CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-503

MICROBIOLOGY REVIEW(S)

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)
MICROBIOLOGY REVIEW
NDA: 21-503 **SN:** N000/B2 **DATE REVIEWED:** 3/3/03
Microbiology Reviewer: Lisa K.Naeger, Ph.D.

Applicant Name and Address:

Agouron Pharmaceuticals, Inc.
10350 North Torrey Pines Road
La Jolla, CA 92037-1020
(858) 622-3000

Initial Submission Dates:

Correspondence Date: 6/28/02
CDER Receipt Date: 7/01/02
Reviewer Receipt Date: 7/08/02
Review Complete Date: 7/12/02

Amendments: Amendment 3 to NDA-21503 Response to Microbiology comments; Update Labeling

Correspondence Date: 2/14/03
CDER Receipt Date: 2/19/03
Reviewer Receipt Date: 2/24/03
Review Complete Date: 4/9/03

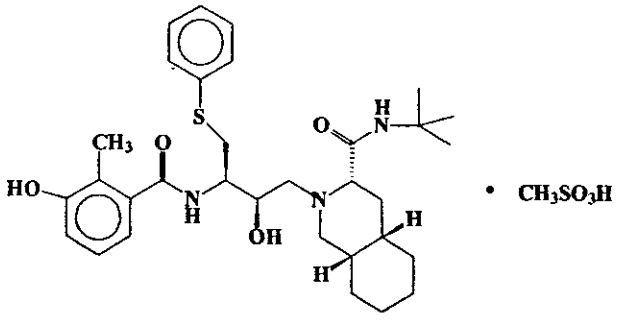
Related/Supporting Documents: IND 48,124 and NDA 20-778/779

Product Name(s):

Proprietary: Viracept™
Non-Proprietary/USAN: nelfinavir mesylate; AG1343
Code Name/Number:

Chemical Name: [3S-[2(2S*,3S*,3α,4aβ,8aβ]]-N-(1,1-dimethylethyl)decahydro-2-[2-hydroxy-3-[(3-hydroxy-2-methylbenzoyl)amino-4-(phenylthio)butyl]-3-isoquinolinecarboxamide, monomethanesulfonate (salt)

Structural Formula:



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Molecular Formula: $C_{32}H_{45}N_3O_4S \bullet CH_3SO_3H$
Molecular Weight: 663.9 (salt); 567.78 (free acid)
Dosage Form: 625-mg capsule-shaped tablet
Route(s) of Administration: Oral
Pharmacological Category: HIV protease inhibitor
Indication: Treatment of HIV infection in combination with other antiretroviral agents

Dispensed: Rx X OTC_

Abbreviations: ABC, abacavir; APV, amprenavir; AZT, zidovudine; BID, *bis in die*; CC, cytotoxic concentration; DAVDP, Division of Antiviral Drug Products; DLV, delavirdine; ddI, didanosine; EC, effective concentration; EFV, efavarenz; FDA, Food and Drug Administration; HAART, highly active antiretroviral therapy; HIV, human immunodeficiency virus; IC, inhibitory concentration; IDV, indinavir; 3TC, lamiviudine; LPV, lopinavir; MOI, multiplicity of infection; NFV, nelfinavir; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; NVP, nevirapine; PI, protease inhibitor; PR, protease; RT, reverse transcriptase; RTI, reverse transcriptase inhibitor; RTV, ritonavir; d4T, stavudine; SQV, saquinavir; TDF, tenofovir; TID, *tris in die*; ddC, zalcitabine;

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

Executive Summary

The original NDA-21503 describes a new formulation for the previously approved HIV protease inhibitor nelfinavir (NFV). The new formulation would reduce the daily pill burden from ten 250-mg tablets (1250 mg BID or 750 mg TID) to four 625-mg tablets (1250 mg BID). Nelfinavir mesylate (NFV) is a selective nonpeptidic inhibitor of HIV protease approved for the treatment of HIV infection in combination with other antiretrovirals. Previous studies have identified the D30N mutation as the predominant mutation in the HIV-1 protease of patients treated with NFV alone or in combination with other antiretrovirals. The L90M mutation is also observed in viruses from patients treated with NFV. Although primary mutations associated with resistance to other PIs (e.g. G48V, I50V, V82A/T/F, I84V) have not been detected in the HIV-1 of patients treated with NFV, these mutations do appear to confer cross-resistance to NFV.

The sponsor was requested by Dr. Jules O'Rear of DAVDP on August 27, 2002 to update the *in vitro* combination activity relationships and cross-resistance (LPV and efficacy of other PIs against NFV resistant virus) sections of the label. This amendment SN000/B2 to NDA-21503 responds to those microbiology comments and proposes updated labeling.

There are currently seventeen FDA-approved anti-HIV drugs including seven NRTIs (zidovudine, lamivudine, stavudine, abacavir, tenofovir, zalcitabine, didanosine), three NNRTIs (efavirenz, delavirdine, nevirapine), six PIs (amprenavir, saquinavir, nelfinavir, ritonavir, lopinavir, indinavir), and one fusion inhibitor (enfuvirtide). To support NFV use in combination with other antiretroviral agents, the *in vitro* combination activity relationships of NFV with all the drugs approved since Viracept's approval were evaluated. Antiviral and cytotoxic effects of NFV in two-drug combinations with PIs, APV and LPV, NRTIs, ABC and TDF, and NNRTIs, DLV, EFV, and NVP, were analyzed by the Pritchard and Shipman method. Results from this study indicated that the combination of NFV with APV, LPV, DLV, EFV, NVP, ABC or TDF resulted in moderate to strongly synergistic interactions in antiviral activity. Minimal to no cellular cytotoxicity was observed with any of these compounds alone are in combination with NFV. The results from this submission in combination with previous results indicate that NFV with other approved anti-HIV agents except for IDV may result in enhanced antiviral activity.

The susceptibility to currently approved protease inhibitors (PIs) of a panel of 44 patient-derived PI-resistant HIV-1 isolates containing clinically relevant PI-resistance associated mutations was analyzed in order to determine the cross-resistance of NFV with other approved PIs. The isolates demonstrated >2.5-fold resistance to at least one of the approved PIs, NFV, APV, SQV, IDV, or LPV. Four of the 44 PI-resistant isolates contained the NFV-associated resistance mutation D30N together with other PI-resistance mutations. All four of these viruses had high-level resistance to NFV but remained susceptible to the other evaluated PIs. Patient-derived recombinant HIV-1 isolates containing the L90M mutation (n=8) demonstrated moderate to high-level

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resistance to NFV and had varying levels of susceptibility to saquinavir, indinavir, lopinavir, and amprenavir *in vitro*. Most patient-derived recombinant isolates with phenotypic and genotypic evidence of reduced susceptibility (>2.5-fold) to lopinavir, amprenavir, saquinavir and/or indinavir demonstrated high cross-resistance to nelfinavir *in vitro*. Mutations associated with resistance to other PIs (e.g. G48V, V82A/F/T, I84V, L90M) appeared to confer high-level cross-resistance to NFV.

1. Recommendations

1.1. Recommendation and Conclusion on Approvability

This NDA for Nelfinavir mesylate 625 mg tablets is approvable with respect to microbiology. NFV was previously approved and the sponsor has provided data updating the *in vitro* combination activity relationships and cross-resistance to recently approved antiretroviral agents.

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

There are no recommendations for Phase 4 marketing commitments at this time.

2. Summary of OND Microbiology Assessments

2.1. Brief Overview of the Microbiological Program

2.2 Non-clinical

Nelfinavir mesylate (NFV) is a selective nonpeptidic inhibitor of HIV protease approved for the treatment of HIV infection in combination with other antiretrovirals. Previous studies have identified the D30N mutation as the predominant mutation in the HIV-1 protease of patients treated with NFV alone or in combination with other antiretrovirals. The L90M mutation is also observed in viruses from patients treated with NFV. Although primary mutations associated with resistance to other PIs (e.g. G48V, I50V, V82A/T/F, I84V) have not been detected in the HIV-1 of patients treated with NFV, these mutations do appear to confer cross-resistance to NFV. The data in this submission confirms that viruses with D30N are highly resistant to NFV but retain susceptibility to other PIs. Viruses with L90M are resistant to NFV and cross-resistant to varying levels with other PIs. In addition, mutations associated with resistance to other approved PIs confer high-level resistance to NFV. Clinical isolates of HIV-1 with phenotypic and genotypic evidence of resistance to LPV, APV, SQV, and IDV demonstrate high cross-resistance to NFV.

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The *in vitro* antiviral and cytotoxic effects of two-drug combinations of NFV with PIs - APV or LPV, NRTIs - tenofovir or ABC, and NNRTIs - DLV, NVP, or EFV were evaluated. The results indicate that the combination of NFV with APV, LPV, DLV, EFV, NVP ABC or TDF is synergistic. Previous results indicate that NFV in combination with IDV is antagonistic. Minimal to no cellular cytotoxicity was observed with any of these compounds alone are in combination with NFV.

3. Administrative

3.1. Reviewer's Signature(s)

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

3.2. Concurrence

HFD-530/Signatory Authority _____Signature _____Date
HFD-530/Micro TL _____Signature _____Date

3.3. CC Block

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DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)
MICROBIOLOGY REVIEW
NDA: 21-503 SN: N000/B2 DATE REVIEWED: 3/3/03
Microbiology Reviewer: Lisa K.Naeger, Ph.D.

OND Microbiology Review

1. Introduction and Background

This original NDA describes a new formulation for the previously approved HIV protease inhibitor nelfinavir (NFV). The new formulation would reduce the daily pill burden from ten 250-mg tablets (1250 mg BID or 750 mg TID) to four 625-mg tablets (1250 mg BID).

1.1. Important Milestones in Product Development

Viracept received accelerated approval on March 14, 1997 (NDAs 20-778/779) and traditional approval on April 25, 2000 (NDAs 20-778 SE7-012 and 20-779 SE7-026). Supporting data for the Microbiology section of the label were previously reviewed by Dr. Lauren Iacono-Connors. No new microbiology data were submitted and no changes to the microbiology section of the label were proposed in the original submission NDA-21503 SN000. The sponsor was requested by DAVDP to update the *in vitro* combination activity relationships and cross-resistance (LPV and efficacy of other PIs against NFV resistant virus) sections of the label. This amendment B2 to NDA-21503 responds to those microbiology comments and proposes updated labeling.

1.1.1. Methodology

Antiviral Assays
The antiviral activity of combinations of compounds was measured by the XTT dye reduction method in CEM-SS cells against HIV-1 RF (Pauwels *et al.*, 1988) Cells were plated in 96-well plates containing dilutions of compounds and were infected at an MOI of 0.47. Six days after infection, XTT tetrazolium and phenazine methosulfate were added to the plate and incubated for 4 hrs. Viability was determined by the amount of XTT formazan produced, quantified spectrophotometrically by absorbance at — nm. The Prichard and Shipman method using MacSynergy software was used to analyze the data from the combination experiments (Pritchard and Shipman, 1990). The difference between the observed combined effects and those expected if the interactions occurred independently are expressed as a volume above or below a plane that represents no interactive effects (plane of additivity). Positive values above an additive effect at 95% confidence interval are indicative of synergy while negative values below the additive effect are indicative of antagonism. Volumes between 25 and 50 μM^2 % suggest minor synergy or antagonism, volumes between 50 and 100 μM^2 % suggest moderate synergy or antagonism, and volumes of >100 μM^2 % suggest strong synergy or antagonism.

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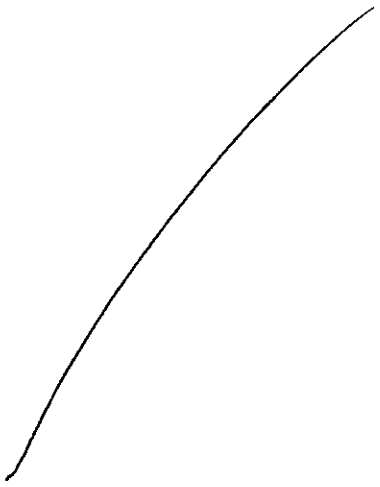
Susceptibility Assays

susceptibility assays were performed at



1.1.2. Prior FDA Microbiological Reviews

IND48124 Serial # 219
Reviewer: Lauren C. Iacono-Connors, Ph.D.
Date reviewed: 10/20/98

RECOMMENDATIONS

Please provide performance information, and supportive data, on the Roche Ultrasensitive assay which sufficiently demonstrates a quantitative limit capability of 50 HIV RNA copies/mL. Assay performance studies should be conducted on control specimens that are from the same tissue reservoir proposed for assessment in this study, and for which HIV RNA concentrations have been independently determined. REMUNE specific IgG should be considered as a possible interfering substance in assay performance studies. Additional discussions with the division on HIV RNA assay performance characteristics studies is encouraged.

It is possible that REMUNE therapy could delay the emergence of HAART-resistant HIV variants. The sponsor may wish to consider monitoring resistance evolution in patient virus variants to treatment drugs.

IND48124 Serial # 252
Reviewer: Lauren C. Iacono-Connors, Ph.D.
Date reviewed: 4/19/99

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NDA: 21-503 **SN:** N000/B2 **DATE REVIEWED:** 3/3/03
Microbiology Reviewer: Lisa K.Naeger, Ph.D.

The sponsor has submitted an information amendment that cross references four study reports submitted to the Agency as part of a supplement to NDA 20-779 (January 26, 1999) for consideration of full approval for Viracept. The four study reports referenced in this amendment are intended to meet four of the Phase IV commitments for Viracept NDA 20-779. The division's concurrence on this matter is requested. The study reports are as follows:

1. #AG1343-VI-001: Susceptibility of nevirapine, AZT, or 3TC resistant HIV-1 isolates to nelfinavir. Intended to meet phase IV commitment number 3a.
2. #AG1343-VI-002: Correlation between HIV genotypic resistance and clinical response in patients receiving nelfinavir monotherapy or nelfinavir in combination with 3TC and AZT. Intended to meet phase IV commitment 3d.
3. #AG1343-VI-003: Virologic response to a ritonavir/saquinavir-containing regimen in patients who had previously failed nelfinavir-containing regimens. Intended to meet phase IV commitment 3cii.
4. #AG1343-VI-004: Correlation of virologic response with genotype of plasma HIV-1 variants in patients treated with nelfinavir in the U.S. expanded access program. Intended to meet phase IV commitment 3bi.

Since these study reports are currently under review in support of the application for full approval of nelfinavir for the treatment of HIV infection, the question of whether they also meet previously agreed upon phase IV commitments for certain questions regarding HIV resistance issues will be addressed in the microbiology review of the NDA supplement number 8.

CONCLUSIONS

A decision on division concurrence as to whether NDA 20-779 phase IV commitment numbers 3a, 3d, 3cii, and 3bi have been satisfied by study reports numbers AG1343-VI-001, AG1343-VI-002, AG1343-VI-003, and AG1343-VI-004 will be made with the division response to NDA 20-779 SE8.

NDA-20779
Reviewer: Lauren C. Iacono-Connors
Date reviewed: 8/31/99

With respect to microbiology phase IV commitment numbers 3a, 3d, 3cii, and 3bi (NDA 20-778/779) have been satisfied by study reports numbers AG1343-VI-001, AG1343-VI-002, AG1343-VI-003, and AG1343-VI-004. The sponsor has provided additional phenotypic nelfinavir susceptibility in vitro data, to date, on a total of 6 clinical isolates, where each isolate had a measurable decrease in susceptibility to an RTI (AG1343-VI-001). Two isolates were "resistant" to zidovudine, two to nevirapine, and two to 3TC. In all cases these isolates remained sensitive to nelfinavir in vitro.

The sponsor evaluated the relationship between treatment associated mutations in selected viral isolates and treatment response in two different clinical studies; Protocol AG1343-505 and Protocol AG1343-511. The data from these studies suggest that patients treated with nelfinavir containing regimens who developed nelfinavir-specific, or other treatment associated mutations, were more likely to loose viral suppression than those who did not develop mutations. In addition, in protocol AG1343-505 baseline HIV RNA levels were significantly higher in the group that acquired the D30N mutation. However, these analyses were conducted with the inclusion of patients whose viral protease genes were not sequenced and therefore were scored as wild type virus. It is recommended to the medical reviewer that they along with the statistical reviewer

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analyze these data and in doing so omit, or reassess how to score, the patients from the analysis who did not have confirmed sequence on their viral isolates.

In study AG1343-VI-003 the virologic response to a ritonavir/saquinavir containing regimen in patients who had previously failed a nelfinavir-containing regimen was evaluated. The results of this study suggest that some patients who have failed a nelfinavir-containing regimen can be switched to a ritonavir/saquinavir containing regimen and achieve a virologic response. In addition, the data suggest that the D30N or the L90M protease gene mutations at baseline are not predictive of a clinical response when the protease(s) in the current treatment regimen are combined ritonavir/saquinavir.

The sponsor initiated a substudy of the VIRACEPT expanded access program to obtain additional information on cross-resistance between nelfinavir and other protease inhibitors. Patients who had previously failed non-nelfinavir, protease inhibitor therapy were treated with nelfinavir monotherapy or in combination with other antiretrovirals. Using the sponsor's criteria for defining treatment responders the results of this study suggest that 33/65 (51%) of subjects virologically responded to therapy. No statistically significant differences between the response groups for either the baseline HIV-1 RNA levels or CD4 cell counts were observed. Likewise, no statistically significant differences were observed between the response groups for the number of prior protease inhibitors used, and no significant differences were detected for the number or type of reverse transcriptase inhibitors used previously. Results from this study do suggest that for this group of pretreated patients, and in the absence of new antiretroviral reverse transcriptase therapy, the number of specific baseline genotypic changes in the protease gene had a significant impact on the virologic response to nelfinavir. However, it is not clear exactly when "baseline" samples were taken for genotyping from participants of this study with respect to their previous treatment history. It is possible that a subject may have been off a previous therapy for an extended period of time prior to baseline genotyping for the nelfinavir expanded access program. Therefore, baseline genotyping results may under-represent the number of actual mutations harbored by a study participant

With respect to the sponsor's proposed microbiology-specific labeling changes, it is recommended that final labeling language be considered during the review of NDAs 20-778 (SE8-012) and 20-779 (SE7-026); the application for traditional approval of nelfinavir.

IND —— Serial # 316
Reviewer: Jules O'Rear, Ph.D.
Date reviewed: 5/3/00

This document from Agouron Pharmaceuticals describes the Final 48-Week Analysis of Study ACTG 372 sent in partial fulfillment of Phase 4 commitment 3b in the accelerated approval letter of March 14, 1997. Phase 4 commitment 3b requested that we "Increase our current efforts to obtain information on cross-resistance between nelfinavir and other protease inhibitors." ACTG 372 is a Phase II study of the prolongation of virologic success and options for virologic failure in HIV-infected subjects receiving Indinavir in combination with NRTIs. Subjects were from a previous ACTG study in which they received ZDV (or d4T), 3TC, and IDV. They were enrolled into four separate groups based upon viral load and prior NRTI use: A, B, C, and D. This report only covers subjects in Groups B-D. Patients in Group B received nelfinavir as part of their anti-retroviral treatment regimen. Groups C and D were observational cohorts.

The current study is a Phase II, randomized, controlled evaluation of Abacavir (1592U89) vs. approved nucleoside analogs and NFV vs. placebo in combination with Efavirenz + adefovir

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dipivoxil in NNRTI-naïve subjects. Subjects enrolled in Group B had plasma HIV-1 RNA concentrations of ≥500 copies/ml, and no prior NNRTI use. The treatment arms were:

- Abacavir (1592U89) 300 mg b.i.d. + Efavirenz 600 mg QD + Adefovir dipivoxil 120 mg QD + NFV 750 mg t.i.d.
- Abacavir (1592U89) 300 mg b.i.d. + Efavirenz 600 mg QD + Adefovir dipivoxil 120 mg QD + NFV placebo t.i.d.
- 2 (or 1) NRTIs + Efavirenz 600 mg QD + Adefovir dipivoxil 120 mg QD + NFV 750 mg t.i.d.
- 2 (or 1) NRTIs + Efavirenz 600 mg QD + Adefovir dipivoxil 120 mg QD + NFV placebo t.i.d.

The primary week 48 endpoint at or after week 16 was defined as the time on or after week 16 but before week 52 that a subject experienced a confirmed virologic failure. A subject who never dropped below 500 copies/ml or who permanently discontinued study treatment during the first 16 weeks was considered a failure at week 16. Otherwise, failure was defined to occur at the earlier of the first of two consecutive measurements above 500 copies/ml and the time of permanent discontinuation of study treatment. If a subject had not experienced a primary endpoint by week 52 (which allows for a 4-week window around week 48), he/she was censored at week 48.

SUMMARY

A statistically significant short-term benefit was observed at week 16 with nelfinavir as detected by a drop in RNA levels and increases in CD4+ T cells (Figures B-9 and –21, Table B-11). This benefit was not evident at week 48 (Figures B-9 and B–21, Table B-16). No phenotypic or genotypic analysis of baseline and endpoint virus isolates is presented.

CONCLUSIONS

The observation that nelfinavir is only of a short-term benefit to patients failing IDV therapy suggests that the IDV resistant virus may have cross-resistance to nelfinavir.

1.1.3. Major microbiological issues that arose during product development.

The sponsor was requested by Dr. Jules O'Rear of DAVDP on August 27, 2002 to update the *in vitro* combination activity relationships and cross-resistance (LPV and efficacy of other PIs against NFV resistant virus) sections of the label. This amendment to the NDA responds to those microbiology comments and proposes updated labeling.

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

An estimated 40 million people were infected with HIV worldwide and 3 million died from AIDS in 2001. Since HAART regimens have been introduced, the number of AIDS cases decreased dramatically. HAART does not eradicate HIV from patients completely and even though the number of HIV RNA copies is reduced to undetectable levels, HIV re-emerges quickly after discontinuation of HAART.

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Therefore, with the currently available regimens, it is likely that most HIV-infected patients will require antiretroviral therapy throughout their lives.

There are currently seventeen FDA-approved anti-HIV drugs including seven NRTIs (zidovudine, lamivudine, stavudine, abacavir, tenofovir, zalcitabine, didanosine), three NNRTIs (efavirenz, delavirdine, nevirapine), six PIs (amprenavir, saquinavir, nelfinavir, ritonavir, lopinavir, indinavir), and one fusion inhibitor (enfuvirtide). NRTIs mimic nucleosides and target HIV-1 RT by competing with natural deoxynucleoside triphosphates for binding to RT and incorporation into newly synthesized viral DNA resulting in chain-termination. NNRTIs inhibit HIV-1 RT by binding near the catalytic site of RT and acting as noncompetitive inhibitors. PIs work at the late stage of viral replication to prevent infectious virus production from infected cells. They block the HIV protease enzyme, which is necessary for the production of mature virions, resulting in defective particles that are unable to infect new cells. Enfuvirtide, also known as T20, is a gp41 membrane fusion inhibitor.

Unfortunately, HIV develops resistance to antiretroviral drugs over time usually from the accumulation of multiple mutations. HAART regimens are also associated with acute toxicities such as diarrhea, kidney stones, rash, central nervous system (CNS) toxicities and hepatotoxicity. Long-term toxicities from antiretroviral therapies include mitochondrial toxicities (lactic acidosis, myopathy, neuropathy, pancreatitis) associated with NRTIs and disorders of lipid metabolism (dyslipidemia) and glucose metabolism (lipodystophy, hypercholesterolemia, hypertriglyceridemia) associated with PIs. These tolerability issues make compliance to therapy more challenging. Compliance is an important determinant of successful virologic suppression for patients on HAART. There is a need for new anti-HIV drugs that are well-tolerated and have reduced pill burden as well as new modes of action and a low likelihood of viral resistance development.

2. Non-clinical Microbiology

The non-clinical microbiology of nelfinavir was previously reviewed for the original NDA. A brief overview is presented here along with new findings.

2.1. Mechanism of Action

Nelfinavir is a selective non-peptidic inhibitor of HIV-1 protease with a K_i of 1.7 nM. Inhibition of the viral protease prevents cleavage of the *gag-pol* polyprotein resulting in the production of immature, non-infectious virus.

2.2. *In vitro* Studies

The *in vitro* antiviral activity of NFV was analyzed in lymphoblastoid cell lines, PBLs and monocytes/macrophages against both acute and chronic HIV infections. NFV has activity against several laboratory strains and clinical isolates of HIV-1 and

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the HIV-2 strain ROD. The EC_{95} of NFV ranged from 7 to 196 nM with a mean EC_{50} of 21 nM and EC_{95} of 58 nM.

To support NFV use in combination with other antiretroviral agents, the *in vitro* combination activity relationships of NFV with all the drugs approved since Viracept's approval were evaluated. Previously, the antiviral and cytotoxic effects of NFV in two-drug combinations with AZT, 3TC, ddC, d4T, or ddI and a three-drug combination of NFV with AZT and 3TC demonstrated additive to synergistic interactions. Minimal cellular cytotoxicity was observed with any of these drugs alone or in combination. Two-drug combination experiments of NFV with IDV, SQV, or RTV demonstrated that the interaction of NFV with SQV or RTV was additive, but the interaction of NFV with IDV was slightly antagonistic. For this submission, antiviral and cytotoxic effects of NFV in two-drug combinations with PIs, APV and LPV, NRTIs, ABC and TDF, and NNRTIs, DLV, EFV, and NVP, were analyzed by the Pritchard and Shipman method. Results from this study indicated that the combination of NFV with APV, LPV, DLV, EFV, NVP, ABC or TDF results in moderate to strongly synergistic interactions in antiviral activity (Table 1, page 017 of submission 000/B2). The combination of NFV with APV resulted in a mean total synergistic volume of 59 μM^2 % with minimal volumes of antagonism suggesting moderate synergy while the combination of NFV with LPV resulted in a mean total synergistic volume of 232 μM^2 % with minimal volumes of antagonism observed indicative of strong synergy. The combination of NFV with the NNRTIs, DLV, EFV, or NVP, resulted in mean synergistic volumes of 217, 158, and 187 μM^2 %, respectively, with minimal volumes of antagonism suggesting strong synergism. The combination of NFV with NRTIs, ABC or TDF, resulted in mean synergistic total volumes of 186 and 295 μM^2 %, respectively, with no volumes of antagonism detected suggesting strong synergism of NFV with these drugs. Minimal to no cellular cytotoxicity was observed with any of these compounds alone are in combination with NFV. These results indicate that NFV with other approved anti-HIV agents except for IDV may result in enhanced antiviral activity.

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Table 1. The Antiviral Effects of the *In Vitro* Combination of NFV and Other Anti-HIV Agents.

Second Compound	Class	Synergy[a] ($uM^2\%$)	Antagonism[a] ($uM^2\%$)
Amprenavir	PI	59	-12
Lopinavir	PI	232	-0.54
Delavirdine	NNRTI	217	-2.8
Efavirenz	NNRTI	158	-2.2
Nevirapine	NNRTI	187	-0.51
Abacavir	NRTI	186	0
Tenofovir	NRTI	295	0

[a]Volumes were calculated by MacSynergy™ II software at 95% confidence interval. Results represent the mean of two experiments. Antiviral effects were determined by XTT dye reduction six days after infection of CEM-SS cells with HIV-1 RF. All experiments were performed in triplicate plates.

2.3. Resistance Studies

2.3.1. Resistance

HIV-1 isolates with reduced susceptibility to NFV have been selected *in vitro*. HIV isolates from selected patients treated with nelfinavir alone or in combination with reverse transcriptase inhibitors were monitored for phenotypic (n = 19) and genotypic (n = 195, 157 of which were evaluable) changes in clinical trials over a period of 2 to 82 weeks. One or more viral protease mutations at amino acid positions 30, 35, 36, 46, 71, 77 and 88 were detected in the HIV-1 of >10% of patients with evaluable isolates. Of 19 patients for which both phenotypic and genotypic analyses were performed on clinical isolates, 9 showed reduced susceptibility (5- to 93-fold) to nelfinavir *in vitro*. All 9 patients possessed one or more mutations in the viral protease gene. Amino acid position 30 appeared to be the most frequent mutation site. The overall incidence of the D30N mutation in the viral protease of evaluable isolates from patients (n=157) receiving nelfinavir monotherapy or nelfinavir in combination with zidovudine and lamivudine or stavudine was 54.8%. The overall incidence of other mutations associated with primary protease inhibitor resistance was 9.6% for the L90M substitution whereas substitutions at 48, 82, or 84 were not observed.

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2.3.2. Cross-Resistance

The — assay was utilized to determine PI susceptibility of a panel of 44 patient-derived PI-resistant HIV-1 isolates containing clinically relevant PI-resistance associated mutations (Table A and B). These isolates demonstrated > 2.5-fold resistance to at least one of the approved PIs, NFV, APV, SQV, IDV, or LPV. Four of the 44 PI-resistant isolates contained the NFV-associated resistance mutation D30N together with other PI-resistance mutations. All four viruses had high-level resistance (>10-fold) to NFV with a median fold-change in NFV susceptibility of 55.5, but remained susceptible to the other evaluated PIs, including LPV (Table A and B). The L90M mutation also conferred resistance to NFV but was not uniquely associated with NFV resistance. Eight of the 44 isolates contained the L90M mutation as well as other PI-resistance mutations. These eight isolates had a median fold-change of 15.5 in NFV susceptibility and none were susceptible to NFV. However, 50%, 38%, and 25%, of the eight L90M containing viruses were susceptible to LPV, APV, and IDV, respectively. Only one (13%) of the eight was susceptible to SQV.

Table A. Cross-resistance of HIV-1 Isolates

	# susceptible isolates (%)					
Compound	Contain D30N N = 4	Contain L90M N = 8	LPV-Resistant N = 30	APV-Resistance N = 27	IDV-Resistant N = 34	SQV-Resistant N = 29
NFV	0 (0)	0 (0)	2 (7)	1 (4)	0 (0)	0 (0)
LPV	4 (100)	4 (50)	0 (0)	1/26 (4)	4/32 (13)	4/27 (15)
APV	4 (100)	3 (38)	5 (17)	0 (0)	8 (24)	6 (21)
IDV	4 (100)	2 (25)	2 (7)	1 (4)	0 (0)	3 (10)
SQV	4 (100)	1 (13)	7 (23)	4 (15)	8 (24)	0 (0)

Table B. Cross-resistance of HIV-1 Isolates (Median Fold-change)

	Median fold-change					
Compound	Contain D30N N = 4	Contain L90M N = 8	LPV-Resistant N = 30	APV-Resistance N = 27	IDV-Resistant N = 34	SQV-Resistant N = 29
NFV	55.5	15.5	22.5	29.5	21	34
LPV	1.2	3.1	13.1	13.2	13	14
APV	1.1	4.3	5.4	6.5	4.9	5.1
IDV	1.8	10.3	17.5	23.8	12.5	22
SQV	2.2	3.8	10.3	15	10.3	13

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Additionally, the cross-resistance of NFV to isolates resistant to recently approved protease inhibitors was evaluated. The evidence from phenotypic analyses of the 44 clinical isolates suggests high cross-resistance between NFV with the other PIs evaluated (APV, LPV, IDV and SQV). Table A shows the number of isolates remaining susceptible to the PIs analyzed and Table B shows the median fold-change in the isolates for each PI. Of the 30 isolates that displayed resistance to LPV, the median fold-resistance to NFV was 22.5 and only two (7%) still remained susceptible to NFV. APV-resistance was demonstrated in 27 isolates and these isolates had a median fold-change in susceptibility to NFV of 29.5 with only one (4%) isolate remaining susceptible to NFV. None of the IDV-resistant (n=34) or SQV-resistant (n=29) isolates retained susceptibility to NFV with median fold-changes in NFV susceptibility of 21 and 34, respectively. Mutations associated with resistance to other PIs (e.g. G48V, V82A/F/T, I84V, L90M) appear to confer high-level cross-resistance to NFV.

3. Conclusions

Drug combination studies with protease inhibitors showed nelfinavir had antagonistic interactions with indinavir, additive interactions with ritonavir or saquinavir and synergistic interactions with amprenavir and lopinavir. Minimal to no cellular cytotoxicity was observed with any of these protease inhibitors alone are in combination with nelfinavir. In combination with reverse transcriptase inhibitors, nelfinavir demonstrated additive (didanosine or stavudine) to synergistic (zidovudine, lamivudine, zalcitabine, abacavir, tenofovir, delavirdine, efavirenz, or nevirapine) antiviral activity *in vitro* without enhanced cytotoxicity.

Patient-derived recombinant HIV-1 isolates containing the D30N mutation (n=4) demonstrated high-level (>10-fold) NFV-resistance but remained susceptible (<2.5-fold resistance) to saquinavir, indinavir, lopinavir, and amprenavir *in vitro*. Patient-derived recombinant HIV-1 isolates containing the L90M mutation (n=8) demonstrated moderate to high-level resistance to NFV and had varying levels of susceptibility to saquinavir, indinavir, lopinavir, and amprenavir *in vitro*. Most patient-derived recombinant isolates with phenotypic and genotypic evidence of reduced susceptibility (>2.5-fold) to lopinavir, amprenavir, saquinavir and/or indinavir demonstrated high cross-resistance to nelfinavir *in vitro*. Mutations associated with resistance to other PIs (e.g. G48V, V82A/F/T, I84V, L90M) appeared to confer high-level cross-resistance to NFV.

4. References

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