viral loads of ≥100,000 were associated with lower response rates (Table 29).

Table 29. Response Rates by Baseline Viral Load and TMC278 Susceptibility in Subset of Subjects below Median IQ (n=330)

Baseline Viral Load	Overall Response	≥2.5 N=38	<2.5 N=292	≥3.0 N=19	<3.0 N=311
≤100,000 n=176 (53%)	149 (85%)	17 (94%)	132 (84%)	7 (100%)	142 (84%)
>100,000 and ≤500,000 n=115 (35%)	84 (73%)	9 (64%)	75 (74%)	4 (50%)	80 (75%)
>500,000 n=39 (12%)	25 (64%)	4 (67%)	21 (64%)	2 (50%)	23 (66%)

This analysis of the effect of both baseline viral load stratification and TMC278 susceptibility in this subset showed that the baseline viral load seem to be a more important factor in determining response than baseline phenotype. Regardless of baseline phenotype, response rates were higher if baseline viral load was ≤100,000 copies/mL and response rates were lower if baseline viral load was >500,000 copies/mL. Additionally, baseline genotypes were examined for this group of subjects, but no substitution or polymorphism seemed to be associated with the lower response.

CROSS-RESISTANCE

In a pooled analysis of the Phase 3 trials TMC278-C209 and TMC278-C215, 40 of the 124 subjects with virologic failure on TMC278 had decreased susceptibility to TMC278. Of these, 85% (n=34) were resistant to etravirine (>3.2 FC) and efavirenz (>3.3 FC), and 60% (n=24) were resistant to nevirapine (>6 FC). In the EFV arm, all 15 of the EFV-resistant virologic failures were still susceptible to etravirine and rilpivirine at failure, but were resistant to NVP.

RESPONSE BY CLADE

There was no significant difference in the proportion of different clades in the virologic failure and responder populations compared to the overall distribution of clades (Table 30).

Table 30. Proportion of Clades in Responder and Failure Populations

Clade	Virologic Failures N=92	Responders n=566	Overall N=686
A1	2 (2%)	9 (2%)	11 (2%)
B	69 (75%)	391 (69%)	485 (71%)
C	12 (13%)	66 (12%)	76 (11%)
D	2 (2%)	2 (0.4%)	4 (0.6%)
F1	1 (1%)	9 (1.5%)	10 (1.5%)
CRF01_AE	5 (5%)	68 (12%)	76 (11%)
CRF02_AG		8 (1.4%)	10 (1.5%)

DISCORDANT AMPLICOR AND TAQMAN VIRAL LOAD DATA RESULTS

The COBAS® TaqMan® HIV-1 assay v1.0 (Roche Diagnostics) was originally used to report viral load results for the Phase III trials C209 and C215. This assay is a relatively new viral load assay with more sensitivity at lower end of viral load. However, the sponsor encountered issues with the TaqMan viral load assay; namely, an increase in blips and persistent low-level viremia (PLLV) with the TaqMan assay while the results were undetectable with the Amplicor viral load assay. This issue with the TaqMan viral load assay has been widely reported [Oliver, 2007; Manavi, 2008; Gatanaga, 2009; Lima, 2009; Montaner, 2009; Smit, 2009; Pas, 2010; Willig, 2010].

On December 15, 2009, Tibotec provided a briefing package for the Division's review and description of the issues encountered with the viral load assays. Tibotec proposed that the COBAS® Amplicor® HIV-1 Monitor (Roche Diagnostics) should be adopted as the primary viral load assay instead of the TaqMan assay. In the January 13, 2010 correspondence, the Division agreed to the use of the Amplicor assay results to report viral load results for the primary efficacy analyses, but requested an analysis using the TaqMan assay results, outcome, and resistance data in patients with "blips" with the TaqMan assay while undetectable with the Amplicor assay. Tibotec was also asked to analyze whether emergence of resistance is occurring in patients with viral load blips and PLLV seen with the TaqMan assay. Viral load blips were defined as intermittent episodes of detectable low-level HIV-1 viremia (≥50 HIV-1 RNA copies/mL) preceded and followed by an undetectable plasma viral load (<50 HIV-1 RNA copies/mL) without any change in therapy. PLLV was defined as a viral load profile in which a confirmed viral load of <50 HIV-1 RNA copies/mL was never achieved and for which, during a series of at least three consecutive visits, the highest VL did not exceed 1,000 HIV-1 RNA copies/mL.

There were 25 and 17 subjects in the TMC278 and EFV arms, respectively, with discordant Amplicor and TaqMan assay results at Week 48 (Table 31). The viral loads of these subjects were <50 copies/mL with the Amplicor assay and >50 copies/mL with the TaqMan assay in the Week 48 window. The median viral load with the TaqMan assay of these subjects was 91 copies/mL (range of 52 - 6363 copies/mL). Only two had detectable phenotypic resistance at the endpoint; one in each arm had TDF resistance. Substitutions emerged in 7 subject isolates from the TMC278 arm and 7 in the EFV arm. However, only two substitutions were common resistance-associated NRTI substitutions.

The endpoint isolate of Subject C209-0195 (EFV arm), with a viral load of 128 copies/mL using the TaqMan assay, developed the D67D/G substitutions, which was not associated with detectable phenotypic resistance. A T69 substitution as detected in the endpoint isolate of Subject C215-0708, who had a viral load of 6363 copies/mL with the TaqMan assay, but this substitution was not associated with detectable phenotypic changes in susceptibility.

The data indicate that viral load blips or PLLV detected with the Taqman viral load assay are not always associated with the emergence of resistance (Non-Responders with Viral Load Blips or PLLV are listed Appendix F). The risk of virologic failure among subjects with viral load blips or PLLV was not different in the TMC278 and control group. Therefore, the difference in virologic failure rate between the two treatment groups does not seem to originate from the subpopulation of patients experiencing viral load blips or PLLV.

Table 31. Subjects with Discordant Viral Load Data (<50 copies/mL with Amplicor and >50 copies/mL with TaqMan; n= 24 TMC278 + 19 EFV)

PID	ARM	RT Substitutions Emerging	Endpoint Resistance	TAQMAN RESULTS
C209-0067	EFV	P14L/P K20K/R W88C/W	NO	95
C209-0069	EFV		NO	88
C209-0113	TMC278	F160F/L D324D/E A360A/T	TDF- 1.8	128
C209-0128	TMC278		NO	78
C209-0141	EFV	N175D/N	TDF- 1.4	106
C209-0185	EFV	E44K D177E	NO	69
C209-0195	EFV	**D67D/G**	NO	128
C209-0235	TMC278		NO	55
C209-0248	TMC278		NO	52
C209-0374	TMC278	V245I	NO	70
C209-0383	TMC278		NO	70
C209-0393	EFV		NO	91
C209-0423	TMC278		NO	53
C209-0489	EFV	S68G	NO	91
C209-0548	TMC278	I329I/V	NO	329
C209-0694	EFV	I135V K166R	NO	271
C209-0713	TMC278		NO	65
C209-0726	EFV		NO	60
C209-0790	EFV	R356K	NO	59
C209-0811	TMC278		NO	63
C209-0818	TMC278	K32R D123E G196R	NO	53
C209-0838	EFV		NO	52
C209-0849	EFV	T139A V292I	NO	227
C209-0857*	TMC278	K311K/R	NO	150
C209-0915*	TMC278		NO	120
C215-0027	EFV		NO	80
C215-0040	TMC278		NO	84
C215-0058	TMC278		NO	126

C215-0077	TMC278		NO	110
C215-0193	EFV		NO	72
C215-0199	TMC278		NO	192
C215-0227*	TMC278		NO	69
C215-0228	EFV		NO	172
C215-0244	EFV		NO	87
C215-0371	EFV		NO	131
C215-0388	TMC278	D192N K220K/R	NO	103
C215-0646*	TMC278		NO	147
C215-0689	TMC278		NO	311
C215-0708	TMC278	**T69A**	NO	6363
C215-0861	EFV		NO	53
C215-0892	TMC278		NO	81
C215-0902	TMC278		NO	146
C215-0933	EFV		NO	162

5. Conclusion

There were twice as many rebounders and never suppressed subjects in TMC278 arm in Study C209, although this was not seen in Study C215. Overall, more genotypic and phenotypic resistance was observed in TMC278 arms than EFV arms with 41% resistant to TMC278 in the TMC278 arms compared to 25% EFV resistance in the EFV arms. Furthermore, resistance to a background drug was 48% in TMC278 arms compared to 15% in EFV arms. The emergent genotypic resistance for both rilpivirine and EFV arms is summarized in Table 32. The most common emerging NNRTI substitutions in the rilpivirine virologic failures were V90I, K101E/P/T, E138K/G/A, V179I/L Y181I/C, V189I, and H221Y (Table 32). The E138K substitution emerged most frequently on rilpivirine treatment commonly in combination with the emtricitabine/lamividine resistance associated substitution M184I.

Table 32. Comparison of Treatment Emergent Substitutions of TMC278 and EFV Arms

	C209 and C215 N=1368	
	TMC278 N=686	**EFV Control N=682**
As-Treated Virologic Failures	14% (93/652)	10% (60/604)
Emergent NNRTI Substitutions in Virologic Failures		
V90I	11% (10/93)	2% (1/60)
K101E	14% (13/93)	2% (1/60)
K101P/T	1	
K103N	0	20% (12/60)
E138K	29% (27/93)	0
E138G	1	
V179I	3% (3/93)	0
V179L/D	1% (1/93)	2% (1/60)
Y181C	6% (6/93)	0

Y181I	1	0
V189I	6% (6/93)	2% (1/60)
H221Y	6% (6/93)	0
F227C/L	2% (2/93)	0
M230L	1% (1/93)	0
E138K M184I	22% (20/93)	0
Emergent NRTI Substitutions in Virologic Failures		
M184I or V	43% (40/93)	13% (8/60)
M184I	27% (25/93)	
M184V	16% (15/93)	13% (8/60)
K65R/N	8% (7/93)	3% (2/60)
K65R	5% (5/93)	3% (2/60)
K65N	2% (2/93)	

The median fold change in rilpivirine susceptibility of the virologic failures with post-baseline resistance data in the rilpivirine arms (n=75) was 3.3 (range: 0.2 – 621) at failure. At virologic failure, the median fold-change in TMC278 susceptibility of the 38 TMC278 virologic failures was 3.5 and the mean fold-change was 18. Of the 38 TMC278 virologic failures with evidence of TMC278 resistance, 89% were resistant to efavirenz and etravirine, and 63% were resistant to nevirapine. Therefore, TMC278 failures are highly likely to be cross-resistant to EFV and ETR.

This supplemental NDA is approvable with respect to virology for the treatment of HIV-1 in combination with other ARV drugs for treatment-naïve HIV-1 infected individuals.

6. Package Insert

<u>Applicant Proposed Package Insert</u>

(b) (4)

Resistance

In Cell Culture

Rilpivirine-resistant strains were selected in cell culture starting from wild-type HIV-1 of different origins and subtypes as well as NNRTI resistant HIV-1. The most commonly observed amino acid substitutions that emerged included: L100I, K101E, V108I, E138K, V179F, Y181C, H221Y, F227C and M230I.

A biological cut-off (BCO) for rilpivirine was determined at the fold change in EC_{50} value (FC) of 3.7, on the basis of the analysis of the susceptibility of a large panel of HIV-1 wild type recombinant clinical isolates.

In Treatment-Naïve Subjects

In the pooled resistance analysis from the Phase 3 trials, 62 (of a total of 72) virologic failures in the [TRADE NAME] arm had resistance data at baseline and time of failure. The amino acid substitutions associated with NNRTI resistance that developed most commonly in these subjects were: V90I, K101E, E138K, E138Q, Y181C, V189I and H221Y. However, in the trials, the presence of the substitutions V90I and V189I, at baseline, did not affect response.

Considering all of the available in vitro and in vivo data, the following amino acid substitutions, when present at baseline, are likely to affect the activity of rilpivirine: K101E, K101P, E138G, E138K, E138R, E138Q, Y181C, Y181I, Y181V and H221Y.

Cross-Resistance

Site-Directed NNRTI Mutant Virus

In a panel of 67 HIV-1 recombinant laboratory strains with one amino acid substitution at RT positions associated with NNRTI resistance, including the most commonly found K103N and Y181C, rilpivirine showed antiviral activity against 64 (96%) of these strains. The single amino acid substitutions associated with a loss of susceptibility to rilpivirine were: K101P, Y181I and Y181V.

Recombinant Clinical Isolates

Rilpivirine retained sensitivity (FC ≤ BCO) against 62% of 4786 HIV-1 recombinant clinical isolates resistant to efavirenz and/or nevirapine.

Treatment-naïve HIV-1-infected patients

In the pooled analysis of the Phase 3 trials TMC278-C209 and TMC278-C215, 31 of the 62 subjects with virologic failure on TRADE NAME™ with phenotypic resistance data lost susceptibility to rilpivirine. Of these, 28 were resistant to etravirine, 27 to efavirenz, and 14 to nevirapine.

Final Approved Package Insert

Indications and Usage

- More TRADE NAME treated subjects with HIV RNA greater than 100,000 copies/mL at the start of therapy experienced virologic failure compared to patients with HIV RNA less than 100,000 copies/mL at the start of therapy.
- The observed virologic failure rate in TRADE NAME treated subjects conferred a higher rate of overall treatment resistance and cross-resistance to the NNRTI class compared to efavirenz [See Microbiology (12.4)].
- More subjects treated with TRADE NAME developed lamivudine/emtricitabine associated resistance compared to efavirenz [See Microbiology (12.4)].

12.4 Microbiology

Mechanism of Action

Rilpivirine is a diarylpyrimidine non-nucleoside reverse transcriptase inhibitor (NNRTI) of human immunodeficiency virus type 1 (HIV-1) and inhibits HIV-1 replication by non-competitive inhibition of HIV-1 reverse transcriptase (RT). Rilpivirine does not inhibit the human cellular DNA polymerases α, β and γ.

Antiviral Activity in Cell Culture

Rilpivirine exhibited activity against laboratory strains of wild-type HIV-1 in an acutely infected T-cell line with a median EC_{50} value for HIV-1_{IIIB} of 0.73 nM (0.27 ng/mL). Rilpivirine demonstrated limited activity in cell culture against HIV-2 with a median EC_{50} value of 5220 nM (range 2510 to 10830 nM) (920 to 3970 ng/mL).

Rilpivirine demonstrated antiviral activity against a broad panel of HIV-1 group M (subtype A, B, C, D, F, G, H) primary isolates with EC_{50} values ranging from 0.07 to 1.01 nM (0.03 to 0.37 ng/ml) and was less active against group O primary isolates with EC_{50} values ranging from 2.88 to 8.45 nM (1.06 to 3.10 ng/ml).

The antiviral activity of rilpivirine was not antagonistic when combined with the NNRTIs efavirenz, etravirine or nevirapine; the N(t)RTIs abacavir, didanosine, emtricitabine, lamivudine, stavudine, tenofovir or zidovudine; the PIs amprenavir, atazanavir, darunavir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir or tipranavir; the fusion inhibitor enfuvirtide; the CCR5 co-receptor antagonist maraviroc or the integrase strand transfer inhibitor raltegravir.

Resistance

In Cell Culture

Rilpivirine-resistant strains were selected in cell culture starting from wild-type HIV-1 of different origins and subtypes as well as NNRTI-resistant HIV-1. The frequently observed amino acid substitutions that emerged and conferred decreased susceptibility to rilpivirine included: L100I, K101E, V106I and A, V108I, E138K and G, Q, R, V179F and I, Y181C and I, V189I, G190E, H221Y, F227C and M230I and L.

In Treatment-Naïve Subjects

In the pooled resistance analysis from the Phase 3 Studies C209 and C215, the emergence of resistance was greater in the rilpivirine arms compared to the efavirenz arms (b) (4) In the combined studies, 41% (38/92) of the virologic failures in the rilpivirine arms had genotypic and phenotypic resistance to rilpivirine compared to 25% (15/60) of the virologic failures in the efavirenz arms who had genotypic and

phenotypic resistance to efavirenz. Moreover, resistance to a background drug (emtricitabine, lamivudine, tenofovir, abacavir or zidovudine) emerged in 48% (44/92) of the virologic failures in the rilpivirine arms compared to 15% (9/60) in the efavirenz arms.

Emerging NNRTI substitutions in the rilpivirine virologic failures included V90I, K101E/P/T, E138K/G, V179I/L Y181I/C, V189I, H221Y, F227C/L and M230L which were associated with a rilpivirine phenotypic fold change range of 2.6 - 621. The E138K substitution emerged most frequently on rilpivirine treatment commonly in combination with the M184I substitution. The emtricitabine and lamivudine resistance-associated substitutions M184I or V and the tenofovir resistance-associated substitutions K65R or N emerged more frequently in rilpivirine virologic failures than in efavirenz virologic failures (See Table 8).

Table 8. Proportion of Frequently Emergent Reverse Transcriptase Substitutions in Virologic Failures from Combined Phase 3 Studies

	C209 and C215 N=1368	
	Rilpivirine N=686	**EFV Control N=682**
Virologic Failures (As-Treated)	92/652 (14%)	60/604 (10%)
Evaluable Post-Baseline Resistance Data	75	37
Emergent NNRTI Substitutions in Virologic Failures		
V90I	12% (9/75)	3% (1/37)
K101E/P/T	19% (14/75)	3% (1/37)
K103N	0	32% (12/37)
E138K/G	36% (27/75)	0
*E138K + M184I	27% (20/75)	0
V179I/L/D	5% (4/75)	3% (1/37)
Y181C/I	9% (7/75)	0
V189I	8% (6/75)	3% (1/37)
H221Y	8% (6/75)	0
Emergent NRTI Substitutions in Virologic Failures		
M184I or V	53% (40/75)	22% (8/37)
K65R/N	9% (7/75)	5% (2/37)

*This combination of NRTI and NNRTI substitutions is a subset of those with the E138K

Cross-Resistance

Site-Directed NNRTI Mutant Virus

Cross-resistance has been observed among NNRTIs. The single NNRTI substitutions K101P, Y181I and Y181V conferred 52-fold, 15-fold and 12-fold decreased susceptibility to rilpivirine, respectively. The combination of E138K and M184I showed 6.7-fold reduced susceptibility to rilpivirine compared to 2.8-fold for E138K alone. The K103N substitution did not show reduced susceptibility to rilpivirine. Combinations of 2 or 3 NNRTI resistance-associated substitutions gave decreased susceptibility to rilpivirine (fold change range of 3.7 - 554) in 38% and 66% of mutant viruses, respectively."

Treatment-naïve HIV-1-infected subjects

Considering all of the available cell culture and clinical data, the following amino acid substitutions, when present at baseline, are likely to decrease the antiviral activity of rilpivirine: K101E, K101P, E138A, E138G, E138K, E138R, E138Q, V179L, Y181C, Y181I, Y181V, H221Y, F227C and M230I or M230L.

Cross-resistance to efavirenz, etravirine and/or nevirapine is likely after virologic failure with a rilpivirine-containing regimen. In the pooled analyses of the Phase 3 clinical trials, 38 rilpivirine virologic failure subjects had evidence of rilpivirine resistance. Of these patients, 89% (n=34) were resistant to etravirine and efavirenz, and 63% (n=24) were resistant to nevirapine. In the EFV arm, none of the 15 EFV-resistant virologic failures were resistant to etravirine at failure. Subjects experiencing virologic failure on TRADENAME developed more NNRTI resistance-associated substitutions conferring more cross-resistance to the NNRTI class and had a higher likelihood of cross-resistance to all NNRTIs in the class than subjects who failed on EFV.

7. References

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8. APPENDICES

APPENDIX A. ANTIVIRAL ACTIVITY OF TMC278 AGAINST HIV-1/HXB2 SDMS HARBORING NNRTI MUTATIONS (n=161)

RT mutations	Median TMC278 FC. (Q1-Q3) n	Median EFV FC (Q1-Q3) n	Median ETR FC (Q1-Q3) n	Median NVP FC (Q1-Q3) n
K101A	2.2 (1.9 - 2.3) (n=5)	4.1 (2.7 - 4.4) (n=5)	1.8 (1.7 - 1.9) (n=4)	12.8 (8.8 - 13.4) (n=3)
K101D	1.4 (1.1 - 1.5) (n=5)	5.7 (2.7 - 6.3) (n=5)	1.0 (0.9 - 1.2) (n=5)	17.0 (13.3 - 18.9) (n=3)
K101N	1.3 (0.7 - 1.6) (n=3)	0.9 (0.9 - 2.8) (n=3)	1.3 (0.8 - 1.4) (n=3)	N.D.
K103H	0.5 (0.4 - 0.7) (n=4)	14.4 (13.5 - 17.1) (n=8)	0.6 (0.6 - 0.8) (n=4)	7.6 (5.8 - 10.8) (n=7)
K103R	1.1 (0.8 - 1.4) (n=8)	1.0 (0.7 - 1.1) (n=12)	1.0 (0.8 - 1.1) (n=8)	1.8 (1.6 - 2.6) (n=10)
K103T	0.4 (0.4 - 0.5) (n=4)	1.5 (1.5 - 1.6) (n=5)	0.6 (0.5 - 0.7) (n=4)	33.7 (31.6 - 37) (n=4)
V106I	1.2 (0.8 - 1.6) (n=4)	1.3 (1.0 - 1.6) (n=4)	1.1 (0.8 - 1.3) (n=4)	1.4 (1.1 - 2.1) (n=4)
V106L	0.2 (0.2 - 0.3) (n=4)	0.8 (0.6 - 0.9) (n=4)	0.3 (0.3 - 0.3) (n=4)	0.5 (0.5 - 0.6) (n=4)
V108L	0.9 (0.6 - 1.0) (n=4)	0.4 (0.4 - 0.5) (n=4)	0.5 (0.5 - 0.7) (n=4)	0.3 (0.3 - 1.0) (n=4)
Y115F	1.0 (1.0 - 1.5) (n=4)	1.7 (0.7 - 2.0) (n=8)	1.1 (0.9 - 1.6) (n=4)	4.3 (2.6 - 9.6) (n=6)
T139K	2.2 (2.1 - 2.7) (n=3)	1.9 (1.2 - 2.3) (n=3)	2.8 (2.4 - 3.0) (n=3)	4.8 (4.4 - 5.8) (n=3)
T139R	1.4 (1.3 - 1.4) (n=3)	2.2 (1.5 - 2.7) (n=3)	1.2 (1.0 - 1.2) (n=3)	7.3 (6.4 - 7.3) (n=3)
I178L	1.7 (1.1 - 2.0) (n=3)	2.0 (1.4 - 2.8) (n=3)	2.1 (1.0 - 3.3) (n=3)	4.4 (3.7 - 11.5) (n=3)
V179G	0.2 (0.2 - 0.4) (n=4)	0.3 (0.3 - 0.4) (n=4)	0.4 (0.3 - 0.5) (n=4)	0.1 (0.1 - 0.2) (n=3)
V179I	0.9 (0.9 - 1.1) (n=4)	1.0 (0.8 - 1.1) (n=5)	0.9 (0.8 - 0.9) (n=5)	1.3 (1.2 - 1.4) (n=4)
V179L	0.8 (0.8 - 0.8) (n=4)	0.7 (0.7 - 0.7) (n=4)	1.0 (0.8 - 1.1) (n=4)	1.7 (1.7 - 1.8) (n=3)
V179M	0.5 (0.4 - 0.6) (n=4)	0.3 (0.3 - 0.4) (n=4)	0.7 (0.5 - 0.9) (n=4)	1.2 (1.1 - 1.5) (n=4)
V179N	0.9 (0.8 - 0.9) (n=3)	1.1 (0.9 - 1.2) (n=3)	0.8 (0.6 - 0.8) (n=3)	1.6 (1.2 - 1.9) (n=3)
V179Y	0.1 (0.1 - 0.2) (n=3)	0.2 (0.2 - 0.2) (n=3)	0.2 (0.1 - 0.2) (n=3)	0.2 (0.2 - 0.5) (n=3)
Y181F	0.3 (0.2 - 0.5) (n=3)	0.4 (0.4 - 0.8) (n=3)	0.4 (0.4 - 0.7) (n=3)	1.5 (1.1 - 2.0) (n=3)
M184V	0.8 (0.7 - 1.0) (n=26)	0.9 (0.7 - 1.1) (n=93)	0.8 (0.7 - 1.0) (n=88)	1.2 (0.8 - 2.6) (n=22)
Y188C	0.1 (0.1 - 0.2) (n=3)	1.5 (1.0 - 1.5) (n=3)	0.2 (0.1 - 0.2) (n=3)	25.5 (23.0 – 34.0) (n=3)
Y188F	0.5 (0.3 - 0.5) (n=3)	0.4 (0.3 - 0.5) (n=3)	0.4 (0.2 - 0.6) (n=3)	1.7 (1.4 - 1.9) (n=3)
V189I	1.0 (0.8 - 1.1) (n=5)	1.0 (0.9 - 1.0) (n=9)	0.8 (0.8 - 0.9) (n=5)	1.4 (1.0 - 1.9) (n=7)
G190T	0.6 (0.6 - 0.7) (n=3)	11.3 (7.8 - 14.9) (n=3)	0.7 (0.6 - 0.7) (n=3)	N.D.
K219D	1.6 (1.1 - 2.1) (n=3)	1.3 (1.3 - 2.2) (n=3)	1.1 (0.4 - 2.0) (n=3)	3.7 (1.4 - 3.8) (n=3)
K219H	1.3 (1.2 - 1.9) (n=3)	1.1 (1.0 - 1.8) (n=3)	1.7 (1.1 - 1.8) (n=3)	2.1 (1.3 - 2.2) (n=3)
H221L	1.3 (1.1 - 2.9) (n=3)	1.6 (1.5 - 1.6) (n=3)	1.1 (1.1 - 1.1) (n=3)	4.3 (4.2 - 5.0) (n=3)
P225H	0.4 (0.4 - 0.8) (n=3)	1.5 (0.9 - 1.6) (n=3)	0.6 (0.6 - 0.9) (n=3)	2.1 (1.2 - 2.7) (n=3)
F227C	3.1 (2.6 - 3.4) (n=4)	4.5 (2.8 - 6.5) (n=3)	2.5 (2.1 - 2.9) (n=4)	N.D.
F227L	0.4 (0.4 - 0.6) (n=4)	0.6 (0.6 - 0.8) (n=4)	0.5 (0.4 - 0.7) (n=4)	5.1 (4.7 - 5.3) (n=3)
F227Y	0.8 (0.7 - 0.8) (n=4)	1.0 (0.7 - 1.3) (n=4)	1.1 (1.0 - 1.1) (n=4)	1.1 (1.1 - 1.3) (n=4)
K238N	0.9 (0.7 - 1.1) (n=4)	1.3 (1.0 - 1.8) (n=4)	1.4 (1.0 - 1.7) (n=4)	3.3 (2.6 - 4.0) (n=3)
K238T	1.4 (1.1 - 1.9) (n=4)	2.1 (1.8 - 2.6) (n=4)	1.3 (1.0 - 1.7) (n=4)	9.2 (6.8 - 9.2) (n=3)
Y318F	1.3 (1.1 - 1.5) (n=4)	0.8 (0.7 - 1.4) (n=5)	1.1 (1.0 - 1.2) (n=4)	2.8 (1.9 - 3.8) (n=4)
Y318W	0.5 (0.4 - 0.8) (n=6)	2.0 (1.2 - 2.5) (n=7)	0.5 (0.3 - 0.8) (n=7)	17.4 (7.3 - 19.6) (n=7)

The results are expressed as the median of 3 independent experiments run in duplicates. If the greatest of the 3 individual FC values was more than 4 times the minimum FC value, the results for the SDM was rejected and the 3 experiments repeated. FC>BCO are shaded in grey

RT mutations	Median TMC278 FC. (Q1-Q3) n	Median EFV FC (Q1-Q3) n	Median ETR FC (Q1-Q3) n	Median NVP FC (Q1-Q3) n
V090I E138K	2.9 (2.7 - 3.2) (n=3)	2.0 (1.3 - 2.3) (n=3)	2.8 (2.4 - 3.0) (n=3)	2.6 (1.7 - 2.7) (n=3)
V090I E138Q	5.5 (4.3 - 7.2) (n=3)	4.1 (3.8 - 5.6) (n=3)	6.1 (4.2 - 8.5) (n=3)	N.D.
V090I Y181C	2.3 (2.2 - 2.6) (n=3)	2.6 (1.0 - 3.0) (n=3)	5.1 (3.0 - 5.7) (n=3)	269.3 (144.1 - 383.0) (n=3)
V090I Y181I	30.2 (20.7 - 43.5) (n=3)	3.7 (1.1 - 4.1) (n=3)	36.8 (18.3 - 45.4) (n=3)	>501.3 (n=3)
V090I H221Y	1.8 (1.8 - 1.9) (n=3)	1.8 (1.6 - 1.9) (n=3)	1.6 (0.7 - 2.4) (n=3)	3.9 (2.8 - 4.8) (n=3)
A098G K101E	6.1 (3.5 - 6.5) (n=3)	12.8 (7.4 - 19.5) (n=3)	3.1 (2.5 - 3.7) (n=3)	53.8 (45.6 - 91.3) (n=3)
L100I T139K	1.2 (1.0 - 1.3) (n=3)	37.1 (18.3-38.7) (n=3)	1.8 (1.2 - 3.9) (n=3)	9.5 (8.6 - 12.5) (n=3)
L100I Y181C	2.0 (1.9 - 2.4) (n=4)	20.3 (10.7 - 23.6) (n=5)	12.2 (10.4 - 15.4) (n=4)	222.7 (192.9 - 436.7) (n=4)
L100I Y188L	293.7 (91.3 - 293.7) (n=3)	12785 (6647.3 - 13739.0) (n=3)	42.8 (27.3 - 44.8) (n=3)	N.D.
K101E E138K	1.9 (1.8 - 2.1) (n=4)	1.8 (1.1 - 2.4) (n=4)	2.6 (2.6 - 2.8) (n=4)	3.6 (3.0 - 4.6) (n=4)
K101E M184I	5.8 (5.1 - 5.9) (n=3)	6.1 (6.1 - 10.6) (n=3)	5.8 (3.3 - 6.9) (n=3)	25.8 (10.7 - 27.5) (n=3)
K101E M184V	2.2 (1.8 - 3.4) (n=3)	4.7 (2.8 - 4.8) (n=3)	2.8 (2.1 - 3.1) (n=3)	10.6 (7.8 - 11.2) (n=3)
K101E V189I	5.0 (4.8 - 5.1) (n=3)	6.1 (4.1 - 7.6) (n=3)	3.3 (2.9 - 4.1) (n=3)	24.2 (17.7 - 24.4) (n=3)
K101E H221Y	8.1 (5.9 - 9.4) (n=4)	9.3 (7.4 - 11.0) (n=4)	5.7 (4.4 - 6.6) (n=4)	N.D.
K101E F227C	10.6 (9.4 - 10.9) (n=4)	28.5 (23.7 - 32.6) (n=4)	5.0 (3.7 - 6.3) (n=4)	106.4 (73.0 - 145.6) (n=4)
K101E M230L	7.0 (6.3 - 7.3) (n=4)	22.7 (18.6 - 27.0) (n=4)	7.5 (7.2 - 7.8) (n=4)	69.0 (50.3 - 91.7) (n=4)
K101H Y181C	11.3 (6.0 - 15.3) (n=3)	8.2 (6.7 - 15.5) (n=3)	13.1 (8.0 - 15.7) (n=3)	>542.4 (n=3)
K101P K103S	429.3 (223.6 - 491.9) (n=3)	1788.5 (1287.3 - 1868.0) (n=3)	20.0 (17.3 - 34.7) (n=3)	>747.9 (n=3)
K101P V108I	41.5 (40.9 - 47.2) (n=3)	61.4 (59.4 - 63.0) (n=3)	2.2 (1.7 - 3.7) (n=3)	>718.1 (n=3)
K101P I167V	31.7 (18.9 - 34.4) (n=3)	27.8 (22.5 - 35.2) (n=3)	2.7 (2.4 - 4.9) (n=3)	237.0 (n=3)
K101P G190A	20.2 (14.1 - 22.2) (n=3)	3414.0 (3159.3 - 13107.8) (n=3)	3.2 (3.2 - 5.7) (n=3)	N.D.
K102L Y188L	10.0 (9.2 - 10.6) (n=3)	230.5 (220.0 - 410.7) (n=3)	2.6 (2.2 - 3.3) (n=3)	>22.7 (n=3)
K103N E138R	1.3 (1.2 - 1.7) (n=4)	11.0 (7.9 - 14.6) (n=4)	1.6 (1.4 - 2.0) (n=4)	116.0 (78.1 - 139.5) (n=4)
K103R V179D	2.2 (1.4 - 3.6) (n=6)	8.7 (8.3 - 9.0) (n=3)	2.7 (1.9 - 3.6) (n=6)	15.2 (10.5 - 19.7) (n=3)
K103N V179I	1.1 (0.9 - 1.3) (n=5)	14.7 (7.1 - 16.8) (n=8)	0.9 (0.7 - 1.5) (n=7)	34.8 (23.8 - 50.8) (n=5)
K103N Y181F	0.3 (0.2 - 0.3) (n=3)	5.8 (4.3 - 8.2) (n=3)	0.2 (0.2 - 0.3) (n=3)	23.7 (23.5 - 35.6) (n=3)
K103N M184V	1.1 (0.7 - 1.2) (n=3)	40.2 (25.5 - 3.2) (n=3)	0.9 (0.6 - 0.9) (n=3)	121.9 (118.3 - 128.8) (n=3)
K103N Y188F	0.9 (0.8 - 0.9) (n=3)	N.D.	0.7 (0.7 - 0.8) (n=3)	>61.8 (n=3)
K103N H221L	1.7 (1.3 - 2.3) (n=3)	N.D.	0.9 (0.8 - 1.1) (n=3)	>79.6 (n=3)
K103N K238T	0.9 (0.7 - 1.2) (n=3)	42.9 (32.8 - 52.3) (n=4)	0.8 (0.7 - 0.9) (n=4)	133.0 (103.2 - 133) (n=4)
K103N Y318F	1.0 (0.6 - 1.4) (n=8)	57.5 (40.1 - 75.3) (n=10)	1.0 (0.9 - 1.0) (n=7)	124.2 (116.8 - 174.5) (n=7)
K103R Y181C	3.4 (3.3 - 3.8) (n=3)	1.3 (1.2 - 1.9) (n=3)	3.9 (2.4 - 4.2) (n=3)	>130.7 (75.4 - 141.9) (n=3)
V106A Y181C	1.8 (1.3 - 2.5) (n=5)	2.8 (1.3 - 3.3) (n=6)	2.2 (2.0 - 2.7) (n=5)	>456.4 (n=4)
V106A F227L	2.4 (1.7 - 3.5) (n=4)	11.0 (8.5 - 14.5) (n=4)	1.2 (1.1 - 1.7) (n=4)	>478.2 (n=4)
V106I E138K	2.8 (2.5 - 3.3) (n=4)	1.9 (1.7 - 2.3) (n=4)	3.9 (3.4 - 4.3) (n=4)	2.2 (2.0 - 2.4) (n=4)
V106I V179I	1.5 (1.3 - 1.6) (n=3)	1.4 (1.1 - 1.5) (n=3)	1.3 (0.9 - 1.5) (n=3)	1.7 (1.2 - 3.4) (n=3)
V108I E138K	2.6 (2.5 - 3.0) (n=4)	2.3 (1.5 - 3.3) (n=4)	2.6 (2.4 - 3.0) (n=4)	4.4 (4.2 - 4.9) (n=4)

RT mutations	Median TMC278 FC. (Q1-Q3) n	Median EFV FC (Q1-Q3) n	Median ETR FC (Q1-Q3) n	Median NVP FC (Q1-Q3) n
V108I E138R	3.0 (2.7 - 3.4) (n=4)	3.1 (2.2 - 4.3) (n=4)	3.4 (3.0 - 3.8) (n=4)	7.6 (6.0 - 9.2) (n=4)
V108I M184V	0.7 (0.6 - 0.9) (n=4)	1.4 (1.3 - 1.5) (n=4)	0.9 (0.8 - 1.1) (n=4)	2.6 (2.1 - 3.9) (n=4)
Y115F M184V	0.9 (0.8 - 1.0) (n=4)	1.0 (0.7 - 1.3) (n=7)	0.9 (0.8 - 1.1) (n=4)	1.8 (1.6 - 3.0) (n=6)
V118I V179L	0.7 (0.6 - 0.8) (n=3)	0.8 (0.5 - 0.9) (n=3)	0.9 (0.8 - 1.1) (n=3)	1.9 (1.6 - 1.9) (n=3)
E138A Y181I	92.1 (41.2 - 107.2) (n=3)	2.7 (2.0 - 3.8) (n=3)	56.4 (43.4 - 70.1) (n=3)	>449.2 (n=3)
E138A M184I	2.8 (1.9 - 3.5) (n=3)	1.4 (1.0 - 1.9) (n=3)	3.3 (3.0 - 3.8) (n=3)	1.3 (1.1 - 2.0) (n=3)
E138G V179G	0.5 (0.4 - 0.6) (n=4)	0.4 (0.3 - 0.4) (n=5)	0.7 (0.6 - 0.8) (n=4)	0.2 (0.2 - 0.2) (n=4)
E138G M184I	1.7 (1.5 - 1.9) (n=3)	1.1 (1.1 - 1.4) (n=3)	2.3 (1.4 - 2.8) (n=3)	N.D.
E138K T139K	3.8 (3.6 - 4.9) (n=3)	3.0 (2.3 - 3.7) (n=3)	4.6 (2.8 - 4.8) (n=3)	N.D.
E138K M184I	6.7 (5.8 - 6.8) (n=6)	2.4 (2.3 - 3.2) (n=5)	5.1 (3.6 - 5.7) (n=6)	3.8 (2.4 - 4.0) (n=5)
E138K M184V	3.1 (2.6 - 3.3) (n=6)	1.9 (1.8 - 2.1) (n=6)	3.2 (2.3 - 3.7) (n=6)	1.8 (1.6 - 2.2) (n=6)
E138K V189I	4.9 (4.4 - 6.9) (n=3)	3.6 (2.5 - 4.2) (n=3)	4.8 (4.3 - 4.9) (n=3)	N.D.
E138R M184V	3.0 (1.7 - 3.9) (n=3)	2.2 (1.6 - 3.5) (n=3)	3.5 (2.7 - 4.4) (n=3)	3.5 (2.5 - 4.2) (n=3)
E138R V189I	3.2 (2.2 - 4.8) (n=3)	3.8 (1.4 - 4.9) (n=3)	5.2 (3.0 - 5.6) (n=3)	7.7 (3.8 - 11.3) (n=3)
I167V Y181C	2.8 (2.0 - 4.2) (n=3)	1.6 (1.4 - 1.6) (n=3)	4.1 (3.9 - 5.6) (n=3)	90.0 (82.8 - 162.4) (n=3)
V179I G190A	1.1 (0.7 - 1.6) (n=5)	4.7 (4.5 - 5.3) (n=5)	1.0 (0.9 - 2.4) (n=6)	203.4 (175.0 - 203.4) (n=5)
V179T Y181C	6.0 (5.2 - 8.3) (n=4)	2.6 (2.0 - 3.3) (n=4)	13.0 (12.1 - 16.4) (n=4)	170.8 (162.7 - 179.2) (n=3)
V179Y G190A	<0.1 (0.1 - 0.1) (n=3)	1.0 (0.8 - 1.3) (n=3)	<0.1(n=3)	16.5 (12.4 - 22.0) (n=3)
Y181C M184I	2.5 (0.9 - 3.1) (n=3)	2.0 (1.0 - 2.6) (n=3)	3.0 (2.4 - 4.5) (n=3)	156.4 (126.9 - 266.9) (n=3)
Y181C Y188C	0.4 (0.3 - 0.4) (n=4)	1.4 (1.0 - 2.8) (n=5)	0.7 (0.6 - 0.8) (n=4)	>373.4 (179.2 - 385.9) (n=4)
Y181C G190A	2.1 (1.7 - 2.8) (n=4)	13.3 (11.3 - 16.8) (n=8)	2.7 (2.5 - 4.7) (n=5)	511.8 (n=8)
Y181C L234I	1.6 (1.3 - 2.0) (n=4)	3.6 (2.9 - 3.6) (n=5)	4.9 (4.1 - 6.4) (n=4)	>43.4 (32.1 - 60.6) (n=4)
M184V T215Y	0.3 (0.3 - 0.5) (n=3)	0.4 (0.4 - 0.5) (n=9)	0.3 (0.3 - 0.3) (n=6)	0.7 (0.5 - 1.2) (n=8)
M184V F227C	1.4 (1.3 - 2.2) (n=3)	2.8 (2.8 - 6.8) (n=3)	2.5 (1.9 - 4.5) (n=3)	N.D.
F223C L234I	2.3 (1.5 - 4.4) (n=73)	30.0 (15.8 - 48.8) (n=102)	4.2 (2.7 - 6.3) (n=113)	>59.6 (47.8 - 67.2) (n=42)
F227C M230L	20.2 (15.3 - 35.4) (n=3)	43.0 (42.9 - 157.2) (n=3)	16.5 (14.5 - 37.5) (n=3)	234.3 (179.7 - 303.8) (n=3)

The results are expressed as the median of 3 independent experiments run in duplicates. If the greatest of the 3 individual FC values was more than 4 times the minimum FC value, the results for the SDM was rejected and the 3 experiments repeated. FC>BCO are shaded in grey

RT mutations	Median TMC278 FC. (Q1-Q3) n	Median EFV FC (Q1-Q3) n	Median ETR FC ((Q1-Q3) n	Median NVP FC (Q1-Q3) n
V090I E138A Y181I	150.9 (79.5 - 158) (n=3)	2.1 (1.5 - 2.3) (n=3)	87.6 (80.3 - 141.8) (n=3)	>501.3 (n=3)
V090I E138A M184I	2.8 (1.2 - 4.1) (n=3)	1.2 (1.2 - 3.7) (n=3)	2.0 (2.0 - 3.1) (n=3)	1.3 (1.0 - 2.9) (n=3)
V090I E138K M184I	4.6 (2.9 - 4.8) (n=3)	3.7 (3.4 - 4.2) (n=3)	3.9 (3.2 - 8.0) (n=3)	2.3 (1.3 - 3.6) (n=3)
V090I E138K M184V	2.9 (2.5 - 6.1) (n=3)	2 (1.8 - 4.9) (n=3)	3.7 (3.3 - 3.9) (n=3)	1.6 (1.4 - 1.7) (n=3)
V090I E138K H221Y	5.6 (4.2 - 7.5) (n=3)	3.6 (3.2 - 4.0) (n=3)	7.2 (6.5 - 8.4) (n=3)	3.9 (3.0 - 7.9) (n=3)
V090I E138Q V189I	6.1 (5.1 - 6.5) (n=3)	6.3 (6.2 - 8.9) (n=3)	9.8 (7.4 - 9.9) (n=3)	20.5 (20.4 - 39.8) (n=3)
V090I Y181C M184I	1.8 (1.1 - 2.2) (n=3)	2.1 (1.1 - 2.9) (n=3)	3.0 (2.9 - 3.3) (n=3)	166.4 (116.9 - 244.6) (n=3)
V090I H221Y M230L	6.9 (3.7 - 7) (n=3)	12.6 (7.8 - 14.4) (n=3)	7.3 (3.5 - 8.1) (n=3)	N.D.
L100I E138K T139K	2.9 (2.8 - 6.1) (n=3)	6.7 (5.4 - 9.9) (n=3)	3.3 (1.5 - 3.3) (n=3)	N.D.
K101E E138K H221Y	4.2 (2.5 - 4.4) (n=3)	3.7 (2.4 - 4.6) (n=3)	3.7 (3.2 - 5.7) (n=3)	9.6 (8.3 - 13.9) (n=3)
E138K V189I H221Y	7.5 (5.3 - 7.9) (n=3)	5.7 (4.6 - 5.7) (n=3)	10.9 (7.4 - 10.9) (n=3)	9.1 (8.9 - 10.3) (n=3)
F227C M230L M184V	19.3 (15.6 - 20.7) (n=3)	56.2 (42.7 - 75.0) (n=3)	14.3 (13.9 - 18.8) (n=3)	352.1 (287.1 - 362.7) (n=3)
C038R V108I Y181C	1.7 (1.5 - 3.4) (n=3)	3.6 (3.5 - 5.8) (n=3)	3.1 (1.8 - 3.2) (n=3)	>22.7 (n=3)
I050T L100I K103N	4.4 (3.2 - 5.3) (n=5)	1511.4 (1161.3 - 2807.3) (n=6)	3.8 (3.2 - 7.8) (n=6)	>85.9 (n=6)
L074V L100I K103N	6.2 (1.8 - 6.3) (n=3)	2354.3 (1192.6 - 2375.4) (n=3)	2.8 (2.7 - 4.5) (n=3)	>105.0 (n=4)
L100I E138K K219E	12.9 (12.8 - 20.6) (n=3)	8.0 (4.1 - 9.8) (n=3)	3.9 (3.2 - 5.6) (n=3)	3.7 (3.6 - 4.3) (n=3)
L100I E138K M184V	1.8 (1.1 - 1.9) (n=3)	4.5 (4.4 - 4.9) (n=3)	1.0 (0.8 - 2.1) (n=3)	1.5 (1.3 - 1.7) (n=3)
L100I K101E F227C	132.3 (124.9 - 175.5) (n=4)	520.3 (488.4 - 667.7) (n=4)	5.1 (4.7 - 6.8) (n=4)	>433.7 (296.9 - 484.9) (n=4)
L100I K103N E138Q	52.9 (35 - 54.6) (n=5)	3334.9 (2557.3 - 3771.7) (n=5)	12.9 (11.5 - 13.7) (n=5)	299.3 (285.6 - 463.4) (n=5)
L100I K103N M184V	4.1 (2.0 - 6.6) (n=3)	1531.1 (1223.2 - 1912.8) (n=3)	1.7 (1.6 - 2.8) (n=3)	>68.6 (67.1 - 105.0) (n=4)
L100I K103N V179I	20.3 (16.2 - 41.1) (n=7)	2396.6 (1902.3 - 2956.3) (n=7)	4.0 (3.7 - 7.4) (n=6)	>118.4 (n=3)
K101E E138K M184I	2.0 (1.7 - 2.2) (n=6)	1.3 (1.1 - 1.6) (n=6)	2.6 (1.7 - 3.0) (n=6)	2.5 (1.0 - 3.7) (n=3)
K101E E138K M184V	1.8 (1.4 - 2.1) (n=3)	1.6 (1.3 - 2.2) (n=3)	1.6 (1.0 - 2.3) (n=3)	N.D.
K101E Y181C H221Y	12.8 (7.6 - 21.6) (n=3)	13.3 (12.7 - 16.5) (n=3)	14.2 (10.2 - 15.4) (n=3)	>628.7 (n=4)
K101E Y181C M184V	4.5 (4.5 - 8.5) (n=3)	4.7 (4.0 - 8.7) (n=3)	2.7 (1.9 - 3.0) (n=3)	247.3 (172.1 - 501.3) (n=3)
K101H K103R Y181C	7.6 (7.6 - 14.9) (n=3)	11.1 (7.6 - 13.5) (n=3)	11.2 (9.9 - 15.5) (n=3)	>503.2 (255.6 - 747.9) (n=3)
K101Q K103N V108I	0.9 (0.9 - 1.2) (n=4)	96.2 (0.8 - 158.7) (n=7)	0.7 (0.6 - 0.9) (n=4)	33.4 (2.2 - 196.1) (n=6)
K103N E138Q Y181C	10.5 (7.2 - 13.6) (n=6)	25.3 (12.3 - 30.4) (n=6)	8.2 (6.1 - 9.4) (n=6)	>85.9 (n=6)
K103N K219D Y181C	13.1 (11.5 - 15) (n=3)	72.9 (48.6 - 116.6) (n=3)	20.2 (15.5 - 21.4) (n=3)	>79.6 (n=3)
K103N T139R Y181C	7.4 (6.2 - 9.5) (n=3)	61.2 (60.2 - 77.0) (n=3)	7.0 (5.6 - 7.4) (n=3)	>22.7 (n=3)
K103N V108I Y181C	4.1 (3.3 - 4.4) (n=4)	110.2 (87.4 - 118.3) (n=4)	2.1 (1.7 - 2.6) (n=4)	>456.4 (n=3)
K103N V179I G190A	0.4 (0.3 - 0.5) (n=4)	208 (122.1 - 249.0) (n=5)	0.6 (0.4 - 0.6) (n=5)	>415.9 (n=4)

RT mutations	Median TMC278 FC. (Q1-Q3) n	Median EFV FC (Q1-Q3) n	Median ETR FC ((Q1-Q3) n	Median NVP FC (Q1-Q3) n
K103N V179T Y181C	5.2 (3.4 - 7.1) (n=4)	21.8 (15.3 - 30.7) (n=4)	8.4 (7.4 - 9.3) (n=4)	>591.5 (n=4)
K103N Y181C E194G	4.3 (3.4 - 5.5) (n=3)	N.D.	3.9 (3 - 4.1) (n=3)	N.D.
K103N Y181C G190A	1.0 (0.9 - 1.0) (n=4)	555.5 (539.3 - 787.5) (n=6)	1.9 (1.8 - 2.1) (n=4)	>456.4 (n=5)
K103N Y181C K219H	11.3 (6.9 - 13.3) (n=3)	49.0 (23.7 - 92.6) (n=3)	14.5 (11.6 - 16.1) (n=3)	>79.6 (n=3)
K103N Y181C Y318F	7.8 (5.2 - 17.4) (n=7)	89.2 (45.7 - 161.6) (n=8)	6.9 (4.8 - 17.2) (n=7)	>376.7 (309.8 - 443.7) (n=6)
K103N Y188S Y318F	0.5 (0.4 - 0.6) (n=4)	32.4 (22.6 - 48.3) (n=5)	0.6 (0.5 - 0.6) (n=4)	241.5 (218.8 - 322.5) (n=4)
K103R V179D Y181C	14.1 (12 - 16.3) (n=3)	60.4 (49.0 - 62.0) (n=3)	46.3 (41.9 - 57.4) (n=3)	>542.4 (n=3)
K103R V179F Y181C	3.5 (2.7 - 3.5) (n=3)	3.4 (3.3 - 7.0) (n=3)	7.3 (6.9 - 12.2) (n=3)	N.D.
V106A E138K F227L	39.9 (38.2 - 41) (n=3)	12.0 (6.6 - 19.4) (n=3)	5.5 (4.1 - 6.6) (n=3)	>501.3 (n=3)
V106A E138K V179I	4.9 (2.8 - 5.0) (n=3)	1.0 (1.0 - 1.8) (n=3)	1.6 (1.5 - 3.3) (n=3)	111.6 (65.5 - 210.1) (n=3)
V106I E138K V179I	2.7 (1.5 - 4.1) (n=3)	1.1 (1.0 - 1.5) (n=3)	2.1 (2.0 - 3.5) (n=3)	1.2 (1.0 - 1.5) (n=3)
V106M V179D Y181C	2.4 (2.3 - 2.9) (n=5)	2704.1 (1953.6 - 3713.9) (n=6)	28.8 (22.1 - 37.9) (n=5)	>484.9 (n=5)
V108I E138K V179I	5.4 (2.3 - 8.9) (n=4)	2.0 (1.1 - 2.6) (n=4)	3.4 (1.5 - 4.9) (n=4)	7.6 (0.5 - 10.2) (n=3)
V108I E138K M184V	2.1 (1.9 - 2.3) (n=4)	1.9 (1.7 - 2.1) (n=4)	2.2 (2.1 - 2.4) (n=4)	3.3 (2.9 - 4.3) (n=4)
V108I Y181C G190A	1.2 (1.2 - 2.0) (n=3)	55.7 (49.3 - 63.5) (n=5)	1.9 (1.6 - 2.4) (n=3)	>456.4 (n=6)
E138A Y181I M184I	33.0 (24.6 - 39) (n=3)	1.2 (1.2 - 1.2) (n=3)	20.0 (12.4 - 20.5) (n=3)	>501.3 (n=3)
E138K M184V M230L	1.8 (1.7 - 2.1) (n=9)	1.9 (1.4 - 2.6) (n=9)	2.7 (2.2 - 3.0) (n=9)	6.0 (4.4 - 8.1) (n=7)
E138K V179I M184V	2.2 (2.1 - 2.4) (n=3)	1.1 (1.0 - 1.2) (n=3)	2.8 (2.0 - 3.1) (n=3)	1.4 (1.1 - 1.5) (n=3)
T139R Y181C Y188L	114.3 (97.1 - 143.7) (n=3)	N.D.	26.6 (20.9 - 33.9) (n=3)	>22.7 (n=3)
V179I Y181C G190A	1.2 (1.0 - 1.5) (n=5)	9.8 (5.4 - 14.2) (n=6)	2.1 (2.1 - 4.1) (n=5)	661.8 (n=5)
Y181C M184V H221Y	2.1 (2.0 - 2.2) (n=3)	3.5 (2.4 - 4.7) (n=3)	3.5 (3.1 - 4.3) (n=3)	>494.3 (n=3)
M184V K219N M230V	4.1 (3.7 - 5.0) (n=4)	2.4 (1.6 - 3.5) (n=11)	3.4 (1.9 - 3.9) (n=9)	2.1 (1.5 - 3.2) (n=8)
L074V L100I K103N M184V	5.5 (4.1 - 6.6) (n=4)	1642.4 (1179.2 - 2307.0) (n=4)	3.2(2.4 - 3.5) (n=4)	>79 (58.4 - 101.7) (n=4)
V090I E138A Y181I M184I	20.5 (13.3 - 33.5) (n=3)	0.8 (0.4 - 0.8) (n=3)	17.3 (17.3 - 25.3) (n=3)	>478.3 (211.9 - 591.5) (n=3)
V090I E138K M184V H221Y	5.2 (4.1 - 14.7) (n=3)	4.2 (3.0 - 4.6) (n=3)	5.1 (3.9 - 7.9) (n=3)	4.3 (3.9 - 7.6) (n=3)
L100I E138K M184V K219E	6.9 (2.4 - 7.3) (n=3)	7.7 (4.3 - 15.2) (n=3)	2.9 (2.1 - 6.8) (n=3)	2.6 (1.2 - 3.4) (n=3)
K101E Y181C M184V H221Y	8.0 (7.6 - 14.4) (n=3)	5.8 (5.6 - 6.8) (n=3)	7.3 (3.8 - 7.8) (n=3)	>470.0(444.3 - 497.1) (n=3)
V106A E138K V179I F227L	47.5 (23 - 66.2) (n=3)	13.2(5.7 - 21.6) (n=3)	9.2 (3.0 - 9.4) (n=3)	>501.3 (n=3)
V106I E138K V179I M184V	2.2 (2.0 - 5.5) (n=3)	1.3 (1.0 - 2.1) (n=3)	3.0 (2.1 - 4.6) (n=3)	1.4 (0.8 - 2.7) (n=3)
E138K V179I M184V K219E	1.7 (1.5 - 2.6) (n=3)	1.3 (0.6 - 1.5) (n=3)	N.D.	1.2 (0.7 - 1.3) (n=3)

The results are expressed as the median of 3 independent experiments run in duplicates. If the greatest of the 3 individual FC values was more than 4 times the minimum FC value, the results for the SDM was rejected and the 3 experiments repeated. FC>BCO are shaded in grey

APPENDIX B. SUBJECT ISOLATES CENSORED (discontinued at or before Week 4 (≤Day 30))

TMC278

TMC278-C209-0072 TMC278 25MG QD
TMC278-C209-0432 TMC278 25MG QD

TMC278-C215-0026

EFV

TMC278-C209-0133 EFAVIRENZ 600MG QD
TMC278-C209-0162 EFAVIRENZ 600MG QD
TMC278-C209-0197 EFAVIRENZ 600MG QD
TMC278-C209-0259 EFAVIRENZ 600MG QD
TMC278-C209-0303 EFAVIRENZ 600MG QD
TMC278-C209-0439 EFAVIRENZ 600MG QD
TMC278-C209-0551 EFAVIRENZ 600MG QD
TMC278-C209-0557 EFAVIRENZ 600MG QD
TMC278-C209-0721 EFAVIRENZ 600MG QD
TMC278-C209-0730 EFAVIRENZ 600MG QD
TMC278-C209-0743 EFAVIRENZ 600MG QD
TMC278-C209-0822 EFAVIRENZ 600MG QD

TMC278-C215-0024
TMC278-C215-0197
TMC278-C215-0221
TMC278-C215-0267
TMC278-C215-0292
TMC278-C215-0297
TMC278-C215-0335
TMC278-C215-0345
TMC278-C215-0386
TMC278-C215-0389
TMC278-C215-0399
TMC278-C215-0438
TMC278-C215-0453
TMC278-C215-0533
TMC278-C215-0620
TMC278-C215-0714
TMC278-C215-0833
TMC278-C215-0855

DC <50
TMC278-C209-0044
TMC278-C209-0050
TMC278-C209-0124
TMC278-C209-0251

TMC278-C209-0277
TMC278-C209-0287
TMC278-C209-0328
TMC278-C209-0329
TMC278-C209-0340
TMC278-C209-0444
TMC278-C209-0459
TMC278-C209-0467
TMC278-C209-0570
TMC278-C209-0576
TMC278-C209-0584
TMC278-C209-0602
TMC278-C209-0700
TMC278-C209-0763
TMC278-C209-0839
TMC278-C209-0855
TMC278-C209-0872
TMC278-C209-0922
TMC278-C209-0948

APPENDIX C. Listing of Virologic Failures As-treated
TMC278 Virologic Failures As-treated (censored) n=92

TMC278-C209-0009
TMC278-C209-0011
TMC278-C209-0023
TMC278-C209-0046
TMC278-C209-0066
TMC278-C209-0079
TMC278-C209-0119
TMC278-C209-0129
TMC278-C209-0146
TMC278-C209-0161
TMC278-C209-0163
TMC278-C209-0199
TMC278-C209-0226
TMC278-C209-0231
TMC278-C209-0256
TMC278-C209-0297
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TMC278-C209-0371
TMC278-C209-0378
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TMC278-C209-0389
TMC278-C209-0405
TMC278-C209-0419
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TMC278-C209-0512
TMC278-C209-0538
TMC278-C209-0548
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TMC278-C209-0573
TMC278-C209-0574
TMC278-C209-0594
TMC278-C209-0612
TMC278-C209-0629
TMC278-C209-0636
TMC278-C209-0667
TMC278-C209-0679
TMC278-C209-0683
TMC278-C209-0703
TMC278-C209-0724
TMC278-C209-0745
TMC278-C209-0750
TMC278-C209-0760
TMC278-C209-0768
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TMC278-C209-0787
TMC278-C209-0807
TMC278-C209-0835
TMC278-C209-0857
TMC278-C209-0871
TMC278-C209-0887

TMC278-C209-0915
TMC278-C209-0935
TMC278-C215-0001
TMC278-C215-0028
TMC278-C215-0032
TMC278-C215-0065
TMC278-C215-0080
TMC278-C215-0089
TMC278-C215-0095
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TMC278-C215-0130
TMC278-C215-0135
TMC278-C215-0181
TMC278-C215-0208
TMC278-C215-0222
TMC278-C215-0227
TMC278-C215-0264
TMC278-C215-0313
TMC278-C215-0330
TMC278-C215-0339
TMC278-C215-0344
TMC278-C215-0387
TMC278-C215-0416
TMC278-C215-0426
TMC278-C215-0439
TMC278-C215-0446
TMC278-C215-0454
TMC278-C215-0466
TMC278-C215-0494
TMC278-C215-0515
TMC278-C215-0534
TMC278-C215-0551
TMC278-C215-0565
TMC278-C215-0592
TMC278-C215-0646
TMC278-C215-0656
TMC278-C215-0707
TMC278-C215-0783
TMC278-C215-0793
TMC278-C215-0914
TMC278-C215-0955

Listing of EFV Virologic Failures As-treated censored n=60
TMC278-C209-0007
TMC278-C209-0054
TMC278-C209-0076
TMC278-C209-0085
TMC278-C209-0092
TMC278-C209-0118
TMC278-C209-0176
TMC278-C209-0285
TMC278-C209-0294
TMC278-C209-0330

TMC278-C209-0333
TMC278-C209-0421
TMC278-C209-0506
TMC278-C209-0542
TMC278-C209-0564
TMC278-C209-0583
TMC278-C209-0648
TMC278-C209-0709
TMC278-C209-0711
TMC278-C209-0726
TMC278-C209-0755
TMC278-C209-0758
TMC278-C209-0908
TMC278-C209-0909
TMC278-C215-0049
TMC278-C215-0066
TMC278-C215-0088
TMC278-C215-0109
TMC278-C215-0134
TMC278-C215-0143
TMC278-C215-0147
TMC278-C215-0164
TMC278-C215-0165
TMC278-C215-0172
TMC278-C215-0228
TMC278-C215-0251
TMC278-C215-0266
TMC278-C215-0281
TMC278-C215-0287
TMC278-C215-0289
TMC278-C215-0317
TMC278-C215-0319
TMC278-C215-0419
TMC278-C215-0472
TMC278-C215-0490
TMC278-C215-0540
TMC278-C215-0591
TMC278-C215-0594
TMC278-C215-0596
TMC278-C215-0621
TMC278-C215-0625
TMC278-C215-0702
TMC278-C215-0773
TMC278-C215-0779
TMC278-C215-0806
TMC278-C215-0835
TMC278-C215-0860
TMC278-C215-0867
TMC278-C215-0871
TMC278-C215-0879

APPENDIX D: ISOLATES WITH POST-BASELINE DATA

TMC278 25MG QD (n=75)
TMC278-C209-0009
TMC278-C209-0011
TMC278-C209-0023
TMC278-C209-0046
TMC278-C209-0066
TMC278-C209-0079
TMC278-C209-0119
TMC278-C209-0129
TMC278-C209-0146
TMC278-C209-0161
TMC278-C209-0163
TMC278-C209-0199
TMC278-C209-0226
TMC278-C209-0231
TMC278-C209-0297
TMC278-C209-0361
TMC278-C209-0371
TMC278-C209-0378
TMC278-C209-0383
TMC278-C209-0389
TMC278-C209-0419
TMC278-C209-0483
TMC278-C209-0495
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TMC278-C209-0548
TMC278-C209-0555
TMC278-C209-0573
TMC278-C209-0574
TMC278-C209-0594
TMC278-C209-0629
TMC278-C209-0636
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TMC278-C209-0683
TMC278-C209-0745
TMC278-C209-0768
TMC278-C209-0779
TMC278-C209-0784
TMC278-C209-0787
TMC278-C209-0807
TMC278-C209-0835
TMC278-C209-0857
TMC278-C209-0871
TMC278-C209-0887
TMC278-C209-0915
TMC278-C215-0001
TMC278-C215-0028
TMC278-C215-0032
TMC278-C215-0065
TMC278-C215-0080

TMC278-C215-0089
TMC278-C215-0095
TMC278-C215-0110
TMC278-C215-0135
TMC278-C215-0181
TMC278-C215-0208
TMC278-C215-0222
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TMC278-C215-0339
TMC278-C215-0344
TMC278-C215-0416
TMC278-C215-0418
TMC278-C215-0439
TMC278-C215-0454
TMC278-C215-0466
TMC278-C215-0494
TMC278-C215-0515
TMC278-C215-0534
TMC278-C215-0551
TMC278-C215-0592
TMC278-C215-0646
TMC278-C215-0783
TMC278-C215-0921

EFAVIRENZ 600MG QD n=37
TMC278-C209-0007
TMC278-C209-0085
TMC278-C209-0092
TMC278-C209-0118
TMC278-C209-0176
TMC278-C209-0195
TMC278-C209-0271
TMC278-C209-0285
TMC278-C209-0294
TMC278-C209-0333
TMC278-C209-0506
TMC278-C209-0564
TMC278-C209-0583
TMC278-C209-0648
TMC278-C209-0711
TMC278-C209-0726
TMC278-C209-0908
TMC278-C215-0042
TMC278-C215-0049
TMC278-C215-0109
TMC278-C215-0134
TMC278-C215-0147
TMC278-C215-0228
TMC278-C215-0266
TMC278-C215-0287
TMC278-C215-0289

TMC278-C215-0419
TMC278-C215-0540
TMC278-C215-0594
TMC278-C215-0596
TMC278-C215-0625
TMC278-C215-0702
TMC278-C215-0773
TMC278-C215-0779
TMC278-C215-0835
TMC278-C215-0860
TMC278-C215-0879

APPENDIX E. PIDs of 40 virologic failures with ≥2.5 fold change in TMC278 susceptibility in Studies C209 and C215.

TMC278-C209-0009
TMC278-C209-0011
TMC278-C209-0066
TMC278-C209-0079
TMC278-C209-0119
TMC278-C209-0129
TMC278-C209-0146
TMC278-C209-0161
TMC278-C209-0163
TMC278-C209-0231
TMC278-C209-0297
TMC278-C209-0361
TMC278-C209-0389
TMC278-C209-0495
TMC278-C209-0512
TMC278-C209-0573
TMC278-C209-0594
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TMC278-C209-0745
TMC278-C209-0768
TMC278-C209-0779
TMC278-C209-0787
TMC278-C209-0807
TMC278-C209-0871
TMC278-C209-0887
TMC278-C215-0001
TMC278-C215-0032
TMC278-C215-0110
TMC278-C215-0135
TMC278-C215-0181
TMC278-C215-0208
TMC278-C215-0330
TMC278-C215-0339
TMC278-C215-0344
TMC278-C215-0416
TMC278-C215-0466
TMC278-C215-0515
TMC278-C215-0534
TMC278-C215-0783

APPENDIX F. Non-Responders with Viral Load Blips or PLLV

***C209 2090199** PLLV TMC278 wk16 5-Jan-09 2.06 POS POS Non Responder Yes
C209 2090236 Blip TMC278 wk40 6-Jul-09 1.93 NEG NEG Non Responder No
C209 2090256 Blip TMC278 wk48 30-Sep-09 2.25 NEG NEG Non Responder No
C209 2090390 PLLV TMC278 wk40 22-Sep-09 2.44 NEG NEG Non Responder No
***C209 2090419** PLLV TMC278 wk48 12-Nov-09 1.75 POS POS Non Responder Yes
*C209 **2090512** Blip TMC278 wk32 3-Aug-09 2.47 POS POS Non Responder Yes
***C209 2090679**
C209 2090703 PLLV TMC278 unscheduled 3-Sep-09 2.6 NEG NEG Non Responder No
C209 2090760 PLLV TMC278 wk32 17-Sep-09 2.79 POS NEG Non Responder Yes
***C209 2090857** Blip TMC278 wk16 4-Jun-09 2.14 POS POS Non Responder Yes
***C215 2150028** Blip TMC278 wk32 6-Mar-09 1.98 POS POS Non Responder Yes
***C215 2150065** PLLV TMC278 wk16 1-Dec-08 2.94 POS POS Non Responder Yes
***C215 2150080** PLLV TMC278 wk16 10-Dec-08 2.44 POS POS Non Responder Yes
***C215 2150089** PLLV TMC278 wk48 5-Aug-09 1.83 POS POS Non Responder Yes
***C215 2150208** PLLV TMC278 wk24 9-Apr-09 2.45 POS POS Non Responder Yes
***C215 2150222** PLLV TMC278 wk24 1-Apr-09 2.26 NEG NEG Non Responder Yes
***C215 2150313** PLLV TMC278 wk48 21-Oct-09 2.33 POS POS Non Responder Yes
C215 2150494 Blip TMC278 wk32 11-Aug-09 2.94 POS POS Non Responder Yes
C215 2150565 Blip TMC278 wk48 23-Dec-09 2.57 NEG NEG Non Responder Yes
C215 2150707 PLLV TMC278 wk40 28-Oct-09 2.18 NEG NEG Non Responder Yes
C215 2150717 PLLV TMC278 wk16 5-May-09 2.19 NEG NEG Non Responder No
C215 2150869 PLLV TMC278 wk40 5-Nov-09 2.05 NEG NEG Non Responder No
C215 2150914 PLLV TMC278 wk48 23-Dec-09 2.22 NEG NEG Non Responder No
C215 2150944 Blip TMC278 wk16 17-Jun-09 1.93 NEG NEG Non Responder No

N=23

Comparing the Taqman viral load curves to the Amplicor VL curves for the subjects with and without virologic failure shows that a cutoff of 100 copies/mL or a more conservative 200 copies/mL could be used for the TaqMan assay to determine if VL rebound was occurring.

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/s/

LISA K NAEGER
03/28/2011

JULIAN J O'REAR
03/29/2011

MICROBIOLOGY FILING CHECKLIST FOR NDA or Supplement

NDA Number: 202022 **Applicant: Tibotec, Inc.** **Stamp Date: 07/23/2010**

Drug Name: TMC278 rilpivirine **NDA Type: Original NDA**

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

	Content Parameter	Yes	No	Comments
1	Is the virology information (nonclinical and clinical) provided and described in different sections of the NDA organized in a manner to allow substantive review to begin?	X		
2	Is the virology information (nonclinical and clinical) indexed, paginated and/or linked in a manner to allow substantive review to begin?	X		
3	Is the virology information (nonclinical and clinical) legible so that substantive review can begin?	X		
4	On its face, has the applicant submitted cell culture data in necessary quantity, using necessary clinical and non-clinical strains/isolates, and using necessary numbers of approved current divisional standard of approvability of the submitted draft labeling?	X		
5	Has the applicant submitted any required animal model studies necessary for approvability of the product based on the submitted draft labeling?		X	Not applicable
6	Has the applicant submitted all special/critical studies/data requested by the Division during pre-submission discussions?	X		
7	Has the applicant submitted the clinical virology datasets in the appropriate format as described in the relevant guidance documents and are the datasets complete?	X		
8	Has the applicant used standardized or nonstandardized methods for virologic outcome measures? If nonstandardized methods were used, has the applicant included complete details of the method, the name of the laboratory where actual testing was done and performance characteristics of the assay in the laboratory where the actual testing was done?	X		
9	Has the applicant submitted draft labeling consistent with current regulation, divisional and Center policy, and the design of the development package?	X		
10	Has the applicant submitted annotated microbiology draft labeling consistent with current divisional policy, and the design of the development package?	X		
11	Have all the study reports, published articles, and other	X		

MICROBIOLOGY FILING CHECKLIST FOR NDA or Supplement

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

IS THE MICROBIOLOGY SECTION OF THE APPLICATION FILEABLE? __YES____

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

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

Lisa K. Naeger 08/24/2010

Reviewing Microbiologist Date

Microbiology Team Leader Date

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-202022	ORIG-1	TIBOTEC INC	TMC278

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

/s/

LISA K NAEGER
08/31/2010

JULIAN J O'REAR
08/31/2010