

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

22-187

MICROBIOLOGY REVIEW(S)

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)

MICROBIOLOGY REVIEW

NDA: 22,187 SN: 000 DATE REVIEWED: 12/3/07

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

NDA#: 22-187

Serial #: 000

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

Sponsor's Name and Address:

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Product Name(s)

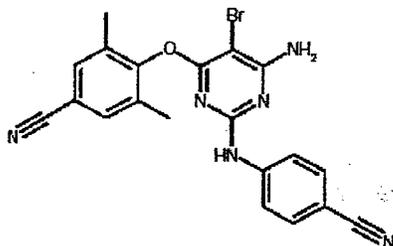
Proprietary: Intelence™
Non-Proprietary/USAN: Etravirine
Code Name/Number: TMC125

Chemical Name: 4-[[6-amino-5-bromo-2-[(4-cyanophenyl)-amino]-4-pyrimidinyl]oxy]-3,5-dimethylbenzonitrile.

Molecular Weight: 435.28

Molecular Formula: C₂₀H₁₅BrN₆O

Structural Formula:



Dosage Form(s): 200 mg (two 100 mg tablets) taken twice daily following a meal.

Route(s) of Administration: Oral

Indication(s): Treatment of HIV-1 infection in ARV treatment-experienced adult patients, _____ co-administered with other antiretroviral (ARV) agents.

Dispensed: Rx OTC _____

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Abbreviations:

AAG, α -1-acid glycoprotein; DRV, darunavir; EC₅₀, effective concentration at 50%; ENF, enfuvirtide; FCS, fetal calf serum; HSA, human serum albumin; IC₅₀, inhibitory concentration at 50%; NNRTI, non-nucleoside analogue reverse transcriptase inhibitor; NRTI, nucleoside analogue reverse transcriptase inhibitor; OBT, optimized background therapy; PBL, peripheral blood lymphocytes; PI, protease inhibitor; PR, protease; PSS, phenotypic sensitivity score; RT, reverse transcriptase; RTV, ritonavir;

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EXECUTIVE SUMMARY

Etravirine is a non-nucleoside analog reverse transcriptase inhibitor (NNRTI) of human immunodeficiency virus type 1 (HIV-1). Etravirine binds directly to reverse transcriptase (RT) and blocks the RNA-dependent and DNA-dependent DNA polymerase activities by causing a disruption of the enzyme's catalytic site. Etravirine does not inhibit the human DNA polymerases α , β , and γ .

Etravirine exhibits activity against laboratory strains and clinical isolates of wild-type HIV-1 in acutely infected T-cell lines, human peripheral blood mononuclear cells, and human monocytes/macrophages with median EC_{50} values ranging from 0.9 to 5.5 nM (i.e., 0.4 to 2.4 ng/mL). Etravirine demonstrates antiviral activity in cell culture against a broad panel of HIV-1 group M (subtype A, B, C, D, E, F, G) with EC_{50} values ranging from 0.29 to 1.65 nM. Less activity was displayed against group O primary isolates with EC_{50} values ranging from 11.5 to 21.7 nM.

No antagonism is observed between etravirine in combination with the studied antiretroviral drugs - the NNRTIs delavirdine, efavirenz, and nevirapine; the N(t)RTIs abacavir, didanosine, emtricitabine, lamivudine, stavudine, tenofovir, zalcitabine and zidovudine; the PIs amprenavir, atazanavir, darunavir, indinavir, lopinavir, nelfinavir, ritonavir, tipranavir, and saquinavir; and the fusion inhibitor enfuvirtide.

Etravirine-resistant strains were selected in cell culture originating from wild-type HIV-1 of different origins and subtypes, as well as NNRTI resistant HIV-1. Development of reduced susceptibility to etravirine typically required more than one amino acid substitution in reverse transcriptase of which the following were observed most frequently: L100I, E138K, E138G, V179I, Y181C, and M230I. In the Phase 3 trials C206 and C216 (DUET-1 and DUET-2), substitutions that developed most commonly in subjects with virologic failure at Week 24 to the etravirine-containing regimen were V179F, V179I, Y181C, and Y181I, which usually emerged in a background of multiple other NNRTI resistance-associated substitutions. In all the trials conducted with etravirine in HIV-1 infected subjects, the following substitutions emerged most commonly: L100I, E138G, V179F, V179I, Y181C and H221Y. Other NNRTI-resistance associated substitutions that emerged on etravirine treatment in <10% of the virologic failure isolates included K101E, K103N, V106I/M, V108I, Y188L, V189I, G190S/C, and R356K. The emergence of NNRTI substitutions on etravirine treatment contributed to decreased susceptibility to etravirine with a median fold-reduction in etravirine susceptibility of 40-fold from reference and a median fold-reduction of 6-fold from baseline.

Etravirine shows antiviral activity against 55 of 65 HIV-1 strains (85%) with single amino acid substitutions at reverse transcriptase positions associated with NNRTI resistance, including the most commonly found K103N. The single amino acid substitutions associated with an etravirine reduction in susceptibility >3 fold were K101A, K101P, K101Q, E138G, E138Q, Y181C, Y181I, Y181T, and Y181V, and of these, the greatest reductions were Y181I (13-fold reduction in EC_{50} value) and Y181V (17-fold reduction in EC_{50} value). Mutant strains containing a single NNRTI resistance-associated substitution (K101P, K101Q, E138Q, or M230L) had cross-resistance between etravirine

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and efavirenz. The majority (39 of 61; 64%) of mutant viruses with 2 or 3 amino acid substitutions associated with NNRTI resistance had decreased susceptibility to etravirine (fold-change >3). The highest levels of resistance to etravirine were observed for HIV-1 harboring a combination of substitutions V179F+Y181C (187 fold-change), V179F+Y181I (123 fold-change), V179F+Y181C +F227C (888 fold-change). In Studies C206 and C216, 35% of the baseline isolates had decreased susceptibility to etravirine (>3-fold change from reference) and >93% of these isolates were cross-resistance to delavirdine, efavirenz, and nevirapine. Cross-resistance to delavirdine, efavirenz and/or nevirapine is expected after virologic failure with an etravirine-containing regimen for the virologic failure isolates.

In Studies C206 and C216, response rates to etravirine decreased as the total number of baseline NNRTI resistance-associated mutations increased. Subjects with three or more IAS-USA-defined (2007) NNRTI mutations at baseline had lower response rates than the overall response rate of 60% for subjects who were taking etravirine and not using or re-using enfuvirtide. In particular, the presence at baseline of the substitutions V179F, V179T, V179D, Y181V, or G190S resulted in a decreased virologic response to etravirine. The presence of K103N, which was the most prevalent baseline NNRTI substitution in Studies C206 and C216, did not affect the response in the etravirine arm.

Response rates assessed by baseline etravirine phenotype showed that a ≤ 3 -fold change in etravirine susceptibility at baseline was associated with >60% response rates. Response rates decreased when baseline etravirine susceptibility was >3-fold. Response rates were 70%, 47% and 34% when baseline etravirine phenotype was 0-3, >3-13, and >13, respectively. These baseline phenotype groups are based on the select subject populations in Studies C206 and C216 and are provided to give clinicians information on the likelihood of virologic success based on pre-treatment susceptibility to etravirine in treatment-experienced subjects. Overall, in the etravirine arms of Studies C206 and C216, the median baseline phenotype was 1.7. The baseline phenotype of responders was 1.4 (n=351) and the median baseline phenotype of virologic failures was 3.4 (n=210).

1. RECOMMENDATIONS

1.1. Recommendation and Conclusion on Approvability

This NDA for TMC125 (etravirine; Intelence™) is approvable with respect to microbiology for the treatment of HIV-1 in NNRTI-treatment experienced subjects.

Intelence™, in combination with other antiretroviral agents, is indicated for the treatment of HIV-1 infection in ARV treatment-experienced adult patients, who have evidence of viral replication and HIV-1 strains resistant to _____

_____ This indication is based on Week 24 analyses from 2 randomized, double-blind, placebo-controlled trials of Intelence™. Both studies were conducted in clinically advanced, 3-class ARV (NNRTI, NRTI, PI) treatment-experienced adults.

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The following points should be considered when initiating therapy with Intelence™.

- Treatment history and resistance testing should guide the use of Intelence™.
- The use of other ARV with Intelence™ is associated with an increased likelihood of treatment response. In patients who have experienced virologic failure on an NNRTI- and NRTI-containing regimen, Intelence™ is not recommended for use in combination with NRTIs only.

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

We have requested that the applicant assess the combination antiviral activity relationships of etravirine with maraviroc and raltegravir; however, this was not a phase IV commitment.

2. SUMMARY OF OND MICROBIOLOGY ASSESSMENTS

2.1 Non-Clinical Microbiology

TMC125 inhibited enzymatic activity of recombinant wild-type HIV-1 reverse transcriptase (RT) with an IC₅₀ value of 38.4 nM. Co-crystallization and molecular modeling studies indicated that TMC125 binds in the non-nucleoside reverse transcriptase inhibitor binding-pocket of the HIV-1 reverse transcriptase in multiple conformations. TMC125 was specific for HIV-1 RT and did not inhibit the human DNA polymerases or other viruses.

TMC125 demonstrated antiviral activity in cell culture against wild-type laboratory and primary HIV-1 isolates of different origins and subtypes in human T-cell lines and in human primary cells, with median EC₅₀ values ranging from 0.87 to 5.46 nM. TMC125 had a 5-day CC₅₀ value greater than 100 μM giving a selectivity index of 62,500 in MT4 cells (CC₅₀ > 100 μM/ EC₅₀ = 1.6 nM). The antiviral activity was not affected by viral subtype within Group M isolates or coreceptor use. Median EC₅₀ values ranging from 5.67 to 7.19 μM were found for wild-type HIV-2, and a median EC₅₀ value = 3.33 μM for simian immunodeficiency virus. In the presence of 50% human serum, the affect of protein binding on the antiviral activity of TMC125 was a median 5.8-fold reduction in the EC₅₀ value ratio.

Cell culture combination experiments showed that TMC125 was not antagonistic with approved HIV-1 antiretrovirals: the non-nucleoside reverse transcriptase inhibitors (delavirdine, efavirenz, and nevirapine), nucleoside (nucleotide) reverse transcriptase inhibitors (abacavir, didanosine, emtricitabine, lamivudine, stavudine, tenofovir, zalcitabine and zidovudine), the protease inhibitors (amprenavir, atazanavir, darunavir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir, and tipranavir), and the fusion inhibitor (enfuvirtide). Combination activity relationships have not been evaluated for the recently approved CCR5 co-receptor antagonist maraviroc or the integrase inhibitor, raltegravir.

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Viruses resistant to TMC125 were selected in cell culture selection experiments starting from wild-type HIV-1 of different origins and subtypes, performed at high and low multiplicity of infection. Viruses with reduced susceptibility to TMC125 selected from HIV-1/IIIB, harvested after 6 to 36 passages, had a wide range of TMC125 EC₅₀ values (ranging from 4.01 nM to > 10,000 nM). The amino acid substitutions L100I, E138K, V179I, Y181C, and M230I were selected. The impact of specific combinations of these emerging reverse transcriptase substitutions, especially those at positions 100, 138, 179, and 181, on the susceptibility of HIV-1 to TMC125 was confirmed using specific HIV-1 variants obtained by site-directed mutagenesis.

Cell culture selection experiments starting from HIV-1 harboring NNRTI resistance-associated mutations Y181C, K103N, or L100I+K103N showed that the number of passages required for virus breakthrough increased when higher fixed concentrations of TMC125 were used. The substitutions L100I, E138G, V179I, and Y181C, emerged in the mutant HIV-1 RT and these mutations were associated with a loss of susceptibility to TMC125. Most of the viruses selected had a TMC125 fold change in EC₅₀ value >100 at endpoint.

TMC125 shows antiviral activity against 55 of 65 HIV-1 strains (85%) with single amino acid substitutions at RT positions associated with NNRTI resistance, including the most commonly found K103N. The single amino acid substitutions associated with an etravirine reduction in susceptibility of >3-fold were K101A, K101P, K101Q, E138G, E138Q, Y181C, Y181I, Y181T, and Y181V, and of these, the greatest reductions were with Y181I (13-fold change in EC₅₀ value) and Y181V (17-fold change in EC₅₀ value). Mutant strains containing a single NNRTI resistance-associated substitution (K101P, K101Q, E138Q, or M230L) had cross-resistance between etravirine and efavirenz. The majority of the NNRTI mutant viruses with 2 or 3 amino acid substitutions (39 of 61; 64%) had decreased susceptibility to etravirine (fold-change >3). The highest levels of resistance to etravirine were observed for HIV-1 harboring a combination of substitutions V179F+Y181C (187 fold-change), V179F+Y181I (123 fold-change), and V179F+Y181C+F227C (888 fold-change).

2.2 Clinical Microbiology

Clinical resistance analyses were performed on 24-Week genotypic and phenotypic data from Phase III Trials TMC125-C206 (DUET-1) and TMC125-C216 (DUET-2). Subjects included in the phase III DUET trials had to be on a stable but failing regimen (confirmed HIV-1 plasma viral load > 5000 HIV-1 RNA copies/mL), had to have at least 1 of the following NNRTI resistance associated mutations, A98G, L100I, K101E/P/Q, K103H/N/S/T, V106A/M, V108I, E138G/K/Q, V179I/F/G, Y181C/I/V, Y188C/H/L, G190A/E/S, P225H, F227C, M230I/L, P236L, K238N/T, and Y318F at either screening or from historical genotype reports, and had to have documentation of IAS-USA primary PI resistance-associated mutations, to be eligible. The baseline median numbers of detectable NNRTI-, NRTI-, and PI-resistance associated mutations were comparable between Studies C206 and C216 and the treatment groups within each trial.

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In Studies C206 and C216, substitutions that developed most commonly in subjects with virologic failure at Week 24 to the TMC125-containing regimen were V179F, V179I, Y181C, and Y181I, which usually emerged in a background of multiple other NNRTI resistance-associated substitutions. Other NNRTI-resistance associated substitutions that emerged on etravirine treatment in <10% of the virologic failure isolates included K101E, K103N, V106I/M, V108I, Y188L, V189I, G190S/C, and R356K. The emergence of NNRTI substitutions in the virologic failure isolates on etravirine treatment contributed to decreased susceptibility to etravirine with a median fold-reduction in etravirine susceptibility of 40-fold from reference and a median fold-reduction of 6-fold from baseline.

In Studies C206 and C216, response rates to etravirine decreased as the number of baseline NNRTI resistance-associated mutations increased. Subjects with 3 or more IAS-USA-defined (2007) NNRTI resistance-associated mutations at baseline had lower response rates than the overall response rate of 60% for subjects who were taking etravirine and not using or re-using enfuvirtide. The presence at baseline of the amino acid substitutions V179F, V179T, V179D, Y181V, or G190S resulted in a decreased virologic response to etravirine. The presence of K103N, which was the most prevalent NNRTI substitution in Studies C206 and C216 at baseline, did not affect the response in the etravirine arm.

Response rates assessed by baseline etravirine phenotype showed that a ≤ 3 fold change in etravirine susceptibility at baseline was associated with >60% response rates. Response rates decreased when the baseline TMC125 phenotypes were >3-fold. The median baseline phenotype in the etravirine arms of Studies C206 and C216 was 1.7. The baseline phenotype of responders was 1.4 (n=351) and the median baseline phenotype of virologic failures was 3.4 (n=210).

The use of enfuvirtide (ENF), number of susceptible drugs in the OBT (measured by phenotypic susceptibility scores (PSS)), DRV susceptibility, and the inhibitory quotient (IQ) were all significant variables affecting response. The percentage of subjects in the TMC125 arms who achieved <50 copies/mL HIV RNA and who used De Novo ENF was 70% compared to 60% of subjects who did not use or re-used ENF. Response rates in the TMC125 arm were greater than the placebo arm if subjects had a PSS of 0-2, but response rates were comparable between the arms if the PSS scores were 3 or more. Since DRV was part of the OBT of all subjects in the DUET trials, factors affecting DRV response (i.e., number of baseline PI substitutions) were assessed. A diminished virologic response was observed in subjects with ≥ 7 FDA-defined protease inhibitor resistance-associated substitutions and when the number of DRV resistance-associated substitutions at baseline was 3 or more showing the contribution of DRV to response. In addition, virologic responses were lower in both the TMC125 and Placebo arms when the fold-reduction of DRV baseline susceptibility was more than 7-fold. The contribution of TMC125 without DRV could be seen in the difference between the TMC125 and Placebo arms in the Re-Used/Not Used ENF subset of subjects; 40% vs. 8% with >3 DRV baseline substitutions and 47% vs. 14% for baseline DRV fold changes >7. Subjects with IQ values above the median 478 had better response rates (73-82%) than subjects with IQ values below 478 (47-62%).

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In Studies C206 and C216, 35% of the baseline isolates had decreased susceptibility to etravirine (>3-fold change from reference) and >93% of these isolates were cross-resistance to delavirdine, efavirenz, and nevirapine. Cross-resistance to delavirdine, efavirenz and/or nevirapine is expected after virologic failure with an etravirine-containing regimen for the virologic failure isolates.

3. ADMINISTRATIVE

3.1. Reviewer's Signature(s)

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

3.2. Concurrence

HFD-530/Micro TL _____ Date _____

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4. OND MICROBIOLOGY REVIEW

4.1 Important Milestones in Product Development

The clinical development of TMC125 focused on treatment-experienced HIV-1 infected subjects with NNRTI-experience and/or resistance. The Phase III program comprised two ongoing Phase III trials DUET 1 and 2, the ongoing Phase III roll-over trial TMC125-C217, 4 completed Phase IIb trials TMC125-C203, TMC125-C209, TMC125-C223, and TMC125-C227, the Phase IIb roll-over trial TMC125-C211 and the 3 completed Proof-of-Principle trials TMC125-C207, TMC125-C208 and TMC125-C201. Four different formulations of TMC125 were used in the efficacy trials of the clinical development program. The recommended dose is 800 mg BID (formulation TF035).

At the time of initiation of the DUET trials, DRV had not received regulatory approval. Therefore, the design of these trials evaluated the combination of 2 new investigational drugs in a treatment-experienced population. Randomization was stratified by the intended use of ENF, previous use of DRV, and screening plasma viral load (< or ≥30,000 copies/mL).

The applicant does not intend to pursue the development of TMC125 for the treatment-naïve patient population.

4.2 Methodology

Genotypes and phenotypes were performed at Virco by default on plasma samples taken at predefined timepoints when the viral load was > 1000 HIV-1 RNA copies/mL. Samples at other timepoints, such as the confirmed virologic failure (VF), were analyzed upon request from the Protocol Virologist if the RT mutations were different from those found at baseline and TMC125 fold-change in susceptibility was ≥4.

GENOTYPIC METHODS

Population Sequencing

Genotypic analyses were performed at Virco by automated population sequence analysis. Individual data were reported as amino acid changes along the PR and RT genes as compared to the HIV-1/HXB2 wildtype. 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.

Single Genome Sequencing

Genotypic analyses were also performed by single genome sequence analysis at Virco. Single genome sequence analysis, used to detect mutations that could be missed by population sequence analysis, was performed on the baseline samples and used to determine whether the emerging mutations were already present, at low prevalence, in the baseline HIV-1 species. Briefly,



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PHENOTYPIC ANALYSES

Phenotypic analyses were performed at Virco using the Antivirogram® assay. Briefly,

The resistance call for each ARV drug was determined based on the biological cut-off or the clinical cut-off (Table 1). A drug was defined as resistant if the fold-change (FC) value \geq biological cut-off or the clinical cut-off for the specific drug indicating that the virus showed decreased susceptibility to the drug.

Table 1. Resistance Call Determination Using Either the Biological or Clinical Cut-Off (Expressed as a FC) from the Antivirogram® Assay

Drug	Biological Cut-Off (FC)	Clinical Cut-Off (FC)
NRTI		
3TC	2.4	3.2
ABC		
AZT	2.7	
d4T	2.3	
ddC	3.0	
ddI	2.2	
FTC	3.5	
TDF		
NNRTI		
DLV	10.5	
EFV	3.4	
NVP	5.5	
PI		
APV	2.2	10.0
ATV	2.4	
IDV	2.4	
LPV		
NFV	2.2	
RTV	2.3	
SQV	1.8	
TFV		

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4.3 Prior FDA Microbiological Reviews

The original IND and subsequent IND submissions were reviewed by Dr. Julian O'Rear, Ph.D.

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 eradicate HIV from subjects and even though the number of serum HIV RNA copies is reduced to undetectable levels, HIV re-emerges quickly after discontinuation of HAART. Therefore, with the currently available regimens, it is likely that HIV-infected subjects will require antiretroviral therapy throughout their lives.

There are currently twenty-two FDA-approved anti-HIV 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 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 protease enzyme, which is necessary for the production of mature virions, resulting in defective particles which are unable to infect new cells.

Unfortunately, HIV develops resistance to antiretroviral drugs over time usually from the accumulation of multiple mutations. HAART regimens are also associated with acute toxicities such as diarrhea, kidney stones, rash, CNS toxicities and hepatotoxicity. Long-term toxicities from antiretroviral therapies include mitochondrial toxicities associated with NRTIs (lactic acidosis, myopathy, neuropathy, pancreatitis), and disorders of lipid metabolism (dyslipidemia) and glucose metabolism (lipodystrophy, hypercholesterolemia, hypertriglyceridemia) associated with PIs. These tolerability issues make compliance to therapy more challenging. Compliance is an important determinant of successful virologic suppression for subjects on HAART. Regimens that are well-tolerated and easy to administer with a few pills once daily are likely to aid in 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.

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4.5 NON-CLINICAL MICROBIOLOGY

MECHANISM OF ACTION

The inhibition of enzymatic activity of recombinant wild-type HIV-1 reverse transcriptase by TMC125 was characterized by an IC_{50} of 38.4 nM. No inhibition of the human DNA polymerases α , β , and γ was observed, indicating the specificity of TMC125 for HIV reverse transcriptase.

Co-crystallization and molecular modeling studies suggested that TMC125 is a flexible molecule able to bind in the non-nucleoside reverse transcriptase inhibitor binding-pocket of the HIV-1 reverse transcriptase in multiple conformations. TMC125 may bind in at least 2 conformationally distinct modes. Within a given binding mode, torsional flexibility of TMC125 could permit access to numerous conformational variants; while the compact design of TMC125 could permit significant repositioning and reorientation within the pocket. The flexibility of TMC125 may increase its ability to adapt the binding to HIV-1 reverse transcriptases containing amino acid substitutions associated with resistance to other non-nucleoside reverse transcriptase inhibitors.

ANTIVIRAL ACTIVITY IN CELL CULTURE

Antiviral Activity against Wild-type HIV

The antiviral activity of TMC125 was determined against wild-type strains of HIV-1 (IIIB, BaL, HXB2D, MN, NL4-3, SF2, and U455) in MT4 cells, PBMCs, and human primary monocytes/macrophages (Table 2; Virology Summary Report 0118488). The EC_{50} values ranged from 0.87 to 5.46 nM. TMC125 was tested against 25 HIV-1 primary isolates from group M (N=22) and group O (N=3) in PBMCs. TMC125 had a median EC_{50} value of 0.71 nM for the group M isolates and 21.7 nM for the group O isolates (Table 3; Virology Summary Report 0118488). Note that one group M isolate, BR/92/014, had an EC_{50} value of 275 nM, considerably higher than the EC_{50} values for the other isolates. The antiviral activity was not influenced by viral subtype or co-receptor use. Median EC_{50} values for wild-type HIV-2 ranged from 5.67 to 7.19 μ M. TMC125 had a median EC_{50} of 3.33 μ M for simian immunodeficiency virus.

Table 2. Antiviral Activity of TMC125 against Wild-type HIV Strains

Virus	Median EC_{50} (nM)
IIIB	0.87-2.75
BaL	2.00-3.61
MN	1.40-3.95
NL4-3	2.08-4.05
HXB2D	1.75
SF2	5.46
U455	2.92
HIV-2/ROD	5673-7190
SIV/mac251	3329

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Table 3: Antiviral Efficacy of TMC125 in HIV-1 Strains and Isolates in PBMCs

Virus	Group/Subtype/Tropism	EC₅₀ Value (nM)
RW/92/020	M/Subtype A/R5	0.47
UG/92/029	M/Subtype A/X4	0.60
UG/92/037	M/Subtype A/R5	0.60
BR/92/014	M/Subtype B/R5X4	275
BR/92/021	M/Subtype B/R5	1.09
JR-CSF	M/Subtype B/R5	1.30
WEJO	M/Subtype B/SI	0.86
BR/92/025	M/Subtype C/R5	0.61
IN/93/101	M/Subtype C/R5	0.62
MW/93/959	M/Subtype C/R5	0.79
UG/92/001	M/Subtype D/R5X4	1.21
UG/92/024	M/Subtype D/X4	1.65
UG/92/035	M/Subtype D/R5	0.42
TH/93/073	M/Subtype E/R5	0.41
CMU06	M/Subtype E/X4	1.19
CMU08	M/Subtype E/X4	1.40
BR/93/019	M/Subtype F/R5	1.01
BR/93/020	M/Subtype F/R5X4	0.36
BR/93/029	M/Subtype F/R5	0.48
JV1083	M/Subtype G/R5	1.05
RU132	M/Subtype G/R5	0.29
G3	M/Subtype G/R5	0.46
BCF01	O/R5	11.5
BCF02	O/R5	36.2
BCF03	O/R5	21.7

The antiviral activity of TMC125 against HIV-1/IIIB was studied at 3 multiplicities of infection (MOIs), representing a low, an intermediate, and a high viral input in the cell cultures. The EC₅₀ values of TMC125 did not change with increasing MOI, which was in agreement with the values observed for the control, EFV.

Antiviral Activity against Other Viruses

TMC125 has specific antiviral activity against HIV-1. No antiviral activity was observed against bovine diarrhea virus, hepatitis B virus, hepatitis C virus, herpes simplex virus 1, herpes simplex virus 2, human coronavirus, influenza virus, moloney murine sarcoma virus, sindbis virus, vaccinia virus, vesicular stomatitis virus, or yellow fever virus.

CYTOTOXICITY

The effects of TMC125 on the cell proliferation and viability of MT4, HeLa (epithelial cervix, adenocarcinoma), HepG2 (epithelial liver, hepatoblastoma), MRC-5 (normal fetal lung fibroblast), and A549 (epithelial lung carcinoma) cells were investigated with EFV as a control. The CC₅₀ values for TMC125, measured at Day 3, ranged from 15 to 40 µM (Table 4; Virology Summary Report 0118488). TMC125 had a 5-day CC₅₀ value greater than 100 µM. The median TC₅₀ value (concentration of a compound that resulted in a

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50% reduction in cell viability on Day 3 compared to Day 0) was greater than 100 μM for all the cell types tested, except for MRC-5 cells for which the TC_{50} value was 24.42 μM . The TC_{50} value of EFV ranged from 72.93 to 116.32 μM .

Table 4. TMC125 CC_{50} Values at Day 3 in Different Cell Types

Cells	TMC125 CC_{50} (μM) ^a		EFV CC_{50} (μM) ^a	
	Median (N)	IQR	Median (N)	IQR
A549	25.30 (1)		N.D.	N.D.
HeLa	14.87 (4)	14.44 – 16.15	54.93 (3)	38.39 – 85.60
HEp-2	16.76 (1)		35.70 (1)	
Hep G2	26.24 (4)	20.53 – 34.59	109.86 (3)	53.07 – 112.56
MRC-5	16.28 (1)		57.65 (1)	
MT4	39.77 (5)	25.79 – 40.00	46.61 (2)	36.43 – 59.62

A selectivity index of > 62,500 was deduced from the antiviral activity and cytotoxicity values in MT4 cells (EC_{50} value = 1.6 nM and CC_{50} value > 100 μM).

The inhibitory activity of TMC125 was tested on human DNA polymerases α , β , γ . TMC125 did not inhibit enzymatic activities of these human polymerases in cell culture (IC_{50} values > 100 μM).

PROTEIN BINDING

The influence of human serum proteins on TMC125 antiviral activity was assessed in assays performed in media containing 10% FCS to which 50% human serum, 45 mg/mL HSA, or 1 mg/mL α -1-acid glycoprotein (AAG) were added (Table 5; Virology Summary Report 0118488). The median ratio in EC_{50} values for TMC125 was 5.8, 4.3, and 1.8 in the presence of 50% human serum, 45 mg of HSA/mL or 1 mg of AAG/mL, respectively. These results indicated that, in human serum, TMC125 is mainly bound to albumin as opposed to AAG.

Table 5. Effect of AAG, HSA, and Human Serum on the Activity of TMC125 against HIV-1/IIIB Compared with EFV and NVP

NNRTI	Cell Culture ^a Media With								
	1 mg/mL AAG			45 mg/mL HSA			50% Human Serum ^c		
	N	EC_{50} Ratio ^b	IQR	N	EC_{50} Ratio ^b	IQR	N	EC_{50} Ratio ^b	IQR
TMC125	31	1.8	1.2 – 4.2	25	4.3	2.0 – 5.6	15	5.8	5.3 – 12.3
EFV	16	3.5	2.3 – 5.6	14	18.7	12.8 – 28.8	4	14.3	11.8 – 17.7
NVP	13	1.5	1.1 – 2.6	12	1.9	1.4 – 3.2	7	3.8	1.8 – 5.1

^b Results were expressed as the ratio between EC_{50} values determined in the presence and the absence of the human serum proteins. Values shown are the median of multiple determinations.

^c Heat inactivated human sera from 2 different lots were used

ANTI-HIV ACTIVITY OF DRUG COMBINATIONS IN CELL CULTURE

The anti HIV-1 activity of the combination of TMC125 with the NNRTIs [delavirdine (DLV), efavirenz (EFV), and nevirapine (NVP)], the NRTIs [AZT, lamivudine (3TC), emtricitabine (FTC), zalcitabine (ddC), didanosine (ddI), stavudine (d4T), and abacavir (ABC)], the NtRTI [tenofovir (TFV)], the PIs [indinavir (IDV), ritonavir (RTV), nelfinavir

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(NFV), SQV, amprenavir (APV), lopinavir (LPV), atazanavir (ATV), darunavir (DRV), and tipranavir (TPV), and the fusion inhibitor [enfuvirtide (ENF)] were studied using MT4 cells with HIV-1/IIIB Table 6; Virology Summary Report 0118488). EC₅₀ values were determined for each combination at 3 molar ratios (MRs), which were calculated according to predetermined EC₅₀ values for the compounds, to give 3:1, 1:1, and 1:3 EC₅₀ value ratios. Additional molar ratios were included for the combinations of TMC125 with AZT and ENF. A combination index (CI) was calculated using the classical isobologram model for combinations.

Cell culture combination experiments showed that TMC125 was not antagonistic with any of the approved HIV-1 antiretrovirals. Additivity was found with the nucleoside reverse transcriptase inhibitors (abacavir, didanosine, stavudine, and zalcitabine), the nucleotide reverse transcriptase inhibitor (tenofovir), the non-nucleoside reverse transcriptase inhibitors (efavirenz, delavirdine, and nevirapine), the protease inhibitors (amprenavir, atazanavir, darunavir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir, and tipranavir), and the fusion inhibitor (enfuvirtide). Evidence of synergy was observed with the nucleoside reverse transcriptase inhibitors zidovudine, lamivudine, and emtricitabine.

Table 6. Determination of the Effect of Combination of TMC125 with Approved ARVs

TMC125 in Combination With	Activity Ratio*	CI*		Score†	
		Median	IQR		
NRTIs/NRTI					
AZT	0.16	0.75	0.68 - 0.75	Synergistic Additive Additive Additive Additive	
	0.39	0.86	0.85 - 0.88		
	0.47	0.67	0.65 - 0.71		
	1.25	0.83	0.80 - 0.93		
	1.42	0.79	0.75 - 0.80		
d4T	3.48	0.85	0.81 - 0.88	Additive Additive Additive	
	0.32	0.99	0.94 - 0.99		
	0.97	0.90	0.80 - 0.92		
ddI	2.91	0.98	0.96 - 1.10	Additive Additive Additive	
	0.23	0.93	0.91 - 0.97		
	0.68	0.85	0.79 - 0.87		
ddC	2.04	0.90	0.90 - 0.90	Additive Additive Additive	
	0.47	0.95	0.95 - 0.99		
	1.41	1.04	0.99 - 1.09		
ABC	4.22	0.94	0.91 - 0.98	Additive Additive Additive	
	0.35	0.91	0.87 - 0.99		
	1.06	0.88	0.86 - 0.92		
BTC	3.19	0.93	0.92 - 1.00	Synergistic Additive Additive	
	0.30	0.77	0.76 - 0.87		
	0.89	0.90	0.85 - 0.94		
FTC	2.66	0.93	0.91 - 0.94	Synergistic Additive Additive	
	0.79	0.78	0.68 - 0.91		
	1.74	0.94	0.91 - 0.96		
TFV	7.37	0.96	0.91 - 1.31	Additive Additive Additive	
	0.51	0.93	0.90 - 0.99		
	1.75	1.00	1.00 - 1.07		
NNRTIs	5.20	0.97	0.90 - 0.97	Additive Additive Additive	
	NVP	0.39	1.13		1.05 - 1.17
		1.17	1.06		1.03 - 1.07
DLV		3.52	1.14	1.11 - 1.23	
		0.16	0.96	0.95 - 1.02	
		0.47	1.19	1.12 - 1.25	
EFV		1.40	1.06	1.00 - 1.12	
		0.46	1.06	0.98 - 1.08	
		1.39	0.96	0.95 - 0.98	
	4.17	1.00	1.00 - 1.06	Additive	

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TMC125 in Combination With	Activity Ratio	CI*		Score
		Median	IQR	
PIs				
IDV	0.32	1.01	0.96-1.02	Additive
	0.97	0.98	0.97-0.99	Additive
	2.91	0.99	0.97-1.01	Additive
RTV	0.37	0.98	0.97-0.98	Additive
	1.12	0.97	0.96-0.98	Additive
	3.37	0.92	0.92-0.95	Additive
SQV	0.39	0.92	0.88-0.99	Additive
	1.17	0.96	0.95-1.00	Additive
	3.52	0.94	0.85-0.98	Additive
NVP*	0.47	0.99	0.97-1.00	Additive
	1.40	0.88	0.88-0.88	Additive
	4.21	0.93	0.88-0.99	Additive
APV	0.24	0.99	0.98-0.99	Additive
	0.73	0.91	0.90-0.92	Additive
	2.18	0.86	0.85-0.88	Additive
LPV	0.48	1.03	1.03-1.12	Additive
	1.60	1.02	1.02-1.14	Additive
	4.83	1.02	1.00-1.09	Additive
ATV	0.41	0.98	0.98-1.02	Additive
	1.18	0.96	0.96-1.02	Additive
	3.99	0.96	0.96-0.99	Additive
TPV	0.29	1.04	1.04-1.05	Additive
	0.90	1.06	1.05-1.07	Additive
	2.66	1.01	0.99-1.07	Additive
DRV	0.24	0.96	0.93-1.00	Additive
	0.72	0.95	0.94-0.96	Additive
	2.12	1.00	0.99-1.02	Additive
Fusion Inhibitor				
ENP	0.38	1.03	0.91-1.07	Additive
	0.49	1.01	0.97-1.05	Additive
	1.18	1.05	1.03-1.07	Additive
	2.07	1.07	1.05-1.11	Additive
	3.15	1.02	1.02-1.03	Additive
	5.39	0.98	0.98-1.06	Additive

RESISTANCE IN CELL CULTURE

Selection experiments in cell culture were performed to determine the rate of emergence of resistant viruses as well as the amino acid substitutions associated with decreased susceptibility to TMC125 upon selective pressure. Two methods were used to isolate viruses resistant to TMC125.

- High multiplicity-of-infection experiments with fixed drug concentrations were conducted to determine the rate of emergence of resistant viruses. This experimental setting allowed for a standardized comparison between different non-nucleoside reverse transcriptase inhibitors.
- Low multiplicity of infection selection experiments with increasing drug concentrations were conducted to identify the mutations that were associated with decreased susceptibility to TMC125 in cell culture.

Selection Experiments with WT HIV-1 at High MOI in the Presence of Fixed Drug Concentrations

HIV-1/IIIIB, HIV-1/NL4-3, HIV-1/HXB2D, and 12 WT recombinant HIV-1 clinical isolates were passaged at high MOI in the presence of TMC125, EFV or NVP, at fixed concentrations of 2, 10, 40, 200, and 1,000 nM. No virus breakthrough was observed at 1,000 nM TMC125 after 9 passages for any of the WT HIV-1 strains, whereas a rapid selection of resistant strains (4 to 7 days) was observed with the same drug concentration of NVP and EFV. For NL4-3, no virus breakthrough was observed in the presence of TMC125 concentrations greater or equal to 40 nM after 9 passages. No

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virus breakthrough after 9 passages was observed at 1,000 nM TMC125 for IIIB, HIV-1/HXB2D and the 12 WT recombinant HIV-1 clinical isolates. Overall the data showed that the emergence of resistant strains from WT HIV-1 was delayed with TMC125 at drug concentrations at which EFV and NVP did not prevent virus breakthrough.

Experiments at Low MOI in the Presence of Increasing Drug Concentrations
HIV-1/IIIB, HIV-1/NL4-3, and 8 recombinant HIV-1 clinical isolates were passaged at low MOI, in the presence of increasing concentrations of TMC125, EFV or NVP. HIV-1/IIIB viruses with reduced susceptibility to TMC125 harvested after 6 to 36 passages had a wide range of TMC125 EC₅₀ values ranging from 4.01 nM to >10,000 nM. NL4-3 viruses with reduced susceptibility to TMC125, harvested after 19 to 88 passages, showed TMC125 EC₅₀ values ranging from 4.31 nM to 1,662.8 nM. The genotypic and phenotypic profiles of the virus strains selected from IIIB, NL4-3 and the recombinant HIV-1 clinical isolates of different subtypes in the presence of TMC125 are shown in Tables 7 and 8 (Virology Summary Report 0118488). NNRTI substitutions at V179, G190, M230 and L100I and Y181C were commonly selected in HIV-1/IIIB experiments. In addition to these NNRTI mutations, E138K was selected in NL4-3 experiments.

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Table 7. Overview of In Vitro Selection Studies Performed With HIV-1/IIIB at Low MOI, in the Presence of TMC125, EFV, and NVP

Experiment ^{a,b}	NNRTI						Final HIV-1 Strain	Mutations Emerging in the RT ^d
	Initial Concentration (nM)	Initial EC50 (nM)	Day	Passage	Final Concentration (nM)	Final EC50 (nM)		
TMC125								
SAL1180	1	0.75	115	33	16001	> 10000.00	T21710	I031L, V179D, V179N, Y181C, F227C, M230I
SAL1486	10	1.60	63	18	15000	9867.57	T13231	E006K, P014S, E040K, L100I, S163R, T165M, V179F, Y181C, M230L
SAL405	2	0.75	125	36	10000	> 2500.00	T20113	L100I, A158T, V179I, Y181C, P225H, T386A
SAL1730	3	0.75	98	28	12288	> 2349.25	T22362	I031L, V075I, L100I, V179F, Y181C, F227C, T386A
SAL1468	10	0.75	50	14	15000	840.50	T13340	I031L, A062V, L074V, V090I, Y181C, G190E, K219R, T386A
SAL1250	2	0.75	80	23	3000	368.41	T15302	V179I, Y181C, P225H, M230I
SAL630	1	0.75	115	33	2000	352.44	T21129	E040K, L100I, T165M, V179I, Y181C, R358K, T386A
SAL1362	2	0.75	21	6	20	7.47	T14947	Y181C
SAL899	2	0.75	41	12	32	4.01	T21265	Y181C
EFV								
SAL1456	10	0.14	52	15	20000	> 10000.00	T13331	E006K, G190E, V276I, T386A
SAL1366	10	0.63	87	25	20000	6767.99	T14915	A062V, L100I, K103N, V108I, P170H
SAL1184	1	0.63	101	29	16001	6187.14	T21685	P069L, L100I, K103N, Y181C, E370K
SAL1730	3	0.63	98	28	12288	4851.10	T22361	L100I, K103N, Y181C, T386A
NVP								
SAL1186	31	17.79	66	19	32000	> 1250.00	T21600	L034I, E036K, E053K, V179D, Y181C, D237N
SAL1361	40	17.79	21	6	200	> 1250.00	T14949	E040K, Y181C, V189I

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Table 8. Overview of Cell Culture Selection Studies Performed With HIV-1/NL4-3 and Recombinant HIV-1 Clinical Isolates at Low MOI, in the Presence of TMC125

Initial HIV-1 Strain ^a	Subtype	Experiment ^b	TMC125						Final HIV-1 Strain	Mutations Emerging in the RT ^c
			Initial Concentration (nM)	Initial EC ₅₀ (nM) ^d	Day	Passage	Final Concentration (nM)	Final EC ₅₀ (nM) ^e		
NL4-3	B	SAL403	5.00	0.64	196	56	400.00	76.66	T15768	V075I, E138K, T165A, M230I
r13813	AG	SAL1527	10.00	0.72	87	25	80.00	6.54	T14498	E006A, E138K, G190E, I195T
r13814	C	SAL1383	2.00	0.95	69	20	40.00	4.88	T15266	E138K, A371V
		SAL1427	10.00	0.95	201	57	5000.00	N.D.	T14985	E138K, G190E, P225H, V276I
r13816	AE	SAL1357	2.00	1.01	76	22	40.00	7.34	T15294	T165K, M230I, E370A
		SAL1610	10.00	1.01	214	62	3000.00	507.17	T20035	V075M, E138K, L187F, K219R, M230I, R356G
r13817	AE	SAL1359	2.00	1.12	66	19	10.00	4.31	T15251	E028K, Y181C, E297K, K390R
V071038	D	SAL409	2.00	0.30	306	88	2000.00	1662.80	T20803	L100I, F116Y, E138K, V179I, M184V, T215S, T286V, E297K, T369I
V071052	AG	SAL411	2.00	0.59	216	62	5000.00	1041.74	T20437	G045E, E138K, Y181C, M230I
V071130	C	SAL413	2.00	1.42	104	30	3000.00	1122.54	T20011	D067N, K102R, E138K, G190E, E297K, E399G
BAA188623	B	SAL407	2.00	2.33	178	51	5000.00	749.43	T20319	G045E, L109M, T165K, I178L, Y181C, M230I, T240K, E370K

HIV-1 site-directed mutant viruses, which harbored NNRTI substitutions Y181C, K103N, K103N+Y181C, L100I+K103N, K101P+G190A and K103N+P225H+K238T were passaged at high MOI in the presence of fixed concentrations of TMC125. Virus breakthrough was observed for the double NNRTI mutant, K103N+Y181C, after 3 to 4 passages at 1,000 nM of TMC125. At 200 nM of TMC125, virus breakthrough was observed after 4 or fewer passages for all the mutants except for K103N and K101P+G190A, where virus breakthrough was observed after 8-9 passages. At 40 nM of TMC125, virus breakthrough was observed after 4 or fewer passages for all four of the HIV-1 site-directed mutant viruses. A single passage corresponds to 3 to 4 days. The time to virus breakthrough observed for the site-directed mutant viruses was comparable to that observed for wild-type HIV-1.

Genotypic analysis of viruses with reduced susceptibility (fold change >3.4) to TMC125 selected at high MOI showed that the most frequently selected substitutions were L100I (10 viruses), V179I (5 viruses), Y181C (3 viruses), M230I (3 viruses), T386A (2 viruses), E194G and V189I. The NNRTI mutations E138G, V179F, and M230L emerged once.

Resistant viruses were selected at low MOI (EC₅₀ values 0.40-134) starting with site-directed NNRTI-resistant HIV-1 mutants harboring 1-3 NNRTI mutations (Table 9). Most of the selected viruses at endpoint had a TMC125 fold change in EC₅₀ value >100. The most frequently selected RT substitutions were V179I (n=6), E138G (n=5), and L100I, Y181C, E297K and N348I that each emerged 4 times. Other substitutions that emerged included K101E, E138K or Q, V179D or F, Y181N or V, Y188C or H, G190E or R, H221Y and P225Y.

The impact of the emerging NNRTI substitutions on TMC125 susceptibility was assessed using site-directed mutant viruses (see Cross-Resistance section below). These data indicated that substitutions at RT amino acid positions 100, 138, 179, 181,

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and 230 play a role in decreased susceptibility to TMC125. The impact of specific combinations of these emerging reverse transcriptase substitutions, especially those at positions 100, 138, 179, and 181, on the susceptibility of HIV-1 to TMC125 was confirmed using specific HIV-1 variants obtained by site-directed mutagenesis. Some of the single amino acid changes observed in these experiments (e.g., Y181I and Y181V) required two mutations within the codon for that amino acid. The low and high multiplicity of infection selection experiments demonstrated that the development of resistance to TMC125 usually required multiple substitutions.

Table 9. Cell Culture TMC125 Selection Studies Starting with NNRTI-Resistant HIV-1 at Low MOI

NNRTI Mutations at Baseline	Initial EC ₅₀ Value (nM)	Final EC ₅₀ Value (nM)	Passage Number	Substitutions Emerging in RT
K103N	0.4	>2500.00	20	T7I, L100I, E138G, V179I, Y181C, L214F
Y181C	1.4	832	29	E6K, L100I, V179I, Y188H, E297K
K103N/P225H/K238T	0.93	290	41	E138G, V179D, P225Y, E399A
K101P/G190A	0.74	223	36	L100I, V179I, Y188C, T397I, E399G
V179I/Y181C	8.84	>2500.00	29	L34I, E122K, E138G, Y181V, E203D, V241A
K101E/K103N	1.4	201	57	E28K, E138G, V179I, Y181N, L214F, H221Y, R277K
E138K	1.3	173	28	E122K, G190E, E297K, N348I
G190A/T240A	1.08	18.5	25	E138K, L214F, H221Y

CROSS-RESISTANCE IN CELL CULTURE

Antiviral Activity against NRTI- and PI-Resistant HIV-1 Isolates

To determine the level of cross-resistance with other compound classes, the antiviral activity of TMC125 was tested on a panel of site-directed mutant viruses harboring multiple NRTI-associated resistant mutations and on a panel of recombinant clinical isolates resistant to RT and/or PR inhibitors. These data showed no decreased susceptibility to TMC125 in viruses harboring all major NRTI or PI RAMs, with EC₅₀ values comparable to that of the HIV-1/IIIB WT reference.

Antiviral Activity against Site-Directed NNRTI HIV-1 Mutants

TMC125 showed antiviral activity in cell culture against 55 of 65 HIV-1 site-directed mutant strains with a single NNRTI resistance-associated substitution such as K103N and Y188C. The single substitutions associated with a TMC125 fold change > 3 were K101A, K101P, K101Q, E138G, E138Q, Y181C, Y181I, Y181T, Y181V and M230L (Table 10). Four of 65 HIV-1 mutant strains containing a single NNRTI resistance associated substitution (K101P, K101Q, E138Q, or M230L) had cross-resistance between etravirine and efavirenz.

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The substitutions which led to the greatest reductions in susceptibility to etravirine in cell culture were Y181I (13-fold change in EC₅₀ value) and Y181V (17-fold change in EC₅₀ value). The amino acid substitutions at K101 and Y181 (K101A, K101P, Y181I, Y181T and Y181V) required two mutations within their codon.

Table 10. Antiviral Activity of TMC125 and EFV against HIV-1 Site-Directed NNRTI Mutant Viruses

Substitution	TMC125 Fold Change in Susceptibility	EFV Fold Change in susceptibility
K101A	5.2	2.4
K101P	6.2	97.4
K101Q	3.4	3.8
K103N	0.7	26.7
E138G	3.8	2.3
E138Q	5.1	7.1
Y181C	3.9	2.2
Y181I	12.5*	1.6
Y181T	3.8	0.7
Y181V	17.4	2.8
M230L	3.4	5.7
L100I+K103N	3.6	845.1
K100I+V106A	5.0	98.6
L100I+Y181C	8.8	20.4
L100I+Y188L	35.0	13253.5
L101P+G190A	5.7	13107.8
K101P+K103N	17.6	>16339.9
K103N+Y181C	3.7	31.9
K103N+Y181I	27.5	6.4
V106M+Y181C	9.1	39.6
E138K+M230L	33.3	26.0
V179D+Y181C	10.8	9.3
V179E+Y181C	43.2	8.6
V179F+Y181C	187.1	6.8
V179F+Y181I	122.8	0.9
V179I+Y181C	9.1	1.8
V179T+Y181C	27.2	3.8
Y181C+F227C	11.9	14.2
Y181C+G190A	4.9	14.6
Y181C+G190S	33.1	723.3
Y181C+L234I	5.6	2.8
Y181C+T386A	3.1	1.3
Y181C+Y188L	7.5	240.5
Y181C+Y318F	3.3	2.8
F227C+234I	3.6	27.2
L100I K103N E138G	24.6	1828.6
L100I K103N T386A	15.3	11978.2
L100I K103N V179I	8.4	2398.1
L100I K103N V179L	18.3	5149.8
L100I K103N Y181C	56.4	2456.3
L100I V179I Y181C	15.1	16.9

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100P K103N V108I	19.6	13451.8
K103N V179T Y181C	7.9	41.7
K103N Y181C Y318F	8.1	111.4
K103N Y181C E194G	3.4	68.4
K103N Y181C Y318F	14.3	43.5
V106M V179D Y181C	45.6	5078.6
V108I Y181C G190A	4.5	9.2
V179F Y181C F227C	887.6	19.3
V179I Y181C G190A	4.3	10.5

Phenotyping was performed using the Antivirogram® assay. The biologic cut-off for EFV was 3.4.

Double NNRTI mutant viruses were tested for susceptibility to TMC125 and EFV and over half (24 of 40) of these mutant viruses had a TMC125 fold change > 3 (Table 10). Many of these isolates had decreased susceptibility for both TMC125 and EFV. The greatest decreases in susceptibility to TMC125 were observed for the HIV-1 site-directed mutants harboring a combination of amino acid changes V179F+Y181C (FC = 187.1) and V179F+Y181I (fold change = 122.8). Interestingly, single changes (D, E, F, G, I, L or T) at amino acid position V179 did not confer susceptibility changes to TMC125 (fold change <1). Results for 21 HIV-1 site-directed mutants with triple NNRTI substitutions, tested for susceptibility to TMC125 and EFV, showed that 15 had a TMC125 fold change > 3 (Table 10). The greatest change in susceptibility to TMC125 (fold change of 887.6) was observed for the triple mutant V179F+Y181C +F227C. Baseline genotypic and phenotypic data will be important in determining patient isolates' susceptibility to TMC125. The data also suggest that treatment with EFV or NVP of subjects failing a TMC125-containing regimen would not be recommended because of the high level of cross-resistance.

Antiviral Activity against Clinical Drug Resistant HIV-1 Isolates

The antiviral activity of TMC125 against NNRTI-resistant HIV-1 was assessed using a panel of 6,171 HIV-1 non-nucleoside reverse transcriptase inhibitor resistant recombinant clinical isolates with loss of susceptibility to at least 1 NNRTI. The TMC125 EC₅₀ values against these isolates ranged from 0.81 and 5.46 nM with a median fold change of 2.1 compared to 65-, 174- and >200-fold for DLV, EFV, and NVP, respectively (Table 11; Virology Summary Report 0118488). TMC125 retained a fold-change ≤ 3 against 60% of 6171 NNRTI-resistant clinical isolates. TMC125 had EC₅₀ values ≤10 nM for 83.2 % of the NNRTI-resistant recombinant HIV-1 clinical isolates, and EC₅₀ values > 100 nM for 2.0 % of the NNRTI-resistant recombinant HIV-1 clinical isolates. The proportion of these clinical isolates resistant to delavirdine, efavirenz and/or nevirapine (defined as a fold-change above their respective biological cutoff values in the assay) was 79%, 87%, and 95%, respectively.

Table 11. Phenotypic Data from the 6171 HIV-1 NNRTI-Resistant Clinical Isolates

NNRTIs	EC ₅₀ (nM) ^a		FC	
	Median	IQR	Median	IQR
TMC125	1.99	0.81 - 5.46	2.1	0.9 - 5.9
DLV	> 1250.00	106.64 - > 1250.00	173.7	15.3 - 392.7
EFV	38.50	5.12 - 1571.27	65.3	8.6 - 2688.6
NVP	> 1250.00	> 1250.00 - > 1250.00	> 202.3	187.0 - 223.1

^a Phenotypic assays were performed using the Antivirogram® method.

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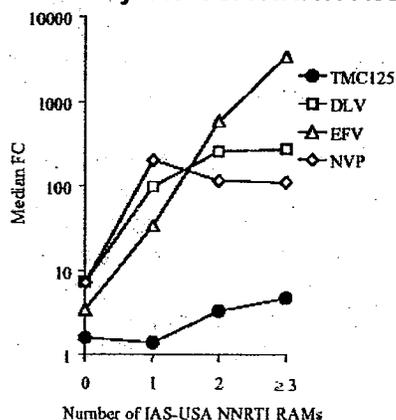
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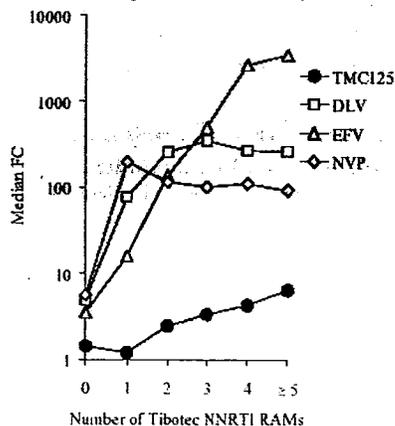
The 4,248 recombinant isolates which had available genotypic data and had up to 2 International AIDS Society-USA-defined or 3 Tibotec-defined NNRTI resistance-associated mutations had median fold changes of <10 to TMC125 (Figures-1 and 2; Virology Summary Report 0118488). The median fold change values to TMC125 increase as the number of IAS and Tibotec-defined NNRTI mutations; however, TMC125 median fold change values for all subgroups were consistently below those of the currently approved NNRTIs. The isolates with ≥ 1 NNRTI resistance-associated mutations had median fold change values that indicated a loss of susceptibility to DLV, EFV, and NVP.

Figure 1. Antiviral Activity against NNRTI-Resistant Recombinant HIV-1 Clinical Isolates by IAS-USA NNRTI Resistance Associated Mutations



IAS-USA list: L100I, K103N, V106A, V106M, V108I, Y181C, Y181I, Y188C, Y188H, Y188L, G190A, G190S, P225H, M230L, and P236L.

Figure 2. Antiviral Activity against NNRTI-Resistant Recombinant HIV-1 Clinical Isolates by Tibotec NNRTI Resistance Associated Mutations



Tibotec list of NNRTI resistant-associated mutations: A098G, L100I, K101E, K101P, K101Q, K103H, K103N, K103S, K103T, V106A, V106M, V108I, E138G, E138K, E138Q, V179D, V179E, V179F, V179G, V179I, Y181C, Y181I, Y181V, Y188C, Y188H, Y188L, G190A, G190C, G190E, G190Q, G190S, P225H, F227C, F227L, M230I, M230L, P236L, K238N, K238T, and Y318F.

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4.6 CLINICAL STUDIES

Trials **TMC125-C201** (n=25) and **TMC125-C208** (n=19) were proof-of-principle trials performed in treatment-naïve subjects who received 7 days of TMC125 monotherapy. Trial **TMC125-C207** (n=16) was an open-label, Phase IIa trial in HIV-1-infected subjects with phenotypically confirmed NNRTI resistance to evaluate the antiviral activity, safety, tolerability, and PK of a functional monotherapy with 900 mg b.i.d TMC125 (formulation TF002) administered during 7 days and as a single dose at Day 8. The primary objective of the trial was to evaluate the effect of the treatment on the viral load decay rates. Subjects had confirmed phenotypic resistance to EFV.

Trial **TMC125-C203** was a Phase IIb, randomized, placebo-controlled, dose-escalating trial, conducted in 2 stages to evaluate safety, tolerability, and efficacy of a 48-week treatment with TMC125 (formulation TF035), when added to an individually optimized background regimen (OBR). Subjects had to be three-class ARV experienced, i.e., having received at least 1 NNRTI, 1 NRTI, and 1 PI in prior treatment regimens, each for a period of at least 3 months. A total of 240 subjects were randomized. In the first stage of the trial 166 subjects were randomized in a 1:1:1 ratio to placebo (n = 55), TMC125 400 mg (n = 57), or TMC125 800 mg (n = 54) b.i.d. In the second stage of the trial, 74 subjects were randomized in a 1:2:4 ratio to placebo (n = 11), TMC125 800 mg (n = 20) or TMC125 1200 mg (n = 43) b.i.d., respectively. After completion of the 48-week treatment period, subjects could extend their treatment for an additional 48 weeks twice to reach up to 144 weeks. Overall, 152 and 55 subjects entered the first and second optional extension periods, respectively.

The **TMC125-C209** trial was an open-label Phase IIb trial in HIV-1-infected, three-class ARV-experienced subjects. Originally, the trial was designed as a 24-week double-blind trial followed by a 24-week open-label follow-up period. Per Protocol Amendment IV the trial design and objective were modified and the recruitment of subjects was stopped. The new goal was to evaluate the sustained safety, tolerability, and efficacy of a 48-week treatment with TMC125 in three-class experienced HIV-1-infected subjects, in combination with an individually OBR, consisting of a maximum of 4 ARVs. The ART had to contain a sensitive ritonavir-boosted (rtv) PI (LPV/rtv or SQV/rtv) and at least 1 sensitive NRTI, based on genotype (virco®TYPE HIV-1) at screening. Six of the seven subjects included in this trial completed the initial 48 weeks of treatment and entered the optional extension period. Five (71.4%) subjects completed the Week 144 extension period in this trial.

Trial **TMC125-C223** was a randomized, controlled, partially blinded, Phase IIb dose-finding trial in HIV-1-infected subjects (n=199) with documented genotypic evidence of resistance to currently available NNRTIs and with at least 3 primary PI resistance-associated mutations, to evaluate the efficacy, tolerability, safety and dose-response of a 400 and 800 mg b.i.d. TMC125 treatment (formulation TF035), administered during 48 weeks.

The **TMC125-C227** trial was a Phase II, randomized, active controlled, open label, 48-week exploratory trial of TMC125 conducted in HIV-1-infected subjects, who were PI-naïve and who had documented genotypic evidence of NNRTI resistance, to evaluate

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the efficacy, safety and tolerability of 800 mg b.i.d. TMC125 (formulation TF035), administered during 48 weeks and added to 2 investigator-selected NRTIs.

Trials **TMC125-C206 (DUET-1)** and **TMC125-C216 (DUET-2)** are Phase III randomized, double-blinded, placebo-controlled trials to investigate the efficacy, tolerability and safety of TMC125 at 48 weeks as part of an antiretroviral therapy including darunavir/rtv, investigator-selected nucleoside reverse transcriptase inhibitors and optional enfuvirtide. Subjects with at least 1 documented non-nucleoside reverse transcriptase inhibitor resistance-associated mutation (either at screening or from historical genotype reports), at least 3 documented primary protease inhibitor mutations, and a plasma viral load > 5,000 HIV-1 RNA copies/mL at screening were eligible. Subjects were randomized in a 1:1 ratio to either TMC125 (200 mg twice daily) or to matching placebo.

Summary of Safety in Duet Studies

A decrease in mortality with etravirine (1.8%) compared to placebo arm (3.3%) was observed in Phase 3 trials. The most frequent cause of death in both treatment groups was an AIDS-defining illness or infection. The most frequent reason for study discontinuation was realization of virologic endpoint (6.8% etravirine compared to 12.9% placebo). Discontinuations due to adverse events were observed in 6.3% of etravirine subjects compared to 4.6% to placebo subjects. Overall, 1.9% of discontinuations in etravirine arm were due to rash compared to none in placebo arm.

The principal safety concerns elucidated were skin reactions, gastrointestinal toxicity and hyperlipidemia. Overall, 15.2% of subjects experienced rash with etravirine use compared to 8.1% in placebo arm. Rash was typically mild to moderate in severity, manifested primarily in the second week of therapy, and infrequently resulted in drug discontinuation. Serious dermatologic entities including Stevens-Johnson syndrome were rare. A female predisposition to development of rash was observed.

The prominent gastrointestinal side-effects attributed to etravirine include nausea and vomiting. A modest increase in LDL cholesterol was observed in etravirine arm compared to placebo arm. With respect to cardiac safety, the frequency of coronary artery disease was comparable in the two treatment groups; the majority of subjects who experienced coronary events had pre-existing coronary artery disease or risk factors for coronary artery disease. A slight imbalance was observed in the frequency of elevations of serum alanine aminotransferase in the etravirine arm compared to placebo arm; however, there were no cases of hepatotoxicity clearly attributable to etravirine use. A slight increase in nasopharyngitis, herpes zoster, herpes simplex, oral candidiasis, and renal failure was noted in the etravirine subjects. The etravirine subjects who experienced renal failure of any type had risk factors with the most prominent risk factor for renal disease being tenofovir use. For a complete review of safety, see Medical Officer Charu Mullick's Clinical Review.

Summary of Efficacy in Duet Studies

The efficacy results of the Week 24 primary analyses were consistent across both DUET trials and demonstrated that TMC125, administered as part of an ART consisting of an OBR including DRV/rtv, was superior when compared with the individually optimized ARV regimens used in the placebo group. Overall, the proportion of subjects in the

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pooled DUET trials with an undetectable viral load (<50 HIV-1 RNA copies/mL) at Week 24 was 41.1% in the placebo and 58.9% in the TMC125 groups. Similar results were obtained in the individual DUET trials. In the individual trials as well as in the pooled analysis, a significant interaction effect was noted between treatment and ENF use ($p = 0.069$). The difference in virologic response rates (<50 HIV-1 RNA copies/mL) between the TMC125 and the placebo treatment arms was statistically significant in the group of subjects who did not use ENF de novo. In subjects using ENF de novo, the effect of adding TMC125 to the individually optimized ART was not statistically significant.

The results of the secondary efficacy parameters were supportive of those for the primary efficacy parameter. For both virological response parameters VL <400 copies/mL and decrease from baseline $> 1 \log_{10}$, the TMC125 group was statistically superior over the placebo group in the subjects reusing or not using ENF. The TMC125 arm was statistical superiority over the control group only for the viral load <400 copies/mL parameter in the subjects using ENF de novo. For a complete review of efficacy, see Dr. Charu Mullick's Clinical Review and Dr. Fraser Smith's Statistics Review.

4.7 CLINICAL VIROLOGY

SUMMARY OF CLINICAL VIROLOGY IN PHASE II STUDIES

Studies C201 and C208

In the proof-of-principle trials C201 and C208, there was no evidence of development of TMC125 resistance or emergence of mutations associated with NNRTI-resistance during the 7-day treatment period. In one subject in trial C208, emergence of the K101N substitution was observed at the final visit. Phenotypic analysis of this isolate did not show decreased susceptibility to TMC125 or other NNRTIs. In addition, the change at position 101 was no longer observed in the follow-up sample 13-weeks after the trial.

Study C207

In open-label, Phase IIa trial C207, 3 subjects acquired additional substitutions in the RT between baseline and end of treatment. However, no decrease in susceptibility to TMC125 or any of the other NNRTIs (DLV, EFV, and NVP) from baseline was observed in any of these subjects. Emerging substitutions were observed between baseline and end of treatment in Subject 2072014 (E6K, E44K, G45E, D76N, D110N, V189I, G196K, E203K, G262E, K287R, E291K, R356K, and D364N), Subject 2072020 (K101Q, R199G, and F346C), and Subject 2072030 (E40G, V90I, K103N, I135V, A360T, and K395R). The K103N NNRTI substitution was also present in the 2 pretreatment samples of Subject 2072030

Studies C203 and C209

Clinical isolates derived from subjects of trials TMC125-C203 and TMC125-C209, who were treated with the recommended dose (800 mg TMC125 b.i.d), experienced virologic failure, and had a endpoint fold change of TMC125 susceptibility ≥ 4.0 were analyzed for genotypic and phenotypic changes ($n=45$ subjects). Virologic failure was defined as either a rebounder (a subject with a viral load $> 0.5 \log_{10}$ HIV-1 RNA copies/mL above nadir for 2 consecutive visits following a virologic response of 2 consecutive (confirmed)

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viral load measurements $> 1 \log_{10}$ HIV-1 RNA copies/mL below baseline), or a non-responder (a subject not achieving a confirmed viral load response of more than $1.0 \log_{10}$ HIV-1 RNA copies/mL below baseline). In Studies C203 and C209, NNRTI – resistance-associated mutations emerged mainly in the group of subjects with a TMC125 fold change ≥ 4.0 , whereas NRTI resistance associated mutations emerged mainly in the group of subjects with a TMC125 fold change < 4.0 . The most frequently observed NNRTI substitutions were E138G, V179F, V179I, and Y181C and V189I. The M184V substitution emerged in subjects who used 3TC as part of their OBR. The phenotypic profiles of the subjects showed that for those with a fold change ≥ 4.0 , the median TMC125 FC increased from 1.3 at baseline to 129 at endpoint and the median number of NNRTI substitutions increased from 2 at baseline to 3 at endpoint for the group of subjects with a TMC125 fold change ≥ 4.0 . The data show that the emergence of the NNRTI substitutions is related to TMC125 resistance. Analysis of genotypes obtained by single genome sequence analysis (SGS) of the RT region of subjects with emerging mutations at endpoint showed that some of these endpoint mutations were also detected at baseline.

Study C223

Subjects from the TMC125-C223 trial were included in the virology analysis if they received the recommended dose of 800 mg b.i.d. TMC125, experienced a virologic failure by rebound and had a TMC125 ratio of fold change ≥ 4.0 . In total, 29 subjects were analyzed with 23 (79%) rebounders having TMC125 fold change ≥ 4.0 . The genotypic profiles for these subjects showed that, in the subjects experiencing virologic failure to the TMC125-containing regimen, the most frequently observed NNRTI substitutions were L100I, K103N, V108I, E122K, S162N, V179I or F, Y181C or I, H221Y, E297K and K366R. The most frequently emerging NRTI substitution was M184V. The emergence of V179F was only observed in combination with Y181C. The phenotypic profiles for this group of subjects showed that the median TMC125 fold change increased from 1.3 at baseline to 73.5 at endpoint showing an association between failure to the treatment and decreased phenotypic susceptibility to TMC125. The median number of NNRTI resistance associated mutations increased from 2 at baseline to 4 at endpoint in these analyzed subjects. Analysis of genotypes obtained by SGS of the RT region pertaining to subjects with emerging mutations at endpoint showed that some of these endpoint mutations were also detected at baseline.

Study C227

Subjects (n=24) in Study C227 who experienced a virologic failure and had TMC125 fold change ≥ 4.0 were analyzed for emerging mutations. Fourteen (58%) (6 rebounders and 8 non-responders) had a TMC125 FCEP/FCBL ≥ 4.0 . The baseline characteristics showed that this group of subjects had a higher median baseline viral load and a higher median baseline TMC125 FC compared to the overall TMC125-C227 800 mg BID population. Comparison of baseline and endpoint genotypic and phenotypic profiles of the 24 subjects showed an increase in both NRTI-and NNRTI-resistance associated mutations from 2 at baseline to 3 at endpoint. Detailed analysis of the genotypic profiles showed the NNRTI substitutions associated with virologic failure were L100I, V179I or F, Y181C, H221Y, L228H and M230L.

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CLINICAL VIROLOGY IN PHASE III STUDIES

Studies C206 (DUET-1) and C216 (DUET-2)

Subjects included in the phase III DUET trials had to be on a stable but virologically failing regimen with a confirmed HIV-1 plasma viral load > 5,000 HIV-1 RNA copies/mL, had to have at least 1 of the following NNRTI resistance-associated mutations, A98G, L100I, K101E/P/Q, K103H/N/S/T, V106A/M, V108I, E138G/K/Q, V179I/F/G, Y181C/I/V, Y188C/H/L, G190A/E/S, P225H, F227C, M230I/L, P236L, K238N/T, and Y318F at either screening or from historical genotype reports, and had to have documentation of IAS-USA primary PI mutations, to be eligible.

Baseline Characteristics Analysis of Duet Trials

The baseline median numbers of detectable NNRTI-, NRTI-, and PI-resistance associated mutations were comparable between Studies C206 and C216 and the treatment groups within each trial (Table 12). The median baseline TMC125 fold change values were 1.6 for both Studies C206 and C216, while the median fold values for the approved NNRTIs (e.g., EFV) were above their biological cut-offs. The data confirms that this was a highly treatment-experience patient population and a NNRTI-resistant population. Darunavir (DRV) was used as a sensitive drug (<10-fold change) in 64% of placebo and 64% of TMC125-treated subjects in Study C206, and in 62% of placebo and 64% of TMC125-treated subjects in each treatment group in Study C216. The applicant states that a high proportion of subjects in Study C206 (placebo: 53.6%; TMC125: 53.8%) and Study C216 (placebo: 55.5%; TMC125: 53.2%) had no sensitive NRTI in their OBR.

Table 12. Baseline Resistance Characteristics

	Study C206		Study C216		Pooled	
	TMC125 N=304	Placebo N=308	TMC125 N=295	Placebo N=296	Study C206	Study C216
FDA-defined NNRTI Mutations	2	2	2	2	2	2
IAS*-defined NNRTI Mutations	1	1	1	1	1	1
IAS*-defined NRTI Mutations	6	6	6	6	6	6
IAS*-defined Primary PI Mutations	4	4	4	4	5	4
Median TMC125 Fold Change	1.6	1.4	1.6	1.7	1.6	1.6
Median EFV Fold Change	102	73	40	28	87	32
Median DRV Fold Change	5.6	6.1	6.7	6.95	5.9	6.8

*IAS-USA 2005 list

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In Studies C206 and C216, 25% and 27% of subjects, respectively, used ENF de novo and it was comparable in each treatment group (Table 13). The proportion of subjects reusing ENF was 16% in Study C206 and 25% in Study C216. The subjects reusing or having never used ENF before was comparable between the studies and treatment arms (approximately 74%).

Table 13. ENF Use in ART in Study C206 and C216

ENF Use	Study C206		Study C216		Combined	
	TMC125 N=304	Placebo N=308	TMC125 N=295	Placebo N=296	TMC125 N=599	Placebo N=604
Total Use	40% (121)	41% (127)	52% (152)	53% (156)	46% (273)	47% (283)
De Novo	24% (74)	26% (79)	27% (79)	27% (81)	26% (153)	26% (160)
Re-Used/Not Used	76% (230)	74% (229)	73% (216)	73% (215)	74% (446)	74% (444)

Note that Re-Use was higher in Study C216

Overall, in the combined DUET studies, 16% of the subjects had no susceptible drugs in their OBT with a PSS of 0 and over half (54%) of the subjects had only 1 or no susceptible drugs in their OBT. About 20% of subjects had 3 or more susceptible drugs in their OBT with PSS ≥3. The phenotypic susceptibility scores were comparable between studies and arms (Table 14).

Table 14. Percentage of Subjects in Studies C206 and C216 by Baseline PSS Score

PSS Score	Study C206		Study C216		Combined	
	TMC125 N=299	Placebo N=303	TMC125 N=293	Placebo N=290	TMC125 N=592	Placebo N=593
0	50 (15%)	50 (17%)	48 (16%)	47 (16%)	17% (198)	16% (97)
1	114 (38%)	107 (35%)	102 (35%)	123 (42%)	36% (216)	39% (230)
2	71 (24%)	95 (31%)	89 (30%)	68 (23%)	27% (160)	27% (163)
3	50 (17%)	42 (14%)	45 (15%)	36 (12%)	16% (95)	13% (78)
4	14 (5%)	7 (2%)	9 (3%)	15 (5%)	4% (23)	4% (22)
5		2 (0.7%)		1 (0.3%)		0.5% (3)

The K103N mutation was the most prominent NNRTI-resistance associated mutation at baseline present in 32% of all subjects in the DUET trials (Table 15).

Table 15. Proportion of NNRTI Mutations at Baseline

NNRTI Mutation	Studies C206 and C216	
	TMC125 N=599	Placebo N=604
K103N	197 (33%)	190 (31%)
V179	178 (30%)	186 (31%)
V179I	112 (19%)	119 (20%)
Y181	199 (33%)	190 (31%)
Y181C	166 (28%)	160 (26%)
Y181I	9 (1.5%)	9 (1.5%)

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Y188L/F	53 (9%)	62 (10%)
L100	49 (8%)	50 (8%)
K101	186 (31%)	170 (28%)
V106	42 (7%)	54 (9%)
V108	102 (17%)	101 (17%)
E138	35 (6%)	35 (6%)
G190	193 (32%)	176 (29%)
M230L	5 (1%)	30 (0.5%)

Baseline Genotype and Virologic Outcome Analyses in DUET Trials

Virologic response analyses of the DUET trials (Studies C206 and C216) at Week 24 based on baseline genotype and phenotype data were performed on a censored as-treated dataset. Subjects who discontinued while suppressed or discontinued at week 2 were censored (See Appendix A). In Studies C206 and C216, 24 and 21 subjects, respectively, who discontinued study treatment before confirmed suppression for reasons including AE, non-compliance, or withdrew consent or who discontinued while suppressed were censored in the analysis, primarily because they discontinued at week 2-4.

Response rates (proportion <50 copies/mL at Week 24) of 565 subjects receiving 800 mg TMC125 in Studies C206 and C216 individually (Table 16) and combined (Table 17) were analyzed by the phenotypic susceptibility score (PSS) and compared to the response rates of the 593 subjects in the control arm. The response rates were generally comparable between the studies (Table 16). Response rates in the TMC125 arm were greater than the placebo arm if subjects had PSS scores of 0-2, but response rates were comparable between the arms if PSS scores were 3 or more (Table 17).

Table 16. Response (<50 copies/mL HIV-1 RNA) by Baseline PSS Score in Studies C206 and C216

PSS Score	Study C206		Study C216	
	TMC125 Arm N=288	Placebo Arm N=300	TMC125 Arm N=277	Placebo Arm N=293
0	44% (21/48)	8% (4/49)	42% (19/45)	7% (3/46)
1	57% (62/108)	22% (23/103)	60% (58/97)	33% (40/122)
2	66% (45/68)	60% (57/95)	82% (67/82)	67% (45/67)
3	69% (33/48)	63% (25/40)	71% (30/42)	75% (27/36)
4	54% (7/13)	71% (5/7)	100% (9/9)	60% (9/15)
5		100% (2/2)		100% (1/1)

*As-treated analysis

Table 17. Response (<50 copies/mL HIV-1 RNA) by Baseline PSS Score in Combined Studies C206 and C216

PSS Score	TMC125 Arms N=565	Placebo Arms N=593
0	43% (40/93)	7% (7/95)
1	59% (120/205)	28% (63/225)
2	75% (112/150)	63% (102/162)

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3	70% (63/90)	68% (52/76)
4	73% (16/22)	64% (14/22)
5		(3/3)
0-1	54% (160/298)	22% (70/320)
2+	73% (191/262)	65% (171/263)

*As-treated analysis

The use of enfuvirtide (ENF) was a significant variable in response (Table 18). Therefore, response rates to baseline genotypic and phenotypic characteristics were examined by subjects who took ENF in their OBT for the first time (De Novo ENF) and by subjects who did not use ENF in their OBT or were re-using ENF (Re-Used/Not Used ENF). Subjects in the placebo arm with PSS scores ≥ 3 had comparable response rates to the TMC125 arms (Table 19). However, response rates in the placebo subjects with PSS scores 0-2 had decreased response rates compared to the TMC125 arm. In the TMC125 arms, subjects with De Novo ENF use had better response rates than subjects without De Novo ENF use if the baseline PSS score was 0-2, but response rates were comparable if the PSS score was ≥ 3 . These results reiterate the fundamental fact that three or more active ARV drugs are required to obtain good response rates in treating HIV infection.

Table 18. Response (<50 copies/mL HIV-1 RNA) by ENF Use in Combined Studies C206 and C216

ENF Use	TMC125 Arms N=565	Placebo Arms N=593
De Novo ENF	70% (102/145)	62% (99/159)
Re-Used/Not Used ENF	60% (251/420)	34% (149/434)

Table 19. Response (<50 copies/mL HIV-1 RNA) by Baseline PSS Score and ENF Use in Combined Studies C206 and C216

PSS Score	TMC125 Arms N=565		Placebo Arms N=593	
	De Novo ENF	Re-Used/Not Used ENF	De Novo ENF	Re-Used/Not Used ENF
0		43% (40/93)		7% (7/95)
1	59% (23/39)	58% (97/166)	37% (13/35)	26% (50/190)
2	76% (44/58)	74% (68/92)	67% (44/66)	60% (58/96)
3	71% (22/31)	69% (41/59)	74% (26/35)	63% (26/41)
4	76% (13/17)	60% (3/5)	65% (11/17)	60% (3/5)
5			3/3	
0-2	69% (67/97)	58% (205/351)	56% (57/101)	30% (115/381)
3+	73% (35/48)	69% (44/64)	73% (40/55)	63% (29/46)

*As-treated analysis

Response rates were analyzed by the number of baseline NNRTI-resistance associated mutations and ENF use using the FDA list of mutations (any change at RT amino acid positions A98, L100, K103, V106, V108, Y181, Y188, G190, P225, M230, P236) (Table 20); the 2005 and 2007 IAS-USA list of mutations (Appendix B and Table 21,

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respectively) and Tibotec-defined NNRTI list of mutations (V90I, A98G, L100I, K101E, K101P, V106I, V179D, V179F, V179T, Y181C, Y181I, Y181V, G190A, and G190S) (Appendix B). Overall, response rates to TMC125 decreased as the number of baseline NNRTI mutations increased (Tables 20 and 21; Appendix B).

Table 20. Proportion of Subjects with <50 copies/mL HIV-1 RNA at Week 24 by Number of Baseline FDA-Defined NNRTI Mutations in an As-Treated Analysis of Combined Studies C206 and C216

#FDA-defined NNRTI	TMC125 Arms N=565		Placebo Arms N=593	
	De Novo ENF	Re-Used/Not Used ENF	De Novo ENF	Re-Used/Not Used ENF
0	95% (21/22)	71% (54/76)	69% (20/29)	46% (32/70)
1	74% (28/38)	65% (79/121)	57% (27/47)	40% (48/121)
2	65% (30/46)	58% (76/132)	67% (36/54)	32% (48/149)
3+	50% (23/39)	54% (42/91)	55% (16/29)	22% (21/94)

FDA-defined: RT mutations at amino acid A98, L100, K103, V106, V108, Y181, Y188, G190, P225, M230, P236

Table 21. Proportion of Subjects with <50 copies/mL HIV-1 RNA at Week 24 by Number of Baseline IAS-USA Defined NNRTI Mutations in an As-Treated Analysis of Combined Studies C206 and C216

#IAS-USA Defined NNRTI*	TMC125 Arms N=565		Placebo Arms N=593	
	De Novo ENF	Re-Used/Not Used ENF	De Novo ENF	Re-Used/Not Used ENF
All ranges	70% (102/145)	60% (251/420)	62% (99/159)	34% (149/434)
0	91% (21/23)	69% (58/84)	71% (22/31)	46% (33/72)
1	71% (24/34)	69% (76/110)	57% (28/49)	41% (52/127)
2	74% (35/47)	61% (78/127)	66% (27/41)	31% (40/128)
3	54% (13/24)	43% (25/58)	69% (11/16)	21% (14/68)
≥4	53% (9/17)	34% (14/41)	50% (11/22)	26% (10/39)
0-2	77% (80/104)	66% (212/321)	64% (77/121)	38% (125/327)
≥3	54% (22/41)	39% (39/99)	58% (22/38)	22% (24/107)

2007 IAS-USA defined mutations = V90I, A98G, L100I, L101E/P, K103N, V106A/I/M, V108I, V179D/F, Y181C/I/V, Y188C/H/L, G190A/S, P225H.

Response rates were high (91-95%) for subjects who were taking De Novo ENF with no baseline NNRTI mutations and 69-71% for subjects re-using/not using ENF with no baseline NNRTI mutations. Response rates for subjects in the TMC125 arm re-using/not using ENF were generally comparable to subjects in the placebo arm taking De Novo ENF. Subjects with 2 or more FDA-defined baseline NNRTI mutations had lower response rates than the overall response rate of 60% for subjects who were taking TMC125 and re-using/not using ENF. Similarly, response rates were lower for subjects with 2 or more 2005 IAS-USA NNRTI mutations at baseline (Appendix B). However,

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when the updated 2007 list of IAS-USA NNRTI mutations was used, response rates were lower when 3 or more mutations were present at baseline (Table 21). The 2007 list contains 21 NNRTI mutations rather than 15 and many of the additional substitutions are etravirine-resistance associated mutations. [Note that mixtures at amino acid positions were included in both the applicant and FDA analyses.] The applicant's analysis differed from the FDA analysis by only a few subject numbers, which did not affect the percentages or overall interpretation. Therefore, the 2007 IAS-USA list of NNRTI mutations was included in the Package Insert.

In addition, response rates were analyzed by the presence of specific NNRTI-resistance associated mutations at baseline (Table 22). The analysis showed that the presence at baseline of the mutations V179F, V179T, V179D, Y181V, or G190S resulted in a decreased virologic response to TMC125. The presence at baseline of mutations V179F or Y181V resulted in decreased response rates to 14%. The presence of any change at V179 and Y181 together resulted in response rates of 33%. According to the applicant, the list of TMC125-resistance associated mutations contains 13 mutations: V90I, A98G, L100I, K101E/P, V106I, V179D/F, Y181C/I/V, and G190A/S.

Table 22. Response (<50 copies/mL HIV-1 RNA) by Baseline Genotype in Re-Used/Not Used ENF Group¹ in Combined Studies C206 and C216

Baseline RT Substitutions	TMC125 Substitution Absent	TMC125 Substitution Present	Placebo ² Substitution Present
V90I	60% (240/397)	48% (11/23)	43% (12/28)
A98G/S	61% (196/323)	57% (55/97)	39% (52/132)
L100I	61% (233/384)	50% (18/36)	45% (22/49)
K101	66% (188/287)	47% (63/133)	37% (62/167)
K103N/S/R/T	58% (158/273)	63% (93/147)	45% (100/220)
V106I/M/A	61% (238/392)	46% (13/28)	26% (14/53)
V108I/M	59% (209/352)	62% (42/68)	33% (33/100)
E138K	60% (237/396)	58% (14/24)	
V179	62% (183/294)	54% (68/126)	39% (72/184)
V179I	62% (183/294)	59% (47/80)	
V179F	62% (183/294)	14% (1/7)	
V179T	62% (183/294)	33% (2/6)	
V179D	62% (183/294)	33% (2/6)	
Y181C/I/V/F	68% (195/287)	42% (56/133)	36% (67/185)
Y181C	68% (195/287)	56% (27/48)	
Y181I	68% (195/287)	50% (4/8)	
Y181V	68% (195/287)	14% (1/7)	
Y188L/F	59% (227/384)	67% (24/36)	29% (18/62)
V189I	60% (238/395)	52% (13/25)	31% (13/42)
G190	67% (187/280)	46% (64/140)	32% (54/171)
G190S	67% (187/280)	11% (1/9)	
P225H/R	60% (248/415)	60% (3/5)	70% (7/10)
M230	60% (249/416)	50% (2/4)	0/2

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P236	60% (251/420)	-	-
V179 +Y181		33% (14/42)	

¹ As-treated analysis; overall response rate in this group was 60% (251/420)

² Overall response for Re-used/Not Used Placebo group = 34% (149/434)

Only one of the 7 subjects with a Y181V substitution at baseline responded to treatment (14%) (Table 23). Three of these seven subjects also had a V179I substitution at baseline and only one of the subjects was taking ENF. The baseline susceptibility to TMC125 of the isolates with Y181V ranged from 3.4 to >1,000.

Table 23. Subjects with Y181V at Baseline

PID	Substitutions at V179	TMC125 Fold Change in Susceptibility at Baseline	# BL NNRTI Substitutions	ENF
TMC125-C206-0219		15.8000	1	N
TMC125-C206-0592		4.7000	1	N
TMC125-C216-0003	I	1099.7	2	N
TMC125-C216-0049	I	356.4000	3	Y
TMC125-C216-0294	I	10.2000	1	N
TMC125-C216-0476*		310.0000	3	N
TMC125-C216-0491		3.4000	2	N

*Responder

Baseline Phenotype and Virologic Outcome Analyses in DUET Trials

An analysis of response of subjects not receiving ENF/ re-using ENF by various fold increments of baseline TMC125 susceptibility (fold change from reference) using a censored as-treated dataset showed that response rates <50 copies/mL at week 24 decreased when baseline change in susceptibility was >3 fold (Table 24). Response rates were 70%, 47% and 34% when baseline etravirine change in susceptibility was 0-3, >3-13, and >13-fold, respectively (Table 24). Overall, in the TMC125 arms of Studies C206 and C216, the median baseline phenotype was a 1.7 fold-change. The baseline phenotype of responders was a 1.4 fold-change (n=351) and the median baseline phenotype of virologic failures was a 3.4 fold-change (n=210). From this data, a baseline phenotype of 3-fold or less was a predictor of a good response to TMC125. In addition, baseline etravirine phenotype correlated with the number of IAS-USA NNRTI Mutations (Appendix D); the proportion of subjects with a baseline etravirine phenotype of 0-3 was 74% if they had 0-2 baseline NNRTI Mutations and 37% if they had ≥3 baseline NNRTI Mutations.

Table 24. Response (<50 copies/mL HIV-1 RNA) by Baseline Etravirine Phenotype and ENF Use in Combined Studies C206 and C216

TMC125 FC	TMC125 Arms N=561	
	De Novo ENF	Re-Used/Not Used ENF
≤2	83% (63/76)	71% (175/248)
>2 - 5	63% (24/38)	49% (29/59)
>5 - 20	41% (9/22)	46% (29/63)
2	72% (13/18)	56% (19/34)

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3	45% (5/11)	53% (10/19)
4	60% (6/10)	27% (4/15)
5-7	50% (5/10)	43% (13/30)
8-10	43% (3/7)	83% (5/6)
10-13	1/1	47% (7/15)
>5	48% (15/31)	41% (45/109)
>3-10	49% (18/37)	45% (31/69)
>3	53% (23/43)	40% (50/125)
>10	56% (9/16)	38% (28/74)
>20	67% (6/9)	35% (16/46)
>300	50% (1/2)	25% (1/4)
0 - 3	82% (75/92)	70% (190/273)
>3 - 13	42% (16/38)	47% (37/78)
>13	53% (8/15)	34% (22/65)

*As-treated analysis

Effect of TMC125 IQ on Response in DUET Trials

The median C_{min} of the subjects in the TMC125 arm was 301 ng/mL and the median inhibitory quotient (IQ; C_{min} /TMC125 fold change from reference) was 478 (mean =1,124). Response rates (<50 copies/mL) were examined by ranges of the IQ in quartiles and below and above the median (Table 25). Subjects with IQ values above the median 478 had better response rates (73-82%) than subjects with IQ values below 478 (47-62%) also depending on ENF use.

Table 25. Response by TMC125 IQ (C_{min} /TMC125 phenotype)

TMC125 IQ	TMC125 Arms N=557	
	De Novo ENF	Re-Used/Not Used ENF
0-162	52% (17/33)	40% (42/106)
>162-478	69% (31/45)	55% (52/94)
>478-1437	84% (27/32)	75% (80/107)
>1437	79% (27/34)	70% (74/105)
0-478	62% (48/78)	47% (94/200)
>478	82% (54/66)	73% (154/212)

Effect of Darunavir-Resistance Associated Mutations and Susceptibility on Response in Duet Trials

Since all subjects in both arms in the DUET trials received DRV, the contribution of DRV to virologic response was analyzed. As shown in the DRV package insert, as the number of baseline PI mutations increases, the response rate to DRV-containing regimens decreases. A diminished virologic response was observed in subjects with ≥ 7 FDA-defined protease inhibitor resistance-associated mutations. This finding was confirmed in the DUET trials as seen in Table 26. The proportion of responders decreased in the TMC125 arms when the number of baseline PI mutations was ≥ 7 . The contribution of TMC125 was more apparent in subjects not receiving De Novo ENF and having ≥ 7 baseline PI mutations. In the ≥ 7 baseline PI mutations subset where the

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contribution of DRV to the treatment response is minimized, 49% of subjects not using De Novo ENF responded in the TMC125 arms compared to 18% in the comparator arm.

Table 26. Response by Number of Baseline PI (FDA-Defined) Mutations

# FDA-Defined BL PI Mutations	TMC125 Arms N=565		Placebo Arms N=593	
	De Novo ENF	Re-Used/Not Used ENF	De Novo ENF	Re-Used/Not Used ENF
0 - 4	67% (16/24)	63% (40/64)	69% (18/26)	47% (31/66)
5 - 6	77% (64/83)	63% (160/252)	68% (67/98)	40% (95/238)
≥7	58% (22/38)	49% (51/104)	40% (14/35)	18% (23/130)

This finding was corroborated in an analysis examining response by the number of DRV-resistance associated mutations (defined by applicant: V11I, V32I, L33F, I47V, I50V, I54L or M, G73S, L76V, I84V or L89V) (Table 27). Response rates decreased when the number of DRV mutations at baseline was 3 or more, but the contribution of TMC125 could be seen in the difference between the TMC125 and Placebo arms; 40% vs. 8% in the Re-Used/Not Used ENF subset of subjects with >3 DRV baseline mutations.

Table 27. Response by Number of DRV-Resistance Associated Mutations¹

Number of DRV BL PI Mutations ¹	TMC125 Arms N=565		Placebo Arms N=593	
	De Novo ENF	Re-Used/Not Used ENF	De Novo ENF	Re-Used/Not Used ENF
0	84% (16/19)	64% (25/39)	83% (10/12)	48% (13/27)
1	71% (17/24)	77% (78/101)	83% (34/41)	50% (54/109)
2	71% (30/42)	62% (76/123)	62% (32/52)	38% (52/136)
3	66% (19/29)	53% (37/70)	56% (14/25)	34% (22/65)
>3	65% (20/31)	40% (35/87)	31% (9/29)	8% (8/97)

¹DRV-Resistance Associated Mutations: V11I, V32I, L33F, I47V, I50V, I54L or M, G73S, L76V, I84V or L89V

Additionally, virologic responses were lower in both the TMC125 and Placebo arms when the DRV susceptibility fold change increased above 7-fold (Table 28). Irrespective of the DRV baseline susceptibility, the responses in the TMC125 arm were higher than the responses in the placebo arm. Again, the contribution of TMC125 could be seen most clearly in the subjects not taking De Novo ENF and those with baseline DRV fold changes >7: 47% compared to 14% for the same subgroup in the Placebo arm.

Table 28. Response by DRV Phenotype

DRV Baseline Phenotype	TMC125 Arms N=560		Placebo Arms N=580	
	De Novo ENF	Re-Used/Not Used ENF	De Novo ENF	Re-Used/Not Used ENF
0 - 2	88% (28/32)	75% (80/106)	77% (33/43)	58% (61/105)

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>2 - 7	65% (26/40)	65% (79/121)	66% (29/44)	46% (54/117)
>7 - 30	70% (30/43)	54% (67/123)	61% (31/51)	20% (27/132)
>30	60% (18/30)	34% (22/65)	18% (3/17)	-3% (2/71)
0 - 2	88% (28/32)	75% (80/106)	77% (33/43)	58% (61/105)
>2 - 7	65% (26/40)	65% (79/121)	66% (29/44)	46% (54/117)
>7	66% (48/73)	47% (89/188)	49% (33/68)	14% (29/203)

Substitutions Emerging on TMC125 Treatment

Examining the isolates from subjects who experienced virologic failure (n = 212) in the censored dataset, the median number of baseline NNRTI substitutions (both FDA-defined and IAS-defined) in these isolates was 2 and the median PSS score was 1. The median baseline susceptibility fold reduction for TMC125 was 3.4 (mean = 37).

Using the non-censored dataset, subjects who were virologic failures (n=239) from Studies TMC125-C206 and TMC125-C216 were analyzed for substitutions that emerged on TMC125 treatment. Substitutions at amino acid position V179 and Y181 emerged most frequently (Table 29). Substitutions at V179 emerged in 20% of the virologic failure isolates with the substitution V179F occurring most frequently (11%). Other NNRTI-resistance associated substitutions that emerged on TMC125 treatment in <10% of the virologic failure isolates included K101E, K103N, V106I/M, V108I, Y188L, V189I, G190S/C, and R356K. The median susceptibility fold reduction for TMC125 of the endpoint isolates of the virologic failures was 40 (mean = 118) and the median fold reduction from baseline was 6 (mean = 43) indicating that the emergence of these mutations on TMC125 treatment contributed to decreased susceptibility to TMC125.

Table 29. Substitutions Emerging in Virologic Failures on TMC125 Treatment

NNRTI Mutations Developing at	Virologic Failures on TMC125 n=239
V179X	48 (20%)
V179F	21 (11%)
V179I	17 (9%)
Y181C/F/S	17 (9%)
Y181C	14 (7%)
K101E/P/H/R	11 (5%)
G190S/C	7 (4%)
R356K	7 (4%)
K103N/R/H	7 (4%)
V189I	7 (4%)
V108I	6 (3%)
G190S	6 (3%)
K101E	4 (2%)
K103N	4 (2%)
Y188F/L	3 (1%)
V106I/M	3 (1%)

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Subject isolates (n=38) that had a substitution at V179 emerge on TMC125 treatment are shown in Table 30. Most of the subjects (33/38; 87%) whose virus developed the V179I or F substitution had the Y181C substitution present at baseline or emerge on TMC125 treatment. Other RT substitutions associated with resistance to NRTIs also emerged on treatment such as M184V, L74V, and K219E in 3% of the virologic failure isolates.

The median number of baseline NNRTI mutations (both FDA-defined and IAS-defined) in these isolates was 2 and the median PSS score was 1. The median baseline susceptibility fold change for TMC125 was 4.5 (mean = 14) and the median fold change from baseline at endpoint was 12.8 (mean = 74). Twenty of the 38 isolates (53%) had a reduced baseline susceptibility to TMC125 of >3-fold. The median IQ value for these 38 failure isolates was 75 and only 4 (10%) of these isolates had an IQ above the median IQ value of 478 (Appendix C). Thirty-two of these 38 isolates (84%) had reduced susceptibility to DRV at baseline (>7-fold change). These findings for the virologic failure isolates confirm that the baseline susceptibility to TMC125, baseline susceptibility to other drugs in OBT (PSS), the number of NNRTI mutations at baseline, IQ, and DRV susceptibility all contributed to the virologic response.

Table 30. Characteristics of the Isolates with Emergent V179I/F (n = 38)

Subject	# BL NNRTI	Baseline NNRTI Substitutions	NNRTI Substitutions Developing	C _{min}	EP* TMC125 FC	BL* TMC125 FC
TMC125-C206-0007	4	K101H K103R V108I Y181C G190A	V179F E194G R356K/R	487	228	7.6
TMC125-C206-0057	2	K101E Y181C G190A	V179F	182	27	2
TMC125-C206-0094	0	V189I	V179I/F Y181C	131	14	1.1
TMC125-C206-0231	3	K101H V106I/V Y181C G190A	V106I V179F	107	63	4.9
TMC125-C206-0309	2	A98S K101Q V108I V118I	V179I/V Y181C H221L	78	31	1.1
TMC125-C206-0353	3	K101H V108I Y181C G190S	V179F	681	473	37
TMC125-C206-0444	4	K101E V106I V108I Y181C V189I G190A	Q174Q/R V179F	279	53	7.8
TMC125-C206-0458	3	K101E V106I Y181C G190A	V179F	207	205	13.4
TMC125-C206-0466	1	K101P K103T R356K	V179I/L	333	619	66
TMC125-C206-0535	1	A98S	V108I/V V179I/V Y181I	115	225	0.2
TMC125-C206-0697	2	A98G K101E G190A	L100I/L V179I/V Y181I V189I/V	411	246	0.8
TMC125-C206-0717	3	A98G K101E Y181C G190A R356K	V179F E194K M357L/M	167	28	1.2

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TMC125-C206-0787	3	K101H V108I Y181C G190A R356K	V179F G190S/A	377		9
TMC125-C206-0793	4	A98S K101K/Q K103K/N Y181C G190A R356K	V179F	310	24	2.1
TMC125-C206-0846	0		V179I Y181C	609	212	0.7
TMC125-C206-0886	4	V106I/V V108I Y181C G190A R356K/R	K101R K102K/Q K103H V179F/V	137	157	2.9
TMC125-C206-0941	2	K101Q Y181C G190S	K101H/Q V179F/V	732	208	20
TMC125-C206-1002	2	K101E V179I Y181C G190S	V179F	253	125	10
TMC125-C206-1065	2	K101S Y181C G190A	V179F R356K M357R	588	170	5.3
TMC125-C206-1145	0		V179I Y181C P236L	231	11.3	0.7
TMC125-C216-0024	1	K103N/S	L100I K103N E138K V179I/V V189I	386	47	0.6
TMC125-C216-0031	3	K103N V108I Y181C	V179I	195	101	18
TMC125-C216-0043	2	Y181C/Y G190G/R	K173E Q174K V179F Y181C G190S Q242H	267	243	57
TMC125-C216-0087	3	A98A/G K101E/K Y181C/F//S G190A/G	K101E V179I Y181I	170	4.5	1
TMC125-C216-0146	2	L100I K103N	V179I/V L228L/R	261	93	47
TMC125-C216-0164	1	K103N	V179I Y181C E203D	196	19	1.3
TMC125-C216-0238	3	K103K/N V108I/V Y181C/Y	V108I V179I Y181I H221Y	167	495	4.9
TMC125-C216-0268	0	R356K	K101H V106I V179I/V Y181C G190S	67	74	1.1
TMC125-C216-0407	2	K101H Y181C G190S R356K	V179F/V	158	218	148
TMC125-C216-0431	2	K101E V179I Y181C G190A R356K	V179F/I	360	26	4.6
TMC125-C216-0451	2	K103N Y181C	V179I	748	18.3	5.8
TMC125-C216-0509	3	A98G K101H Y181C G190A R356K	V179F	328	81	20
TMC125-C216-0511	4	A98S K101Q V108I Y181C G190A R356K	V179F	90	9.1	-
TMC125-C216-0678	2	K101Q Y181C	K101E V179F	288	89	2.7

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		G190A R356K	G190S			
TMC125-C216-0690	4	A98G K101Q V106I/V Y181C G190A	V106I V179F	132	17	1.5
TMC125-C216-0769	2	L100I K103N R356K	V179I/V Y181C/F/I/S	604	512	1.9
TMC125-C216-0839	2	K101E Y181C G190A	V179F	234	20	2.9
TMC125-C216-0900	1	A98G K102N	K101E/K V179I Y181C G190S/G	72	16.2	0.9

EP= endpoint; BL=baseline; FC= fold change

Clinical Cross-Resistance

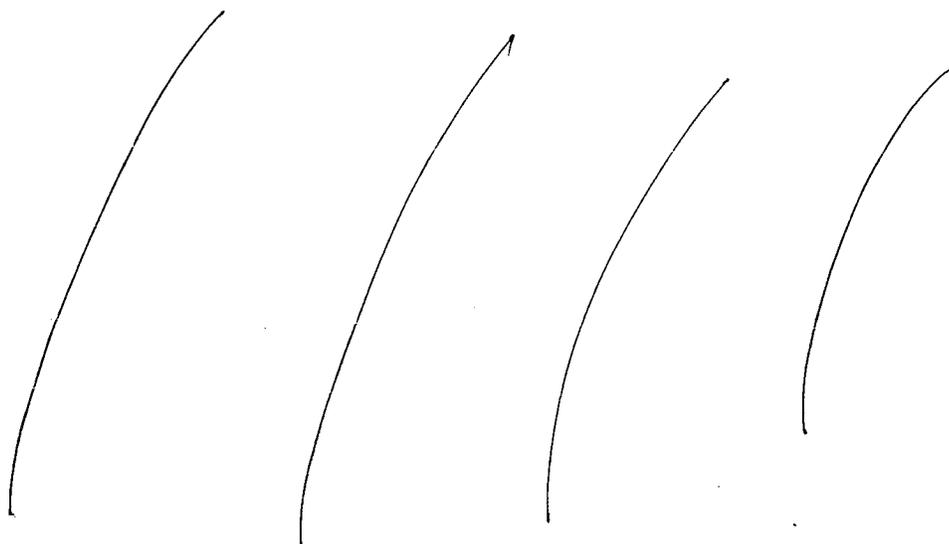
In Studies C206 and C216, 35% of the isolates had decreased susceptibility to etravirine (>3-fold change from reference) at baseline and 61%, 71%, and 79% of these isolates were resistant to delavirdine, efavirenz, and nevirapine, respectively. Cross-resistance to delavirdine, efavirenz and/or nevirapine is expected after virologic failure with an etravirine-containing regimen.

5. CONCLUSION

This NDA is approvable with respect to microbiology for the treatment of HIV.

We have requested that the applicant assess the combination antiviral activity relationships of etravirine with the recently approved CCR5 co-receptor antagonist, maraviroc, and integrase inhibitor, raltegravir.

6. PACKAGE INSERT



4 Page(s) Withheld

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✓ Draft Labeling

 Deliberative Process

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7. APPENDICES

APPENDIX A: Subjects Censored for As-Treated Analysis

Study C206 Censored:

N=16

TMC125-C206-0116	TMC125	DC while suppressed
TMC125-C206-0158	TMC125	DC while suppressed/NC
TMC125-C206-0190	TMC125	DC while suppressed/AE
TMC125-C206-0263	TMC125	DC while suppressed/AE
TMC125-C206-0569	TMC125	DC while suppressed/AE
TMC125-C206-0649	TMC125	DC while suppressed/AE
TMC125-C206-0687	TMC125	DC while suppressed/AE
TMC125-C206-1046	TMC125	DC while suppressed
TMC125-C206-0074	TMC125	DC before suppressed/AE
TMC125-C206-0252	TMC125	DC before suppressed/AE
TMC125-C206-0379	TMC125	DC before suppressed/AE
TMC125-C206-0457	TMC125	DC before suppressed/AE
TMC125-C206-0469	TMC125	DC before suppressed/AE
TMC125-C206-0689	TMC125	DC before suppressed/AE
TMC125-C206-0712	TMC125	DC before suppressed/AE (WK2)
TMC125-C206-1116	TMC125	DC before suppressed/AE (WK2)

N=8

TMC125-C206-0145	Placebo	DC while suppressed/withdrew consent
TMC125-C206-0244	Placebo	DC while suppressed/withdrew consent
TMC125-C206-0623	Placebo	DC while suppressed/AE
TMC125-C206-0646	Placebo	DC while suppressed/AE
TMC125-C206-1056	Placebo	DC while suppressed/sponsor's decision
TMC125-C206-0095	Placebo	DC before suppressed/AE (Week 2)
TMC125-C206-0511	Placebo	DC before suppressed/AE (Week 2)
TMC125-C206-1047	Placebo	DC before suppressed/AE (Week 4)

Kept

TMC125-C206-0057 - 1 log₁₀ decrease by Week 2; 0.5 decrease Week 4/DC
TMC125-C206-0174 (PCB)-DC at Week 8/never suppressed
*TMC125-C206-0193 (PCB)-DC at Week 4 (2 log₁₀ drop WK 2; 0.8 at WK4)/NC
TMC125-C206-0424-DC at Week 8

*For subject TMC125-C206-0193, drug intake start date is 5/4/06 and end date is 6/9/06 because this subject was non-compliant. It appears that the drug was dispensed for the period up until 6/9/06.

- 24 subjects in Study C206 who discontinued study treatment before confirmed suppression for reasons including AE, non-compliance, or withdrew consent or who discontinued while suppressed were censored in the analysis, primarily because they discontinued at week 2-4.

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Study C216 Censored:

N=18

TMC125-C216-0215	TMC125	DC while suppressed
TMC125-C216-0235	TMC125	DC while suppressed
TMC125-C216-0368	TMC125	DC while suppressed
TMC125-C216-0369	TMC125	DC while suppressed
TMC125-C216-0044	TMC125	DC before suppressed/AE
TMC125-C216-0045	TMC125	DC before suppressed/AE (Week 2)
TMC125-C216-0073	TMC123	DC before suppressed/AE
TMC125-C216-0092	TMC125	DC while suppressed
TMC125-C216-0206	TMC125	DC before suppressed/AE (Week 4)
TMC125-C216-0222	TMC125	DC while suppressed/NC
TMC125-C216-0228	TMC125	DC before suppressed/AE (Week 2)
TMC125-C216-0304	TMC125	DC before suppressed/AE
TMC125-C216-0329	TMC125	DC before suppressed/AE
TMC125-C216-0409	TMC125	DC while suppressed/AE
TMC125-C216-0499	TMC125	Not suppressed at Week 4/AE
TMC125-C216-0542	TMC125	DC before suppressed/AE (Week 2)
TMC125-C216-0838	TMC125	DC before suppressed WK4/Lost
TMC125-C216-0909	TMC125	DC before suppressed/AE

N=3

TMC125-C216-0815	Placebo	DC while suppressed
TMC125-C216-0172	Placebo	DC while suppressed
TMC125-C216-0748	Placebo	DC before suppressed/AE (Week 2)

Kept

TMC125-C216-0809	TMC125	Not suppressed at WK 8/Withdrew Consent
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- 21 subjects in Study C216 who discontinued study treatment before confirmed suppression for reasons including AE, non-compliance, or withdrew consent or who discontinued while suppressed were censored in the analysis, primarily because they discontinued at week 2-4.

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APPENDIX B:

Proportion of Subjects with <50 copies/mL HIV-1 RNA at Week 24 by Number of Baseline IAS-USA* Defined NNRTI Mutations* in an As-Treated Analysis of Combined Studies C206 and C216

#IAS-USA* Defined NNRTI*	TMC125 Arms N=565		Placebo Arms N=593	
	De Novo ENF	Re-Used/Not Used ENF	De Novo ENF	Re-Used/Not Used ENF
All ranges	70% (102/145)	60% (251/420)	62% (99/159)	34% (149/434)
0	89% (26/29)	67% (72/108)	59% (26/44)	44% (44/101)
1	70% (32/46)	69% (94/137)	56% (31/55)	37% (55/147)
2	66% (29/44)	47% (61/129)	79% (34/43)	29% (41/140)
3+	58% (15/26)	52% (24/46)	47% (8/17)	20% (9/46)
0-1	77% (58/75)	68% (166/245)	58% (57/99)	40% (99/248)
≥2	63% (44/70)	49% (85/175)	70% (42/60)	27% (50/186)

2005 IAS-USA defined mutations = L100I, K103N, V106A, V106M, V108I, Y181C, Y181I, Y188C, Y188H, Y188L, G190A, G190S, P225H, M230L, and P236L.

Proportion of Subjects with <50 copies/mL HIV-1 RNA at Week 24 by Number of Baseline Tibotec Defined NNRTI Mutations* in an As-Treated Analysis of Combined Studies C206 and C216

#Tibotec- Defined NNRTI*	TMC125 Arms N=565		Placebo Arms N=593	
	De Novo ENF	Re-Used/Not Used ENF	De Novo ENF	Re-Used/Not Used ENF
All ranges	70% (102/145)	60% (251/420)	62% (99/159)	34% (149/434)
0	80% (44/55)	70% (125/178)	67% (44/66)	43% (74/174)
1	72% (36/50)	62% (77/124)	67% (36/54)	35% (53/153)
2	58% (15/26)	54% (35/65)	65% (11/17)	24% (15/63)
3+	50% (7/14)	26% (14/53)	36% (8/22)	16% (7/44)
0-1	76% (80/105)	67% (202/302)	67% (80/120)	39% (127/327)
≥2	55% (22/40)	42% (49/118)	49% (19/39)	21% (22/107)

Tibotec defined mutations = V90I, A98G, L100I, K101E, K101P, V106I, V179D, V179F, V179T, Y181C, Y181I, Y181V, G190A, and G190S.

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APPENDIX C:

TMC125 Susceptibility, C_{min} and IQ of the Isolates with Emergent V179I/F (n = 38)

Subject	C_{min}	EP	BL	IQ
		TMC125 FC	TMC125 FC	
TMC125-C206-0007	487	228	7.6	64.07895
TMC125-C206-0057	182	27	2	91
TMC125-C206-0094	131	14	1.1	119.0909
TMC125-C206-0231	107	63	4.9	21.83673
TMC125-C206-0309	78	31	1.1	70.90909
TMC125-C206-0353	681	473	37	18.40541
TMC125-C206-0444	279	53	7.8	35.76923
TMC125-C206-0458	207	205	13.4	15.44776
TMC125-C206-0466	333	619	66	5.045455
TMC125-C206-0535	115	225	0.2	575
TMC125-C206-0697	411	246	0.8	513.75
TMC125-C206-0717	167	28	1.2	139.1667
TMC125-C206-0787	377		9	41.88889
TMC125-C206-0793	310	24	2.1	147.619
TMC125-C206-0846	609	212	0.7	870
TMC125-C206-0886	137	157	2.9	47.24138
TMC125-C206-0941	732	208	20	36.6
TMC125-C206-1002	253	125	10	25.3
TMC125-C206-1065	588	170	5.3	110.9434
TMC125-C206-1145	231	11.3	0.7	330
TMC125-C216-0024	386	47	0.6	643.3333
TMC125-C216-0031	195	101	18	10.83333
TMC125-C216-0043	267	243	57	4.684211
TMC125-C216-0087	170	4.5	1	170
TMC125-C216-0146	261	93	47	5.553191
TMC125-C216-0164	196	19	1.3	150.7692
TMC125-C216-0238	167	495	4.9	34.08163
TMC125-C216-0268	67	74	1.1	60.90909
TMC125-C216-0407	158	218	148	1.067568
TMC125-C216-0431	360	26	4.6	78.26087
TMC125-C216-0451	748	18.3	5.8	128.9655
TMC125-C216-0509	328	81	20	16.4
TMC125-C216-0511	90	9.1	-	9.89011
TMC125-C216-0678	288	89	2.7	106.6667
TMC125-C216-0690	132	17	1.5	88
TMC125-C216-0769	604	512	1.9	317.8947
TMC125-C216-0839	234	20	2.9	80.68966
TMC125-C216-0900	72	16.2	0.9	80

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APPENDIX D:

Median etravirine phenotype for Subjects in TMC125 arms with ≥ 3 IAS-USA NNRTI Mutations (2007) = 4.5 (mean=48)

Association of the Number of IAS-USA NNRTI Mutations and Etravirine Baseline Susceptibility

Number of IAS* NNRTI Mutations	Etravirine Baseline Phenotype 0-3	Etravirine Baseline Phenotype >3
0-2	74% (314/422)	26% (108/422)
≥ 3	37% (51/139)	63% (88/139)

*2007 IAS-USA list of NNRTI Mutations

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NDA microbiology review

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