CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER 20-972

MICROBIOLOGY REVIEW

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

NDA#: 20-972
IND#: 49,465

REVIEWER: Lauren Iacono-Connors
CORRESPONDENCE DATE: 06/11/98
CDER RECEIPT DATE: 06/12/98
REVIEW ASSIGN DATE : 06/15/98
REVIEW COMPLETE DATE: 08/27/98

SPONSOR: DuPont Merck Pharmaceutical Company
DuPont Merck Plaza, MR2110
Centre Road
Wilmington, DE 19880-0721

SUBMISSIONS REVIEWED: NDA 20-972 (N-000)
IND 49,465 (N-240, N-268)

DRUG CATEGORY: Antiviral

INDICATION: For the treatment of HIV-1

DOSAGE FORM: Capsule (50 mg, 100 mg, and 200 mg)

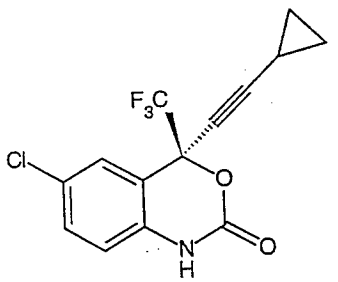
PRODUCT NAMES:

a. **PROPRIETARY:** SUSTIVA™

b. **NONPROPRIETARY:** Efavirenz

c. **CHEMICAL:** (S)-6-Chloro-4-(cyclopropylethynyl)-1,4-dihydro-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one

STRUCTURAL FORMULA:



SUPPORTING DOCUMENTS: IND 49,465

BACKGROUND

Introduction

Efavirenz is a non-nucleoside benzoxazinone that has been demonstrated to inhibit HIV-1 reverse transcriptase (RT) activity and hence HIV-1 replication. Efavirenz is specific for HIV-1 RT, and does not inhibit other retroviral RTs or human DNA polymerases. The sponsor seeks marketing approval for efavirenz (SUSTIVA), in combination with other antiretroviral agents, for the reduction of HIV-1 RNA in infected individuals.

HIV-1 Biology

The human immunodeficiency virus-1 (HIV-1), the etiologic agent of acquired immune deficiency syndrome (AIDS) in humans, is a member of the virus family *Retroviridae*. HIV-1 is an enveloped, single-stranded RNA virus. Infectious virions contain two positive polarity RNA molecules which, upon infection of cells, are transcribed into a double-stranded DNA molecule by the HIV-1 RT. The resultant viral cDNA molecule is subsequently integrated into the host cell genomic DNA.

HIV has been detected in a variety of human tissues, and can infect an even greater variety of human cells in culture. In particular, CD4+ helper T lymphocytes appear to be a major target for HIV infection and are most efficient at supporting viral replication. The major cell surface receptor for HIV is the CD4 molecule. The following is a summary of the steps in the life-cycle of an HIV infection in a competent cell. The virus surface glycoprotein (gp120) attaches to a host-cell surface receptor and likely undergoes a conformational shift and subsequent displacement and/or proteolytic cleavage of gp120 such that a second HIV structural protein, the transmembrane glycoprotein (gp41), facilitates the fusion of the HIV envelope with the plasma cell membrane in a pH independent fashion. The HIV core particle enters the cell cytoplasm and cDNA is directly synthesized by RT using the genomic viral RNA as template. Viral cDNA is then transported into the nucleus, and integrated into chromosomal DNA material. Viral messenger and genomic RNAs are transcribed from the integrated proviral DNA and shuttled back to the cytoplasm where virus-specific translation, virus packaging, budding of the viral capsid through the cell membrane and viral protease (PR)-dependent virus maturation completes the cycle.

Antiviral Therapy Against HIV-1

Clinical treatment of HIV is designed to target certain critical events in the virus infection life-cycle. Therapies under investigation include approaches employing antiviral agents (i.e.; RT inhibitors, viral protease inhibitors (PRIs), and viral integrase inhibitors), virus entry inhibitors (i.e.; neutralizing polyclonal and/monoclonal antibodies), and a broad spectrum of immune-based therapies. Determination of treatment efficacy is assessed primarily by clinical status of the patient, CD4+ cell counts, and plasma/serum associated HIV RNA levels.

Nucleoside analogue inhibitors of HIV-1 RT represent a major class of anti-HIV therapies. Once phosphorylated by cellular enzymes, nucleoside analogues compete for incorporation into viral DNA (as catalyzed by RT) and thus often act as chain terminators for DNA synthesis. One caveat in nucleoside analogue antiviral therapy has been the emergence of drug-resistant HIV strains. The phenomena of nucleoside analogue-resistant HIV development *in vitro* and *in vivo* has been well characterized for a number of FDA-approved nucleoside analogues, however, the clinical utility of the knowledge of the phenomena has not been well defined. Currently there are two hypotheses which describe the mechanism for the development of resistant HIV under drug pressure. It is thought that a treatment naive HIV(+) individual has a heterogenous population of circulating HIV. Once treatment is initiated, de novo virus-replication cycles are down modulated due, at least in part, to RT inhibition. Over time, genetically altered HIV emerges as a result of low fidelity HIV-RT replication, and/or the selection pressure and passive expansion of agent-resistant HIV present in the heterogenous virus population. The rate at which this occurs clinically is likely to be dependent upon a variety of factors, including the therapeutic agent, dose and schedule, length of therapy, disease stage at treatment initiation, viral load before and during therapy, and phenotypic/genotypic HIV characteristics present before and during therapy.

In contrast to nucleoside analogues, non-nucleoside RT inhibitors (NNRTIs) (including efavirenz) are designed to inhibit one of the three enzymatic functions of RT, either the RNA-dependent DNA polymerase activity, the RNase H activity, or DNA-dependent DNA polymerase activity.

SUMMARY

Mechanism of Action

Efavirenz is a non-nucleoside, predominantly non-competitive, inhibitor of the HIV-1 RT activity. Both the RNA-dependent and DNA dependent DNA polymerase activities of recombinant RT are inhibited by efavirenz. Using four different combinations of template-primers in polymerization assays it was demonstrated that inhibition was template dependent; with IC_{50}s ranging from 1.1 (± 0.24) nM to 22 (± 1.3) nM (Enclosure 1). Inhibition of purified recombinant RT mutants were also evaluated. The RT mutations were selected for evaluation based on their known *in vitro* effects on non-nucleoside RT inhibitor class agents. The results demonstrate that these RT mutants are less inhibited by efavirenz than the wild-type RT, and that the inhibition, as measured by a loss in agent susceptibility (IC_{50}), was from 3- to 28-fold less efficient depending upon the mutations tested (Enclosure 1).

Efavirenz is specific for HIV-1 RT. Activities of human DNA polymerases alpha, beta, and gamma are not affected by efavirenz (IC_{50}s >300 uM). The IC_{50} of human DNA polymerase delta was 260 (±19) μM. HIV-2 RT is inhibited by efavirenz with an IC_{50} of 81 μM (Enclosure 2). Finally, efavirenz (concentrations up to 300 uM) did not inhibit the activities of molony murine leukemia virus RT, avian myeloblastosis virus RT, *E. coli* RNA polymerase, or the klenow fragment (Enclosure 2).

Inhibition of recombinant HIV-1 RT activity by efavirenz is largely non-competitive with nucleoside triphosphates or template/primer. Steady state kinetic studies utilizing a homopolymer RNA template (rC) and dGTP determined that the K_i for efavirenz was 2.93 (±0.17) nM (Enclosure 3). Inhibition of recombinant RT mutants were also evaluated. Ki values for single-site and double-site RT mutants ranged from 2.97 (±0.46) to 17.60 (±2.31) nM, and 26.05 (±1.28) to 56.50 (±27.60) nM, respectively (Enclosure 3). The RT mutations were selected based on their known *in vitro* effects on non-nucleoside RT inhibitor class agents. Values for both Ki and Kii were generated. Two- to three-fold differences between the Ki and Kii values for some of the mutant enzymes suggests a small degree of competitive inhibition for the substrate (Enclosure 3).

Antiviral Activity of Efavirenz *In Vitro*

The effect of efavirenz on HIV-1 replication in cell culture has been assessed in several studies utilizing various methodologies. Activity was assessed in T cell lines (lymphoblastoid), human peripheral blood mononuclear cells (PBMCs), and human macrophage/monocyte cultures. Methodology used included production of p24 antigen, production of viral RNA, or reduction in yield of infectious virus particles. The data from these studies are summarized and referenced in enclosure 4. $IC_{90/95}$ values ranged from 1.7 nM to 25 nM, depending upon the strain of HIV-1 (laboratory strains or clinical isolates) and methodology used, not including "L-697,661-resistant isolates." Efavirenz was also active against HIV-1 zidovudine (ZDV)-resistant clinical isolates. Efavirenz activity ($IC_{90/95}$) against HIV-1 ZDV-resistant clinical isolates was from 2.8 nM to 25 nM.

The concentration of efavirenz needed to inhibit (IC_{90}) HIV-1_{RF} replication in MT-2 cells was 1.7 (±0.5) nM. The concentration of efavirenz needed to reduce MT-2 cell viability by 50% (TC_{50}) was 18.9 (±5.5) μM. Therefore, demonstrating an *in vitro* therapeutic index of >11,000 in this cell system (Enclosure 5).

Activity of Efavirenz in the Presence of Human Plasma Proteins

Many antiviral therapies have been shown to be predominantly protein bound in human serum. Efavirenz has been reported to be >99% protein-bound in normal human plasma. In order to evaluate the effect of human plasma protein binding on the antiviral activity of efavirenz human serum albumin (HSA) at 45 mg/mL and α-1-acid glycoprotein (AAG) at 1 mg/mL were added to MT-2 cell growth media. The concentration of HSA and AAG in normal human serum is 45 mg/mL and 0.4-0.7 mg/mL, respectively. Under these conditions the antiviral potency of efavirenz was assessed by a viral RNA production assay using the HIV-1_{RF} strain to acutely infect MT-2 cells. IC_{90} results indicate that efavirenz antiviral activity was reduced approximately 16-fold in the presence of both HSA and AAG (enclosure 6). These data demonstrate a decrease in antiviral activity due to the presence of certain plasma proteins *in vitro*, and suggest that the absolute efavirenz antiviral activity will likely be effected *in vivo*. However, the degree to which this may occur and the clinical ramifications of such events are not predictable.

The effect of HSA and AAG on the antiviral activity (IC_{90}) of other NNRTIs was assessed in a similar experiment as that described above. The IC_{90} shift for nevirapine and delavirdine was 2-fold and 38-fold, respectively (Enclosure 7). The activity shift observed for two NNRTIs under investigation, [redacted] (b) (4) respectively.

Antiviral Activity of Efavirenz in Combination With Other Anti-HIV Agents

The effect of efavirenz, in combination with ZDV, didanosine (ddI), or indinavir (IND) (b) (4) on HIV-1 replication *in vitro* was assessed in MT-4 cells using laboratory variants of HIV-1. Antiviral activity *in vitro* was assessed by measurement of p24 antigen levels produced in HIV-1 infected cells after 4 days in culture, the latter 3 days under drug pressure. Combination drug interactions for activity were analyzed by the method of Chou and Talalay (1984) and reported as combination index (CI) values. A graphic display of the results of these analyses are shown in enclosure 8, however, the actual CI values were not provided. In this system, values <1 indicate synergy; =1 indicate additive effects; and >1 indicate antagonism. Efavirenz was reported to have a synergistic activity relationship with ZDV, ddI, and IDV.

In Vitro Antiviral Activity Against Recombinant Mutants of an HIV-1 Laboratory Strain

Activity of efavirenz was assessed against a panel of recombinant, site-directed HIV-1 mutants of strain NL4-3, RF, and HXB2. These mutant viruses contain amino acid substitutions in the RT gene region that have been previously reported to correlate with a decrease in susceptibility to other agents in the NNRTI drug class. A p24 reduction assay in MT-4 cells was employed to evaluate changes in efavirenz activity *in vitro* ($IC_{90/95}$). MT-4 cells were infected with matched-pair HIV-1 (wild-type and corresponding mutants). The results of this study are presented enclosure 9. The efavirenz $IC_{90/95}$s for the single NL4-3 mutants were from 1.9 nM to 120 nM, and depended upon the mutation and the strain background. The fold-change $IC_{90/95}$ from that of the respective backgrounds was from 0.6 to 50 (enclosure 9). The fold-changes observed in HIV-1 mutants with multiple amino acid mutations in the RT was as high as >1000-fold loss in susceptibility 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*.

Development and Analysis of Efavirenz-Resistant HIV-1 Variants *In Vitro*

The rapid emergence of NNRTI-resistant HIV-1 isolates *in vitro* has been well established. In an effort to address whether efavirenz can select for HIV-1 in cell culture that has lost a measurable degree ($IC_{50/90/95}$) of efavirenz susceptibility the sponsor conducted several studies. The following is a brief description of the virologic studies which defined the potential for development of an efavirenz-resistant phenotype in HIV-1 when studied *in vitro*.

In the first study the ramifications on the virus population of a constant concentration of efavirenz pressure was evaluated. $HIV\text{-}1_{RF}$ was used to infect PBMCs at an MOI of 0.001.

Infection was allowed to establish for one week. At that time efavirenz was introduced into the cell culture medium at a concentration of 3.2 nM, 9.5 nM, 95 nM, or 950 nM. The cultures were incubated for 10 weeks under constant efavirenz pressure at the identified concentrations. Cultures were maintained with fresh medium and compound, and PBMCs twice a week or weekly, respectively. At week 10 compound was removed and the cultures were refed and allowed to propagate efavirenz-free for an additional 3 weeks. Efavirenz negative cultures were produced for control HIV-1_{RF} material. HIV-1_{RF} specific to each culture was evaluated at selected times for sensitivity to efavirenz *in vitro* by viral p24 antigen production in fresh PBMCs. The results of these analyses are provided in enclosure 10. It is assumed that virus materials from the supernatants were expanded, titered and evaluated for infectivity prior to testing for their drug-susceptibility phenotype. The data contained in enclosure 10 reveal that efavirenz 3.2 nM (approximately one IC_{95}: reference enclosure 4) selection produced a virus population that harbored a 9-fold shift in efavirenz susceptibility as early as week four. That virus population retained that phenotype through week 12 with an IC_{90} increase of 22-fold as compared to wild-type. The selectivity of efavirenz for phenotypically altered HIV-1 *in vitro* appeared to be dose-dependent in these studies. Efavirenz at 9.5 nM selected for virus isolates with a greater decrease in susceptibility at the same time point; 30-fold decrease in efavirenz-susceptibility at week four. Efavirenz selection for HIV-1 variants at 95 nM did not differ from the those found in the 9.5 nM cultured virus populations as studied here (enclosure 10); suggesting that the maximally selective, but non-virucidal, concentration of efavirenz was between 9.5 and 95 nM in these studies. At an efavirenz concentration of 950 nM no HIV-1 variants were detected at any time point. Limited data on genetic RT information were provided, but in those data included in enclosure 10 a single mutant variant was reported in the week four 95 nM population with a genotype of L100I (leucine to isoleucine at amino acid 100 in the RT). In the week six and twelve 95 nM virus populations two mutations were observed (L100I, V108I). Sequencing templates were PCR amplified, and the technology was population-based; for an RT range of amino acid 1 through 250.

In the second study the ramifications on the cultured virus population of a step-wise increasing concentration of efavirenz pressure were evaluated. HIV-1_{RF} was used to infect MT-2 cells at an MOI of 0.001 and efavirenz was added at a concentration beginning at 0.32 nM. Virus-containing undiluted culture supernatants were passaged to fresh MT-2 cells every 3 to 4 days or when CPE was evident. The sponsor has provided information on HIV variants passaged up to 24 times with a maximum efavirenz concentration of 317 nM. At each passage virus was isolated and evaluated for efavirenz sensitivity using a virus yield reduction assay as compared to untreated controls. Selected results are provided in enclosure 11. By passage 12 the variant HIV-1_{RF} population was approximately 6-fold less susceptible to efavirenz than that of parent virus. The passage 23 (efavirenz at 158 nM) virus population was > 380-fold less susceptible to efavirenz than parent virus, and harbored three mutations (L100I/V179aspartic acid [D]/tyrosine[Y]181cysteine[C]). These data demonstrate that it is biologically possible for a laboratory strain to develop a measurable degree of efavirenz resistance. The clinical ramifications of these data are not predictable at this time.

Genotypic Analysis of Laboratory-Derived Efavirenz-Resistant HIV-1 Isolates

Genotypic analysis of the RT genes from representative virus clones from each of the efavirenz-resistant virus populations described above were performed. As stated, or presented, above a prominent nucleotide substitution mutation which should alter the deduced RT amino acid sequence at position L100I or V179D was observed as a single-mutation in an HIV-1_{RF} background. One double-mutant genotype was identified, L100I/V108I, and one triple-mutant genotype was identified, L100I/V179D/Y181C.

To address the question of whether a causative relationship exists between these genetic mutations and a phenotypically expressed efavirenz resistance *in vitro*, site-directed mutagenesis was used to construct infectious clone HIV-1 variants. Activity of efavirenz was assessed against a panel of recombinant, site-directed HIV-1 mutants of strain NL4-3, and RF. A p24 reduction assay in MT-4 cells was employed to evaluate changes in efavirenz activity *in vitro* ($IC_{90/95}$). The results of these experiments are contained in a table displayed in enclosure 9. An HIV-1_{NL4-3} recombinant with the L100I mutation had an approximately 20-fold decrease in susceptibility ($IC_{90/95}$). Conversely, an HIV-1_{NL4-3} recombinant with the V179D mutation had no detectable change in susceptibility as compared to wild-type *in vitro*. It appears that the double-mutant observed in the selection studies described above was not constructed for this study. Finally, a triple mutant, L100I/V179D/Y181C, selected for in the studies described above, was constructed into an HIV-1_{RF} background. This recombinant virus had a substantial shift in efavirenz susceptibility *in vitro*, harboring an IC_{90} increase of approximately 1000-fold compared to wild-type parent (Enclosure 9).

These data demonstrate that certain point mutations in the HIV-1 RT can cause a shift in efavirenz susceptibility and that these changes are likely involved, the degree of which is unknown, in the changes in susceptibility observed in the efavirenz-selected isolates produced *in vitro*.

Cross-Resistance Analysis of Laboratory-Derived Efavirenz-Resistant HIV-1 Isolates

The potential for HIV-1 cross-resistance between non-nucleoside RT inhibitors has been evaluated in one *in vitro* derived efavirenz-resistant laboratory isolate. A recombinant RT enzyme which contained the point mutation L100I was cloned into an HIV-1_{NL4-3} infectious clone. The HIV-1 variant was tested for NNRTI drug susceptibility *in vitro* using a p24 reduction assay in acutely infected MT-4 cells. The single mutation conferred some loss of susceptibility to each of the NNRTIs tested (Enclosure 12); efavirenz with a 22-fold shift in IC_{90}, nevirapine with a 4.8-fold shift in IC_{90}, and delavirdine with a 34-fold shift in IC_{90}.

A variety of other RT recombinants were evaluated for the relationship between a RT mutation(s) and the potential causative effect it has on the activity of one of the three NNRTIs tested here. Not surprisingly, phenotypic analyses of these cloned variants demonstrated that certain RT mutations can confer cross-resistance, as measured *in vitro*, to all three specific NNRTIs tested (enclosure12). Genotypic analysis of the variants and the correlative or causative

relationships between those RT point mutations and the degree of phenotypic resistance to a given alternate NNRTI appear to be somewhat complex. Additional studies are needed in order to unequivocally define the impact of one or more RT mutations on drug susceptibility. The simplest interpretations of these studies are that single agent selection using efavirenz and a HIV-1 wild type isolate can produce an efavirenz-resistant variant, the variant could possess some degree of cross-resistance to other NNRTIs, and the biological relevance, other than the fact that variant selection is biologically possible, is unclear.

Phenotypic and genotypic analysis of HIV-1 isolates from patients during therapy with efavirenz

The sponsor has conducted three studies on patient specimen analyses for the presence of treatment associated RT-specific mutations, and the impact of those mutations on susceptibility to efavirenz, and other NNRTIs, *in vitro*. Study I assessed the RT and viral protease genotype in selected patients from clinical phase II studies DMP 266-003 and -004. Efavirenz- treated patients received either efavirenz monotherapy or efavirenz in combination with other antiretrovirals (no other NNRTIs). Study II assessed the *in vitro* drug susceptibility of selected clinical isolates from the efavirenz- treated cohorts. The sponsor's intention was to determine whether viruses with reduced susceptibility to efavirenz emerged in patients experiencing "viral rebound" while on efavirenz therapy. Study III was designed to assess the potential for selection of "resistant virus" *in vivo* during efavirenz therapy and to compare the frequency of the mutation event with that observed in non-efavirenz treatment cohorts. A brief discussion of the salient observations from each study is provided below.

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. Any additional information contained in these study reports that attempts to draw a relationship between the appearance of these mutations as they relate to a clinical event will not be discussed in this review.

STUDY I:

Virus isolates (plasma associated) have been evaluated in patients participating in phase II clinical trials (DMP 266-003 and -004); efavirenz monotherapy or efavirenz in combination with other antiretrovirals (not including other NNRTIs). The presence and frequency of RT-specific mutations (amino acid positions 1-229) were evaluated in virus isolates from the efavirenz

treatment cohorts. Of the 86 patients selected for genotypic analyses 62 (72%) had evaluable virus material from both a baseline and at least one co/post-efavirenz treatment time point, thus, matched pairs. At at least one time point after efavirenz treatment initiation all 62 patients harbored HIV material with one or more novel, RT-specific mutation as compared to a population baseline (Enclosure 13 and 14). RT mutations at amino acid positions 100, 101, 103, 108, 190, and 225 were detected in patients from both clinical studies with a study-specific minimum frequency of 10% per site but was as high as 100% at the predominant 103 site (Enclosure 13). The templates used for sequence determination were of clonal populations. If study patients are pooled the mutation frequency observed at RT site 103 was 58/62 (94%). Eleven additional RT amino acid positions, listed in enclosure 13, were also mutable under efavirenz pressure but with a frequency typically less than 10% depending upon the study. All of the mutations listed in enclosure 13 represent NNRTI-induced genotypic changes that have been associated with NNRTI resistance and have been previously reported (Schinazi, 1996; Pelemans, 1997). These results suggest that efavirenz can select for HIV-1 RT-specific amino acid substitutions in HIV-1 infected humans.

The sponsor used a sequence protocol that included a cloning step of the relevant viral genetic elements per isolate prior to sequence determination. Therefore, reporting a mutation profile as linked mutations per specimen was possible. The summary of the frequency of multiple mutations was provided. The data presented revealed that only the predominant K103N mutation is seen as both a single-site mutation (unlinked mutation) and in combination with other efavirenz-specific mutations (linked mutations) (Enclosure 15). The relevance of this observation is not clear at this time.

STUDY II

The sponsor assessed the *in vitro* drug susceptibility of selected clinical isolates from efavirenz-treated cohorts. The sponsor's intention was to determine whether viruses with reduced susceptibility to efavirenz emerged in patients experiencing "viral rebound" while on efavirenz therapy. Virus isolates were obtained from PBMCs of actively treated patients in study DMP 266-003 (n=16) and DMP 266-004 (n=10) where possible who were identified as those with viral rebound. Virus isolates were then tested for susceptibility to efavirenz and other selected antiretrovirals using a p24 measurement assay in PBMCs (ACTG consensus protocol).

Baseline $IC_{50/90}$ values for efavirenz, ddC, and IDV were determined for 23 patient isolates intended to be representative of all patients at baseline. The mean $IC_{50/90}$ values are provided in enclosure 16. Sixteen patient's isolates were evaluated for susceptibility $IC_{50/90}$ to the same three drugs at some point after therapy initiation and after a virus rebound was identified. The mean efavirenz $IC_{50/90}$ for representative baseline isolates was 0.7 (±0.9) and 1.8 nM (± 2.1), respectively (Enclosure 16). The mean efavirenz $IC_{50/90}$ for representative efavirenz-treated isolates was 50 (±45) and 70 (±44) nM, respectively (Enclosure 16). Thus, a shift in IC_{90} of approximately 40-fold was reported. Again, the sponsor suggests that viral rebound while on efavirenz active therapy is related to these *in vitro* detected decreases in efavirenz susceptibility. Since virus isolates from efavirenz treatment responders are not available for the same analysis it

is difficult to assume that they in fact would have retained full susceptibility to efavirenz if tested *in vitro*. Since these data are not available it cannot be logically deduced or assumed that the patient specific specimen's *in vitro* measured susceptibility profiles are or are not related to viral load responses. The mean ddC $IC_{50/90}$ for representative efavirenz-treated isolates was 26 (±26) and 130 (±140) nM, respectively (Enclosure 16). The mean IDV $IC_{50/90}$ for representative efavirenz-treated isolates was 160 (±240) and 430 (±430) nM, respectively (Enclosure 16). Thus, a shift in ddC and IDV IC_{90} was observed to be approximately 2-fold and 12-fold, respectively. It should be noted that the degree of shift in IDV susceptibility observed between the efavirenz treatment cohort and the placebo cohort (IDV) appeared to be similar (Enclosure 16).

Of the 16 samples from efavirenz treated patients that were analyzed for susceptibility to efavirenz 8 had actual matching baseline isolates. Therefore, both genotyping and *in vitro* susceptibility testing were attempted for all 8 matched pair isolates. Sequence information was obtainable for 5 of the 8 patient isolate matched pairs. The individual patient-specific sample analyses for both genotyping and phenotyping are presented in enclosure 17. These data show the following. First, only 6 of the 8 patient isolates (patients identified as virologic rebounders) showed a decrease in susceptibility to efavirenz *in vitro* (Enclosure 17). Of the two patients with "fully susceptible" HIV neither had RT complimentary sequence data generated. Second, the range of decrease of efavirenz susceptibility as measured *in vitro* compared to specific baseline values seen in the 6 patient's isolates was from >9-fold to >312-fold (Enclosure 17). Third, of those 6 matched pair isolates 5 had the RT sequence analyzed for treatment-associated (efavirenz) mutations. The genotyping results are also presented in enclosure 17 and show that in all 5 patient's treatment isolates at least one of the RT mutations (amino acid site 103 and 108) previously shown to be prevalent in efavirenz treated patient isolates was detected.

A similar analysis of patient isolates from study DMP 266-004 was conducted as that described above for study DMP 266-003. Briefly, baseline $IC_{50/90}$ values for efavirenz, ZDV, and epivir were determined for 13 patient's isolates intended to be representative of all patients at baseline. The mean $IC_{50/90}$ values are provided in enclosure 18. Ten patient's isolates were evaluated for susceptibility, $IC_{50/90}$, to ZDV, epivir, and efavirenz at some point after therapy initiation and after a virus rebound was identified. The mean efavirenz $IC_{50/90}$ for representative baseline isolates was 0.6 (±0.5) and 1.3 (± 1.1) nM, respectively (Enclosure 18). The mean efavirenz $IC_{50/90}$ for representative efavirenz-treated isolates was 80 (±42) and 80 nM (±42), respectively (Enclosure 18). It seems odd that both the IC_{50} and IC_{90} for efavirenz-treated patient specimens were identical. However, a mean shift in IC_{90} of approximately 62-fold was reported. Again, the sponsor suggests that viral rebound while on efavirenz active therapy is related to these *in vitro* detected decreases in efavirenz susceptibility. As discussed above, since virus isolates from efavirenz treatment responders are not available for the same analysis it is difficult to assume that they in fact would have retained full susceptibility to efavirenz if tested *in vitro*. Since these data are not available it cannot be logically deduced or assumed that the patient specific specimen's *in vitro* measured susceptibility profiles are or are not related to viral load responses. Of the 10 samples from efavirenz treated patients that were analyzed for susceptibility to efavirenz 5 had actual matching baseline isolates. Therefore, *in vitro* susceptibility testing was attempted for all 5 matched pair isolates. The results of this analysis are shown in enclosure 19. Basically,

all 5 co/post-efavirenz treated virus isolates had a shift in efavirenz susceptibility compared to baseline that ranged from >26-fold to >312-fold, *in vitro*. The clinical significance of this observation is not known. The genotype of these 5 virus isolates, at baseline or co/post- efavirenz treatment, has not been determined.

In order to assess whether efavirenz-experienced clinical isolates were fully susceptible to the other well studied NNRTIs in this class of drugs, nevirapine and delavirdine, the sponsor determined the $IC_{50/90}$ values for efavirenz, nevirapine and delavirdine using clinical isolates from study DMP266-003/004. Again, baseline $IC_{50/90}$ values for efavirenz, nevirapine and delavirdine were determined for 11 patient isolates intended to be representative of all patients at baseline. The mean $IC_{50/90}$ values are provided in enclosure 20. The *in vitro* susceptibility of post viral load rebound isolates (n=16) from the two studies were determined (enclosure 20). A mean IC_{90} decrease in efavirenz susceptibility *in vitro* ranged from 216-fold to 312-fold. A mean IC_{90} decrease in nevirapine susceptibility *in vitro* ranged from 42-fold to 67-fold. A mean IC_{90} decrease in delavirdine susceptibility *in vitro* ranged from 70-fold to 127-fold. In enclosure 20 the sponsor presented the evaluated isolates as those that were simply acquired from post-treatment viral load rebounders, n=16, and as only those rebounder isolates that were found to be "efavirenz-resistant," n=13. It is assumed that efavirenz-resistant isolates were defined here based on *in vitro* susceptibility analyses. Therefore, of the 16 viral rebound patient isolates presented 13 actually had a measurable shift in *in vitro* susceptibility to efavirenz, suggesting that a viral rebound in efavirenz-treated patients is not uniformly dependent upon a decrease in *in vitro* susceptibility. The clinical significance of this observation is not known.

STUDY III

Virus isolates (plasma associated) have been evaluated in patients participating in phase II clinical trials (DMP 266-003 and -004); efavirenz monotherapy or efavirenz in combination with other antiretrovirals (not including other NNRTIs). Similar to STUDY I, presented above, the presence and frequency of RT-specific mutations (amino acid positions 1-229) was evaluated in the efavirenz treatment cohorts.

Twenty nine patients were analyzed for genotypic analyses of the RT gene after reaching viral loads in excess of 1000 copies/mL; Study DMP 266-003. However, genotyping in these studies was conducted using population-based sequencing of nucleic-acid-amplified virus material directly from patient plasma. Although this method of analysis of frequency of RT-specific mutations associated with efavirenz therapy is technically different than that used above the sponsor reports that the basic result was similar but not identical to that reported previously in study report PRR97-20. Essentially, RT mutations at amino acid positions 101, 103, and 225 were detected in patients with a minimum frequency of 13% per site but was as high as 86% at the predominant 103 site (Enclosure 21). Not seen in these analyses were >10% mutation frequency at the 100 and 108 RT amino acid positions. Of the 29 patients evaluated for RT-specific, efavirenz treatment-associated, mutations 27/29 (93%) had novel efavirenz-associated RT mutations. Therefore, these data demonstrate that the method of generating sequence information, clonal- or population-based, did not alter the initial observation, that treatment of

patients with efavirenz can result in the majority (93 to 100%) of evaluable patient isolates retaining one or more RT-specific, novel amino acid substitution.

Performance Characteristics for the [redacted] (b) (4) Test

There are virologic endpoints (HIV-1 RNA levels) designed in the pivotal trials, and the method for measurement of HIV-1 RNA levels in study participants was an [redacted] (b) (4) [redacted] (b) (4) an experimental/investigational assay. As requested by the Division the sponsor has provided an assay performance package that should be designed to address the performance characteristics of this assay that are relevant to study design. After a general review of the performance package, and in conjunction with the statistical and clinical reviewer, it has been determined that the [redacted] (b) (4) experimental assay performance characteristics have not been sufficiently demonstrated in order to support use of data generated by this method to further demonstrate the efficacy of DMP 266 in the pivotal studies.

With respect to assay limit of detection the information provided was based on assay results of the well characterized ACTG VQA standard (Layne et al., 1992; Lin et al., 1994). However, no analyses were subsequently conducted on independently quantitated clinical isolates for performance confirmation. Precision studies were conducted with the VQA standard, at both a low positive (250 copies/mL) and a high positive (25,000 copies/mL) concentration. In addition, an undefined (origin and characterization unclear) "clinical pool" with a proposed HIV RNA concentration of 500 and 25,000 copies of RNA copies/mL were also used to demonstrate assay precision. First, it is not clear how the HIV RNA concentration of the "clinical pool" was determined, and second, the low positives are not representative of the HIV RNA concentrations intended to be used as the qualitative limit (surrogate endpoint) of the [redacted] (b) (4) assay. It is expected that the precision studies would encompass those HIV RNA concentrations that are intended as treatment efficacy discriminators (below 50 HIV RNA copies/mL) in the DMP 266 pivotal studies. In addition to these study design limitations the statistician indicated that the number of samples tested in each of the performance characteristics studies was not adequate. Finally, the range of specificity, the assays reported observation from 500 seronegative blood donors was not provided. This information is needed to establish that the 95% confidence interval of observations around a negative sample and that around the qualitative limit (range of sensitivity) are distinguishable.

It is recommended to the clinical reviewer that a list of regulatory considerations for experimental HIV RNA measurement assays be forwarded to the sponsor if the data generated using the experimental [redacted] (b) (4) assay are required to achieve marketing approval for Sustiva. Otherwise, it is recommended to the clinical reviewer that the division follow-up with the sponsor on the issue of "[redacted] (b) (4) RNA data," reference the DMP 266 controlled studies, after the initial NDA review is completed.

The following list of HIV RNA measurement assay performance characteristics of interest to the Division, provided below, could be provided to the sponsor, at the discretion of the medical Team Leader, to assist the sponsor in preparing nucleic acid-based performance characteristics reports for future submissions.

Regulatory Considerations for Experimental HIV RNA Measurement Assays:

When experimental/investigational HIV RNA measurement assays are used to support clinical trials sufficient assay performance characteristics data should be provided such that it permits an independent evaluation of an assay's limitations. Review of assay performance by the division will focus on the interpretability of data generated by the assay in the particular clinical trials. Thus, the review of assay performance data does not imply that the given assay is validated or FDA-approved for patient prognosis and/or monitoring. Further, this review does not imply that the given assay is automatically acceptable for future clinical trials.

a. The assay that is used in the clinical trial must be identical to the assay that is used to assess the performance characteristics. If the assay is used both quantitatively and qualitatively in the clinical trials then both qualitative and quantitative performance information should be provided.

b. Assay design rationale, essential methodology, and performance characteristics must be provided to the Division of Antiviral Drug Products for review.

c. Assay performance characteristics studies should be conducted on specimens that are representative of the same virus type, and are from the same tissue reservoirs (serum, plasma, other) as that proposed in the clinical trial.

d. Specimen stability (handling, processing and storage protocols) should be demonstrated to result in no significant change of HIV RNA material as measured by the assay.

e. It is important that the experimental performance data generated to support the use of the assay derive from a protocol-based experiment, and this protocol should be submitted in conjunction with the assay data. In addition to the experimental data, information should also be provided regarding the quality assurance/quality control of the assay in the particular clinical trials under consideration.

f. Quantitative Assay Performance Studies

    1. In order to permit quantitative clinical virology data to be considered when interpreting antiviral drug trial results the methodology/technology used to generate those data must be adequately described in the application. A HIV RNA quantitative assay should be able to accurately and precisely report HIV RNA material over a defined range. Assay performance characteristics must

include, but are not limited to, information/data that defines the assay accuracy, precision, sample stability, and effects of certain interfering substances.

2. Accuracy may be assessed by calculating the mean of repeated observations of a given known sample and comparing the mean to the known input value. Assay accuracy should be determined across the proposed range of the assay. Precision may be assessed by calculating the mean square error (MSE) and converting to a percent CV. Precision should be determined across the proposed range of the assay. The quantitative limit of the assay will be determined by the lowest input value where the assay maintains its accuracy and precision. A quantitative upper limit may be similarly defined.

3. Ultimately, the quantitative limit must be supported by and derived from the assay performance characteristics data. Laboratory strains and unique clinical HIV "test" specimens should be used to derive the performance characteristics of the assay. Each of these "test" specimens should first be independently and adequately quantitated prior to being used to define the performance characteristics of the new assay.

g. Qualitative/Screening Assay Performance Studies

1. In order to permit qualitative clinical virology data to be considered when interpreting antiviral drug trial results the methodology/technology used to generate those data must be adequately described in the application. These assays should have the ability to distinguish between known HIV seropositive clinical specimens and known HIV seronegative specimens with 95% confidence. A threshold or screening cut-off value (qualitative limit), expressed in HIV RNA material per mL, should be determined. An assay result would be expressed as either a $\geq$ or a $<$ the HIV RNA qualitative limit, however, a result that is below the screening cut-off value does not imply that the specimen is HIV negative, it implies only that the specimen has less HIV RNA material than that needed to distinguish the specimen from a known negative with 95% confidence.

2. Assay performance characteristics must include, but are not limited to, information/data that defines the assay range of specificity, range of sensitivity, sample stability, and effects of certain interfering substances. The range of specificity of the assay is defined as the 95% confidence interval of reported observations from 500 random seronegative blood or plasma donors. The range of sensitivity of the assay is defined as the 95% confidence interval of reported observations from 200 unique seropositive samples. Each of these seropositive samples should be quantified by an independent method and then diluted to the proposed qualitative limit prior to assessing the assay range of

sensitivity. It is expected that the two ranges will not overlap for the proposed qualitative limit to be acceptable.

3. Ultimately, the qualitative limit must be supported by and derived from the assay performance characteristics data. Laboratory strains and unique clinical HIV "test" specimens should be used to derive the performance characteristics of the assay. Each of these "test" specimens must first be independently and adequately quantitated prior to being used to define the performance characteristics of the new assay.

Definitions for assay terminology:

Quantitative Assay: An assay that is accurate and precise over a defined range.

Qualitative Assay: An assay that can distinguish between a known HIV positive specimen and a HIV negative specimen.

Range of Specificity: A 95% confidence interval of reported observations from 500 seronegative random blood or plasma donors.

Range of Sensitivity: A 95% confidence interval of reported observations from a dilution of 200 unique seropositive samples to the proposed qualitative limit, each quantified prior to dilution by an independent method.

Quantitative Limit: The lower boundary of the accurate and precise defined range.

Qualitative Limit: The lowest concentration of HIV RNA that the assay can reliably distinguish from negative samples.

Interfering Substances: Any Substance/infectious agent that may be present in a clinical sample, and effect a performance characteristic of the new assay.

Precision: The variability in terms of the mean square error (MSE) converted to a percent CV (CV= the square root of MSE divided by the expected value x 100%) within the proposed range.

Accuracy (Bias): The mean of repeated observations of a given known sample compared to the expected value for knowns within the proposed range of the assay.

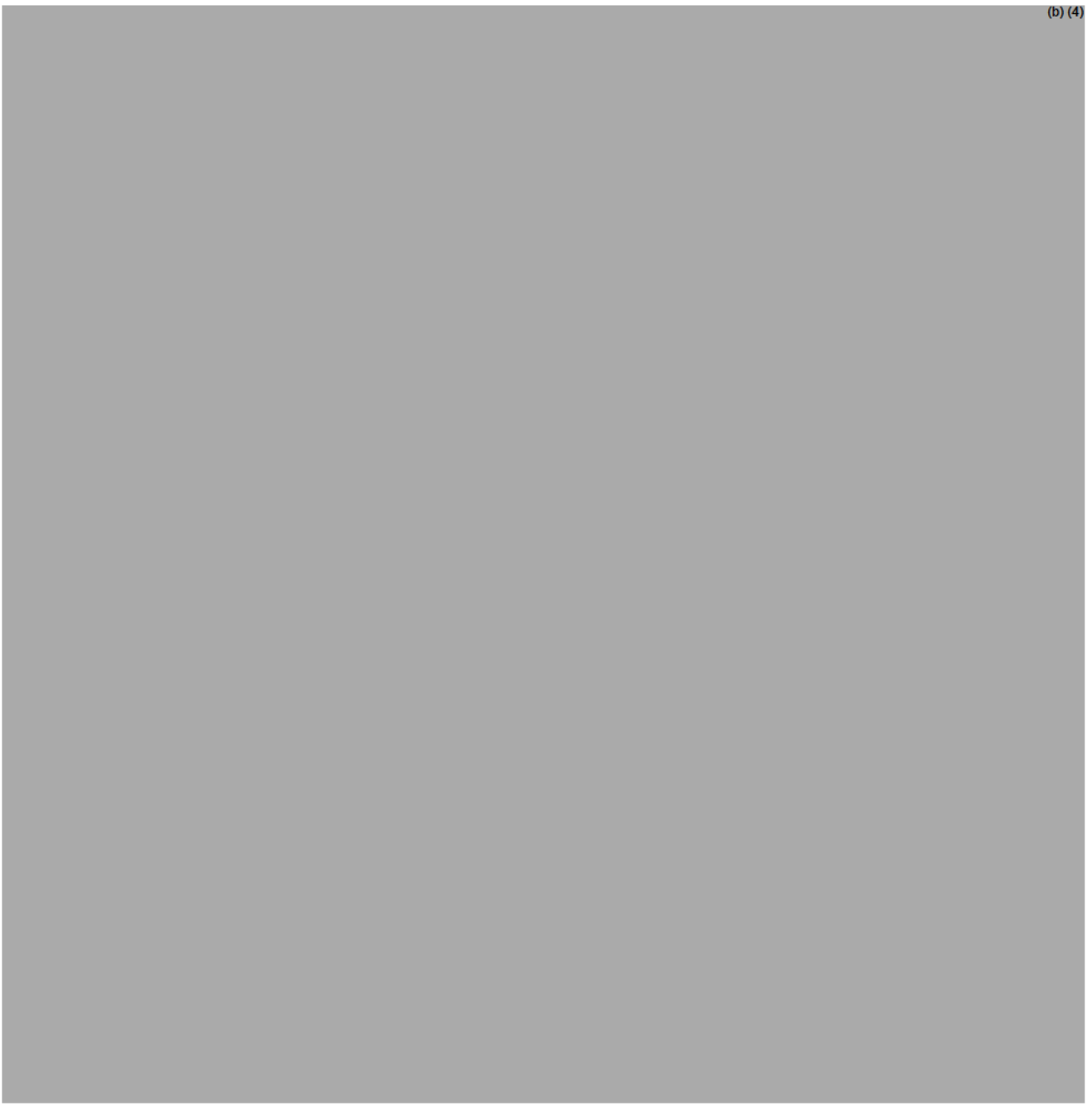
Sample stability: Specimen handling, processing and storage procedures that result in no significant changes in expected HIV RNA concentrations

References

1. Chou T.C. and Talalay P. Adv. Enzyme Regul. 1984: 22: 27-55.
2. Layne S.P. et al., Virol. 1992: 189: 695-714.
3. Lin et al., J. Infect. Dis. 1994: 70: 553-562.
4. Pelemans H. et al., J Virol. 1997: 71(11): 8195-8203.
5. Schinazi R.F. et al., Int Antiviral News. 1996: 4: 95-107.

Microbiology Labeling Proposed by the Sponsor.

(b) (4)



(b) (4)

FDA Proposed Microbiology Labeling

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 and macrophage/monocyte cultures. The 90-95% inhibitory concentration (IC_{90-95}) 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).

(b) (4)

(b) (4)

[redacted] (b) (4)

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

1. With respect to microbiology, SUSTIVA is approved.

2. Efavirenz is a non-nucleoside RT inhibitor that has marked activity *in vitro* against HIV-1 clinical and laboratory isolates.

3. Drug interaction studies *in vitro* with zidovudine, didanosine or indinavir have demonstrated synergistic anti-HIV-1 activity.

4. HIV-1 isolates with reduced susceptibility to efavirenz emerge *in vitro*. Efavirenz-induced phenotypic resistance has been demonstrated to be at least partially related to the development of one or more amino acid point mutations in the RT.

5. Emergence of HIV strains that are cross-resistant to non-nucleoside RT inhibitors has been observed *in vitro*.

6. Emergence of efavirenz-resistant HIV-1 has been observed following combination therapy with other antiretrovirals in phase II clinical trials.

7. Preliminary data from five patients who had received efavirenz in combination with other antiretrovirals revealed that all 5 individuals possessed HIV-1 isolates that were efavirenz-resistant, and that all 5 patient's isolates expressed a measurable degree of phenotypic resistance to both nevirapine and delavirdine. The clinical significance of this observation is not known.

8. The performance characteristics of the experimental [redacted] (b) (4) assay have not been sufficiently demonstrated.

RECOMMENDATIONS:

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

[redacted] (b) (6)

Microbiologist

CONCURRENCES:

HFD-530/Deputy Dir. [redacted] (b) (6) **Signature** 9/10/98 **Date**
HFD-530/SMicro **Signature** 9/8/98 **Date**
cc:
HFD-530/Original NDA
HFD-530/Division File
HFD-530/Div Dir Reading file
HFD-530/Pre-Clin Dep
HFD-530/MO
HFD-530/Pharm
HFD-530/Chem
HFD-530/SMicro
HFD-530/Review Micro
HFD-530/CSO Crescenzie

Reference: NDA 20-972: Sustiva™ (Efavirenz)
Microbiology Section

Report Number PRR 97-14

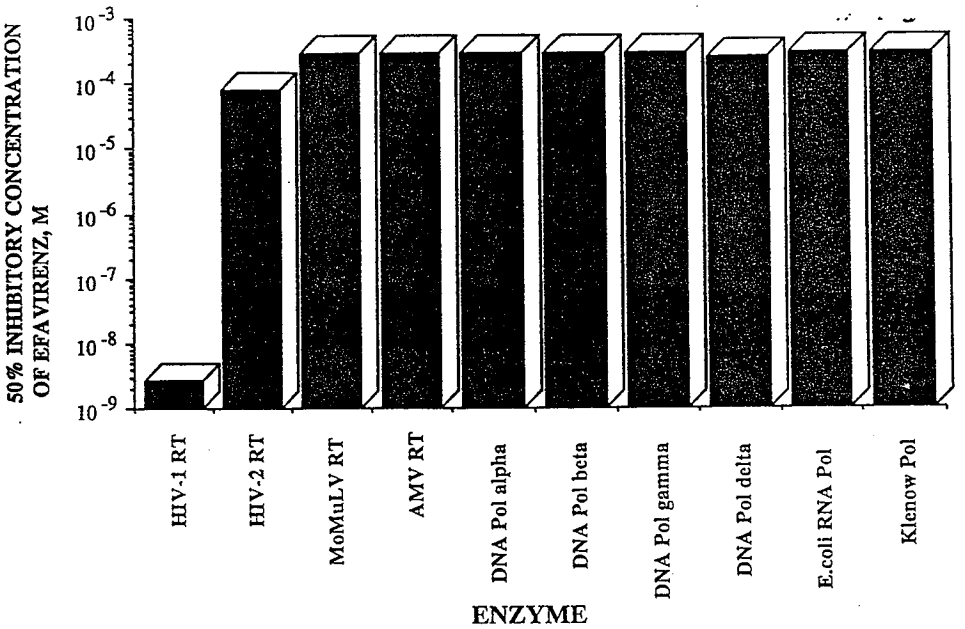
Table 1. Inhibition of HIV-1 RT by Efavirenz

Enzyme	IC_{50} (nM) against			
	Poly r(C)•oligo d(G)	Poly d(A)•Oligo d(T)	rRNA.15 mer	Poly r(A)•oligo d(T)
Wild type RT	2.6 ± 0.25	1.7 ± 0.28	1.1 ± 0.24	22 ± 1.3
K103N	30 ± 3.0	ND	ND	ND
Y181C	7.7 ± 0.36	ND	ND	ND
K103N/Y181C	72 ± 12	ND	ND	ND

ND = Not determined
See Appendix B.

Reference: NDA 20-972: Sustiva™ (Efavirenz)
Microbiology Section

Figure 1. Specificity Of Efavirenz For The HIV-1 Reverse Transcriptase



Reference: NDA 20-972: Sustiva™ (Efavirenz)
Microbiology Section

Table 1. Inhibition of wild-type and mutant HIV-1 RT by L-743,726

Enzyme	K_i (nM)	K_{ii} (nM)
wild-type	2.93 ± 0.17	2.93 ± 0.17
98 (A → G)	3.85 ± 0.60	4.48 ± 0.63
100 (L→ I)	17.13 ± 3.57	25.30 ± 8.95
101 (K →E)	7.27 ± 1.08	4.17 ± 0.04
103 (K →N)	17.60 ± 2.31	31.24 ± 4.02
106 (V → A)	6.89 ± 3.37	6.75 ± 1.96
108 (V → I)	2.97 ± 0.46	3.22 ± 0.22
179 (V → D)	3.60 ± 1.51	3.68 ± 1.65
179 (V → E)	9.68 ± 1.57	3.51 ± 0.13
181 (Y→ C)	7.62 ± 1.12	3.74 ± 0.68
101 (K→ E) + 103 (K → N)	56.50 ± 27.60	92.94 ± 0.68
103 (K→ N) + 181 (Y→ C)	26.05 ± 1.28	40.41 ± 10.94

Reference: NDA 20-972: Sustiva™ (Efavirenz)
Microbiology Section

Table 3. Antiviral Activity Of Efavirenz Against Laboratory And Clinical Isolates Of HIV-1

Virus/ Phenotype	Cell Type	Assay	IC_{90}, nM	IC_{95}, nM	Reference
Laboratory Strains					
RF	MT-4	p24		≦3.0	PRR 97-12
RF	PBMC	p24	2.8		PRR 97-05
RF	MT-2	Yield	2.4 ± 0.5, N=4		PRR 97-19
RF	MT-2	RNA	1.7 ± 0.5, N=38		PRR 97-05
IIIB	MT-4	p24		6.0	PRR 97-12
IIIB	PBMC	p24	2.8		PRR 97-05
MN	MT-4	p24		6.0	PRR 97-12
AL-1	MT-4	p24		6.0	PRR 97-12
HXB2	PBMC	p24	2.8		PRR 97-05
HXB2	MT-4	p24	3.2 ± 0.32, N=4		PRR 97-19
NL4-3	PBMC	p24	6.0		PRR 97-05
SF-162	Monocytes/ macrophages	p24		≦12.0	PRR 97-12
Wild Type Clinical Isolates					
5024-0	PBMC	p24		≤25.0	PRR 97-12
A012B	PBMC	p24	3.2		PRR 97-05
A018A	PBMC	p24	7.0		PRR 97-05
Thai-9466	PBMC	p24	8.5		PRR 97-05
ZDV-resistant Clinical Isolates					
A012D	PBMC	p24	2.8		PRR 97-05
A018C	PBMC	p24	2.8		PRR 97-05
WR10983	PBMC	p24	8.2		PRR 97-05
WR15935	PBMC	p24	6.6		PRR 97-05
WR16231	PBMC	p24	6.3		PRR 97-05
D2	PBMC	p24	7.3		PRR 97-05
E	PBMC	p24	2.8		PRR 97-05
116-0	PBMC	p24		≤25.0	PRR 97-12
421-7	PBMC	p24		≤25.0	PRR 97-12
L-697,661-Resistant Isolates					
116-7	PBMC	p24		≤25.0	PRR 97-12
5002-8	PBMC	p24		≤25.0	PRR 97-12
5024-16	PBMC	p24		200.0	PRR 97-12

Reference: NDA 20-972: Sustiva™ (Efavirenz)
Microbiology Section

Report Number PRR 97-04

Table 2. Antiviral Potency and Cytotoxicity of DMP 266 in MT-2 Cells

IC_{90}, nM	TC_{50}, µM
1.7 ± 0.5	18.9±5.5
N=38	N=39

Notebook References: Appendix C

Reference: NDA 20-972: Sustiva™ (Efavirenz)
Microbiology Section

Table 4. Antiviral Potency Of Efavirenz In The Presence Of Human Serum Components

Condition[a]	IC_{90} +/- SD, nM	N	Fold Increase Relative to Control condition	% Bound by Equilibrium Dialysis at 16.2 μM efavirenz, N=4
DMEM + FCS	1.95 ± 0.66	11	Control	72.8
DMEM + AAG	(1X) 6.97	1	3.6	88.8
DMEM + AAG + HSA	(16X) 32.08 ± 8.89	11	16.5	99.2

a All conditions contained 5% fetal calf serum (FCS)

Reference: NDA 20-972: Sustiva™ (Efavirenz)
Microbiology Section

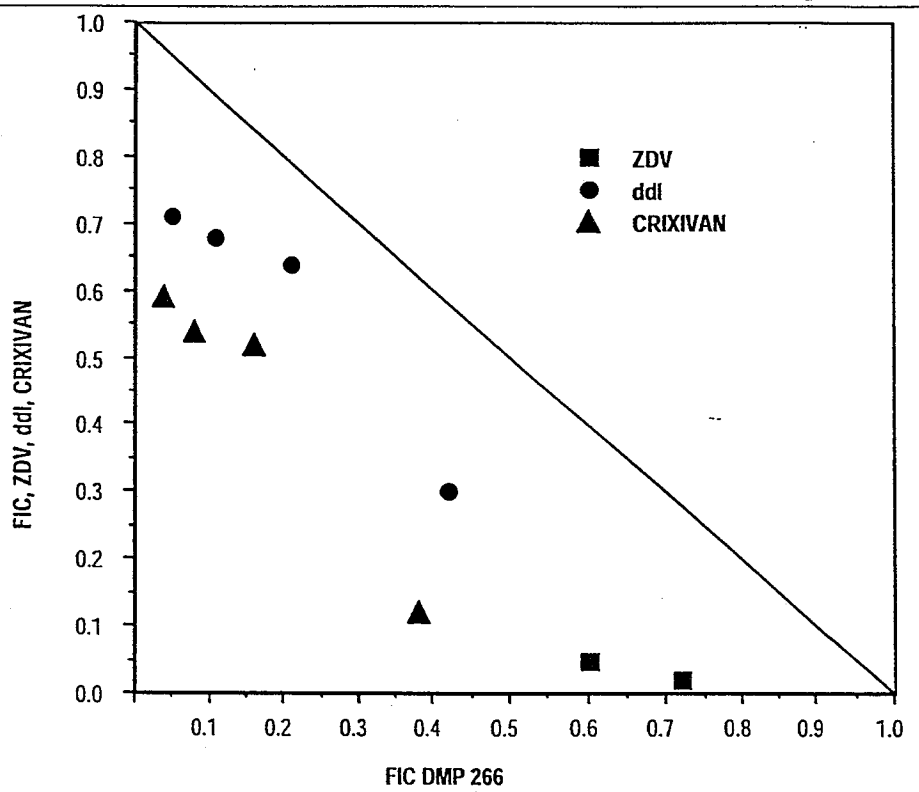
Table 5. Antiviral Potency Of Non-Nucleoside Reverse Transcriptase Inhibitors In The Presence of Human Serum Components

Inhibitor	Condition[a]	IC_{90}, nM	N	Fold Difference from Control
Efavirenz	DMEM	1.95	11	Control
	DMEM + HSA + AAG	32.08	11	16.5
Nevirapine	DMEM	50.3	9	Control
	DMEM + HSA + AAG	100.5	6	2.0
Delavirdine	DMEM	36.6	9	Control
	DMEM + HSA + AAG	1392.4	4	38.0
[redacted]				(b) (4)

[a] DMEM is [redacted] (b) (4)

Report Number PRR 97-12

Figure 1. Combination Activity of DMP 266 (L743,726) With ZDV, ddI and Crixivan



Appears this way on original

Table 7. Potency Of Efavirenz Against Mutant Variants Of HIV-1

Virus	IC_{90}, nM	Fold Resistance	IC_{95}, nM	Fold Resistance
Single Mutants				
NL4-3	3.5	Wild Type	3	Wild Type
A98G	ND		12	4.0
L100I	77	22	50	17
K101E	24	6.9	≤25	≤8.3
K103N	64	18	100	33
V106A	11	3.1	12	4.0
V108I	3.8	1.1	3	1.0
V179D	ND		3.0	1.0
Y181C	4.1	1.2	6.0	2.0
Y188C	13	3.7	ND	
G190A[a]	14	4.0	ND	
HXB2	3.2	Wild Type	ND	
S48T	3.5	1.1	ND	
P236L	1.9	0.6	ND	
RF	2.4	Wild Type	ND	
L100I in RF	120	50	ND	
Multiple Mutants				
NL4-3	3.5	Wild Type	3.0	Wild Type
Y188L	480	140	1500	500
K101E+K103N	ND		1500	500
Y181C+K103N	ND		400	133
L100I+K103N	ND		>3000	>1000
G190A+K103N[b]	450	130	ND	
HXB2	3.2	Wild Type	ND	
S48T+G190S	310	97	ND	
S48T+K103N+G190S	12000	3800	ND	
RF	2.4	Wild Type	ND	
L100I+V179D+Y181C	2400	1000	ND	
Protease Mutants				
NL4-3	5.8	Wild Type	ND	
L10R+M46I+L63P+V82T+I84V	5.7	1.0	ND	
HXB2	11	Wild Type	ND	
V32I/V82I	4.1	0.4	ND	

a (b) (4)

b

ND=Not determined

Reference: NDA 20-972: Sustiva™ (Efavirenz)
Microbiology Section

Table 1. Antiviral Potency of DMP 266 in Continuous Long-Term Cultures of Peripheral Blood Mononuclear Cells

Passage	Selecting Concentration of DMP 266, nM	Observed IC_{90} for DMP 266, nM	Fold Increase Relative to Week 0	Mutations Observed
Week 0	Pre-treatment	2.9	Control	
Week 4	0	7.0	2.4	ND
	3.2	26.0	9.0	ND
	9.5	89.0	30.7	L100I ←
Week 6	0	7.3	2.5	ND
	3.2	28.5	9.8	ND
	9.5	83.9	28.9	ND
	95.0	79.5	27.4	L100I, V108V+I ←
Week 12	0	8.2	2.8	ND
	3.2	64.6	22.3	ND
	9.5	95.7	33.0	ND
	95.0	>3167.8	>1092	L100I, V108I ←
Weeks 1-13	950	No HIV detected at any time		←

ND=Not determined.
NB reference: DMP 3870:68,92,125, DMP 5469:48, DMP 3583:181

Table 2. Antiviral Potency of DMP 266 in Successive Passages of MT-2 Cells

Passage Number	Concentration of DMP 266 present, nM	IC90, nM	Fold Increase Relative to Week 0	Mutations Observed
0	0	2.5	Control	Wild Type
12	6.3	15.8	6.3	ND
15	15.8	26.9	10.8	V179D
23	158.0	>950	>380	ND
24	317.0	ND	ND	L100I/V179D/Y181C

ND=Not Determined
NB Reference: DMP 3870:68, 92, 125, DMP 4569:48; DMP 3814:1-13,29-30,51057; DMP 4392:1-21, 165-166.

Reference: NDA 20-972: Sustiva™ (Efavirenz)
Microbiology Section

Appears this way on original

Table 2. Potency of Efavirenz, Nevirapine and Delavirdine Against HIV-1 Mutant Variants
Page 1 of 2

Recombinant Virus	Efavirenz Avg IC_{90} (nM)±SD	N	Efavirenz Fold Resistance[1]	Nevirapine Avg IC_{90} (nM)±SD	N	Nevirapine Fold Resistance	Delavirdine Avg IC_{90} (nM)±SD	N	Delavirdine Fold Resistance
Single Mutations									
S48T	3.5 ± 0.95	3	1.1	280 ± 66	3	2.3	51 ± 23	2	1.9
L100I	77 ± 26	19	22	630 ± 170	3	4.8	1300 ± 290	2	34
K101E	24 ± 5.4	3	6.9	1600 ± 67	2	12	190	1	5.0
K103N	64 ± 24	20	18	5100 ± 830	3	39	1000 ± 270	3	26
V106A	11 ± 1.9	2	3.1	14000	1	110	580	1	15
V108I	3.8 ± 1.6	2	1.1	330 ± 67	2	2.5	39 ± 1.7	2	1.0
Y181C	4.1 ± 1.9	5	1.2	12000 ± 3800	2	92	980 ± 130	2	26
Y188C	13 ± 5.4	3	3.7	5300 ± 530	2	41	100 ± 7.2	2	2.6
BIRR027[2]	14 ± 6.0	2	4.0	4700 ± 1200	2	36	6.3 ± 1.7	2	0.17
P236L	1.9 ± 0.32	2	0.59	260 ± 5.3	2	2.2	2400 ± 730	2	89
Multiple Mutations									
Y188L	480 ± 62	2	140	>190000	1	>1500	760 ± 230	2	20
BIRR028[3]	450 ± 46	2	130	60000	1	460	140	1	3.7
S48T/G190S	310 ± 13	2	97	89000	1	740	29	1	1.1
S48T/K103N/G190S	12000 ± 4600	2	3800	180000 ± 38000	2	1500	3500 ± 770	2	130

[1] The average IC_{90} of each mutant was divided by the corresponding wild type average IC_{90} to obtain the average fold resistance. All mutants have been constructed in the NL4-3 background with the exception of: S48T, P236L, S48T/G190S and S48T/K103N/G190S which were constructed in the HXB2 background.
[2] Corresponds to G190A mutation.
[3] Corresponds to a multiple mutation (M41L, D67N, V75M, K103N, G190A, L210W and T215Y) derived from a patient isolate RT gene subsequently cloned into the NL4-3 background.
[4] Potency was assessed against the L100I mutant selected from RF-infected PBMCs, and the triple mutant from *in vitro* selection experiments using RF-infected MT-2 cells carrying the mutations L100I/V179D/Y181C (Reid, *et al.*, 1997).
The above footnotes apply to the entire table

Appears this way on original

Table 14. Prevalence Of NNRTI-Related RT Gene Mutations In Plasma Virus Of Efavirenz-Exposed Patients With Significant Rebounds In Viral Load

	% of Patients (# of Patients, # of Clones)	
	Study 003	Study 004
	40 Patients	22 Patients
NNRTI Mutation	747 Clones	416 Clones
L74V	2.5% (1,1)	0
V75I	2.5% (1,1)	0
A98G	5.0% (2,2)	13.6% (3,6)
→ L100I	10.0% (4,7)	→18.2% (4,17)
→ K101E	10.0% (4,16)	→13.6% (3,13)
→ K103N	90.0% (36,503) (36/40)	→100.0% (22,369) (22/22)
V106I	2.5% (1,5)	9.1% (2,2)
V106A	0	9.1% (2,2)
→ V108I	30.0% (12,37)	→ 45.5% (10,43)
E138K	7.5% (3,3)	4.5% (1,1)
T139I	2.5% (1,1)	0
G141E	2.5% (1,1)	4.5% (1,1)
V179E	0	0
V179D	0	0
Y181I	0	0
Y181C	5.0% (2,2)	9.1% (2,2)
Y188C	0	9.1% (2,2)
Y188L	7.5% (3,54)	4.5% (1,13)
Y188H	7.5% (3,10)	0
Y189I	2.5% (1,1)	4.5% (1,2)
→ G190S	12.5% (5,39)	4.5% (1,2)
G190A	2.5% (1,1)	→18.2% (4,11)
G190E	0	4.5% (1,1)
→ P225H	35% (14,76)	→ 27.3% (6,26)

The % of patients with one or more clones at one or more timepoint (all timepoints after exposure to efavirenz considered) carrying the specified mutation is listed. Linkage of mutations is not considered. NNRTI mutations include all mutations described as associated with resistance to any non-nucleoside reverse transcriptase inhibitor in Schinazi, 1996 and Pelemans, 1997. Some NNRTI mutations which were not detected in any sample are excluded from the table.

Appears this way on original

Table 15. Prevalence Of NNRTI Mutations In Plasma Virus From Patients In Study DMP 266-003: Comparison Of Baseline, Placebo And Efavirenz-Exposed Patients.

	% of Patients (# of Patients, # of Clones)			
		Patients with Significant Rebounds in Viral Load		
NNRTI Mutation	Baseline[a]	Indinavir Monotherapy[b]	Efavirenz[c]	Switched to Efavirenz + D4T or 3TC[d]
	46 Patients 303 Clones	13 Patients 187 Clones	32 Patients 637 Clones	8 Patients 110 Clones
L74V	0	0	3.1 (1,1)	0
V75I	4.4 (2,7)	0	3.1 (1,1)	0
A98G	0	0	3.1 (1,1)	12.5 (1,1)
L100I	0	0	9.4 (3,6)	12.5 (1,1)
K101E	0	7.7 (1,1)	12.5 (4,16)	0
K103N	0	0	90.6 (29,424)	87.5 (7, 79)
K103T	0	0	0	0
V106I	2.2 (1,3)	15.4 (2,2)	3.1 (1,5)	0
V106A	0	0	0	0
V108I	0	0	31.3 (10,27)	25.0 (2,10)
E138K	0	0	9.4 (3,3)	0
T139I	0	0	3.1 (1,1)	0
G141E	0	0	3.1 (1,1)	0
V179E	0	0	0	0
V179D	0	0	0	0
Y181I	0	0	0	0
Y181C	0	0	6.3 (2,2)	0
Y188C	0	0	0	0
Y188L	0	0	6.3 (2,47)	12.5 (1,7)
Y188H	2.2 (1,1)	0	6.3 (2,3)	12.5 (1,7)
Y189I	0	0	3.1 (1,1)	0
G190S	0	0	15.6 (5,39)	0
G190A	0	0	3.1 (1,1)	0
G190E	2.2 (1,1)	0	0	0
P225H	0	0	34.4 (11,69)	37.5 (3,7)

[a]Baseline is defined as samples collected on Day A0 for Cohorts I, II and III and Day B0 for Cohorts IV-VIII of Study DMP 266-003.

[b]Indinavir monotherapy samples are those collected after day A0 or B0 from patients initially randomized to efavirenz placebo and before any switch to active efavirenz-containing drug combinations.

[c]Efavirenz samples are those collected after day 0 (A0 in cohorts I, II and III, B0 in cohorts IV-VII) from patients initially randomized to active efavirenz-containing drug combinations.

[d]Switched to efavirenz samples were those collected after patients initially randomized to efavirenz placebo (indinavir monotherapy) were switched to active efavirenz plus 3TC or D4T.

Linkage of mutations is not considered. NNRTI mutations include all mutations described as associated with resistance to any non-nucleoside reverse transcriptase inhibitor in Schinazi *et al.*, 1996 and Pelemans et. al., 1997. Some NNRTI mutations which were not detected in any sample are excluded from the table.

Appears this way on original

Table 16. Mutant Viral Genotypes In Plasma Virus Of Efavirenz-Exposed Patients

RT Genotype	% of Patients Carrying Viral Genotype in Study	
	DMP 266-003, N=40	DMP 266-004, N=22
L100I	0	0
L100I+K103N	10.0	13.6
L100I+K103N+Y188L	0	4.5
K101E	2.5	0
K101E+K103N	5.0	9.1
K101E+P225H	2.5	4.5
K103N	85.0 (34/40)	100.0 (22/22) ~90%
K103N+G190S	10.0	0
K103N+V108I	27.5	31.8
K103N+P225H	30.0	22.7
K103N+V108I+P225H	7.5	4.5
V108I	0	4.5
Y188L	7.5	4.5
G190S	12.5	4.5
G190A	0	13.6
P225H	0	0

Table 18. *In Vitro* Susceptibility Of Clinical Isolates From Study DMP 266-003 To Efavirenz, Indinavir And ddC

Isolate	N	ddC		Efavirenz (EFV)		Indinavir (IDV)	
		IC_{50}, nM	IC_{90}, nM	IC_{50}, nM	IC_{90}, nM	IC_{50}, nM	IC_{90}, nM
Baseline	23	19 ± 33	71 ± 120	0.7 ± 0.9	1.8 ± 2.1	15 ± 24	37 ± 43
Post Viral Load Rebound Samples							
EFV+IDV	16	26 ± 26	130 ± 140	50 ± 45	70 ± 44	160 ± 240	430 ± 430
IDV	7	32 ± 38	110 ± 120	3.9 ± 4.8	21 ± 36	270 ± 420	420 ± 480
Control Viruses[a]							
NL4-3	5	37 ± 43	180 ± 290	3.0 ± 4.5	12 ± 16	80 ± 160	180 ± 300
K103N	5	33 ± 44	150 ± 200	42 ± 37	71 ± 43	60 ± 96	130 . ± 200
MSV	5	57 ± 66	330 ± 450	3.8 ± 5.0	9.5 ± 12	130 ± 100	480 ± 430

[a] NL4-3 corresponds to wild type HIV-1, K103N is a recombinant virus in the NL4-3 background with a single K103N mutation, MSV is a recombinant virus in the NL4-3 background with five mutations in the protease gene, yielding a gene product with L10R, M46L, L63P, V82T and I84V substitutions.

Appears this way on original

Table 19. *In Vitro* Drug Susceptibility And Virus Genotype Of Clinical Isolates In Study DMP 266-003 With Paired Baseline Samples (Page 1 of 2)

Patient	Timepoint[a]	*In vitro* Sensitivity to:				Virus Genotype	
		Efavirenz (EFV)		Indinavir (IDV)		RT Gene	Protease Gene
		IC_{50}, nM	IC_{90}, nM	IC_{50}, nM	IC_{90}, nM		
Efavirenz + indinavir							
1302	A000	<0.32	<0.32	4.3	20	WT	L10V,L63P,V77I
	B112	6.8	>100 (63/2)	120	250	K103N	L10V,L63P,V77I
	B252	0.72	2.4	230	640	WT	L10V,L63P,V77I
	B336	>100	>100	12	>1000	ND	ND
4104	A000	<0.32	1.7 Ø	8.4	32	ND	ND
	B420	0.9	2.1 Ø	8.6	20	ND	ND
4204	A000	0.33	1.5 (>67)	3.8	21	ND	ND
	B112	>100	>100	>1000	>1000	K103N, E138A, Y188L	V32I,L63P,A71 VV82A
4206	A000	0.58	1.7 Ø	9.7	23	ND	ND
	B420	<0.32	0.73 Ø	7.8	19	ND	ND
6306	A000	0.4	1.5 (>67)	8.2	22	WT	L63P
	B112	>100	>100	30	91	K103N	L63P
7101	A000	0.4	1.4 (>67)	4.3	17	WT	L63P
	B168	47	>100 (>67)	280	>1000	V108I	L10R,L63P
13402	B000	<0.32	<0.32	24	61	ND	ND
	B380	>100	>100 (>3/2)	23	44	ND	ND
16403	B000	4.6	11	120	220	WT	L63P
	B112	>100	>100 (>9)	46	88	K103N	L63H

[a] Indicates the phase (A= monotherapy, B= combination therapy period) and the day of sample collection. Patient 4204, initially randomized to indinavir monotherapy, added efavirenz on day 194. Patient 6302, initially randomized to indinavir monotherapy, added efavirenz on day 168.
The above footnotes and/or abbreviations apply to the entire table.

Reference: NDA 20-972: Sustiva™ (Efavirenz)
Microbiology Section

Table 20. *In Vitro* Susceptibility Of Clinical Isolates From Study DMP 266-004

Virus Isolate	N	*In vitro* Sensitivity to:					
		AZT (ZDV)		Efavirenz (EFV),		3TC	
		IC_{50}, nM	IC_{90}, nM	IC_{50}, nM	IC_{90}, nM	IC_{50}, µM	IC_{90}, µM
Baseline Isolates	13	15 ± 15	140 ± 270	0.6 ± 0.5	1.3 ± 1.1	7.3 ± 4.3	7.7 ± 4.4
Post Viral Load Rebound							
ZDV+EFV+3TC	10	240 ± 400	640 ± 470	80 ± 42	80 ± 42	6.4 ± 4.7	8.1 ± 4.0
ZDV+3TC	3	450 ± 580	1000	0.5 ± 0.3	2.1 ± 0.8	5.0 ± 7.0	6.1 ± 5.5
Control Viruses							
NL4-3[a]	4[b]	47 ± 88	260 ± 490	0.7 ± 0.6	2.0 ± 1.0	0.03 ± 0.02	0.31 ± 0.4
K103N	4[b]	11 ± 15	51 ± 86	14 ± 13	35 ± 34	0.034 ± 0.005	0.12 ± 0.05
ZDV-sensitive	4[b]	64 ± 70	380 ± 450	1.6 ± 1.5	5.1 ± 5.3	0.51 ± 0.86	2.9 ± 4.8
ZDV-resistant	4[b]	800 ± 400	1000	0.4 ± 0.1	1.4 ± 0.7	0.075 ± 0.02	0.36 ± 0.1

[a] NL4-3 corresponds to wild type HIV-1. K103N is a recombinant virus in the NL4-3 background carrying the single mutation K103N. ZDV-resistant refers to known clinical isolates previously phenotyped as to ZDV resistance.
[b] Indicates the number of assays performed with the same virus stock.
[c] For calculation of mean IC50 and IC90, data for individual isolates was truncated at the upper and lower limits of the assay, which were $3.2x10^{-4}$ µM to 0.1 µM for efavirenz, $3.2X10^{-3}$ µM to 1 µM for ZdV, and $3.2x10^{-2}$ µM to 10µM for 3TC.

Reference: NDA 20-972: Sustiva™ (Efavirenz)
Microbiology Section

Table 21. *In Vitro* Drug Susceptibility And Virus Genotype Of Clinical Isolates In Study DMP 266-004 With Paired Baseline Samples

Patient	Timepoint	ZDV		Efavirenz (EFV)		3TC	
		IC_{50}, nM	IC_{90}, nM	IC_{50}, nM	IC_{90}, nM	IC_{50}, µM	IC_{90}, µM
1101	Day 0	<3.2	11	1	2.5	>10.0	>10.0
	Day 140	11	32	>100	>100 (>40)	>10.0	>10.0
4106	Day 0	11	32	<0.32	0.44	>10.0	>10.0
	Day 140	12	>1000	>100	>100 (>227)	1.2	>10.0
	Day 224	<3.2	160	>100	>100	>10.0	>10.0
12103	Day 0	16	59	<0.32	0.52	>10.0	>10.0
	Day 168	≥1000	>1000	>100	>100 (>192)	>10.0	>10.0
	Day 252	97	>1000	>100	>100	>10.0	>10.0
15303	Day 0	26	150	<0.32	<0.32 (>312)	>10.0	>10.0
	Day 112	>1000	>1000	>100	>100	10.0	>10.0
17304	Day 0	<3.2	12	2	3.8 (>26)	>10.0	>10.0
	Day 112	6.9	56	>100	>100	3.1	>10.0

The upper and lower limits of the assay were $3.2x10^{-4}$ µM to 0.1 µM for efavirenz and $3.2x10^{-3}$ µM to 1 µM for Zd, and $3.2x10^{-2}$ µM to 10µM for 3TC.

Appears this way on original

Table 22. Cross Resistance Of NNRTIs To Efavirenz-Resistant Clinical Isolates

Isolate	N	*In vitro* Sensitivity to:					
		Efavirenz		Nevirapine		Delavirdine	
		IC_{50}, nM	IC_{90}, nM	IC_{50}, nM	IC_{90}, nM	IC_{50}, nM	IC_{90}, nM
Baseline, Study DMP 266-003	6	<0.3	1.6 ± 0.5	46 ± 18	150 ± 58	6.6 v 4.4	60 ± 59
Baseline, Study DMP 266-004	5	<0.3	2.5 ± 4.2	50 ± 55	160 ± 150	20 ± 34	70 ± 120
All post-Viral Load Rebound Isolates							
Study DMP 266-003	7	120 ± 140	440 ± (275) 320	5700 ± 5000	(57) 8600 ± 3700	3800 ± 4400	(108) 6500 ± 4100
Study DMP 266-004	9	360 ± 490	540 ± (216) 490	5000 ± 5500	(42) 6700 ± 5100	2200 ± 3500	(70) 4900 ± 4300
Efavirenz-resistant Isolates Only							
Study DMP 266-003	6	130 ± 140	500 ± (372) 282	6700 ± 4800	(67) >10000	4400 ± 4500	(127) 7600 ± 3200
Study DMP 266-004	7	460 ± 510	700 ± (280) 440	7500 ± 5000	(63) >10000	2900 ± 3800	(90) 6300 ± 3800

For calculation of mean IC_{50} and IC_{90} individual values were truncated at the upper and lower limits of the assay which were $3.2x10^{-4}$ to 1μM for efavirenz and $3.2x10^{3}$ to 10μM for nevirapine and delavirdine.

Appears this way on original

Table 24. Specific NNRTI Mutations In The HIV-1 RT Gene Of Plasma Virus From Patients In Study DMP 266-003 With Viral Loads≥1000 After 16 Or 28-40 Weeks

NNRTI Mutation	Number of Patient Sequences with Specified Mutation (%)				
	Baseline [a]	Efavirenz/Indinavir Treated[b]		Indinavir Treated[b]	
		16 weeks	28-40 weeks	16 weeks	28-40 weeks
	N=30	N=15	N=14	N=19	N=2
L100I	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
K101E	0 (0%)	2 (13.3%)	0 (0%)	0 (0%)	0 (0%)
K103N	0 (0%)	12 (80%) (93%)	12 (86%)	1 (5%)[d]	0 (0%)
V108I	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Y188L	0 (0%)[c]	2 (13%)	0 (0%)	0 (0%)[c]	0 (0%)
G190E	0 (0%)	0 (0%)	1 (7.1%)	0 (0%)	0 (0%)
P225H	0 (0%)	0 (0%)	3 (21%)	0 (0%)	0 (0%)

[a] Baseline plasma samples selected for sequencing were derived from patients who subsequently had viral loads ≥1000 copies/ mL plasma at weeks 8-16, and may not be representative of all patients enrolled in the study.
[b] Samples selected for sequencing had plasma viral loads≥1000 copies HIV RNA/ mL plasma.
[c] One patient (Patient ID # 7102) has a Y188Y/F mixed virus population both at baseline and after 112 days of indinavir monotherapy
[d] One patient (Patient ID #1202) was randomized to indinavir monotherapy but was dispersed active efavirenz for approximately one month prior to day B112.