

OCT 29 1999

MICROBIOLOGY REVIEW
DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

NDA 20-972 ~~SE~~⁷-001 Reviewer: Lauren C. Iacono-Connors

Date submitted: 05-26-99 Date received: 05-26-99

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Sponsor: DuPont Pharmaceuticals Company
Chestnut Run Plaza, MR 2136
974 Centre Road
Wilmington, DE 19805

Proprietary Name(s): SUSTIVA™

Nonproprietary: Efavirenz

Chemical Name: (S)-6-Chloro-4-(cyclopropylethynyl)-1,4-dihydro-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one

Molecular Formula: C₁₄H₉ClF₃NO₂ Molecular Weight: 315.68

Structural formula: N/A

Indication: Treatment of HIV infection

Dosage Form/route of administration: Capsule/Oral

Drug Category: Non-Nucleoside Reverse Transcriptase Inhibitor

Supporting Documents: IND 49,465

BACKGROUND

Sustiva™ was granted accelerated approval on September 17, 1998, based on data that demonstrated the suppression of plasma associated HIV-1 RNA in study participants on treatment up to 24 weeks duration. The sponsor has submitted a supplemental NDA (sNDA) seeking traditional approval of Sustiva™ based on the observed suppression of plasma-associated HIV-1 RNA in study participants two clinical studies, DMP- 266-006 and ACTG 364, each with at least 48 weeks of therapy. In addition, the available results to date from studies further characterizing the resistance profile of viral mutations associated with Sustiva™ treatment failures are included with this sNDA and reviewed below. These data are the basis for the sponsor's proposed changes to the *Resistance* paragraph in the *Microbiology* section of the current Sustiva™ label.

SUMMARY

Study Report Number PRR 99-01: Antiviral activity of efavirenz against recombinant mutants of HIV-1 reverse transcriptase and protease

This study serves as an update of data submitted to the original efavirenz NDA submission. The activity of efavirenz was assessed against a panel of recombinant, site-directed HIV-1 mutants of strain NL4-3, and HXB2. These mutant viruses contain amino acid substitutions in the RT or the PR gene region that have been previously reported to correlate with a decrease in susceptibility to efavirenz, to other agents in the NNRTI drug class, and to the protease inhibitor drug class, respectively. A p24 reduction assay in MT-4 cells was employed to evaluate changes in efavirenz activity *in vitro* (IC₉₀). The results of this study are presented enclosures 1 through 5. The efavirenz IC₉₀s for the single site mutants were from — nM, and depended upon the mutation and the strain background (enclosure 1). The fold-change IC₉₀ from the respective background levels — (enclosures 2 and 3). The fold-changes observed in HIV-1 mutants with multiple amino acid mutations in the RT was as high as 3800-fold loss in susceptibility to efavirenz in these studies. The data demonstrate that of the mutant laboratory HIV-1 strains tested under these assay conditions certain specific RT mutations provided a mechanism by which those mutant viruses could escape the full antiviral activity of efavirenz *in vitro*. In addition, these data suggest that the multiple mutations in the protease gene tested here had no effect on the antiviral activity of efavirenz *in vitro*.

The IC₉₀s for nevirapine, delavirdine, MKC442, loviride and HBY097 were also determined on both the single and multiply mutated recombinant viruses in parallel (enclosures 2 through 5). In general, a number of single-site and multiple mutation combinations specific to NNRTI pressure imparted some degree of decreased susceptibility to the other drugs evaluated here. However, uniform cross-resistance per mutation *in vitro* was not always observed.

Genotypic analysis of HIV-1 isolates from patients during therapy with efavirenz:

The sponsor has conducted four studies on patient specimens to analyze for the presence of efavirenz-associated RT-specific mutations. The objectives of these studies were to assess the potential for selection of virus variants *in vivo* during efavirenz therapy, to identify and characterize the variants selected, and to examine the time course of emergence of specific mutations as they relate to viral rebound in plasma. These data serve as an update of data submitted to the original NDA and should be used to update the package insert for Sustiva. The clinical studies that contributed viral isolates for these analyses at baseline and during treatment were DMP 266-003, DMP 266-004, DMP 266-005, and DMP 266-024.

It should be noted here that the sponsor did not randomly select patients for sequence evaluation from the various study cohorts. Instead the sponsor selected those patients that were identified as having experienced a “significant viral rebound” in viral load hence they referred to

those persons as treatment failures. It is not the intention of this reviewer to consider these data in the context of clinical relevance however, it should be noted that the sponsor did not evaluate "treatment responders" in a similar fashion. It is understood that "treatment responders" are likely to have HIV RNA levels in the blood that are below the level needed to successfully amplify genetic material for cloning and sequencing analysis, but that should not be considered a sufficient excuse for assuming that the responders do not have the same RT mutations as the treatment failures. Therefore, reporting the prevalence of RT mutations associated with efavirenz therapy in treatment failures should not allow the reader to assume the converse, that the absence of mutations should be the expected observation in treatment responders.

Study Report Number PRR 99-03: Genotypic characterization of plasma virus from patients in study DMP 266-003

This study assessed the RT and PR genotype in selected patients from clinical phase II study DMP 266-003. Subjects enrolled in this study had CD4 cell counts between 100 and 500 cells/mm³, and had plasma HIV-1 RNA levels >20,000 copies/mL (Amplicor HIV-1 Monitor™ Test). Patients were required to be NNRTI and PR inhibitor (PI) naïve. Plasma samples were collected at baseline and at intervals before and after a significant rebound in plasma virus load was observed for genotype determination. The selection and categorization of patients for on-treatment genotyping was based on their responses to treatment. Patients with "significant rebounds" in viral load were defined as those patients whose HIV-1 RNA levels rose above 1000 copies/mL on two successive occasions after reaching a nadir of ≤400 copies/mL or those patients with a rise of — (in those who never achieved an HIV-1 RNA of <400 copies/mL) on two successive visits relative to the lowest RNA copy number previously measured. Efavirenz-treated patients received either efavirenz monotherapy or efavirenz in combination with other retroviral agents (no other NNRTIs).

Plasma samples were collected for genotyping at baseline, and at intervals before and after a significant rebound in plasma virus load of efavirenz-exposed patients. Genotyping was carried out using a clonal sequencing technique, and encompassed a major segment of the RT gene (RT amino acids 1-229), and the entire PR gene. The frequency of individual RT gene mutations known to be associated with NNRTI class resistance found in samples from DMP 266-003 efavirenz treatment failures is shown in enclosure 6. Thirty two subjects were on initial efavirenz therapy and an additional 14 who started out on indinavir monotherapy were switched to efavirenz plus d4T or 3TC. At baseline none of the 94 subjects tested harbored NNRTI-significant mutations, however, in the indinavir monotherapy cohort, one of the twelve subjects failing that initial monotherapy appeared to have the efavirenz-specific K103N mutation actually prior to the initiation of efavirenz-containing therapy. The sponsor offers no explanation for this anomaly.

Of the 46 patients selected for genotypic analysis RT mutations at amino acid positions 74, 100, 101, 103, 108, 138, 181, 188, 190, 225 and 227 were detected with a minimum frequency of 9% per site. In patients with "significant" rebounds in viral load while on active

efavirenz-containing regimens 30/32 of those treated with efavirenz in combination with other antiretrovirals (93.8%) had the K103N mutation. Likewise, 14/14 (100%) subjects failing efavirenz-containing regimens after an initial virologic failure on indinavir monotherapy harbored the K103N mutation (enclosure 6). Other mutations frequently observed in failing patients initially randomized to active efavirenz therapy or switched from indinavir to active efavirenz containing regimens included V108I (37.5%) and P225H (40.6%), and P225H (50%), K101Q (28.6%), respectively (enclosure 6).

It may be concluded that the 103 RT mutation appears to be associated with virologic failure in patients receiving efavirenz-containing treatment regimens, however, we don't have information on the mutation status of treatment responders. Therefore, the likely causal relationship between the frequency of the observed mutation and that of virologic failure remains unclear.

Study Report Number PRR 99-04: Genotypic characterization of plasma virus from patients in study DMP 266-004

This study assessed the RT and viral PR genotype in selected patients from clinical phase II study DMP 266-004. Subjects enrolled in this study had CD4 cell counts >50 cells/mm³, and had plasma HIV-1 RNA levels >2500 copies/mL (Amplicor HIV-1 Monitor™ Test) and ≥8 weeks of prior zidovudine/3TC treatment. Patients were required to be NNRTI and PI naïve. Plasma samples were collected at baseline and at intervals before and after a significant rebound in plasma virus load was observed for genotype determination. The selection and categorization of patients for on-treatment genotyping was based on their responses to treatment. Patients with "significant rebounds" in viral load were defined as those patients whose HIV-1 RNA levels rose above 1000 copies/mL on two successive occasions after reaching a nadir of ≤400 copies/mL or those patients with a rise of — (in those who never achieved an HIV-1 RNA of <400 copies/mL) on two successive visits relative to the lowest RNA copy number previously measured. Efavirenz-treated patients received either efavirenz or placebo in combination with zidovudine/3TC (no other NNRTIs).

Plasma samples were collected for genotyping at baseline, and at intervals before and after a significant rebound in plasma virus load of efavirenz exposed patients. Genotyping was carried out as described above. The frequency of individual RT gene mutations known to be associated with NNRTI class resistance found in samples from DMP 266-004 efavirenz treatment failures is shown in enclosure 7. Thirty six subjects were on initial efavirenz therapy and an additional 8 started out on efavirenz placebo plus ZDV/3TC and were switched to efavirenz plus ZDV/3TC. At baseline several of the 51 subjects harbored NNRTI-significant mutations, however none appeared to have the efavirenz-specific K103N mutation actually prior to the initiation of efavirenz-containing therapy.

Of the 44 patients selected for genotypic analysis RT mutations at amino acid positions 74, 98, 100, 101, 103, 106, 108, 188, 190, 225 and 227 were detected with a minimum frequency

of 9% per site. In patients with "significant" rebounds in viral load while on active efavirenz-containing regimens forty (91%) had a mutation at RT amino acid position 103. (enclosure 7). Other mutations frequently observed in failing patients initially randomized to active efavirenz therapy or switched to active efavirenz containing regimens included were similar to those observed in study DMP 266-003 with following additional mutations observed A98G (13.6%) and V106I (15.9%) (enclosure 7).

These data further support the above conclusion that the 103 RT mutation appears to be associated with virologic failure in patients receiving efavirenz-containing treatment regimens, however, the likely causal relationship between the frequency of the observed mutation and that of virologic failure remains unclear.

Study Report Number PRR 99-05: Plasma virus genotypes of patients in study DMP 266-005

This study assessed the RT and PR genotype in selected patients from clinical phase II study DMP 266-005. Subjects studied were asymptomatic or mildly symptomatic and were antiretroviral naive. Subjects were treated with various doses of efavirenz in combination with double NRTIs (ZDV/3TC). Plasma samples were collected at baseline and at intervals before and after a significant rebound in plasma virus load was observed for genotype determination. Plasma samples were selected for sequencing based on viral load data; a minimum of 1000 copies HIV RNA/mL plasma was required before sequencing was attempted. Patients with "significant rebounds" in viral load were defined as those patients whose HIV-1 RNA levels rose above 1000 copies/mL on two successive occasions after reaching a nadir of ≤ 400 copies/mL or those patients with a rise of ≥ 1000 copies/mL (in those who never achieved an HIV-1 RNA of < 400 copies/mL) on two successive visits relative to the lowest RNA copy number previously measured. In addition, baseline samples from some patients who experienced a sustained suppression in viral load were selected for sequencing.

Plasma samples were collected for genotyping at baseline, and at intervals before and after a significant rebound in plasma virus load of efavirenz exposed patients. Genotyping was carried out as described above. The frequency of individual RT gene mutations known to be associated with NNRTI class resistance found in samples from DMP 266-005 efavirenz treatment failures is shown in enclosure 8. Ten subjects were on initial efavirenz therapy and an additional 4 started out on efavirenz placebo plus ZDV/3TC and were switched to efavirenz plus ZDV/3TC. At baseline several of the 16 subjects tested harbored a few NNRTI-significant mutations, none appeared to have the efavirenz-specific K103N mutation actually prior to the initiation of efavirenz-containing therapy.

Of the 14 patients selected for genotypic analysis RT mutations at amino acid positions 100, 101, 103, 106, 108, 188, 189, 190, 225 and 227 were detected with a minimum frequency of 9% per site. In patients with "significant" rebounds in viral load while on active efavirenz-containing regimens 12 (85.7%) had a mutation at RT amino acid position 103 (enclosure 8). These data further support the above conclusion that the 103 RT mutation appears to be

associated with virologic failure in patients receiving efavirenz-containing treatment regimens, however, the likely causal relationship between the frequency of the observed mutation and that of virologic failure remain unclear.

Study Report Number PRR 99-06: Genotypic characterization of plasma virus from patients in study DMP 266-024

This study assessed the RT and PR genotype, and the nucleotide sequence covering the p7/p1 and p1/p6 PR cleavage sites in selected patients from clinical phase II study DMP 266-024. Subjects enrolled in this study had CD4 cell counts ≥ 50 cells/mm³, and had plasma HIV-1 RNA levels $\geq 10,000$ copies/mL and were either NRTI-experienced (n=30) or naïve (n=32).

Subjects were treated with efavirenz in combination with nelfinavir. Plasma samples were collected at baseline and at intervals before and after a significant rebound in plasma virus load was observed for genotype determination. Plasma samples were selected for sequencing based on viral load data; a minimum of 1000 copies HIV RNA/mL plasma was required before sequencing was attempted. Patients with "significant rebounds" in viral load (Amplicor HIV-1 Monitor™ Test) were defined as those patients whose HIV-1 RNA levels rose above 1000 copies/mL on two successive occasions after reaching a nadir of ≤ 400 copies/mL or those patients with a rise of — (in those who never achieved an HIV-1 RNA of < 400 copies/mL) on two successive visits relative to the lowest RNA copy number previously measured or a less than — decrease from baseline in HIV RNA by week 8.

Plasma samples were collected for genotyping at baseline, and at intervals before and after a significant rebound in plasma virus load of efavirenz exposed patients. Genotyping was carried out using a population-based method, therefore, mutation linkage information was not captured. The frequency of individual RT gene mutations known to be associated with NNRTI class resistance found in samples from DMP 266-024 efavirenz/nelfinavir treatment failures is shown in enclosure 9. At baseline several subjects harbored NNRTI-specific mutations, however, none appeared to have the efavirenz-specific K103N mutation prior to the initiation of efavirenz/nelfinavir therapy.

Of the 17 subjects selected for genotypic analysis RT mutations at amino acid positions 98, 100, 101, 103, 106, 179, 188, 190, and 225 were detected in NRTI-experienced subjects with a minimum frequency of 9% per site. Of the 17 subjects selected for genotypic analysis RT mutations at amino acid positions 101, 103, 106, 108, 181, 190, and 225 were detected in NRTI-naïve subjects with a minimum frequency of 9% per site. In patients with "significant" rebounds in viral load while on active efavirenz-containing regimens 15 (88.2%) had a mutation at RT amino acid position 103 (enclosure 9). More specifically, the NRTI treatment-naïve subjects had the 103 mutation at failure with a frequency of 66.7% while that observed for the NNRTI treatment-experienced group was 100% (enclosure 9).

The frequency of individual PR relevant mutations known to be associated with PR resistance found in samples from DMP 266-024 efavirenz/nelfinavir treatment failures is shown

in enclosure 9. At baseline a number of subjects harbored PR inhibitor-specific polymorphic mutations, however, none appeared to have the nelfinavir-specific D30N mutation prior to the initiation of efavirenz/nelfinavir therapy. Of the 17 subjects selected for genotypic analysis PR-relevant mutations at amino acid positions LP1'F, 10, 30, 46, 60, 63, 71, 77, 82, 88, and 90 were detected in NRTI-experienced subjects with a minimum frequency of 9% per site. Of the 17 subjects selected for genotypic analysis PR mutations at amino acid positions LP1'F, 30, 36, 63, 71, 77, 88, and 90 were detected in NRTI-naive subjects with a minimum frequency of 9% per site. In patients with "significant" rebounds in viral load while the efavirenz/nelfinavir regimen 7 (41.2%) had a mutation at PR amino acid position 30. (enclosure 9).

The objectives of this study were to determine the spectrum of HIV-1 mutations associated with "resistance" to efavirenz/nelfinavir combination therapy. The RT mutation at position 103 was the predominant NNRTI mutation detected in virologic failures and the PR mutation at position 30 was the predominant PI mutation detected. The relative contributions of these mutations to virologic failure is unclear. However, the data suggest that even in the presence of additional non-RT targeted therapy efavirenz still selects for the 103 mutation and that this mutation is predominant among treatment failures.

Microbiology Labeling Proposed by the Sponsor.

MICROBIOLOGY

FDA Proposed Microbiology Labeling (29 October 1999)

MICROBIOLOGY:

Mechanism of Action: Efavirenz is a non-nucleoside reverse transcriptase (RT) inhibitor of human immunodeficiency virus type 1 (HIV-1). Efavirenz activity is mediated predominantly by non-competitive inhibition of HIV-1 RT. HIV-2 RT and human cellular DNA polymerases alpha, beta, gamma, and delta are not inhibited by efavirenz.

***In Vitro* HIV Susceptibility:** The clinical significance of *in vitro* susceptibility of HIV-1 to efavirenz has not been established. The *in vitro* antiviral activity of efavirenz was assessed in lymphoblastoid cell lines, peripheral blood mononuclear cells (PBMCs) and macrophage/monocyte cultures. The 90-95% inhibitory concentration (IC₉₀₋₉₅) of efavirenz for wild type laboratory adapted strains and clinical isolates ranged from 1.7 to 25 nM. Efavirenz demonstrated synergistic activity against HIV-1 in cell culture when combined with zidovudine (ZDV), didanosine, or indinavir (IDV).

Resistance: HIV-1 isolates with reduced susceptibility to efavirenz (>380-fold increase in IC₉₀) compared to baseline can emerge *in vitro*. Phenotypic (N=26) changes in evaluable HIV-1 isolates and genotypic (N=104) changes in plasma virus from selected patients treated with efavirenz in combination with IDV, or with ZDV plus lamivudine were monitored. One or more RT mutations at amino acid positions 98, 100, 101, 103, 108, 188, 190 and 225, were observed in 102 of 104 patients with a frequency of at least 9% compared to baseline. The mutation at RT amino acid position 103 (lysine to asparagine) was the most frequently observed (90%).¹

¹ A mean loss in susceptibility (IC₉₀) to efavirenz of 47-fold was observed in 26 clinical isolates. Five clinical isolates were evaluated for both genotypic and phenotypic changes from baseline. Decreases in efavirenz susceptibility (range

from 9 to >312-fold increase in IC₉₀) were observed for these isolates *in vitro* compared to baseline. All 5 isolates possessed at least one of the efavirenz-associated RT mutations. □

Cross-resistance: Rapid emergence of HIV-1 strains that are cross-resistant to non-nucleoside RT inhibitors has been observed *in vitro*. Thirteen clinical isolates previously characterized as efavirenz-resistant were also phenotypically resistant to nevirapine and delavirdine *in vitro* compared to baseline. Clinically derived ZDV-resistant HIV-1 isolates tested *in vitro* retained susceptibility to efavirenz. Cross-resistance between efavirenz and HIV protease inhibitors is unlikely because of the different enzyme targets involved.

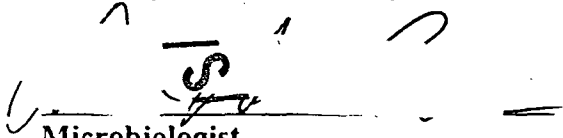
CONCLUSIONS

The data presented in the five study reports further supports the hypothesis that efavirenz selects for specific RT mutations and that these mutations, one in particular at amino acid position 103, are associated with virologic failure in patients receiving efavirenz in combination with other NRTIs or with the protease inhibitor nelfinavir. To date the sponsor has evaluated virus isolates from 104 efavirenz/RTI treated subjects and 17 efavirenz/nelfinavir treated subjects.

It should be noted here that the sponsor did not randomly select patients for sequence evaluation from the various study cohorts. Instead the sponsor selected those patients that were identified as having experienced a "significant viral rebound" in viral load, hence they referred to those persons as treatment failures. The sponsor did not evaluate "treatment responders" in a similar fashion. It is understood that "treatment responders" are likely to have HIV RNA levels in the blood that are below the level needed to successfully amplify genetic material for cloning and sequencing analysis, but that should not be considered a sufficient excuse for assuming that the responders do not have the same RT mutations as the treatment failures. Therefore, reporting the prevalence of RT mutations associated with efavirenz therapy in treatment failures should not allow the reader to assume the converse, that the absence of mutations should be the expected observation in treatment responders.

RECOMMENDATIONS:

With respect to microbiology, NDA 20-972 (SUSTIVA) is approved.


Microbiologist