CENTER FOR DRUG EVALUATION AND RESEARCH

APPROVAL PACKAGE for:

APPLICATION NUMBER: 020779

TRADE NAME: Viracept Tablets 250 mg

GENERIC NAME: Nelfinavir mesylate

SPONSOR: Agouron Pharmaceutical, Inc.

APPROVAL DATE: 03/14/97

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville MD 20857

NDA 20-778
NDA 20-779

MAR 14 1997

Agouron Pharmaceuticals, Inc
Attention: Barry Quart, Pharm.D.
10350 North Torrey Pines Road
La Jolla, California 92037-1020

Dear Dr. Quart:

Please refer to your new drug applications dated December 20, 1996, received December 26, 1996, submitted under section 505(b) of the Federal Food, Drug, and Cosmetic Act for VIRACEPT® (nelfinavir mesylate) Oral Powder 50 mg/g and VIRACEPT® (nelfinavir mesylate) Tablets 250 mg.

We acknowledge receipt of your submissions dated:

January 17, 1997
January 20, 1997
January 31, 1997
February 11, 1997
February 17, 1997
February 20, 1997
February 25, 1997
February 26, 1997
February 27, 1997
March 1, 1997
March 3, 1997
March 4, 1997
March 6, 1997
March 10, 1997
March 14, 1997

The User Fee goal date for these applications is June 26, 1997.

These new drug applications provide for the use of VIRACEPT for the treatment of HIV infection when therapy is warranted.

We have completed the review of these applications, including the submitted draft labeling, according to the regulations for accelerated approval and have concluded that adequate information has been presented to approve VIRACEPT (nelfinavir mesylate) Oral Powder 50 mg/g and VIRACEPT (nelfinavir mesylate) Tablets 250 mg for use as recommended in the draft labeling in the submissions dated March 14, 1997. Accordingly, these applications are approved under 21 CFR 314.520. Approval is effective on the date of this letter.

The final printed labeling (FPL) must be identical to the draft labeling submitted on March 14, 1997. Marketing the products with FPL that is not identical to this draft labeling may render the products misbranded and unapproved new drugs.

Please submit 20 copies of the FPL as soon as it is available, in no case more than 30 days after it is printed. Please individually mount ten of the copies on heavy-weight paper or similar material. For administrative purposes, this submission should be designated "FINAL PRINTED LABELING" for approved NDAs 20-778, 20-779. Approval of this submission by FDA is not required before the labeling is used.

Should additional information relating to the safety and effectiveness of the drugs become available, revision of that labeling may be required.

Products approved under the Accelerated Approval Regulations (21 CFR 314.510) require further adequate and well-controlled studies to verify and describe clinical benefit. We acknowledge and concur with your Accelerated Approval commitments outlined in your letter dated March 13, 1997. Your commitments are as follows:

We remind you of your Phase 4 commitments specified in your submission dated March 13, 1997. These commitments, along with any completion dates agreed upon, are listed below.

Protocols, data, and final reports should be submitted to your IND for this product and a copy of the cover letter sent to this NDA. Should an IND not be required to meet your Phase 4 commitments, please submit protocol, data, and final reports to this NDA as correspondences. In addition, we request under 21 CFR 314.81(b)(2)(vii) that you include in your annual report to this application, a status summary of each commitment. The status summary should include the number of patients entered in each study, expected completion and submission dates, and any changes in plans since the last annual report. For administrative purposes, all submissions, including labeling supplements, relating to these Phase 4 commitments must be clearly designated "Phase 4 Commitments."

We also remind you that, under 21 CFR 314.550, after the initial 120 day period following this approval, you must submit all promotional materials, including promotional labeling as well as advertisements, at least 30 days prior to the intended time of initial dissemination of the labeling or initial publication of the advertisement.

Validation of the regulatory methods has not been completed. At the present time, it is the policy of the Center not to withhold approval because the methods are being validated. Nevertheless, we expect your continued cooperation to resolve any problems that may be identified.

Please submit one market package of the drug product when it is available.

We remind you that you must comply with the requirements for an approved NDA set forth under 21 CFR 314.80 and 314.81.

If you have any questions, please contact Kimberly A. Struble, R.Ph., Regulatory Management Officer, at (301) 827-2335.

Sincerely yours,

David Feigal, M.D., M.P.H.
Director
Office of Drug Evaluation IV
Center for Drug Evaluation and Research

FINAL PRINTED LABELING HAS NOT BEEN SUBMITTED TO THE FDA.

DRAFT LABELING IS NO LONGER BEING SUPPLIED SO AS TO ENSURE ONLY CORRECT AND CURRENT INFORMATION IS DISSEMINATED TO THE PUBLIC.

DRUG STUDIES IN PEDIATRIC PATIENTS
(To be completed for all NME's recommended for approval)

NDA # 20-778 and 20-779 Trade (generic) names VIRACEPT (nelfinavir mesylate) Tablets and Oral Powder

Check any of the following that apply and explain, as necessary, on the next page:

___ 1. A proposed claim in the draft labeling is directed toward a specific pediatric illness. The application contains adequate and well-controlled studies in pediatric patients to support that claim.

___ 2. The draft labeling includes pediatric dosing information that is not based on adequate and well-controlled studies in children. The application contains a request under 21 CFR 210.58 or 314.126(C) for waiver of the requirement at 21 CFR 201.57(f) for A&WC studies in children.

___ a. The application contains data showing that the course of the disease and the effects of the drug are sufficiently similar in adults and children to permit extrapolation of the data from adults to children. The waiver request should be granted and a statement to that effect is included in the action letter.

___ b. The information included in the application does not adequately support the waiver request. The request should not be granted and a statement to that effect is included in the action letter. (Complete #3 or #4 below as appropriate).

___ 3. Pediatric studies (e.g., dose-finding, pharmacokinetic, adverse reaction, adequate and well-controlled for safety and efficacy) should be done after approval. The drug product has some potential for use in children, but there is no reason to expect early widespread pediatric use (because, for example, alternative drugs are available or the condition is uncommon in children.

___ a. The applicant has committed to doing such studies as will be required.

_X_ (1) Studies are ongoing. (***See explanation on page 2)***
___ (2) Protocols have been submitted and approved.
___ (3) Protocols have been submitted and are under review.
___ (4) If no protocol has been submitted, on the next page explain the status of discussions.

___ b. If the sonsor is not willing to do pediatric studies, attach copies of FDA's written request that such studies be done and of the sponsor's written response to that request.

___ 4. Pediatric studies do not need to be encouraged because the drug product has little potential for use in children.

__ 5. If none of the above apply, explain.

Explain, as necessary, the foregoing items:_____

Kimberly A. Struble 3/14/97

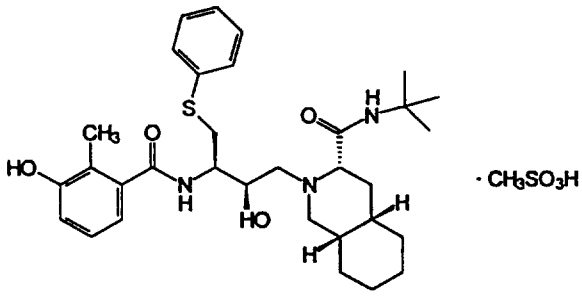
Signature of Preparer Date

cc: Orig NDA
HFD-5?/Div File
NDA Action Package

NDA 20-778 and NDA 20-779

MEDICAL OFFICER'S REVIEW OF NEW DRUG APPLICATION

1 GENERAL INFORMATION

DATE OF SUBMISSION:	December 20, 1996
DATE OF COMPLETION:	April 28, 1997
DRUG BRAND NAME:	Viracept®
DRUG GENERIC NAME:	Nelfinavir Mesylate
DRUG CODE NAME:	AG1343
DRUG CLASS:	HIV protease inhibitor
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).
STRUCTURE:	
INDICATION:	HIV infection when antiretroviral therapy is warranted.
FORMULATIONS:	Viracept (nelfinavir mesylate) 250 mg tablets (NDA 20-779) Viracept Oral Powder containing 50 mg (as free base) in each level scoopful (1 gram) (NDA 20-778)
DOSAGE:	Adults: 750 mg orally three times per day. Pediatric Patients: 20-30 mg/kg/dose TID
APPLICANT:	Agouron Pharmaceuticals, Inc. 10350 North Torrey Pines Road La Jolla, CA 92037-1020 (619) 622-8000

2 RESUME

The applicant submitted six clinical studies supporting NDA 20-779 (Viracept tablets), and preliminary data from two pediatric studies in support of NDA 20-778 (Viracept oral powder). Studies 511, 506, and 505 were double-blind, placebo-controlled studies of Viracept as monotherapy or in combination with nucleoside analogs in the treatment of HIV-infected patients. The applicant considered these three studies as the main studies in support of Viracept activity and safety.

Study 511 was a phase 3, randomized, double-blind, placebo-controlled study of Viracept in combination with zidovudine plus lamivudine versus zidovudine plus lamivudine alone in HIV-infected patients who were antiretroviral naive. The effects of nelfinavir in combination with zidovudine and lamivudine on the surrogate markers of HIV disease (viremia and CD4 cell count) were superior to the effects of zidovudine plus lamivudine alone (this is best summarized in figures 3, 4, and 5; pages 46, 47 and 48). The most common adverse event associated with nelfinavir therapy was diarrhea. Diarrhea appeared to be tolerated by most patients either without treatment or with symptomatic treatment with antimotility agents. Other adverse events, clinical or laboratory, do not appear to be particularly associated with nelfinavir use.

Study 506 was a phase 3, randomized, double-blind, placebo-controlled study of Viracept in combination with stavudine versus stavudine alone in HIV-infected patients. The effects of nelfinavir in combination with stavudine on surrogate markers of HIV disease (plasma HIV-RNA and CD4 cell count) were superior to the effects of stavudine alone (this is best summarized in figures 7, 8, and 9; pages 85 and 86). The most common drug associated adverse event was diarrhea. Diarrhea appeared to be tolerated by most patients either without treatment or with symptomatic treatment with antimotility agents. Other adverse events, clinical or laboratory, do not appear to be particularly associated with nelfinavir use.

Study 505 was a phase 2, randomized, double-blind, placebo-controlled, dose range-finding study of Viracept as monotherapy in HIV-infected patients. Patients in the placebo arm were re-randomized to either nelfinavir 750 mg or nelfinavir 500 mg arm after four weeks of treatment. Nelfinavir administered as monotherapy at 750 mg and 500 mg TID was more effective than placebo in reducing HIV RNA and increasing CD4 lymphocyte counts during the first four weeks of treatment. The effects of nelfinavir monotherapy in these surrogate markers decreased with time. By 16 weeks, the majority of patients had either switched therapy or added other antiretrovirals to their regimen. Diarrhea was the most common adverse event found with a higher incidence in patients receiving nelfinavir 750 mg TID.

Results of these and other supportive studies demonstrate that Viracept is both active in the treatment of HIV infection as measured by surrogate marker changes and safe at the recommended doses in adults and pediatric patients. Therefore, approval of Viracept tablets and Viracept Oral Powder formulations is recommended for accelerated approval as stipulated in CFR 314.510.

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4 CHEMISTRY, MANUFACTURING, AND CONTROL INFORMATION

(Please refer to Chemistry review by Dr. Paul Liu dated March 14, 1997)

Nelfinavir mesylate is a white to off-white amorphous powder, slightly soluble in water at pH $\leq$ 4, and freely soluble in methanol, ethanol, isopropanol and propylene glycol. Viracept® is presented as a light blue, capsule-shaped tablet in a 250 mg strength (as nelfinavir base). Viracept® is also presented as oral powder in bottles of 50 mg/g strength (as nelfinavir base). The chemical name for nelfinavir mesylate is [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). Its molecular weight is 663.90 (567.79 as the free base). Dr. Liu did not identify issues precluding approval. Chemistry recommendations by Dr. Liu have been incorporated in the proposed labeling by the applicant.

5 PHARMACOLOGY AND TOXICOLOGY INFORMATION

(Please refer to Pharmacology review by Dr. Ken Hastings dated March 6, 1997).

A series of GLP pharmacology and toxicology studies have been done both *in vitro* and *in vivo* using mice, rats, beagle dogs, and cynomolgos monkeys to support the clinical evaluation of nelfinavir. Studies of mutagenicity, toxicity and reproductive toxicity were conducted by the applicant. Results of these studies are discussed in Dr. Hastings review. Dr. Hastings did not identify issues precluding approval. Pharmacology and Toxicology recommendations by Dr. Hastings have been incorporated in the proposed labeling by the applicant.

6 MICROBIOLOGY INFORMATION

(Please refer to Microbiology review by Drs. Shukal Bala and Lauren Iacono-Connors dated March 13, 1997).

Nelfinavir is an inhibitor of the HIV-1 protease. Inhibition of the viral protease prevents cleavage of the gag-pol polyprotein resulting in the production of immature, non-infectious virus. The antiviral activity of nelfinavir *in vitro* has been demonstrated in both acute and/or chronic HIV infections in lymphoblastoid cell lines, macrophages, peripheral blood mononuclear cells. HIV isolates with reduced susceptibility to nelfinavir have been selected *in vitro*. Drs. Bala and Iacono-Connors did not identify issues precluding approval. Microbiology recommendations by Drs. Bala and Iacono-Connors have been incorporated in the proposed labeling by the applicant.

7 CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS INFORMATION

(Please refer to Biopharmaceutics review by Dr. Kellie Reynolds which is pending at this time)

Absorption: The applicant conducted studies that demonstrated that the administration of nelfinavir with food prolongs and increases absorption. However, the effects of different meals on the pharmacokinetics of nelfinavir was not investigated. **Distribution:** Nelfinavir is highly protein bound (>98%) to serum proteins. **Metabolism:** Nelfinavir is extensively converted to many oxidative metabolites. The predominant circulating metabolite was the hydroxy-*t*-butylamide, M8 (12-14%). In vitro antiviral activity tests indicated that M8 was of comparable potency to nelfinavir in acute HIV-1 infection models, suggesting that M8 may contribute to antiviral effects in patients. Using human liver microsomes, cDNA-expressed cytochrome P450 isoforms, and specific chemical inhibitors of cytochrome P450 isoforms, it was determined that the major enzyme metabolizing nelfinavir is CYP3A4. CYP3A4 appears to be responsible for approximately 50% of nelfinavir metabolism. Other isoforms appear to be involved: CYP2C19, CYP2D6, and CYP2C9. **Elimination:** Over a period of seven days, the median radiochemical recovery after a single dose of [^{14}C]nelfinavir was 89%, with 87% recovered in feces and 1.6% recovered in urine. **Drug Interaction Studies:** A series of drug interaction studies of nelfinavir with drugs likely to be used by HIV-infected patients were conducted by the applicant. Results of these studies are discussed in detail by Dr. Reynolds in her review.

8 STATISTICAL INFORMATION

(Please refer to Statistical review by Dr. Michael Elashoff, dated March 19, 1997)

Controlled studies 511, 506, and 505 were analyzed by applicant following the Intent-to-Treat approach specified in the protocols. For noncontrolled, open label studies, a descriptive statistical analysis was done. These statistical approaches to the data are discussed in detail in Dr. Elashoff's review. The statistical review also discusses in detail the numerical interpretations of the laboratory assay used in the study to quantify plasma HIV RNA.

9 LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

3TC:	Lamivudine
AIDS:	Acquire Immunodeficiency Syndrome
ADC:	AIDS Defining Condition
ADE:	Adverse Drug Event
ANC:	Absolute Neutrophil Count
BID:	Two times per day
CDC:	Center for Disease Control and Prevention
CPCRA:	Community Programs for Clinical Research on AIDS
CRF:	Case Report Form
d4T:	Stavudine
dL:	Deciliter
DSMB:	Data Safety Monitoring Board
FDA:	Food and Drug Administration
g:	Gram
GCP:	Good Clinical Practice
HIV:	Human Immunodeficiency Virus
IRB:	Institutional Review Board
ITT:	Intent To Treat
mL:	Milliliter
mm^3:	Cubic milliliter
NDA:	New Drug Application
TID:	Three times per day
RNA:	Ribonucleic Acid
RTIs:	Reverse Transcriptase Inhibitors
ULN:	Upper Limit of Normal
ZDV:	Zidovudine

10 ETHICS

According to the sponsor, all studies were conducted in accordance with the Declaration of Helsinki and United States Good Clinical Practice. The protocol, consent documents, and protocol amendments were approved by each site's Institutional Review Board (IRB). Written informed consent from each patient who participated in these studies, or his/her authorized representative, was required before study enrollment.

11 INVESTIGATORS AND STUDY SITES

Study AG1343-511: This study was conducted at 28 geographically dispersed sites across the United States.

Study AG1343-506: This study was conducted at 26 geographically dispersed sites across the United States.

> **Comment: Because Site 38 (Chicago, Illinois) had numerous protocol violations, patients enrolled at this site were not included in the applicants final analysis of the data. Twenty-four patients in study 511 and eight in study 506 were excluded from analysis.**

Study AG1343-505: This study was conducted at nine geographically dispersed sites across the United States.

> **Comment: The applicant included a list of all investigators and study sites in appendix 2 of each study report.**

12 INTRODUCTION

The applicant, Agouron Pharmaceuticals, Inc., submitted its first IND for nelfinavir in June, 1995. In December, 1996, Agouron submitted to the FDA two NDAs in support of accelerated approval of nelfinavir tablets and nelfinavir oral powder. The applicant followed a very rapid drug development process in which several clinical trials were ongoing simultaneously.

The applicant's studies used surrogate markers of response to therapy to assess activity, especially changes in plasma HIV RNA as measured by a bDNA assay, and changes in CD4 lymphocyte counts. The sponsor is currently conducting a clinical confirmatory trial in collaboration with CPCRA.

Agouron Pharmaceuticals, Inc., has also developed a formulation suitable to pediatric patients who cannot swallow tables. This formulation is presented as an oral powder to be mixed with food. The applicant provided dosing information for pediatric patients based on a study of nelfinavir single- and multiple-dose. Safety information in pediatrics is limited and the Applicant is collecting more safety information in pediatric patients. However, the perceived need of drugs of this class and the potential benefits to the pediatric patients outweigh the risks of recommending approval of this drug for use in children with the relatively small safety data base available at this time.

Nelfinavir is a non-peptidic inhibitor of HIV-1 protease. The compound was designed using knowledge of the three dimensional structure of the active site of the protease. This activity is specific for the viral protease since no activity ($K_i > 1000nM$) was recorded against a variety of human aspartyl proteases, e.g., cathepsin E, pepsin and renin. Antiviral efficacy of nelfinavir was tested in a panel of acute infection models. The mean ED_{50} value was 22 nM with a range of 2.5-60 nM and the mean ED_{95} value was 59 nM with a range of 7-130 nM. The effect of nelfinavir on the cleavage of the precursor polyprotein p55 gag to its processed product, p24, has also been examined in a chronically infected cell model (HIV1 IIIB in CEM-SS cells). Western blot analysis of solubilized virions from nelfinavir-treated cells indicated a dose-related inhibition of p55 processing to p24. According to the applicant these results confirm that the antiviral efficacy of nelfinavir is due to its inhibition of HIV-1 protease. The resistance profile of nelfinavir to mutations in HIV-1 protease is currently under study. Nelfinavir is highly bound to protein, more than 98% in human serum. There did not appear to be a significant reduction in the antiviral efficacy of nelfinavir in the presence of increasing concentrations (up to 50%) of either human or bovine serum. Clinical studies of nelfinavir started in Europe after the preclinical toxicology and pharmacokinetic studies were completed.

The first clinical study was **Protocol AG1343-501-01** conducted in the United Kingdom. "A Phase I, Double-Blind, Placebo-Controlled, Ascending Single Oral Dose Safety, Tolerability, Pharmacokinetic and Food Interaction Study in 12 Healthy Male Volunteers." Two groups of 6 volunteers received the following single doses of nelfinavir:

Group A	100 mg with food
Group B	200 mg with food
Group A	400 mg with food
Group B	800 mg with food
Group A	400 mg fasted
Group B	800 mg fasted

In each group, two male volunteers received placebo and four volunteers received nelfinavir. Results are summarized in the Table 1:

TABLE 1
Nelfinavir Pharmacokinetics in Fed and Fasted States

Dose (mg)	Condition	C_{max} Mean (ng/ml)	t_{max} Mean (h)	AUC Mean (ng.h/mL)	$t_{½}$ Mean (h)
100	Fed	313	3.25	1140	1.83
200	Fed	440	2.75	1754	2.21
400	Fed	1351	4.00	7820	2.41
400	Fasted	995	1.63	4030	1.87
800	Fed	3165	3.50	23212	3.42
800	Fasted	1567	1.63	6168	2.40

Administering nelfinavir on an empty stomach resulted in AUC values 27-50% of those observed when the drug was administered with food. Plasma concentrations up to 4 μg/ml were not associated with any significant toxicity.

Protocol AG1343-502-01, "A Phase I, Double-Blind, Placebo-Controlled, Multiple Oral Dose Safety, Tolerability and Pharmacokinetic Study in 14 Healthy Male Volunteers," was completed on December 17, 1994. This study included two groups of 7 volunteers: two volunteers received placebo and five received nelfinavir in a blinded manner. Each group received drug for seven days, with serial blood sampling on Day 1 and Day 7. The two groups of volunteers received the following multiple dose regimens of nelfinavir:

Group A	400 mg every 12 hours with food
Group B	300 mg every 8 hours with food

In Group A, steady-state was achieved by Day 4. Mean trough plasma levels were 586 ng/ml by day 7, which is approximately 15 times the ED_{95} of nelfinavir (40 ng/ml). Only mild adverse experiences were reported during the first four days of dosing, including headache (3), flatulence (2), dizziness (1), and lightheadedness (1). No adverse experiences were reported during the last three days of dosing by volunteers receiving active drug. In group B, steady-state levels appeared to be achieved by Day 5. Mean trough plasma concentrations in excess of 600 ng/ml (15 times the ED_{95}) were observed on Day 7. Peak concentrations of over 2000 ng/ml were well tolerated. Only one volunteer reported adverse experiences during the study and these were

not considered drug related: runny nose, cough and earache. The sponsor concluded that one week of multiple dosing with AG1343 was well tolerated at doses up to 900 mg/day.

Protocol AG1343-504-01, "A Phase II, Open-Label Study of AG1343 Given Orally in HIV Positive Patients." This study was designed to evaluate the pharmacokinetics, antiretroviral activity and safety of AG1343 HIV positive patients for 28 days. Preliminary data from 8 of the 10 patients enrolled in this study demonstrated antiretroviral activity by decreases in viral load as measured by PCR and bDNA.

Protocol AG1343-503, "A Pilot Phase II, Open-Label, Dose Range-Finding Study of Nelfinavir in HIV Positive Patients." This was the original protocol submitted under an IND to the Food and Drug Administration in the USA. This was a dose finding escalation study using 500, 600, and 750 mg BID and 500, 750, and 1,000 mg TID of Nelfinavir. According to the sponsor, patients tolerated the drug well. Diarrhea was the most common adverse event observed and appeared to be dose dependent. Nelfinavir mesylate demonstrated antiretroviral activity and was well tolerated as monotherapy in all regimens. Nelfinavir 1000 mg TID did not appear to provide a significant advantage over 750 mg TID to reduce plasma HIV RNA levels and this dose produced a higher incidence of $\geq$ grade 2 diarrhea (50%). After this study, the Applicant decided to continue development of the following treatment regimens: nelfinavir 750 mg TID and nelfinavir 500 mg TID.

Protocol AG1343-506, "A Phase III, Randomized, Double-Blind, Placebo-Controlled Study of VIRACEPT in Combination with Stavudine (d4T) versus Stavudine (d4T) Alone in HIV Positive Patients." Stavudine-naive patients were randomized to one of three treatment arms: stavudine (d4T) with placebo, stavudine (d4T) in combination with VIRACEPT 500 mg TID, or stavudine (d4T) in combination with VIRACEPT 750 mg TID. Patients received this therapy for 24 weeks. Patients were stratified according to previous zidovudine (ZDV) used. The primary endpoints were change from baseline in CD4 cell counts and quantitative HIV RNA titer. Results of this study are discussed in detail in Section 13.2 of this review.

Study AG1343-511, "A Phase III, Randomized, Double-Blind, Placebo-Controlled Study of VIRACEPT in Combination with Zidovudine (ZDV) + Lamivudine (3TC) Versus ZDV + 3TC Alone in HIV Positive Patients with $<$ 1 Month or No Prior Antiretroviral Treatment." The sponsor enrolled 316 patients; however, 19 patients were considered non-evaluable. According to the sponsor 297 patients are evaluable. The primary endpoints were change from baseline in CD4 cell counts and quantitative HIV RNA titer. Results of this study are discussed in detail in Section 13.1 of this review.

Study AG1343-505 was a monotherapy study of nelfinavir 500 mg TID and 750 mg TID compared to placebo. Results of this study are discussed in detail in Section 13.3 of this review.

The applicant is conducting (or has conducted) several other clinical trials with this drug: **Study AG1343-519** was a pharmacokinetic study of the interaction between Viracept and Terfenadine. **Study AG1343-520** was a pharmacokinetic study of the interaction between Viracept and Ketoconazole. **Study AG1343-521** was a pharmacokinetic study of the interaction between Viracept and Rifampin. **Study AG1343-525** is a safety study of Viracept in combination with other antiretroviral therapy. In the fall of 1996, the sponsor also initiated an expanded access program for patients unable to tolerate other protease inhibitors already on the market.

The three primary clinical studies in support of the application are: A double-blind, randomized, placebo-controlled monotherapy study of Nelfinavir 500 mg TID, Nelfinavir 750 mg TID and Placebo **[Study 505]**; A double-blind, randomized, placebo-controlled study of Nelfinavir 500 mg TID, Nelfinavir 750 mg TID, and placebo in patients receiving d4T (Stavudine) **[Study 506]**; and A double-blind, randomized, placebo-controlled study of Nelfinavir 500 mg TID, Nelfinavir 750 mg TID, and placebo in patients receiving ZDV (zidovudine) and 3TC (lamivudine) **[Study 511]**.

13 CLINICAL STUDIES

Indication: Treatment of HIV-infection when antiretroviral therapy is warranted.

13.1 STUDY NUMBER 1: STUDY 511

A Phase 3 Randomized, Double-Blind, Placebo-Controlled Study of Viracept™ In Combination with Zidovudine (ZDV) + Lamivudine (3TC) Versus ZDV+3TC Alone in HIV Positive Patients with < 1 Month or No Prior Antiretroviral Treatment.

13.1.1 STUDY OBJECTIVES

a) To evaluate the efficacy and safety of nelfinavir administered in combination with ZDV+3TC in HIV positive patients who had received ZDV for less than one month or had no prior antiretroviral treatment and who had quantitative plasma HIV RNA $\geq$ 15,000 copies/mL. The primary efficacy endpoints were change from baseline in quantitative plasma HIV RNA and CD4 lymphocyte counts.

b) To assess the potential for viral resistance by assessing changes in genotype, phenotype, and viral sensitivity.

Comment: The second objective and the viral resistance results obtained by this study are discussed in the microbiology review written by Drs. Bala and Iacono-Connors.

13.1.2 INVESTIGATIONAL PLAN

13.1.2.1 OVERALL STUDY DESIGN

This was a double-blind, placebo-controlled, randomized phase 3 study designed to compare the efficacy and safety of two different dose levels of nelfinavir in combination with ZDV and 3TC versus placebo+ZDV+3TC.

Two-hundred ten patients who were antiretroviral naive with quantitative plasma HIV RNA $\geq$ 15,000 copies/mL were to be randomized to one of three treatment groups:

Nelfinavir 500 mg three times daily (TID) + ZDV + 3TC (Treatment Group A)

Nelfinavir 750 mg TID + ZDV + 3TC (Treatment Group B)

Placebo + ZDV + 3TC (Treatment Group C)

TABLE 2
Study Design

Treatment Group	Number of Patients	Dose (mg) TID	Total Daily Dose (mg)
A	70	500	1500
B	70	750	2250
C	70	Placebo	Placebo
Total	210		

Patients were stratified by CD4 lymphocyte counts ($\leq$100, >100 and $\leq$ 300, or >300 cells/mm^3) in the randomization procedure to balance treatment groups.

Comment: The study accrued more patients than originally planned. A total of 605 patients were screened. Three-hundred sixteen patients were randomized into the study. Of the 316 patients randomized into the study, 18 patients were excluded from study for a variety of reasons. Two-hundred ninety-eight patients were considered evaluable: 100 in the nelfinavir 750 mg TID + ZDV + 3TC group, 97 in the nelfinavir 500 mg TID + ZDV + 3TC group, and 101 in the placebo + ZDV + 3TC group.

This was a 24-week study with an administrative analysis performed at 16 weeks. This administrative analysis was performed and submitted as part of the nelfinavir New Drug Application (NDA). The applicant agreed that other than for safety reasons, no changes in sample size or study design were to be made as a result of this analysis. For this reason, the FDA and the applicant agreed that no adjustments were to be made to the alpha level for the final analysis.

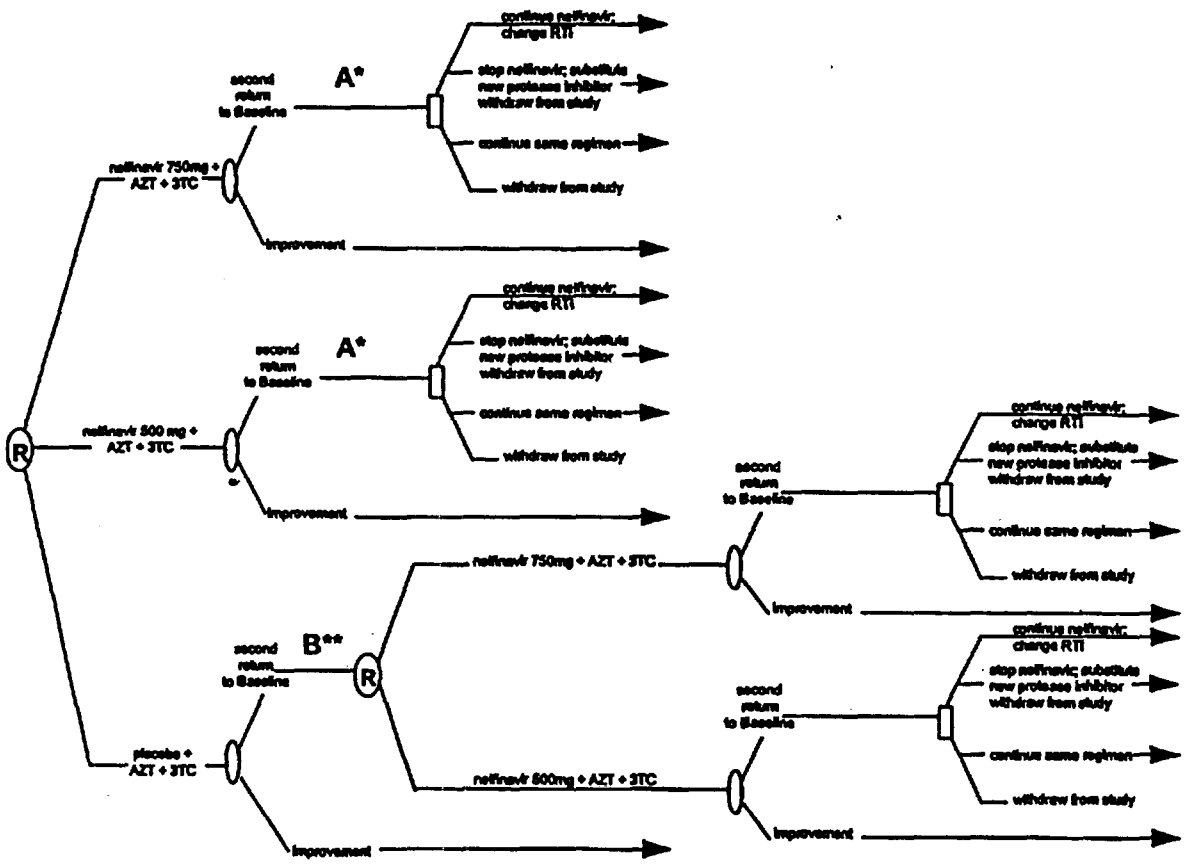
The primary activity variables were plasma HIV RNA and CD4 lymphocyte counts. An independent, unblinded DSMB monitored the ongoing safety of patients during the study and initiated changes in treatment based on objective failure criteria applied to the primary endpoints. Treatment failure is defined as the return to calculated baseline plasma HIV RNA or CD4 lymphocyte count on two consecutive study visits after at least four weeks of study drug treatment (this is referred as "second return to baseline" in Figure 1, page 20).

13.1.2.2 DISCUSSION OF STUDY DESIGN, INCLUDING THE CHOICE OF CONTROL GROUPS

Based on information obtained from phase 1 and 2 clinical trials, the sponsor had already chosen both 500 mg TID and 750 mg TID of nelfinavir as the doses and schedule to be used in larger clinical trials. In the design of this study, the sponsor was looking for information on the differences between dose regimens, if any. However, the main objective of the study was to compare both nelfinavir regimens to placebo. For ethical and current medical practice reasons all patients were receiving active treatment with a combination of nucleoside analogs (ZDV and 3TC). Therefore, at the time the study was conducted, this three arms were considered appropriate to answer the questions raised and also appropriate medical practice.

A risk of the study design was that the ZDV + 3TC combination alone could have demonstrated a significant effect on surrogate markers; therefore, making more difficult for nelfinavir to show an added benefit. However, the sponsor did not have many alternatives because at the time of this study combination therapy with nucleoside analogs had become the standard of care.

Figure 1: Schematic Study Design: Study 511



A* DSMB notifies Agouron and Agouron works with the investigators to decide a new RTI regimen or treatment.

B** DSMB notifies , to re-randomize the patient to either 750 mg or 500 mg nelfinavir and ship active drug.

R Indicates patients are randomized.

13.1.2.3 SELECTION OF STUDY POPULATION

13.1.2.3.1 INCLUSION CRITERIA

Patients eligible for this study were HIV positive patients 13 years of age or older. These patients had to have the following characteristics: a quantitative plasma HIV RNA level of $\geq$ 15,000 copies/mL at Screen 1, a Karnofsky Performance Status $\geq$ 70, and antiretroviral naive (no prior antiretroviral treatment or less than one month of cumulative lifetime exposure to ZDV).

Comment: CD4 cell counts were not a requirement for entry because it was expected that the major drug effect was going to be in viral load and not change of CD4 cell counts. CD4 cell count changes were expected as a consequence of significant decreases in viral load.

13.1.2.3.2 EXCLUSION CRITERIA

Patients were excluded if they had received prior antiretroviral therapy. Patients who had received immune modulators or vaccines within a month of baseline were also excluded. Patients with the following characteristics were also excluded: Pregnant or nursing patients, patients with neoplastic disease requiring systemic cytotoxic or radiation treatment, patients with acute pancreatitis or hepatitis, patients with opportunistic infections at baseline, patients who are active substance abuser, patients with clinically significant malabsorption syndrome (chronic diarrhea [four to ten stools per day of 30 days or greater duration]), patients with renal insufficiency (serum creatinine of > 2.5 mg/dL at screening visit 2).

Patients with the following laboratory abnormalities at screening visit 2 were also excluded: Serum bilirubin > 2.5 times the upper limit of normal, platelet count $\leq$ 50,000 cells/mm^3, absolute neutrophil count $\leq$ 750 cells/mm^3, hemoglobin < 8 g/dL, and liver enzymes > 5 times the upper limit of normal.

Comment: Exclusion of antiretroviral experienced patients from this study may make the study results difficult to generalize. In the future, the sponsor may need to conduct clinical studies in patients who have received other antiretroviral therapy including other protease inhibitors in order to demonstrate how patients who have received antiretroviral therapy respond to nelfinavir therapy. Patients with advanced HIV disease typically have one or more characteristics that would have

made them ineligible to enter into this study. Therefore, patients with one or more of these characteristics may not have the same safety and efficacy profile of the selected population of study 511. It has been recognized with other drugs that patients with advanced HIV disease have a different safety and efficacy profile than the selected patients included in clinical trials. Spontaneous reporting of Adverse Drug Events (ADE) may not be sufficient to develop a safety profile of the drug that mimics more closely its use in general practice.

13.1.2.3.3 REMOVAL OF PATIENTS FROM THERAPY OR ASSESSMENT

Patients were going to be removed from therapy for any of the following reasons:

A. The development of a toxicity or concurrent illness which precluded further study treatment.

B. Treatment failure.

C. Concurrent use of another antiretroviral drug, including a protease inhibitor, or unapproved use of an investigational agent.

Patients who discontinued study drug for any reason were to continue all study visits unless they were unable to do so or withdrew consent for further participation. At a minimum, patients who discontinued therapy were to have the Week 24 (final visit), Week 2 post study (Safety Follow Up), and Month 4 Vital Status Follow Up assessment completed.

Patients were to be removed permanently from the study for any of the following reasons:

D. The patient withdrew consent and refused further contact with the study (some patients withdrew consent but agreed to continue follow up).

E. The investigator was shown to be non-compliant with protocol requirements or GCP.

F. Termination of the study by sponsor.

13.1.2.4 TREATMENTS

13.1.2.4.1 TREATMENTS ADMINISTERED

All patients received ZDV + 3TC therapy throughout the study. Both ZDV and 3TC are nucleoside analogs approved by the FDA for the treatment of HIV infection, and are specifically approved as combination therapy.

Zidovudine and lamivudine were provided in commercial packaging. Lamivudine tablets were provided in bottles containing a four-week supply of 150 mg dosage strength (i.e., 60 tablets per bottle). ZDV capsules were provided in bottles containing a two-week supply of 100 mg dosage strength (i.e., 100 capsules per bottle). Commercial package labeling contained the following information: Drug identification, dosage strength, number of capsules/tablets per bottle, directions for use per labeling, lot number, expiration date, caution statement, manufacturer, storage instructions (ZDV was to be stored at 59°F to 77°F, 3TC was to be stored at 36°F to 86°F).

An additional label for ZDV and 3TC contained the following information:

- Protocol, site, and patient number
- Directions for 3TC: As recommended by the manufacturer, take one (1) tablet two (2) times daily or as directed by physician.
- Directions for ZDV: As recommended by the manufacturer, take two (2) capsules three (3) times daily or as directed by physician.
- Sponsor name and address.

Nelfinavir/placebo was packaged in blinded blister packs. Each blister pack consisted of seven daily cards containing sixty-three tablets of Viracept and/or placebo for one week (i.e., three doses per day for seven days). Each dose consisted of three tablets. For patients assigned to:

- Nelfinavir 750 mg, each dose consisted of three 250-mg tablets of nelfinavir.
- Nelfinavir 500 mg, each dose consisted of two 250-mg tablets of nelfinavir and one placebo tablet.
- Placebo, each dose consisted of three placebo tablets.

Directions for use were printed on each blister pack. Individual blister packs were labeled with a single-panel label containing the following information:

- Protocol, site, and patient number.
- Unique package number.
- Directions: Take as directed.
- Store at room temperature (59° to 86°F)
- Sponsor name and address.
- CAUTION: New Drug - Limited By Federal Law To Investigational Use

Space was provided on the label for patient initials and dispensing date. All drugs were for oral administration only.

Nelfinavir or matching placebo was to be administered with food and eight to 12 ounces of water. Zidovudine 200 mg TID and Lamivudine 150 mg BID were to be administered in combination with nelfinavir and placebo. Patients were to take ZDV at the same time as nelfinavir and 3TC with their morning and night doses of nelfinavir.

13.1.2.4.2 IDENTITY OF INVESTIGATIONAL PRODUCT

In Table 2, section 5.3, Volume 2, of the applicant's study report, there is a list of shipment dates and lot numbers of nelfinavir and placebo.

13.1.2.4.3 METHOD OF ASSIGNING PATIENTS TO TREATMENT GROUPS

At enrollment, patients were to be randomized to one of three treatment groups.

- nelfinavir 750 mg TID + ZDV + 3TC
- nelfinavir 500 mg TID + ZDV + 3TC
- placebo TID + ZDV + 3TC

The objective of the randomization was to provide a balance of treatment group assignments within each of three pre-defined patient strata, at each investigative site. The strata were:

- CD4 lymphocyte count $\leq$ 100 cells/mm^3
- CD4 lymphocyte count > 100 and $\leq$ 300 cells/mm^3
- CD4 lymphocyte count > 300 cells/mm^3

The randomization was implemented by the use of permuted blocks of the three treatments, with pre-assignment of a series of these blocks to each stratum separately at each study site. Patients within each stratum were sequentially assigned treatment according to the order of treatments within these blocks.

Patients were randomized through a central randomization center established -, using an interactive voice response (IVR) system. Upon receipt of a telephone call from an authorized study site, identifying a potential study patient, the IVR solicited identification and stratification information. A patient number was then assigned and provided to the caller, the assignment was documented in the randomization database, and a confirmation fax was sent to the site with the identification information and the study number assigned. At the same time, the system automatically provided faxed notification of the unblinded treatment assignment to ProClinical, initiating the drug shipment process. The randomization list is provided in appendix 5 of the study report.

13.1.2.4.4 SELECTION OF DOSES IN THE STUDY

Dose selection was justified by results from Study AG1343-503. In this open label dose-ranging monotherapy study, nelfinavir demonstrated antiviral activity and was tolerated by patients at doses of 500 mg BID, 600 mg BID, 750 mg BID, 500 mg TID, and 1,000 mg TID. The most frequently occurring grade 2 or greater adverse event was diarrhea. The lowest incidence of grade 2 or greater diarrhea was seen in patients receiving nelfinavir 500 mg or 750 mg TID. Nelfinavir 1,000 mg TID did not appear to provide a significant advantage over 750 mg TID in the reduction of plasma HIV RNA and this dose produced a higher incidence of grade 2 or greater diarrhea. Therefore, both doses 500 mg TID and 750 mg TID were chosen for the larger clinical trials and potential use in clinical practice.

13.1.2.4.5 SELECTION AND TIMING OF DOSE FOR EACH PATIENT

Patients were randomized to one of three treatment groups for 24 weeks: nelfinavir 750 mg TID + ZDV + 3TC, nelfinavir 500 mg TID + ZDV + 3TC, and placebo + ZDV + 3TC.

Nelfinavir or matching placebo was to be administered with food and eight to 12 ounces of water. Zidovudine 200 mg TID and 3TC 150 mg BID were to be administered in combination with nelfinavir or placebo. Patients were to take ZDV at the same time as nelfinavir and 3TC with their morning and night doses of nelfinavir.

Comment: Early in development, the sponsor conducted a pharmacokinetic study (Study 501) to compare dosing in fasting and fed state. Results of this study are summarized in the synopsis of this review (Section 2 of this review). Results of this study demonstrated that administering AG1343 on an empty stomach resulted in AUC values 27-50% of those observed when the drug was administered with food.

13.1.2.4.6 BLINDING

Nelfinavir/placebo was packaged in blinded blister packs. Each blister pack consisted of seven cards containing study drug for one week (i.e., three doses per day for seven days).

To ensure complete blinding of study drug supplied study drug to the site on a per patient basis. Upon randomization of a patient via the interactive voice response (IVR) system, fax notification automatically went to ProClinical to trigger the initial drug shipment for the patient. In an effort to maintain the study blind, all plasma HIV RNA results after Screen 1 were concealed from the investigator until after all of the patients at his/her site had completed their Week 24 evaluations.

13.1.2.4.7 PRIOR AND CONCOMITANT THERAPY

Patients were to have no prior protease inhibitor experience and no exposure to ddI, ddC, d4T, 3TC, nevirapine, delavirdine, or loviride.

At Screen 1, any patients who took ZDV within the preceding two weeks and who received less than one month of cumulative lifetime exposure participated in a two-week washout period.

Patients who received systemic cytotoxic or radiation treatment, or who received immune modulators or vaccines within one month of study entry were not allowed into the study.

All other medications were permitted during the study.

13.1.2.4.8 TREATMENT COMPLIANCE

Drug dispensing and return records were to be maintained for each patient for nelfinavir/placebo, ZDV, and 3TC.

Each time study drug was dispensed, the date and number of tablets dispensed to the patient were to be recorded on the CRFs. At each study visit, the date and number of tablets returned by the patient were to be recorded. If any tablets were unaccounted for, an explanation was to be provided. In addition, site staff were to record the number of missed doses of study drug.

Comment: The applicant did not provide data on treatment compliance other than a listing of patients who were found to be noncompliant by their investigator. There is no description on how patients were found to be noncompliant.

13.1.2.5 EFFICACY AND SAFETY VARIABLES

13.1.2.5.1 EFFICACY AND SAFETY MEASUREMENTS ASSESSED AND FLOW CHART

Virologic and Immunologic Endpoints: Efficacy of two different doses of VIRACEPT, administered in combination with ZDV + 3TC, versus ZDV + 3TC alone, were evaluated using virologic and immunologic markers of HIV disease progression. The primary endpoints of this study are the magnitude and duration of the changes from baseline between treatment arms of the primary virologic and immunologic markers (HIV RNA titer levels and CD4 cell count). The secondary endpoints included ADC (as defined in Appendix VII of the protocol), p24 antigen levels, percent CD4, percent and absolute CD8, CD4/CD8 ratio and QoL.

Changes of HIV RNA using the net change from baseline of the $\log_{10}$ transformation were evaluated as well as the durability of the decrease over the 24-week study period. Change from baseline in absolute CD4 cell counts was followed. The observed increase over the 24 week study period was assessed.

Adverse events including those suggesting disease progression (AIDS-Defining Condition by CDC criteria [ADC]) and changes from baseline in laboratory parameters were monitored and evaluated during the study (see schedule of events on next page). Patients were evaluated after 24 weeks of drug administration and, if eligible, allowed to continue for a 6-month extension period receiving open label nelfinavir. The vital status of patients was evaluated at 4 (3-6) months after the end of therapy. Adverse events and changes from baseline in laboratory parameters were graded using the Adverse Event Severity Grading Scale in Appendix II of protocol. All events were summarized, reported, and analyzed.

Table 3 (page 29) summarizes the schedule of events. This table was reproduced from the applicant's study protocol.

TABLE 3: Flow Chart of Study Procedures*

Parameter	Screen 1[a]	Washout	Screen 2 (Day -7)	Lab Visit[b] (Day -3)	Baseline (Day 0) Study Enrollment	Week 1	Week 2	Week 4	Week 6	Week 8	Week 12	Week 16	Week 20	Week 24[c] Last Visit	2 weeks post Rx[d]	Study Exten[e] (6 mo)	4 Mo (3-6 mo) Post Rx
Demographics			X														
Medical History			X														
Physical Exam			X[l]		X[l]	X	X	X	X	X	X	X	X	X	X	X	
Vital signs & weight			X[l]		X[l]	X	X	X	X	X	X	X	X	X	X	X	
Karnofsky Status			X[l]		X[l]	X	X	X	X	X	X	X	X	X	X	X	
Hematology			X		X		X	X	X	X	X	X	X	X	X	X	
Chemistry			X		X		X	X	X	X	X	X	X	X	X	X	
Urinalysis			X		X		X	X	X	X	X	X	X	X	X	X	
HIV RNA Titer	X		X	X	X	X	X	X	X	X	X	X	X	X		X	
CD4 and CD8			X	X	X	X	X	X	X	X	X	X	X	X		X	
p24[f]			X	X	X	X	X	X	X	X	X	X	X	X		X[f]	
Plasma Sample[g]					X					X		X		X		X[g]	
Plasma PBMC Sample[h]					X					X		X		X		X[h]	
Concomitant Meds			X		X	X	X	X	X	X	X	X	X	X	X	X	
Adverse Events/HIV Event					X	X	X	X	X	X	X	X	X	X	X	X	
QOL[i]/Symptom Survey					X						X			X		X[i]	
ECG[j]					X									X		X	
Vital Status Update																	X

*Flow chart is the same for all doses and placebo arms of the study.

[a] Patients will qualify for Washout with Screen #1 and will qualify for enrollment with results from Screen #2.

[b] The Lab Visit on Day -3 will be needed only for those patients requiring washout from prior antiretroviral therapy.

[c] These measurements should be performed on the last study visit if the last visit is prior to Week 24 and again at Week 24. Patients who discontinue prior to Week 24 will be asked to return at Week 24 for assessments.

[d] Visit should occur two weeks after the end of therapy for safety assessments.

[e] Study Extension procedures and assessments are performed monthly, on a months-end schedule (e.g., when a patient who is eligible for the study extension completes the first month of study drug, evaluations will be summarized as 'Month End 1')

[f] After baseline, p24 assessments will be performed only in patients antigen positive at baseline.

[g] Time population pharmacokinetic samples will be drawn at Week 2 and Week 8.

[h] PBMC samples are to be drawn pre-dose at baseline, Week 12 and Week 24 or exit and every 6 months during extension therapy, and on all patients who fail therapy.

[i] Quality of Life assessments will be completed every three months including during extension and exit. These assessments should be completed prior to any clinical evaluation.

[j] Baseline ECGs must be completed within 30 days prior to baseline, at Week 24 or patient's last visit, and at end of extension.

[l] Physical and neurologic exams, vital signs, weight and Karnofsky may be performed either at Screen 2 or baseline.

[f] Serum pregnancy test will be required for female patients of child bearing potential.

Notes:

1. Patients will have up to four plasma concentrations of Study Drug(s) determined during the study.
2. All blood samples drawn for laboratory studies (especially CD cell subsets) should be drawn at the same time of day for every patient during the study. This standardization of draw-time is an effort to control for diurnal fluctuations.
3. Plasma samples for potential virology studies will be aliquoted from the HIV RNA titer sample.

13.1.2.5.2 APPROPRIATENESS OF MEASUREMENTS

The primary activity endpoints of this study are surrogate markers (HIV RNA and CD4 lymphocyte counts) commonly used in clinical practice. These surrogate markers have been used in multiple clinical trials of other drugs. Some of these trials have served as the basis of FDA-approval for other antiretroviral drugs. Accelerated approval requires sponsor's conduct of confirmatory clinical trials. It is also believed that surrogate markers changes reasonably predict clinical outcome. Therefore, the use of surrogate markers in this trial as primary efficacy endpoints is acceptable within the accelerated approval regulations.

CD4 lymphocyte counts are commonly performed in hematology laboratories. The techniques used in the collection, handling, and interpretation of CD4 lymphocyte counts appear to be standard across different laboratories.

HIV RNA values obtained from the different techniques employed have not been standardized. Some of the techniques have not been validated by independent parties. Therefore, there is still uncertainty about the meaning of the magnitude changes obtained and the intercorrelation of the different assays. However, HIV RNA values are useful to clinicians and investigators in the management of patients and clinical trial, respectively.

Comment: The sponsor used This assay has not been approved by the FDA. By our request, the applicant submitted data provided by on the validation of the assay. Unfortunately, the small amount of data provided in response to FDA requests was not sufficient to understand all the characteristics of the assay, including its limitations. Although the data submitted provided evidence that below 1,200 estimated copies/mL the assay was not able to accurately quantify HIV RNA. The Statistical review written by Dr. Michael Elashoff, and the Microbiology review written by Drs. Lauren Iacono-Connors and Shukal Bala will comment further in the validation of the assay used in this clinical trial. Overall, the conclusion in these reviews was the lower limit of quantification for this assay for use in this application was 1,200 copies/mL.

13.1.2.5.3 PRIMARY EFFICACY VARIABLES

Plasma HIV RNA by bDNA: Samples for plasma HIV RNA were collected at Screen 1, Screen 2, Day -3 (if applicable), baseline, and at Weeks 1, 2, 4, 6, 8, 12, 16, 20, and 24 (or last visit). Samples for plasma HIV RNA were also collected on Day -3 for patients who required antiretroviral washout. Calculated baseline plasma HIV RNA values were established by averaging the results from the baseline sample with the results of the most recent pre-dose sample (Day -3 or Screen 2) collected before baseline; the Screen 1 plasma HIV RNA value was not used.

Comment: Plasma samples for HIV RNA were analyzed using an assay

CD4 Lymphocyte Count: Samples for CD4 lymphocyte counts were to be collected at Screen 2, Day -3 (if applicable), baseline, and at Weeks 1, 2, 4, 6, 8, 12, 16, 20, and 24 (or last visit). Samples for CD4 lymphocyte counts were also collected on Day -3 for patients who required antiretroviral washout. Calculated baseline CD4 lymphocyte counts were established by averaging the results from the baseline sample with the results of the most recent pre-dose sample (Day -3 or Screen 2) collected before baseline.

Comment: Both HIV RNA and CD4 lymphocyte counts, are surrogate measurements of disease status. Both measurements are used in clinical practice to follow patients' response to therapy or lack thereof.

13.1.2.5.4 DRUG CONCENTRATION MEASUREMENTS

The sponsor collected blood samples at Week 2 and Week 8 with the objective of conducting population pharmacokinetic analysis. Patients also had four plasma concentrations of study drug (nelfinavir, ZDV and/or 3TC) drawn during the study for population pharmacokinetics. Analysis of these samples will be done in the future.

Comment: Results of the population pharmacokinetic study were not submitted in this NDA.

13.1.2.6 DATA QUALITY ASSURANCE - AUDIT CERTIFICATE

The applicant contracted to performed an audit and compare the database to the CRFs for 10% of patients selected at random. If a data table in the sample failed the audit (error rate $\geq$ 0.5%), all of the data for that table were audited. Data support services performed a 100% audit for all patients for HIV related events, AEs, and concomitant medications. Clinical data managers reviewed all data listings for outliers, data inconsistencies, and spelling errors.

In addition to the audits, Agouron conducted an audit for a random sample of 10% of the patients. Problems identified were forwarded to and/or the study site for resolution. Additional quality checks were performed on key study endpoints. Agouron also employed the services of another contract research organization, to perform an independent audit on the programming used to generate key tables, listings, and figures.

Comment: Before the end of the study, Agouron disqualified site 38 and its investigator for violations to the protocol. This incident was reported to the Division of Scientific Investigations of the FDA several months before the NDA filing.

13.1.2.7 STATISTICAL METHODS PLANNED IN THE PROTOCOL AND DETERMINATION OF SAMPLE SIZE

13.1.2.7.1 STATISTICAL AND ANALYTICAL PLANS

The applicant planned and proposed an intent-to-treat analysis. All patients for whom data was available were to be included in the efficacy analysis. All patients exposed to drug were to be included in the safety analysis.

The primary efficacy endpoints of the study were the change in the virologic and immunologic markers of disease progression, as assessed by HIV RNA and CD4 cell count. The $\log_{10}$ transformation of the HIV RNA titer data was planned for use in the analysis of plasma HIV RNA changes. The $\log_{10}$ HIV RNA titer and CD4 cell count data was to be listed and summarized across time by treatment arm. The change from baseline was to be summarized for each evaluation period by treatment arm and compared using analysis of variance.

Analyses were to include all protocol specified intervals during the 24 week study period. For patients who failed the Study treatment or switched to therapy other than Study treatments, their data prior to that point were to be included for treatment comparison. Data collected after that point were to be summarized to describe the changes.

A Data Safety Monitoring Board was formed by Agouron. The DSMB were to monitor the safety of the study and make recommendations regarding changes in therapy to Agouron's Medical Monitor.

13.1.2.7.2 DETERMINATION OF SAMPLE SIZE

The sponsor planned to enroll 70 subjects per treatment arm based on the following criteria: Sixty-one evaluable subjects provide 90% power to identify the mean difference of 60 absolute CD4 cells between patients receiving active Viracept and placebo assuming significance level of 0.05 adjusted for multiple comparisons, and standard deviation of 90 cells. This sample size also provide 90% power to identify the mean difference of 0.4 log HIV RNA when comparing treatment arms with respect to viral load, assuming standard deviation of ± 0.3 log. To allow for attrition, 70 subjects were planned per treatment arm.

13.1.2.8 CHANGES IN THE CONDUCT OF THE STUDY OR PLANNED ANALYSES

Due to the rapid accrual process, the sponsor enrolled more patients than originally expected. This change is not expected to affect the study results adversely. Although not a change in planned analyses, the sponsor was instructed that a true intent-to-treat analysis focuses on the original treatment assignments regardless of treatment changes that may occur after randomization. The applicant argued that this approach may bias the results in favor of patients randomized to placebo but who later were switched to nelfinavir. The applicant was advised that the intent-to-treat analysis was required but did not preclude other analysis it considered appropriate or necessary.

13.1.3 STUDY PATIENTS

13.1.3.1 DISPOSITION OF PATIENTS

Patient enrollment began on February 8, 1996, and closed on April 16, 1996. A total of 605 patients had samples drawn for Screen 1 plasma HIV RNA. Of these, 340 were randomized into the study. According to the sponsor, early during the study, Site 38 was withdrawn from participation due to GCP violations, including irregularities in source document record keeping and other inconsistencies in patient accrual. The sponsor summarized data from this site in a separate listing in Appendix 12, Data Listing 22. Site 38 patients (n=24) are not included in the number of patients in the statistical tables. Therefore, only 316 patients appear as randomized into the study.

Comment: The sponsor reported these irregularities on Site 38 to the FDA while the study was ongoing. The Division of Scientific Investigation received a report and will be looking into the issues.

Of the 316 patients randomized into the study, 19 patients were excluded and considered by the applicant not treated. Table 4 summarizes patient disposition for patients excluded for analysis. These patients were excluded because they did not have a single follow up visit after the initial randomization visit.

Three of these patients had limited exposure to study drug: Patient 13-04 (one day), 29-09 (four days), and 49-06 (two days). Since there is no follow up data for these patients, the sponsor considered them randomized but not treated.

Comment: This reviewer disagrees with sponsor on patient 29-09. Patient 29-09 discontinued because he could not tolerate medications. This patient should be included in the analysis and counted as withdrawn from study due to adverse events. Therefore, in the table above only 18 patient appear as excluded from statistical analysis. Patient 29-09 started therapy on 4/17 and returned on 4/20 stating that "the medications made him sick".

Two hundred ninety-eight patients were randomized and treated as follows: 100 patients (33%) in the nelfinavir 750 mg + ZDV + 3TC group, 97 patients (33%) in the nelfinavir 500 mg + ZDV + 3TC group, and 101 patients (34%) in the ZDV + 3TC alone group.

TABLE 4
Patients Randomized but not Included in the Statistical Analysis

Patient ID	Gender	Age (years)	Race	Treatment Group	Reason for non-inclusion
	Male	46	White	750mg + ZDV + 3TC	Consent withdrawn
	Male	41	White	750mg + ZDV + 3TC	Consent withdrawn
	Male	57	Black	750mg + ZDV + 3TC	Lost to follow up
	Male	40	White	750mg + ZDV + 3TC	No baseline visit
	Male	36	White	750mg + ZDV + 3TC	Did not meet entry criteria[2]
	Male	29	Other	750mg + ZDV + 3TC	Lost to follow up
	Male	47	White	500mg + ZDV + 3TC	Consent withdrawn
	Male		White	500mg + ZDV + 3TC	Lost to follow up
	Male	42	White	500mg + ZDV + 3TC	Death before therapy[3]
	Female	50	White	500mg + ZDV + 3TC	Lost to follow up
	Male	34	Black	500mg + ZDV + 3TC	No baseline visit
	Female	37	White	500mg + ZDV + 3TC	Did not meet entry criteria
	Male	41	White	500mg + ZDV + 3TC	Consent withdrawn
	Male	30	Other	500mg + ZDV + 3TC	Consent withdrawn
	Male	24	White	ZDV + 3TC Alone	Consent withdrawn
	Male	29	White	ZDV + 3TC Alone	Did not meet entry criteria
	Male	35	White	ZDV + 3TC Alone	Violation of protocol[4]
	Male	24	Asian	ZDV + 3TC Alone	Consent withdrawn

[1] The numbering convention (049.004) indicates this patient was the fourth patient randomized at Site 49.

[2] According to investigator, patient refused to curtail his drinking. Patient did not receive drug.

[3] Patient died of PCP before he received drug.

[4] Patient was given drug on 5/16; however, in a subsequent visit on 8/6, he reported that he had not taken the drug because he was afraid.

Table 5 summarizes patients who discontinued and the reasons for discontinuation for the three arms of the study. A total of 53 patients discontinued therapy: Sixteen in the nelfinavir 750 mg TID arm, nineteen in the nelfinavir 500 mg TID arm, and eighteen in the ZDV + 3TC alone arm. All these patients were off-drug and off-study.

Comment: The sponsor and investigators made assessments of relationship to drug. However, these assessments are entirely subjective. Criteria for assessment of relationship to study drug were not defined before the study started. Therefore, this reviewer only summarized ADEs observed during the study regardless of relationship to drug. Table 5 was adapted from Table 5 of applicant's submission (section 8, volume 6, page 83).

Fifteen patients were lost to follow up. These patients failed to return to their scheduled visits and the investigators were not able to contact them. Thirteen patients requested withdrawal from study. Their request was unrelated to study medication. Eleven patients were non compliant with the study medication regimens. Patient 29-09, in the nelfinavir 750 mg arm was excluded in the applicants analysis and included in this analysis; although he discontinued study drug because according to him the study drugs made him sick. This patient did not agreed to be followed. Only three patients discontinued study due to diarrhea; two in the nelfinavir 750 mg arm and one in the zidovudine + lamivudine alone arm.

TABLE 5
PATIENTS WHO DISCONTINUED

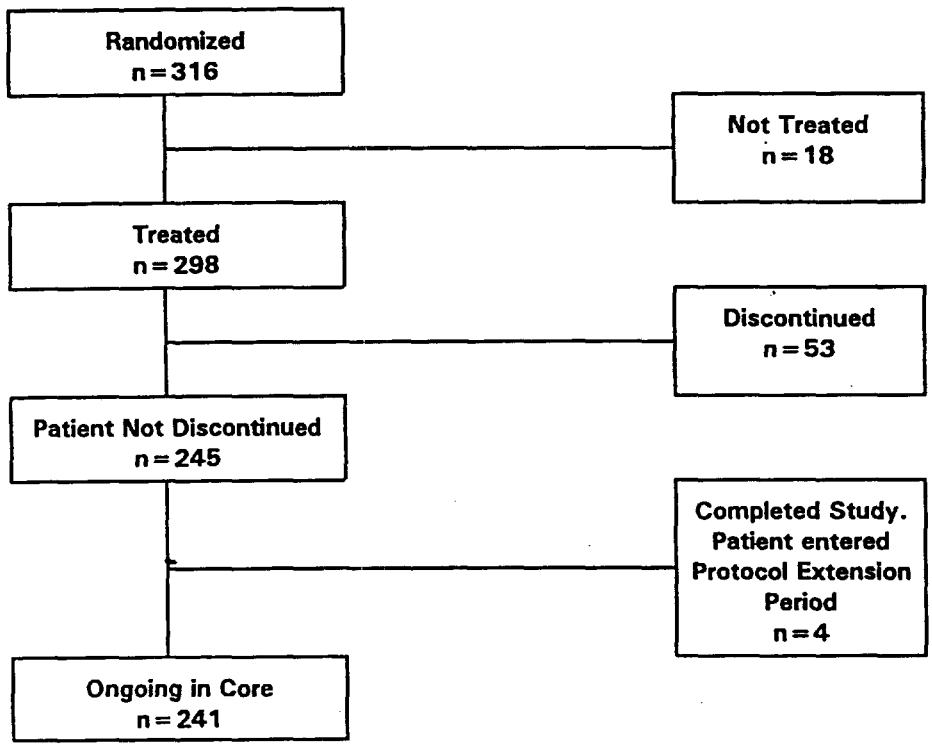
Reason	Nelfinavir 750 mg + ZDV + 3TC	Nelfinavir 500 mg + ZDV + 3TC	ZDV + 3TC alone	Total
Lost to follow up	4	6	5	15
Patient Request	3	5	5	13
Non Compliance	4	2	5	11
Intolerance to Study Medications	1	2	1	4
Diarrhea	2	0	1	3
Nausea, Abdominal discomfort[1]	1	1	0	2
Headache, Fatigue and Lethargy[2]	0	2	0	2
Pancreatitis	0	0	1	1
Skin Rash	0	1	0	1
Progression of Disease	1	0	0	1
TOTAL	16	19	18	53

[1] Patients who have GI symptoms other than diarrhea are included in the row of "Nausea and Abdominal Discomfort".

[2] Patients who had other non-GI adverse events are included in the row "Headache, Fatigue, and Lethargy".

At the data cutoff date, 241 patients (76%) were ongoing and four (1%) had entered the protocol extension period. Figure 2 is the patient disposition flow chart.

Figure 2. Patient Disposition Flow Chart



13.1.3.2 PATIENTS WITH PROTOCOL DEVIATIONS

During the study, a total of 21 (7%) patients had eligibility violations. Patient 49-04 (ZDV + 3TC alone arm) withdrew consent before starting therapy. Sixteen patients were granted exemptions. Five patients had received immune modulators or vaccines within one month before baseline. Three patients had HIV RNA values below 15,000 copies/mL at Screen 1. Three patients had absolute neutrophil counts (ANC) less than 750 cells/mm^3 at Screen 2. Two patients had prior antiretroviral therapy that was prohibited by the protocol. Seven patients had taken ZDV for more than one month. One patient had liver enzymes greater than 5 times ULN. One patient had acute pancreatitis or hepatitis at entry. Patient 39-09 had two violations to protocol (immune modulators or vaccines within one month prior to study and ANC $\leq$ 750 cells/mm^3).

Table 6 summarizes the number of patients with protocol violations by treatment group.

TABLE 6
PROTOCOL VIOLATIONS

Protocol Violation	Nelfinavir 750 mg +ZDV+3TC N=100	Nelfinavir 500 mg +ZDV+3TC N=97	ZDV+3TC Alone N=101	Total N=298 (%)
Immune modulators or vaccine within one month	1	3[1]	1	5 (1.7)
HIV RNA < 15,000	0	1	2[2]	3 (1.0)
ANC $\leq$ 750 cells/mm^3	0	2[1]	1	3 (1.0)
Prior antiretroviral therapy	0	1	1	2 (0.7)
ZDV > 1 month	4	2	1	7 (2.3)
Liver enzymes > 5X ULN	1	0	0	1 (0.3)
Pancreatitis or hepatitis	0	0	1	1 (0.3)
TOTAL	6	9	7	22* (7.4)

[1] Patient 39-09 is counted twice because she had two protocol violations (immune modulators or vaccines within one month prior to study and ANC $\leq$ 750 cells/mm^3).

[2] Patient 49-04 had HIV RNA < 15,000 at screening, however he withdrew consent before initiation of therapy.

* The total number of patients is 20 if both the patient entered twice and the patient who withdrew consent are excluded.

Comment: Data in this Table is different than the data reported by the sponsor in the text of its submission, in Table 9 of Appendix 10, and in Data Listing 1B (Appendix 12). The sponsor only reports in the table and data listing sixteen patients with protocol violations. This discrepancy occurred because the sponsor did not include four additional patients who had protocol violations: Patients 03-02, 23-03, 27-09, of the 750 mg arm, and patient 47-12 of the 500 mg arm received ZDV for more than one month prior entry in this study.

The sponsor considered all the violations to the protocol to be minimal. Of the 20 patients with protocol violations 16 were granted exceptions. Once enrolled, no patient received the wrong treatment.

Comment: Ideally no violations to the protocol should occur. Upon carefully reviewing these patients, this reviewer accepts the sponsor's conclusion that the violations to the protocol were minor and were not likely to affect the study results. Patients with violations to the protocol are distributed almost equally in the three study arms.

13.1.4 EFFICACY EVALUATION

13.1.4.1 DEMOGRAPHIC AND OTHER BASELINE CHARACTERISTICS

Demographic data of patients in study 511 is presented in Table 7 (see page 40).

Two-hundred sixty-four patients were male (88.6%), two-hundred thirty-three (78.2%) were white, forty-three (14.4%) were black, and twenty-two (7.4%) were of other ethnic group. The age, CD4 cell counts, and Plasma HIV RNA at baseline had a similar distribution in the three study arms.

TABLE 7
DEMOGRAPHIC CHARACTERISTICS
AND BASELINE CD4 CELL COUNTS AND HIV RNA

Characteristics	Nelfinavir 750mg +ZDV+3TC n=100 (%)	Nelfinavir 500mg +ZDV+3TC n=97 (%)	ZDV+3TC Alone n=101 (%)	TOTAL n=298 (%)
Gender				
Male	87 (87)	88 (91)	89 (88)	264 (89)
Female	13 (13)	9 (9)	12 (12)	34 (11)
Race				
White	80 (80)	74 (76)	79 (78)	233 (78)
Black	11 (11)	14 (14)	18 (18)	43 (14)
Other	9 (9)	9 (10)	4 (4)	22 (8)
Age (years)				
Mean	36.46	37.32	36.86	36.87
Range	21 - 63	21 - 57	25 - 56	21 - 63
Baseline CD4 cell count (cells/mm^3) - Number of Patients				
$\leq$ 100	20 (20)	19 (20)	23 (23)	62 (21)
> 100 to $\leq$ 300	35 (35)	33 (34)	30 (30)	98 (33)
> 300	45 (45)	45 (46)	48 (47)	138 (46)
Baseline Plasma HIV RNA (Log_{10} copies/mL)				
Mean	4.85	4.90	4.84	4.86
Standard Deviation	0.46	0.48	0.57	0.51
Time since HIV Diagnosis (months)				
Mean	39.64	37.78	39.29	38.89
Range	1 - 169	0 - 159	0 - 162	0 - 169

13.1.4.2 MEASUREMENTS OF TREATMENT COMPLIANCE

Compliance was to be monitored by tablet count at each visit. Some patients were discontinued due to lack of compliance (see Table 3 of this review). However, there is no description of compliance results in the analysis of the data.

13.1.4.3 EFFICACY RESULTS AND TABULATIONS OF INDIVIDUAL PATIENT DATA

13.1.4.3.1 ANALYSIS OF DRUG ACTIVITY

The applicant is requesting approval of nelfinavir tablets based on changes of the two surrogate markers of HIV infection most commonly used at the present time: Plasma HIV RNA and CD4 cell counts. The sponsor conducted an Intent To Treat (ITT) analysis on changes of these surrogate markers as specified in the protocol. The sponsor also conducted analyses of the data using different criteria:

1. Patients who switched from the nelfinavir placebo group to one of the nelfinavir doses (500 mg TID or 750 mg TID) were analyzed in the group to which they switched. The sponsors rationale for this type of analysis is that the ITT analysis biased the results in favor of the placebo group because patients who switched to nelfinavir showed changes in the surrogate markers that tend to approach those of the patients originally randomized to one of the nelfinavir doses. Therefore, the difference between the groups would tend to disappear. Fortunately, most patients assigned to ZDV + 3TC remained in that arm of the study. As of twenty-four weeks of study, only fourteen patients switched from ZDV + 3TC alone to one of the nelfinavir doses.

2. The sponsor also conducted an analysis with the Last Observation Carried Forward.

Comment: The different statistical analysis types are summarized in section 13.1.4.4.2 of this review (page 43).

These different analysis techniques were not expected to yield significant differences. The results presented by the sponsor in all three analysis are very similar. FDA traditionally has requested sponsor to performed ITT analysis of the data. Therefore, this review will only refer to results obtained by the Intent To Treat analysis.

The ITT analysis was performed on data based on original treatment assignment, disregarding treatment changes or switches, and includes all efficacy data obtained through Week 24 or study discontinuation. Some patients in the nelfinavir groups were allowed to change to another RTI due to adverse events or intolerance of ZDV or 3TC. There were fourteen patients who switched from ZDV + 3TC alone arm to either nelfinavir 500 mg or 750 mg arm.

Comment: According to the sponsor, this type of analysis (i.e., that does not account for treatment switches), may not show the actual contribution of the investigational agent to the differences observed. HIV RNA and CD4 cell count in the placebo group tend to approach the values obtained in the active drug treatment groups.

To allow patients the possibility of changing therapy before the end of the study without compromising their health status, a clause was introduced in the protocol that qualified patients as failures if they had a return to baseline in their CD4 cell count or plasma HIV RNA in two consecutive visits after at least four weeks of treatment. Of the 26 patients who experienced treatment failure on nelfinavir (14 in the nelfinavir 750 mg group and 12 in the nelfinavir 500 mg group), only 3 patients in the 500 mg group had a change in RTI regimen due to ADEs attributed to ZDV. All 14 patients who experienced treatment failure in the ZDV+3TC alone group were randomized and switched from placebo to nelfinavir. Table 8 (page 43) summarizes patients' characteristics and time of switching.

The proportion of patients who experience treatment failure was similar among treatment groups. The minimum time to treatment failure was 21, 28, and 26 days in the nelfinavir 750 mg+ZDV+3TC, nelfinavir 500 mg+ZDV+3TC, and ZDV+3TC alone groups, respectively.

TABLE 8
PLACEBO PATIENTS WHO SWITCHED TO NELFINAVIR

Patient ID	Age (years)	Race	Gender	Date Switched	Time (days) on ZDV+3TC	Nelfinavir Dose
-	31	White	Male	6/4/96	92	500mg+ZDV+3TC
-	33	White	Male	7/10/96	113	500mg+ZDV+3TC
-	31	White	Male	10/1/96	169	500mg+ZDV+3TC
-	33	White	Male	7/2/96	82	750mg+ZDV+3TC
-	30	White	Male	8/13/96	169	750mg+ZDV+3TC
-	27	White	Male	9/16/96	169	500mg+ZDV+3TC
-	42	White	Male	7/10/96	141	750mg+ZDV+3TC
-	32	White	Male	7/16/96	113	500mg+ZDV+3TC
-	25	Hispanic	Male	7/3/96	83	750mg+ZDV+3TC
-	35	White	Male	5/30/96	85	750mg+ZDV+3TC
-	45	White	Male	6/26/96	74	750mg+ZDV+3TC
-	26	White	Female	7/12/96	120	500mg+ZDV+3TC
-	34	Black	Male	8/29/96	141	500mg+ZDV+3TC
-	47	White	Male	7/1/96	113	500mg+ZDV+3TC

13.1.4.3.2 STATISTICAL/ANALYTICAL ISSUES

As previously noted, the statistical analysis of the data was done using Intent-To-Treat analysis. The sponsor also conducted two other types of analysis. The protocol specified analysis was performed on data up to the time of the second return to baseline for patients who were treatment failures or up through Week 24 or study discontinuation for patients who were not treatment failures. The other analysis carried the last observation forward. The carry forward analysis incorporates, for patients who fail therapy, the surrogate marker data at confirmation of treatment failure as if that value had been observed at all subsequent time points up through and including Week 24. With either approach, the results of this study arrived to the same conclusion: Nelfinavir appears to be an active antiretroviral drug demonstrated by the changes in CD4 cell counts and HIV RNA over time. Other details of the statistical analysis are discussed in Dr. Michael Elashoff's review of the data.

13.1.4.3.2.1 DROPOUTS AND/OR MISSING DATA

Table 9 summarizes the status of the patients at 16-week analysis of the data. Of the 298 patients, 257 were still enrolled in the study, three had completed the study and continued receiving drug as part of the extension program the sponsor created, and 38 (13%) patients withdrew from study for different reasons.

TABLE 9
PATIENTS ON AND OFF-STUDY AT SIXTEEN WEEKS

Status of Patients	Nelfinavir 750 mg +ZDV+3TC	Nelfinavir 500 mg +ZDV+3TC	ZDV+3TC alone	TOTAL
Ongoing	88	82	87	257
Completed Study	1	1	1	3
Did not complete study	11	14	13	38
TOTAL	100	97	101	298

Table 10 summarizes the reasons for discontinuation of the 38 patients did not complete the study. Ten (3%) patients who did not complete study requested to be withdrawn. Some of the assessments that led to the numbers included in this table are different than the assessments made by sponsor: Three patients listed by sponsor as going off study for reasons other than drug related toxicity were changed to going off study for reasons related to drug toxicity. These three patients 26-17, 36-06, and 03-02, had diarrhea while taking nelfinavir.

All these patients were included in the safety and efficacy analysis. The efficacy analysis is an intent-to-treat analysis. The safety analysis includes all patients exposed to drug and for whom at least one visit of follow-up is available.

TABLE 10
REASONS FOR DISCONTINUATION

Reason for Discontinuation	Nelfinavir 750 mg + ZDV+3TC	Nelfinavir 500 mg + ZDV+3TC	ZDV+3TC alone	TOTAL
Patient Request	4	3	3	10
Other[1]	1	4	2	7
Non-Compliance	2	1	4	7
Study Drug Toxicity	2	4	0	6
Intercurrent Illness[2]	0	0	3	3
Drug Intolerance	0	1	1	2
Other Antiretroviral toxicity	1	1	0	2
Progression of Disease[3]	1	0	0	1
TOTAL	11	14	13	38

[1] Of the seven patients in this category, six were lost to follow-up and one move out of the country.

[2] Patient 4-17 developed pancreatitis and died.

[3] Patient 19-04 died of progressive multi focal leukoencephalopathy.

13.1.4.3.2.2 MULTICENTER STUDIES

Twenty-eight centers enrolled patients in this study. One center was disqualified due to serious irregularities with the data. This center and investigator were reported to the Division of Scientific Investigations. All centers followed the same protocol and were monitored by the sponsor. The center with the largest number of patients in the study enrolled 24 patients. The center with the smallest number of patients in the study enrolled only 2 patients.

Comment: This reviewer did not do a by-center analysis of the data. The number of patients in each center is a small fraction of the total. It is unlikely that one center alone will have a major impact in the overall study results.

13.1.4.3.2.3 EFFICACY RESULTS

The ITT analysis was performed on data based on original treatment assignment, disregarding treatment changes or switches, and includes all available efficacy data obtained through Week 24 or study discontinuation.

Comment: Treatment failure is defined by patients return to baseline in CD4 cell count or plasma HIV RNA in two consecutive visits after at least four weeks of treatment.

By Week 16, thirteen of 100 (13%), 10 of 97 (10%), and 11 of 101 (11%) of patients in the nelfinavir 750 mg + ZDV + 3TC, nelfinavir 500 mg + ZDV + 3TC, and ZDV + 3TC alone groups, respectively, had experienced treatment failure. By Week 24 the number of failures per group were 14 of 100 (14%), 12 of 97 (12%), and 14 or 101 (14%) in the nelfinavir 750 mg + ZDV + 3TC, nelfinavir 500 mg + ZDV + 3TC, and the ZDV + 3TC alone groups, respectively. All but two of the treatment failure events were determined by CD4 lymphocyte count criteria (i.e., a return to calculated baseline of CD4 lymphocyte count on two consecutive study visits after at least four weeks of treatment).

Comment: Most patients in the nelfinavir treatment groups did not change therapy even after reaching the protocol defined treatment failure. Therefore, all three methods of analysis of the data (ITT, last observation carried forward, and censoring of patients) showed similar results.

HIV RNA ASSAY:

For measurements of plasma HIV RNA levels, the Applicant use a which is currently an unapproved investigational assay. The Applicant proposed as the lower limit of detection for this assay a value of The FDA requested that the sponsor submit data demonstrating the validity of this assay across the entire spectrum of viral detection. The Applicant submitted data to the FDA, which they obtained \fter reviewing the data, the FDA concluded that this assay, under a well controlled testing environment, is not able to quantify HIV RNA copies with an acceptable level of confidence below At levels below the assay is very variable and no longer linear. It should also be noted that the units measured may not represent actual viral particles and may not directly correlate with other HIV RNA assays at this level of HIV RNA quantification.

Therefore, the correct way to express the numbers obtained from this assay will be in Units particular to this assay. Due to this change, the figures, tables, and conclusions in the FDA efficacy analysis are different than those presented by the Applicant. For more details about assay validity, please see Microbiology Review written by Drs. Shukal Bala and Lauren Iacono-Connors and Statistical Review written by Dr. Michael Elashoff.

Figure 3 presents the changes from baseline in mean $\log_{10}$ transformed plasma HIV RNA observed during the 24-Week treatment period, as determined by bDNA, for the three treatment groups. This figure shows the difference between the nelfinavir containing groups and the ZDV + 3TC alone arm. Although the initial decrease of HIV RNA was similar in all groups, at Week 4 the mean HIV RNA values in the ZDV + 3TC alone arm start to increase and they plateau at about Week 8 of study. The differences among treatment groups were statistically significant at all timepoints after Week 4.

Figure 3 and 4 were generated by FDA reviewers using electronic data submitted by the applicant. The lower limit of HIV RNA quantification for the generation of these figures was

Figure 3:

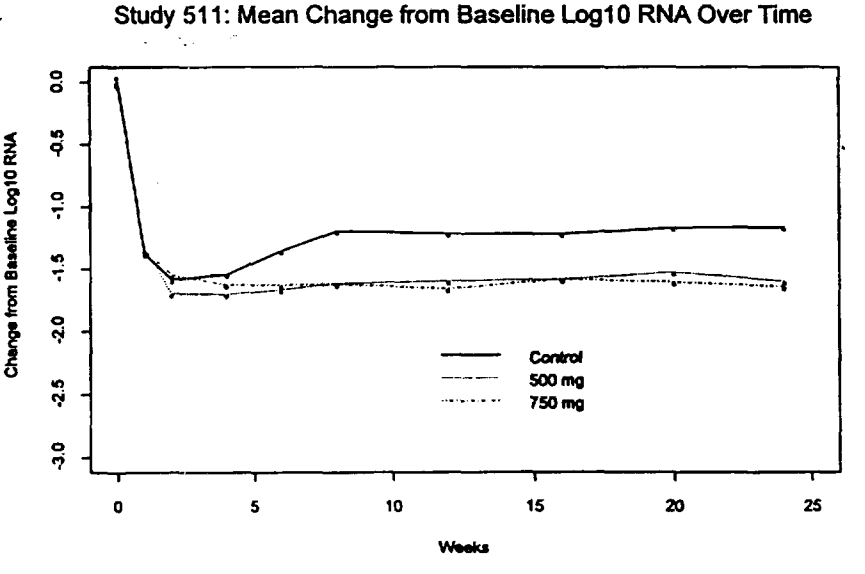
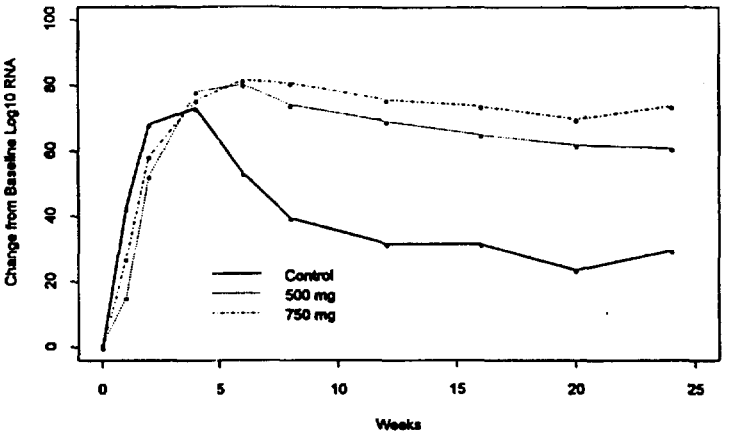


Figure 4 presents the proportion of patients with plasma HIV RNA values below the limit for which the assay can acceptably estimate viral particles at each timepoint during the study.

Figure 4: Study 511: Percent Below 1200 RNA Over Time

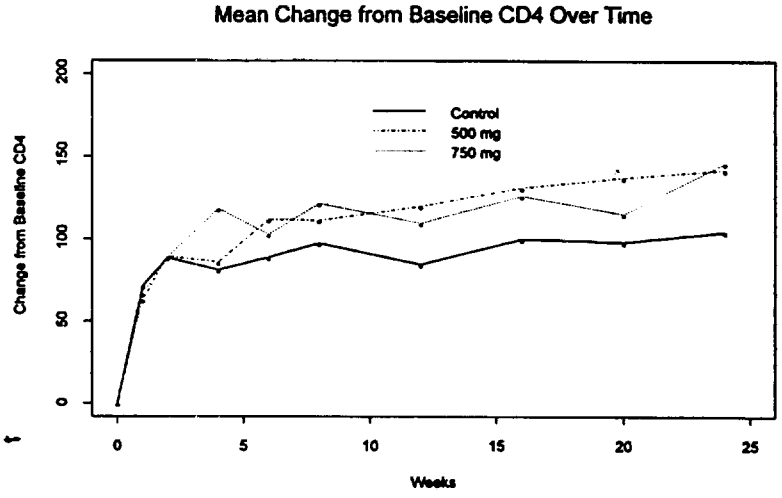


Patients with decreases in estimated plasma HIV RNA to below copies/mL were seen in all three treatment groups by Week 4. At this timepoint, 74%, 76%, and 73% of patients in the nelfinavir 750 mg + ZDV + 3TC, nelfinavir 500 mg + ZDV + 3TC, and ZDV + 3TC alone groups, respectively, were below the estimated RNA value of By Week 12, 74%, 67%, and 32% of patients in nelfinavir 750 mg + ZDV + 3TC, nelfinavir 500 mg + ZDV + 3TC, and ZDV + 3TC alone groups, respectively, were below th estimated RNA value. By Week 16, 70%, 60%, and 30% of patients in nelfinavir 750 mg + ZDV + 3TC, nelfinavir 500 mg + ZDV + 3TC, and ZDV + 3TC alone groups, respectively, were below estimated RNA value of

Comment: The percentages in the paragraph above are based on the total number of patients randomized to treatment groups. The sponsor presented data in percentages using as a denominator the total number of patients still in study at the time of the analysis. However, this reviewer considers the sponsor approach potentially misleading because the sponsor does not take into account all the patients in the study. All these patients had the same potential to reach a value below estimated copies/mL. All patients randomized should remain in the denominator because they had the same potential to contribute to the numerator as did the patients who remain in the study.

Figure 5 presents the changes from baseline of CD4 lymphocyte counts observed during the 24-Week treatment period for the three treatment groups. This figure shows the difference between the nelfinavir arms and the ZDV + 3TC alone arm. The differences between the nelfinavir groups and the control group were statistically significant at Weeks 8 ($p = 0.0444$) and 24 ($p = 0.0023$); at Week 24, mean increases were 155, 161, and 105 cells/mm^3 in the nelfinavir 750 mg + ZDV + 3TC, nelfinavir 500 mg + ZDV + 3TC, and ZDV + 3TC control groups, respectively.

Figure 5:



13.1.4.3.2.4 EXAMINATION OF SUBGROUPS

The Applicant did not specify analysis of subgroups in the original protocol; however, a post hoc analysis of a subgroup of patients who at 24 weeks had an estimated HIV RNA value below 1,200 copies/mL was performed. These patients were divided in four groups:

- HIV RNA > 100,00 and CD4 > 300
- HIV RNA > 100,00 and CD4 $\leq$ 300
- HIV RNA $\leq$ 100,00 and CD4 > 300
- HIV RNA $\leq$ 100,00 and CD4 $\leq$ 300

Table 11 (page 49) summarizes the findings. It is of note that the percentage of patients below the HIV RNA estimated value of 1,200 copies/mL is similar for both doses of nelfinavir for patients with baseline HIV RNA $\leq$ 100,000 (67% for each dose group in patients with CD4 cell count $\leq$ 300; and 80% and 71% for patients in the nelfinavir 750 mg arm, and nelfinavir 500 mg arm, respectively). In the group of patients with baseline HIV RNA >

100,000 and baseline CD4 > 300, there is a difference that appears to favor the nelfinavir 500 mg arm (80%) over the nelfinavir 750 mg arm (60%). However, the number of patients in this subgroup is very small. Therefore, it will not be appropriate to draw generalizable conclusions from this subgroup of patients. In patients with HIV RNA baseline values > 100,000 and CD4 cell counts ≤ 300, the difference between the nelfinavir containing regimens appears to be large. Seventy-nine percent of patients in the nelfinavir 750 mg arm were below the estimated HIV RNA level of 1,200 copies/mL, whereas only 39% of patients in the nelfinavir 500 mg arm were below the estimated HIV RNA level of 1,200 copies/mL. Although it can be inferred that patients with baseline "high" levels of viremia and baseline "low" levels of CD4 cell counts may benefit more from the higher nelfinavir dose (750 mg TID), caution should be exercised because this data is based on a small number of patients (24 patients in the nelfinavir 750 mg arm and 31 patients in the nelfinavir 500 mg arm).

TABLE 11
PROPORTION OF PATIENTS BELOW THE ESTIMATED HIV RNA LEVEL OF 1,200 COPIES/mL AT WEEK 24 OF STUDY BY BASELINE CD4 AND BASELINE PLASMA HIV RNA

	CD4 > 300			CD4 ≤ 300		
	Nelfinavir 750mg +ZDV+3TC n*=10 (%)	Nelfinavir 500mg +ZDV+3TC n=10 (%)	ZDV+3TC alone n=8 (%)	Nelfinavir 750mg +ZDV+3TC n=24 (%)	Nelfinavir 500mg +ZDV+3TC n=31 (%)	ZDV+3TC alone n=33 (%)
HIV RNA >100,000	6 (60)	8 (80)	2 (25)	19 (79)	12 (39)	2 (6)
	Nelfinavir 750mg +ZDV+3TC n=35 (%)	Nelfinavir 500mg +ZDV+3TC n=35 (%)	ZDV+3TC alone n=40 (%)	Nelfinavir 750mg +ZDV+3TC n=31 (%)	Nelfinavir 500mg +ZDV+3TC n=21 (%)	ZDV+3TC alone n=20 (%)
HIV RNA ≤100,000	28 (80)	25 (71)	17 (42.5)	20 (65)	14 (67)	9 (45)

*n = number of patients randomized

Gender Analysis: Thirty four women were included in this study, 13 in the nelfinavir 750 mg arm, 9 in the nelfinavir 500 mg arm, and 12 in the ZDV + 3TC alone arm. The number of female patients in this study is too small to be able to appreciate possible differences between males and females in drug efficacy and activity. The safety profile of all treatment regimens studied in this trial appear to be similar in female and male patients.

Ethnic Analysis: The majority of patients included in this study were white. There does not appear to be a biologically plausible reason to suspect that the drug safety and efficacy will be different in another ethnic group.

Age Groups: The age range of patients enrolled in this study was 21 to 63 years old. No patients over 65 years of age were enrolled; therefore, we are not able to comment on the use of nelfinavir in the geriatric population. The pediatric population is being studied in separate studies which are summarized in section 13.4 of this review.

13.1.4.3.3 EFFICACY CONCLUSIONS

Changes on CD4 lymphocyte counts and HIV RNA shortly after starting therapy with nelfinavir in combination with ZDV + 3TC clearly showed activity of this treatment regimen. The comparator regimen of ZDV + 3TC has also been shown to be an effective regimen in the treatment of HIV infection. The triple combination therapy of nelfinavir + ZDV + 3TC produced an initial response in CD4 lymphocyte count and HIV RNA similar to the double combination ZDV + 3TC. However, this similar response was only observed in the first four weeks of the study. From 4 to 24 weeks, the triple combination on average maintained a level of viral suppression greater than the viral suppression achieved with the ZDV + 3TC combination. Patients in both nelfinavir containing arms had also greater increases in CD4 lymphocyte counts than patients in the ZDV + 3TC alone arm. These changes in surrogate markers were sustained up to the predetermined cut-off date for this study (Week-24). Although, the applicant has not demonstrated that these changes in surrogate markers translate into clinical benefits for patients, the changes observed in this study are considered evidence of antiretroviral activity and have been previously felt to be consistent with the regulatory requirements stated in 21 CFR 314.510.

13.1.5 SAFETY EVALUATIONS

All patients who received drug and had at least one follow up visit are included in the analysis of safety. Patients were exposed to different dosage levels and different lengths of treatment. Data is analyzed taking in consideration length of exposure and total dose received. A comparison is made for patients receiving the two different nelfinavir doses and those receiving nelfinavir-placebo. Data from patients who discontinued drug due to adverse events were reviewed in an attempt to identify possible risk factors associated with their ADEs. All patients with serious ADEs, including those who died, were also reviewed individually in an effort to recognize possible risk factors associated with the ADEs.

13.1.5.1 EXTENT OF EXPOSURE

According to sponsor, mean days on study were similar among treatment groups. Mean days on study treatment for the three treatment groups were 155 (range, 22 to 192), 151 (range, 14 to 181), and 101 days (range, 12 to 177) for the nelfinavir 750 mg+ZDV+3TC, nelfinavir 500 mg+ZDV+3TC, and ZDV+3TC alone groups, respectively. The mean days on study for patients who completed study was slightly higher (167 days), and the mean days on study among patients who discontinued was lower (85 days). Table 12 summarizes the exposure to nelfinavir at 24 weeks. This table is an adaptation of Table 13 of applicant's study report (Section 8, Volume 6, page 110).

TABLE 12
EXPOSURE TO NELFINAVIR OVERALL

Drug Exposure	Nelfinavir 750 mg +ZDV+3TC n=100	Nelfinavir 500 mg +ZDV+3TC n=97
Cumulative dose (g)[1]		
Mean	330.8	214.3
Min	31.5	8.0
Max	432.7	271.5
Average daily dose (mg/day)[2]		
Mean	2,147.2	1,437.8
Min	1,417.5	5,71.4
Max	2,654.2	2,800

[1] Cumulative dose = (total tablets dispensed-total tablets returned)Xdose strength

[2] Average daily dose = cumulative dose/number of days in study before the time of drug discontinuation

Comment: The sponsor did not include patient 29-09 in the nelfinavir 750 mg arm. This patient withdrew from study because after 3 days on study drug he stated that the medications made him ill. Although this patient is an outlier, his inclusion is not likely to affect the results in any meaningful way.

13.1.5.2 ADVERSE EVENTS

13.1.5.2.1 BRIEF SUMMARY OF ADVERSE EVENTS

The most common adverse event observed in this clinical trial was diarrhea. Diarrhea appears to be associated to the use of nelfinavir. Most patients appear to tolerate this adverse event without the need to modify the dose or schedule of drug administration. Some patients successfully used antimotility agents to treat this nelfinavir-associated diarrhea. All other clinical and laboratory adverse events do not appear to be associated with nelfinavir therapy.

13.1.5.2.2 DISPLAY OF ADVERSE EVENTS

The sponsor summarized Adverse Events by investigator's assessment of severity and causality. Unfortunately, causality of Adverse Events is a complex process that goes beyond the perception of individual investigators. Therefore, in this review Adverse Events will be summarized by treatment group regardless of perceived causality with the drug.

Table 13, summarizes the adverse events reported by $\geq$ 3% of patients who had at least grade 2 severity. Some of these patients had more than one occurrence of the same adverse event. Some patients also had more than one concomitantly adverse event. Table 11 summarizes only the number of patients with adverse events by treatment group. The most common grade 2 or above adverse event associated with nelfinavir was diarrhea. Diarrhea appears to be dose related. Ten percent of patients in the ZDV + 3TC alone arm, 19% in the nelfinavir 500mg + ZDV + 3TC arm, and 26% in the nelfinavir 750 mg + ZDV + 3TC arm had diarrhea. Nausea (severity grade 2 or above) was the most common adverse event reported. However, nausea does not appear to be only associated with nelfinavir and its dose. Twenty two percent of patients in the nelfinavir 750 mg + ZDV + 3TC arm and 21% in the ZDV + 3TC alone arm had nausea. Only 13% in the nelfinavir 500 mg + ZDV + 3TC arm had nausea reported as severity 2 or greater. All other adverse events shown in table 8 do not appear to be particularly associated with nelfinavir with the exception of depression. However, the number of patients with depression is small and any presumed relationship needs to be considered in context of the large number of comparisons being made.

TABLE 13
NUMBER OF PATIENTS WITH ADVERSE EVENTS OF AT LEAST GRADE 2 SEVERITY REPORTED BY ≥ 3% OF PATIENTS DURING THE STUDY

	nelfinavir 750mg +ZDV+3TC n=100 n (%)	nelfinavir 500mg +ZDV+3TC n=97 n (%)	ZDV+3TC alone n=101 n (%)	Total n=298 n (%)
Gastrointestinal System				
Nausea	22 (22)	13 (13)	21 (21)	56 (19)
Diarrhea	26 (26)	19 (19)	10 (10)	55 (18)
Abdominal Pain	2 (2)	4 (4)	10 (10)	16 (5)
Vomiting	4 (4)	3 (3)	6 (6)	13 (4)
Flatulence	3 (3)	6 (6)	0 (0)	9 (3)
Body as a Whole				
Headache	13 (13)	7 (7)	10 (10)	30 (10)
Asthenia	4 (4)	8 (8)	9 (9)	21 (7)
Pain	4 (4)	3 (3)	3 (3)	10 (3)
Fever	2 (4)	3 (3)	4 (4)	9 (3)
Nervous System				
Depression	9 (9)	2 (2)	2 (2)	13 (4)
Anxiety	5 (5)	4 (4)	2 (2)	11 (4)
Hematology				
Leukopenia	9 (9)	5 (5)	13 (13)	27 (9)
Anemia	6 (6)	5 (5)	9 (9)	20 (7)
Skin and Appendages				
Rash	6 (6)	5 (5)	4 (4)	15 (5)

13.1.5.2.3 ANALYSIS OF ADVERSE EVENTS

The number of patients with grade 2 or greater adverse events are summarized in Table 13. The sponsor reported all adverse events that occurred during the study. The sponsor also assigned causality of adverse events to the study drug and/or the other antiretrovirals used during the

study. The assignment of causality is based on the opinions of the investigators. Unfortunately there is no evidence that the investigators follow the same patterns of reasoning for causality assignment. Therefore, this reviewer considers more appropriate to list all adverse events occurring during the study. The results of this summary data indicates that diarrhea is particularly associated to the use of nelfinavir. It also appears that this adverse event may be dose dependent. More patients receiving the highest nelfinavir dose had diarrhea. This finding is congruent with the findings in pre-clinical and previous clinical studies. All other adverse events listed do not appear to follow a pattern of association backed by biological plausibility and/or frequency of events.

13.1.5.2.4 LISTING OF SERIOUS ADVERSE EVENTS BY PATIENT

Table 14 lists all patients having serious adverse events at the 24-week evaluation point. A serious adverse event is an event that is life threatening, that results in severe or permanent disability, cancer, or a congenital anomaly, that requires or prolongs hospitalization, or that is due to a study drug overdose, regardless of relationship to nelfinavir. The seriousness of an adverse event is independent of its severity.

TABLE 14
PATIENTS EXPERIENCING SERIOUS ADVERSE EVENTS

Site and Patient No.	COSTART Term	Severity Grade	Action Taken
Nelfinavir 750 mg TID + ZDV + 3TC			
03-18	Skin Carcinoma	2	Other
04-12	Tachycardia	1	Hospitalization (required or prolonged)
06-01	Herpes Zoster	3	Therapy required, hospitalization
19-04	Encephalopathy	4	Other (Patient died)
23-03	Pericarditis Pneumonia Viral Infection	3 3 3	Therapy required, hospitalization (required or prolonged)
39-06	Bacterial Infection Fever Carcinoma	3 2 2	Therapy required, hospitalization (required or prolonged)
40-07	Aphasia	3	Therapy required, hospitalization (required or prolonged)

Site and Patient No.	COSTART Term	Severity Grade	Action Taken
40-08	Lymphoma (Liver Masses)	3	Therapy required, hospitalization
41-13	Appendicitis	2	Hospitalization (required or prolonged)
Nelfinavir 500 mg + ZDV + 3TC			
29-08	Anemia	3	Hospitalization (required or prolonged)
39-09	Diarrhea	4	Therapy required, hospitalization
43-01	Diarrhea	4	No action
47-11	kidney Calculus Pneumonia	2 3	No action Therapy required, hospitalization
ZDV + 3TC alone			
03-12	Skin Carcinoma	3	No action
03-20	Deep Thrombophlebitis	3	Therapy required, hospitalization
04-17	Pancreatitis Cholecystitis Sepsis	3 3 4	Hospitalization (required or prolonged) Other No action
13-01	Tuberculosis aggravated	3	Therapy required, hospitalization
23-06	Fever	2	Therapy required, hospitalization
24-04	Herpes Zoster	3	Therapy required, hospitalization
24-07	Anxiety	3	Therapy required, hospitalization
27-05	Abdominal pain	3	Hospitalization (required or prolonged)
42-09	Anemia	2	Hospitalization (required or prolonged)
47-03	Convulsion Liver enzymes increased CPK increased	3 3 3	Therapy required Other No action

Nine patients in the nelfinavir 750 mg arm had serious adverse events. None of these patients had diarrhea or other gastrointestinal ADEs. Four patients in the nelfinavir 500 mg arm had serious adverse events. Two of these patients had diarrhea (the most common ADE associated with nelfinavir). Ten patients in the ZDV + 3TC alone arm had serious ADEs. The study drug does not appear to cause or be associated with increase number of patients presenting with serious ADEs.

Other Adverse Events Not Considered Serious but That Led Patients to Discontinue Therapy:

Thirteen of 298 patients (4%) discontinued the study because of treatment related Adverse Events other than death and serious adverse events.

In the nelfinavir 750 mg + ZDV + 3TC group, **patient 29-09** started therapy on 4/17 and returned on 4/20 stating that "the medications made him sick", **patient 04-07** discontinued because of nausea and asthenia, **patient 26-17** and **patient 03-02** discontinued because of diarrhea.

In the nelfinavir 500 mg + ZDV + 3TC group, **patient 03-07** discontinued due to nausea, flatulence, and asthenia, **patient 26-19** discontinued because of abdominal pain, anxiety, chest pain, and diarrhea, **patient 29-03** discontinued due to headache, **patient 36-06** discontinued due to nausea and vomiting, **patient 41-15** discontinued because of a rash, and **patient 43-01** discontinued because of asthenia.

In the ZDV + 3TC group, **patient 27-05** discontinued because of diarrhea and nausea, and **patient 47-03** discontinued because of hepatitis related to alcohol consumption.

13.1.5.3 DEATHS

Only three patients died during the 24-week study:

Patient 13-02, randomized to nelfinavir 500 mg TID + ZDV + 3TC, died of complications of PCP before taking study drug.

Patient 19-04, assigned to nelfinavir 750 mg TID + ZDV + 3TC took study drug for 73 days and died of progressive multifocal leukoencephalopathy.

Patient 04-17, assigned to placebo + ZDV + 3TC, took study drugs for 57 days and died of severe pancreatitis and septic shock.

Comment: PCP and leukoencephalopathy are diseases associated with HIV-infection and AIDS. Therefore, it is likely that the deaths of patients 13-02 and 19-04 were not associated to the drugs they were receiving but to progression of their HIV disease. Although pancreatitis is a known adverse event associated to 3TC, the investigator and drug sponsor stated that this Adverse Event was not related to study drugs.

There was only one more patient with pancreatitis (patient 51-06). This patient was assigned to the nelfinavir 750 mg + ZDV + 3TC arm. He had an elevated serum amylase and his pancreatitis was graded as severity 2 and lasted only two days. He continued therapy with all three drugs.

13.1.5.4 CLINICAL LABORATORY EVALUATION

All the information presented in this section is based on the analysis of data at week sixteen of study.

Hematology

Anemia was an expected adverse event in patients receiving ZDV and 3TC. There were ten patients who had anemia (hemoglobin less than 8 g/dL) at some point during the study. Two patients were in the nelfinavir 750 mg arm, three were in the nelfinavir 500 mg arm, and five were in the ZDV + 3TC alone arm. A description of the patients and events follows.

Patients in the nelfinavir 750 mg + ZDV + 3TC arm:

Patient 33-12, is a 34 year old, white male, diagnosed with HIV infection one month prior enrollment. His baseline CD4 count was 11 cells/mm^3, and his baseline Log_{10} HIV RNA was 5.1. He had a hemoglobin value of 11.2 g/dL at baseline. At day 28 of study his hemoglobin value was 5.6 g/dL. At his next visit (day 42), his hemoglobin was 9.9 g/dL. However, there is no information indicating if the patient received a blood transfusion. At his last visit (day 140), his hemoglobin value was 12.3 g/dL.

Patient 39-06, is a 41 year old, white male, diagnosed with HIV infection more than 10 years prior enrollment. His baseline CD4 count was 10 cells/mm^3, and his baseline Log_{10} HIV RNA was 5.2. He had a hemoglobin value of 11 g/dL at baseline. At day 42 of study his hemoglobin value was 7.7 g/dL. At his next visit (day 56), his hemoglobin was 9.2 g/dL. At his last visit (day 140), his hemoglobin value was 9.6 g/dL.

Patients in the nelfinavir 500 mg + ZDV + 3TC arm:

Patient 14-10, is a 33 year old, Hispanic male, diagnosed with HIV infection one month prior enrollment. His baseline CD4 count was 192 cells/mm^3, and his baseline Log_{10} HIV RNA was 4.7. He had a hemoglobin value of

14.9 g/dL at baseline. At day 42 of study his hemoglobin value was 6.5 g/dL. At his next visit (day 56), his hemoglobin was 9.7 g/dL. At his last visit (day 112), his hemoglobin value was 13.4 g/dL.

Patient 29-08, is a 34 year old, white male, diagnosed with HIV infection one month prior enrollment. His baseline CD4 count was 60 cells/mm^3, and his baseline Log_{10} HIV RNA was 6.13634. He had a hemoglobin value of 10.8 g/dL at baseline. At days 28, 42, 56, and 84 his hemoglobin values were 7, 5.8, 7.4, and 6.8, respectively. There is no information indicating if the patient received a blood transfusion. At the last visit (day 112), his hemoglobin value was 13.3 g/dL.

Patient 41-18, is a 35 year old, white male, newly diagnosed with HIV infection. His baseline CD4 count was 123 cells/mm^3, and his baseline Log_{10} HIV RNA was 5.1. He had a hemoglobin value of 14.2 g/dL at baseline. At day 84, he had five different hemoglobin values 5.7, 6.1, 6.3, 6.7, and 8.7 g/dL. Although it is not specified in the presentation of the data by sponsor, it is probable that this patient had a blood transfusion at day 84. At his next and last visit at day 112, his hemoglobin value was 8.1 g/dL.

Patients in the nelfinavir placebo + ZDV + 3TC arm:

Patient 04-10, is a 42 year old, Hispanic male, diagnosed with HIV infection ten months prior enrollment. His baseline CD4 count was 10 cells/mm^3, and his baseline Log_{10} HIV RNA was 5.10857. He had a hemoglobin value of 15.1 g/dL at baseline. At day 56, he had three different hemoglobin values: 7.9, 8.5, and 9.7 g/dL. Although it is not specified in the presentation of the data by sponsor, it is possible that this patient had a blood transfusion at day 56. At his last visit (day 140), his hemoglobin value was 15.4 g/dL.

Patient 23-06, is a 29 year old, white male, diagnosed with HIV infection five months prior enrollment. His baseline CD4 count was 12 cells/mm^3, and his baseline Log_{10} HIV RNA was 5.9. He had a hemoglobin value of 9.7 g/dL at baseline. At day 42, he had a hemoglobin value of 7.8 g/dL. At his next visit (day 84), his hemoglobin value was 10 g/dL. It is not known whether the patient had a blood transfusion or if his hemoglobin increased spontaneously between days 42 and 84. At his last visit (day 112), his hemoglobin value was 5.9 g/dL.

Patient 27-01, is a 51 year old, white male, diagnosed ten years prior enrollment. His baseline CD4 count was 231 cells/mm^3, and his baseline Log_{10} HIV RNA was 4.52016. He had a hemoglobin value of 15.7 g/dL at

baseline. At day 84, his hemoglobin value was 6.4 g/dL. On visit days 112 and 140, his hemoglobin values were 12.1 and 12.6 g/dL, respectively. It is not known whether the patient had a blood transfusion or if his hemoglobin increased spontaneously between days 84 and 112.

Patient 42-09, is a 41 year old, native American male, diagnosed four years prior enrollment. His baseline CD4 count was 167 cells/mm^3, and his baseline Log_{10} HIV RNA was 5.0. He had a hemoglobin value of 12.8 g/dL at baseline. At day 84, his hemoglobin value was 6 g/dL. His last hemoglobin value was recorded at the next visit (day 112) and it was 10.7 g/dL.

Patient 47-07, is a 47 year old, white male, diagnosed four years prior enrollment. His baseline CD4 count was 58 cells/mm^3, and his baseline Log_{10} HIV RNA was 6.20412. He had a hemoglobin value of 13.4 g/dL at baseline. At day 56, he had three different hemoglobin values: 7.1, 7.3, and 8.4 g/dL. His last hemoglobin values were 16.6 and 13.7 g/dL on days 112 and 140, respectively.

Neutropenia (absolute neutrophil count less than 750 cells/mm^3) is another hematological adverse event associated with both ZDV and 3TC use. Neutropenia was not associated to nelfinavir monotherapy use in previous studies. However, the monotherapy use of nelfinavir has been relatively of short duration. In this trial, there were 24 patients who had neutropenia at some point during the study. There were 5 patients in the nelfinavir 750 mg arm, seven patients in the nelfinavir 500 mg arm, and 12 patients in the ZDV + 3TC alone arm.

Serum Chemistry

Serum Alanine Aminotransferase (ALT) was greater than five times the upper limit of normal in 19 patients. The upper limit of normal for the purpose of this review is 30 U/L. There may be some variability from laboratory to laboratory that are not accounted for in this review. Five patients were in the group receiving 750 mg of nelfinavir, six patients were in the group receiving 500 mg of nelfinavir, and 8 patients were in the group receiving ZDV + 3TC alone.

Serum Aspartate Aminotransferase (AST) was greater than five times the upper limit of normal in 10 patients. The upper limit of normal for the purpose of this review is 35 U/L. There may be some variability from laboratory to laboratory that are not accounted for in this review. One patient was in the group receiving 750 mg of nelfinavir, two patients were in the

group receiving 500 mg of nelfinavir, and seven patients were in the group receiving ZDV + 3TC alone.

Serum Gamma-Glutamyl Transferase (GGT) was greater than five times the upper limit of normal in 10 patients. The upper limit of normal for the purpose of this review is 50 U/L. Three patients were in each the nelfinavir 750 mg group and the ZDV + 3TC alone group. Four patients were in the nelfinavir 500 mg group.

Serum Lactate Dehydrogenase (LDH) was greater than five times the upper limit of normal in only one patient: Patient 47-03, is a 47 years old, white male, randomized to the ZDV + 3TC alone arm but who after 4 months switched to the nelfinavir 500 mg arm. He experienced LDH increase while on ZDV + 3TC alone. His LDH values had returned to normal by the day he switched to the nelfinavir 500 mg arm (visit day 112).

Serum creatinine, BUN, and electrolytes were not affected by administration of these drugs in a clinically significant way.

13.1.5.5 SAFETY CONCLUSIONS

The overall safety of nelfinavir two dosages in this group of patients appears to be acceptable. The only adverse event that is clearly associated to study drug is diarrhea. Diarrhea appears to be dose related. However, the causal mechanism of diarrhea is unknown. Most patients tolerated diarrhea with antimotility agents for symptomatic relief. Most of the serious adverse events observed during this trial do not appear to be causally related to study drug. Thirteen of 298 patients (4%) discontinued therapy due to treatment related adverse events: Four patients in the nelfinavir 750 mg group discontinued therapy. Two of these discontinued due to diarrhea. Six patients in the nelfinavir 500 mg group discontinued therapy. One of these discontinued due to diarrhea. Two patients in the ZDV + 3TC alone group discontinued therapy. One of these discontinued due to diarrhea. Overall, only four patients (1.3%) discontinued therapy due to diarrhea.

Only three patients died during the study. One of these patients died before receiving the first dose of study medication. The other two patients died of clinical syndromes associated with advance HIV-infection.

The hematologic abnormalities observed (anemia and neutropenia) are known adverse events associated with ZDV and 3TC use. It is impossible to determine if nelfinavir contributed to neutropenia and/or anemia because the number of patients with these adverse events is very small. Therefore, even if nelfinavir contributes to these adverse events, it may be a very small contributory effect.

The abnormalities observed in serum chemistry markers do not appear to be particularly associated with any of the three regimens used in this trial. Even if nelfinavir contributes to some of the serum chemistry abnormalities, the effect of the drug is probably very small.

The safety profile of nelfinavir in children, pregnant women, elderly patients, and people with abnormalities of drug metabolism or excretion were not tested during this clinical trial. As with most clinical trials, the population that participated in this study are fairly homogenous. Therefore, there are limitations in the extrapolation of these data to the general population. This drug has the potential to be use in combination with drugs other than ZDV and 3TC. In an earlier study, the sponsor used nelfinavir in combination with stavudine. However, the safety profile of nelfinavir in combination with other reverse transcriptase inhibitors and protease inhibitors have not been tested. Physicians prescribing nelfinavir should be made aware of the limitations of the known safety of nelfinavir. Pharmacovigilance and future clinical trials may contribute to a better understanding of the safety profile of nelfinavir in clinical use.

13.1.6 OVERALL CONCLUSIONS

The effects of nelfinavir in combination with ZDV and 3TC on the surrogate markers of HIV disease (viremia and CD4 cell count) is best summarized in figures 3, 4, and 5. Nelfinavir in combination with ZDV and 3TC decreases viremia to levels that are difficult or impossible to measure with accuracy with the current available technology. Low viremia is perceived by sponsors, investigators, and practicing clinicians as the goal of therapy with current drug combinations for HIV infection. Although the clinical meaning of changes in these surrogate markers has not been validated, physicians in clinical practice are using viremia to modify and monitor their patients treatment. Therefore, the results observed in this study are relevant to current medical practice. The safety profile of nelfinavir in combination with ZDV and 3TC was summarized in section 13.1.5 of this review. The most common drug associated adverse event was diarrhea. Diarrhea appears to be

tolerated by most patients either without treatment or with symptomatic treatment with antimotility agents. Other adverse events, clinical or laboratory, do not appear to be particularly associated to nelfinavir use. Therefore, the potential benefits of nelfinavir in combination with ZDV and 3TC appear to outweigh the risks of using this drug as part of this combination.

13.2 STUDY NUMBER 2: STUDY 506

A Phase 3 Randomized, Double-Blind, Placebo-Controlled Study of Viracept™ In Combination with Stavudine (d4T) Versus Stavudine (d4T) alone in HIV Positive Patients

13.2.1 STUDY OBJECTIVES

a) To evaluate the efficacy and safety of nelfinavir administered in combination with d4T in HIV positive patients who had CD4 lymphocyte counts $\geq$ 50 cells/mm^3 and quantitative plasma HIV RNA levels $\geq$ 15,000 copies/mL. The primary end points were change from baseline in CD4 lymphocyte counts and quantitative plasma HIV RNA level.

b) To assess the potential for viral resistance by assessing changes in genotype, phenotype, and viral sensitivity.

Comment: The second objective and the viral resistance results obtained by this study will be discussed in the microbiology review written by Dr. Bala and Dr. Iacono-Connors.

13.2.2 INVESTIGATIONAL PLAN

13.2.2.1 OVERALL STUDY DESIGN

This was a double-blind, placebo-controlled, randomized phase 3 study designed to compare the efficacy and safety of two different dose levels of nelfinavir in combination with d4T versus placebo plus d4T.

Two-hundred forty HIV positive, d4T naive patients with baseline CD4 lymphocyte counts $\geq$ 50 cells/mm^3 and quantitative plasma HIV RNA levels $\geq$ 15,000 copies/mL were to be enrolled and participate in this study for 24 weeks. Patients were required to enroll in a two week washout period during which antiretroviral drugs were to be discontinued if they had taken antiretroviral drugs in the two weeks before enrollment.

Doses and Schedule:

Nelfinavir 500 mg three times daily (TID) + d4T
Nelfinavir 750 mg TID + d4T
Placebo + d4T

TABLE 15
DOSES AND SCHEDULE

Treatment Group	Number of Patients Stratum I (less than 6 mo ZDV)	Number of Patients Stratum II (6 mo or greater ZDV)	Nelfinavir Dose (mg) TID	Nelfinavir Total Daily Dose (mg)
A	20	60	500	1500
B	20	60	750	2250
C	20	60	Placebo	Placebo
Total	60	180		

CD4 lymphocyte counts (50 $\leq$ 300 or > 300 cells/mm^3) were used in the randomization to balance treatment groups.

Comment: The study accrued more patients than planned. A total of 750 patients had samples drawn for Screen 1 plasma HIV RNA. Of these, 329 were randomized into the study.

13.2.2.2 DISCUSSION OF STUDY DESIGN, INCLUDING THE CHOICE OF CONTROL GROUPS

Based on information obtained from phase 1 and 2 clinical trials, the sponsor had already chosen both 500 mg TID and 750 mg TID of nelfinavir as doses and to be studied in larger clinical trials. For ethical reasons all patients were to receive treatment with at least one active drug. If patients had two successive CD4 measurements showing a return to baseline, they were

offered the opportunity to change their reverse transcriptase inhibitor, stop nelfinavir and change to another protease inhibitor, continue the same regimen or withdraw from study therapy. Patients in the d4T alone arm were offered a re-randomization to the nelfinavir 500 mg TID arm or nelfinavir 750 mg TID arm. This design gave the opportunity to clinicians and patients to have flexibility in their choices of therapy if the study therapy was perceive to be ineffective. For this reason, this clinical trial may have been attractive to patients who wanted to participate in a clinical trial and minimize their personal risk of receiving ineffective therapy. Therefore, this design was appropriate for the planned objectives and facilitated the accrual process.

13.2.2.3 SELECTION OF STUDY POPULATION

13.2.2.3.1 INCLUSION CRITERIA

Patients eligible for this study were HIV positive patients 13 years of age and older. These patients had to meet the following characteristics: a quantitative plasma HIV RNA level of $\geq$ 15,000 copies/mL at Screen 1, a Karnofsky Performance Status $\geq$ 70, CD4 lymphocyte count $\geq$ 50 cells/mm^3, and d4T naive.

13.2.2.3.2 EXCLUSION CRITERIA

Patients with the following characteristics were excluded: patients who had received immune modulators or vaccines within a month of baseline, pregnant or nursing patients, patients with neoplastic disease requiring systemic cytotoxic or radiation treatment, patients with acute pancreatitis or hepatitis, patients with opportunistic infections at baseline, patients who are active substance abusers, patients with clinically significant malabsorption syndrome (chronic diarrhea [four to ten stools per day of 30 days or greater duration]), patients with renal insufficiency (serum creatinine > 2.5 mg/dL at screening visit 2).

Patients with the following laboratory abnormalities at screening visit 2 were also excluded: Serum bilirubin > 2.5 times the upper limit of normal, platelet count $\leq$ 50,000 cells/mm^3, absolute neutrophil count $\leq$ 750 cells/mm^3, hemoglobin < 8 g/dL, and liver enzymes > 5 times the upper limit of normal.

Comment: Patients with advanced HIV disease typically have one or more characteristics that would have made them ineligible to enter into this study. Therefore, patients with one or more of these characteristics may not have the same safety and efficacy profile as patients enrolled in this study. Patients with advanced HIV disease may have a different safety and efficacy profile to a given drug than the selected patients included in clinical trials. Spontaneous reporting of Adverse Drug Events (ADE) may not be sufficient to develop a safety profile of the drug that mimics more closely its use in general practice.

13.2.2.3.3 REMOVAL OF PATIENTS FROM THERAPY OR ASSESSMENT

Patients were to be removed from therapy for any of the following reasons:

A. The development of a toxicity or concurrent illness which precluded further study treatment.

B. Treatment failure (patients were given the opportunity to switched to other therapies)

C. Concurrent use of another antiretroviral drug, including a protease inhibitor, or unapproved use of an investigational agent.

Patients who discontinued study drug for any reason were to continue all study visits unless they were unable to do so or withdrew consent for further participation. At a minimum, patients who discontinue were to have the Week 24 (final visit), Week 2 Safety Follow Up, and Month 4 Vital Status Follow Up assessment completed.

Patients were to be removed permanently from the study for any of the following reasons:

D. Withdrawal of consent and refused further contact with the study.

E. The investigator was shown to be non-compliant with protocol requirements or GCP.

F. Termination of the study by sponsor.

13.2.2.4 TREATMENTS

13.2.2.4.1 TREATMENTS ADMINISTERED

All patients were to receive d4T therapy. One third of the patients were to be randomized to the nelfinavir 750 mg TID arm, one third of the patients were to be randomized to the nelfinavir 500 mg TID arm, and the remaining third of the patients were to be randomized to nelfinavir-placebo.

Nelfinavir/placebo was packaged in blinded blister packs. Each blister pack consisted of seven cards containing enough study drug for one week (i.e., three doses per day for seven days). Each dose consisted of three tablets.

For patients assigned to:

- Nelfinavir 750 mg, each dose consisted of three 250-mg tablets of nelfinavir.
- Nelfinavir 500 mg, each dose consisted of two 250-mg tablets of nelfinavir and one placebo tablet.
- Placebo, each dose consisted of three placebo tablets.

Directions for use were printed on each blister pack. Individual blister packs were labeled with a single-panel label containing the following information:

- Protocol, site, and patient number.
- Unique package number.
- Directions: Take as directed.
- Store at room temperature (59° to 86°F)
- Sponsor name and address.
- CAUTION: New Drug - Limited By Federal Law To Investigational Use

Space was provided on the label for patient initials and dispensing date. All drugs were for oral administration only.

Stavudine was provided in clinical packaging. Stavudine capsules were provided in bottles containing a two-week supply of 15-mg, 20-mg, 30-mg, or 40-mg dosage strength.

13.2.2.4.2 IDENTITY OF INVESTIGATIONAL PRODUCT

In table 2, section 5.3 of the study report, the sponsor lists shipment's dates and lot numbers of nelfinavir and placebo.

13.2.2.4.3 METHOD OF ASSIGNING PATIENTS TO TREATMENT GROUPS

At enrollment, patients were to be randomized to one of three treatment groups.

- nelfinavir 750 mg TID + d4T
- nelfinavir 500 mg TID + d4T
- nelfinavir-placebo + d4T

The objective of the randomization was to provide a balance of treatment group assignments within each of four pre-defined patient strata, at each investigative site. The strata were:

- < 6 months of prior ZDV treatment and CD4 lymphocyte count 50 cells/mm^3 to 300 cells/mm^3.
- < 6 months of prior ZDV treatment and CD4 lymphocyte count > 300 cells/mm^3.
- $\geq$ 6 months of prior ZDV treatment and CD4 lymphocyte count 50 cells/mm^3 to 300 cells/mm^3.
- $\geq$ 6 months of prior ZDV treatment and CD4 lymphocyte count > 300 cells/mm^3.

The randomization was implemented by the use of permuted blocks of the three treatments, with pre-assignment of a series of these blocks to each stratum separately at each study site. Patients within each stratum were sequentially assigned treatment according to the order of treatments within these blocks.

Patients were randomized through a central randomization center established , using an interactive voice response (IVR) system. Upon receipt of a telephone call from an authorized study site, identifying a potential study patient, the IVR solicited identification and stratification information. A patient number was then assigned and provided to the caller, the assignment was documented in the randomization database, and a confirmation fax was sent to the site with the identification information and the study number assigned. At the same time, the system automatically provided faxed notification of the unblinded treatment assignment to ProClinical, initiating the drug shipment process. The randomization list is provided in appendix 5 of the study report.

13.2.2.4.4 SELECTION OF DOSES IN THE STUDY

Dose selection was justified by results from Study AG1343-503. In this open label dose-ranging monotherapy study, nelfinavir demonstrated antiviral activity and was tolerated by patients at doses of 500 mg BID, 600 mg BID, 750 mg BID, 500 mg TID, and 1,000 mg TID. The most frequently occurring grade 2 or greater adverse event was diarrhea. The lowest incidence of grade 2 or greater diarrhea was seen in patients receiving nelfinavir 500 mg or 750 mg TID. Nelfinavir 1,000 mg TID did not appear to provide a significant advantage over 750 mg TID in the reduction of plasma HIV RNA and this dose produced a higher incidence of grade 2 or greater diarrhea. Therefore, both doses 500 mg TID and 750 mg TID were chosen for the larger clinical trials and potential use in clinical practice.

13.2.2.4.5 SELECTION AND TIMING OF DOSE FOR EACH PATIENT

Patients were randomized to one of three treatment groups for 24 weeks.

- nelfinavir 750 mg TID + d4T
- nelfinavir 500 mg TID + d4T
- nelfinavir-placebo + d4T

Nelfinavir or matching placebo was to be administered with food and eight to 12 ounces of water.
Patients weighing less than 60 kg were to receive 30 mg d4T BID and patients weighing 60 kg or more were to receive 40 mg d4T BID.

13.2.2.4.6 BLINDING

Nelfinavir/placebo was packaged in blinded blister packs. Each blister pack consisted of seven cards containing enough study drug for one week (i.e., three doses per day for seven days). Each dose consisted of three tablets. For patients assigned to:

- Nelfinavir 750 mg, each dose consisted of three 250-mg tablets of nelfinavir.
- Nelfinavir 500 mg, each dose consisted of two 250-mg tablets of nelfinavir and one placebo tablet.
- Placebo, each dose consisted of three placebo tablets.

To ensure complete blinding of study drug, supplied study drug to the site on a per patient basis. Upon randomization of a patient via the interactive voice response (IVR) system, fax notification automatically went to trigger the initial drug shipment for the patient. In an effort to maintain the study blind, all plasma HIV RNA results after Screen 1 were concealed from the investigator until after all of the patients at his/her site had completed their Week 24 evaluations.

13.2.2.4.7 PRIOR AND CONCOMITANT THERAPY

Patients were to have no prior HIV protease inhibitor or d4T exposure. Upon qualifying for Screen 2 (based on a Screen 1 plasma HIV RNA titer of 15,000 copies/mL or greater), patients who were on antiretroviral drug therapies were to begin a two-week washout period. Patients who had taken investigational or non-conventional agents for the treatment of HIV related diseases within one month before study entry were to be evaluated on a case by case basis to determine the impact of these treatments on study results. Such patients were to be included or excluded on a case by case basis after discussion with the Agouron medical monitor.

Patients were not to have taken immune modulators or vaccines within one month before baseline. Patients who had received systemic cytotoxic or radiation treatment within one month of study entry were to have fully recovered from the effects of treatment before study entry.

13.2.2.4.8 TREATMENT COMPLIANCE

Drug dispensing and return records were to be maintained for each patient for nelfinavir/placebo and d4T.

Each time the study drug was dispensed, the date and number of tablets or capsules dispensed to the patient were to be recorded on the CRFs. At each study visit, the date and number of tablets or capsules returned by the patient were to be recorded. If any tablets or capsules were unaccounted for, an explanation was to be provided. In addition, site staff were to record the number of missed doses of study drug.

13.2.2.5 EFFICACY AND SAFETY VARIABLES

13.2.2.5.1 EFFICACY AND SAFETY MEASUREMENTS ASSESSED AND FLOW CHART

Virologic and Immunologic Endpoints: Efficacy of two different doses of VIRACEPT, administered in combination with D4T, versus D4T alone, were evaluated using virologic and immunologic markers of HIV disease progression. The primary endpoints of this study are the magnitude and duration of the changes from baseline between treatment arms of the primary virologic and immunologic markers (HIV RNA titer levels and CD4 cell count). The secondary endpoints included ADC (as defined on Appendix VII of the protocol), p24 antigen levels, percent CD4, percent and absolute CD8, CD4/CD8 ratio and QoL.

HIV RNA titer (using the net change from baseline of the $\log_{10}$ transformation), and the durability of the HIV RNA decrease over the 24-week study period were evaluated.

Change from baseline in absolute CD4 cell counts was followed. The observed increase over the 24 week study period was assessed. The change in concentration of p24 antigen was evaluated for patients determined to be antigen positive at baseline.

Adverse events, including those suggesting disease progression (AIDS-Defining Condition [ADC]), and changes from baseline in laboratory parameters were monitored and evaluated during the study (see schedule of events on next page). Patients were evaluated after 24 weeks of drug administration and, if eligible, allowed to continue, for a 6-month period. The

vital status of patients was evaluated at 4 (3-6) months after the end of therapy. Adverse events and changes from baseline in laboratory parameters were graded using the Adverse Event Severity Grading Scale in Appendix II of protocol. All events were summarized, reported, and analyzed. Schedule of events for this trial are summarized on Table 16 (page 73 of this review)

13.2.2.5.2 APPROPRIATENESS OF MEASUREMENTS

The primary efficacy endpoints of this study are surrogate markers (HIV RNA and CD4 lymphocyte counts) commonly used in clinical practice. These surrogate markers have been used in multiple clinical trials of other drugs. Some of these trials have served as the basis of FDA-approval for other antiretroviral drugs. The regulatory term for the early approval of drugs based on surrogate markers is called "accelerated approval". Accelerated approval is contingent on the sponsor's conduction of confirmatory clinical trials. Therefore, the use of surrogate markers in this trial as primary efficacy endpoints is acceptable within the accelerated approval regulations.

CD4 lymphocyte counts are commonly performed in hematology laboratories. The technics used in the collection, handling, and interpretation of CD4 lymphocyte counts appear to be standard across different laboratories. It is almost certain that there is some variability in the measurements obtained from different laboratories. However, the values obtained are considered standard across different centers.

HIV RNA values obtained from the different techniques employed have not been standardized. Some of the techniques have not been validated by independent parties. Therefore, there is still uncertainty about the meaning of the magnitude changes obtained and the intercorrelation of the different assays. However, HIV RNA values are useful to clinicians and investigators in the management of patients and clinical trial, respectively.

Comment: The sponsor usec **This assay has not been approved by the FDA. By our request, the applicant submitted data provided** **on the validation of the assay. Unfortunately, the small amount of data provided in response to FDA requests was not sufficient to understand all the characteristics of the assay, including its limitations. Although the data submitted provided evidence that below the assay was not able to accurately quantify HIV RNA. The Statistical review written by Dr. Michael Elashoff, and the Microbiology review written by Drs. Lauren Iacono-Connors and Shukal Bala will comment further in the validation of the assay used in this clinical trial. Overall, the conclusion in these reviews was the lower limit of quantification for this assay for use in this application wa.** copies/mL.

TABLE 16: Flow Chart of Study Procedures*

Parameter	Screen 1[a]	Washout	Screen 2 (Day -7)	Lab Visit[b] (Day -3)	Baseline (Day 0) Study Enrollment	Week 1	Week 2	Week 4	Week 6	Week 8	Week 12	Week 16	Week 20	Week 24[c] Last Visit	2 weeks post Rx[d]	Study Exten[e] (6 mo)	4 Mo (3-6 mo) Post Rx
Demographics			X				f										
Medical History			X														
Physical Exam			X[g]		X[g]	X	X	X	X	X	X	X	X	X	X	X	
Vital signs & weight			X[g]		X[g]	X	X	X	X	X	X	X	X	X	X	X	
Karnofsky Status			X[g]		X[g]	X	X	X	X	X	X	X	X	X	X	X	
Neurological Exam			X[g]		X[g]	X	X	X	X	X	X	X	X	X	X	X	
Hematology			X		X		X	X	X	X	X	X	X	X	X	X	
Chemistry			X		X		X	X	X	X	X	X	X	X	X	X	
Urinalysis			X		X		X	X	X	X	X	X	X	X	X	X	
HIV RNA Titer	X		X	X	X	X	X	X	X	X	X	X	X	X		X	
CD4 and CD8			X	X	X	X	X	X	X	X	X	X	X	X		X	
p24[i]			X	X	X	X	X	X	X	X	X	X	X	X		X[i]	
Plasma Sample[g]					X					X		X		X		X[g]	
Plasma PBMC Sample[h]					X					X		X		X		X[h]	
Concomitant Meds			X		X	X	X	X	X	X	X	X	X	X	X	X	
Adverse Events/HIV Event					X	X	X	X	X	X	X	X	X	X	X	X	
QOL[k]/Symptom Survey					X						X			X		X[k]	
ECG[l]					X									X		X	
Vital Status Update																	X

*Flow chart is the same for all doses and placebo arms of the study.

[a] Patients will qualify for Washout with Screen #1 and will qualify for enrollment with results from Screen #2.

[b] The Lab Visit on Day -3 will be needed only for those patients requiring washout from prior antiretroviral therapy.

[c] These measurements should be performed on the last study visit if the last visit is prior to Week 24 and again at Week 24. Patients who discontinue prior to Week 24 will be asked to return at Week 24 for assessments.

[d] Visit should occur two weeks after the end of therapy for safety assessments.

[e] Study Extension procedures and assessments are performed monthly, on a months-end schedule (e.g., when a patient who is eligible for the study extension completes the first month of study drug, evaluations will be summarized as 'Month End 1')

[i] After baseline, p24 assessments will be performed only in patients antigen positive at baseline.

[g] Time population pharmacokinetic samples will be drawn at Week 2 and Week 8.

[h] PBMC samples are to be drawn pre-dose at baseline, Week 12 and Week 24 or exit and every 6 months during extension therapy, and on all patients who fail therapy.

[k] Quality of Life assessments will be completed every three months including during extension and exit. These assessments should be completed prior to any clinical evaluation.

[l] Baseline ECGs must be completed within 30 days prior to baseline, at Week 24 or patient's last visit, and at end of extension.

[f] Physical and neurologic exams, vital signs, weight and Karnofsky may be performed either at Screen 2 or baseline.

[j] Serum pregnancy test will be required for female patients of child bearing potential.

Notes:

1. Patients will have up to four plasma concentrations of Study Drug(s) determined during the study.
2. All blood samples drawn for laboratory studies (especially CD cell subsets) should be drawn at the same time of day for every patient during the study. This standardization of draw-time is an effort to control for diurnal fluctuations.
3. Plasma samples for potential virology studies will be aliquoted from the HIV RNA titer sample.

13.2.2.5.3 PRIMARY EFFICACY VARIABLES

Plasma HIV RNA by bDNA: Samples for plasma HIV RNA were collected at Screen 1, Screen 2, Day -3 (if applicable), baseline, and at Weeks 1, 2, 4, 6, 8, 12, 16, 20, and 24 (or last visit). Samples for plasma HIV RNA were also collected on Day -3 for patients who required antiretroviral washout. Calculated baseline plasma HIV RNA values were established by averaging the results from the baseline sample with the results of the most recent pre-dose sample (Day -3 or Screen 2) collected before baseline; the Screen 1 plasma HIV RNA value was not used.

CD4 Lymphocyte Count: Samples for CD4 lymphocyte counts were to be collected at Screen 2, Day -3 (if applicable), baseline, and at Weeks 1, 2, 4, 6, 8, 12, 16, 20, and 24 (or last visit). Samples for CD4 lymphocyte counts were also collected on Day -3 for patients who required antiretroviral washout. Calculated baseline CD4 lymphocyte counts were established by averaging the results from the baseline sample with the results of the most recent pre-dose sample (Day -3 or Screen 2) collected before baseline.

Comment: Both HIV RNA and CD4 lymphocyte counts, are surrogate measurements of disease status. Both measurements are used in clinical practice to follow patients' response to therapy or lack thereof. Although a correlation of changes in surrogate markers with clinical progression has not yet been established, these surrogate endpoints are considered reasonably likely to predict clinical benefit (see 21 CFR 314.510).

13.2.2.5.4 DRUG CONCENTRATION MEASUREMENTS

The sponsor collected blood samples at Week 2 and Week 8 with the objective of conducting time population pharmacokinetic analysis. Patients also had four plasma concentrations of study drug (nelfinavir and d4T) drawn during the study for population pharmacokinetics.

13.2.2.6 DATA QUALITY ASSURANCE - AUDIT CERTIFICATE

The applicant contracted to performed an audit and compare the database to the CRFs for 10% of patients selected at random. If a data table in the sample failed the audit (error rate $\geq$ 0.5%), all of the data for that table were audited. Data support services performed a 100% audit for all patients for HIV related events, AEs, and concomitant medications. Clinical data managers reviewed all data listings for outliers, data inconsistencies, and spelling errors.

In addition to the audits, Agouron conducted an audit for a random sample of 10% of the patients. Problems identified were forwarded to and/or the study site for resolution. Additional quality checks were performed on key study endpoints. Agouron also employed the services of another contract research organizatic to perform an independent audit on the programming used to generate key tables, listings, and figures.

Comment: Before the end of the study, Agouron disqualified site 38 and its investigator for violations to the protocol. This incident was reported to the Division of Scientific Investigations of the FDA.

13.2.2.7 STATISTICAL METHODS PLANNED IN THE PROTOCOL AND DETERMINATION OF SAMPLE SIZE

13.2.2.7.1 STATISTICAL AND ANALYTICAL PLANS

The sponsor planned and proposed an intent-to-treat analysis. All patients for whom data was available were to be included in the efficacy analysis. All patients exposed to drug were to be included in the safety analysis.

The primary efficacy endpoint of the study is the change in the virologic and immunologic markers of disease progression, as assessed by HIV RNA and CD4 cell count. The $\log_{10}$ transformation of the HIV RNA titer data was planned for used in the analysis of viral load. The $\log_{10}$ HIV RNA titer and CD4 cell count data was to be listed and summarized across time by treatment arm. The change from baseline was to be summarized for each evaluation period by treatment arm and compared using analysis of variance.

Analysis were to include all protocol specified intervals during the 24 week study period. For patients who have failed the Study treatment or switched to therapy other than Study treatments, their data prior to that point were to be included for treatment comparison. Data collected after that point were to be summarized to describe the changes.

The Data Safety Monitoring Board was formed by Agouron. The DSMB was to monitor the safety of the study and make recommendations regarding changes in therapy to Agouron's Medical Monitor.

13.2.2.7.2 DETERMINATION OF SAMPLE SIZE

Sample size calculations were made under the assumption that the overall sample size should be sufficient to perform independent analysis of the strata containing patients with $\geq$ 6 months of ZDV experience.

For the stratum containing patients with greater than or equal to six months of ZDV experience, a total of 43 evaluable patients per treatment group was calculated to provide 80% power to identify a mean difference of 60 CD4 lymphocytes, between patients receiving nelfinavir and placebo, assuming a significance level of 0.05 adjusted for multiple comparisons and a standard deviation (SD) of 90 cells. This sample size also was to provide 80% power to identify the mean difference of 0.41 $\log_{10}$ copies/mL plasma HIV RNA when comparing treatment groups with respect to plasma HIV RNA levels, assuming an SD of 0.7 $\log_{10}$ copies/mL. To allow for attrition, 60 patients were to be randomized to each treatment group in this stratum. Up to 60 additional patients (approximately 20 patients per treatment group) were to be enrolled with less than six months of ZDV experience. These patients were to be evaluated descriptively for treatment effects.

13.2.2.8 CHANGES IN THE CONDUCT OF THE STUDY OR PLANNED ANALYSES

Due to the rapid accrual process, the sponsor enrolled more patients than originally expected. This change is not expected to affect the study results adversely. Although not a change in planned analyses, the sponsor was instructed that a true intent-to-treat analysis focuses on the original treatment assignments regardless of treatment changes that may occur after randomization. The sponsor argued that this approach may bias the results in favor of patients randomized to placebo but who later were switched to nelfinavir. The sponsor was advised that the intent-to-treat analysis was required but this does not preclude the sponsor of doing any other analysis it considers appropriate or necessary.

Comment: This reviewer agrees with the applicant on that an ITT analysis may be problematic in this type of trials in which a "escape" clause allows patients to cross over. An ITT analysis for a study allowing patients to cross over will tend to make the study arms appear equal when they may be different. Other types of analysis may be more appropriate to present results of trials in which cross over is allowed.

13.2.3 STUDY PATIENTS

13.2.3.1 DISPOSITION OF PATIENTS

This study was conducted at 26 geographically dispersed sites across the United States. Patient enrollment began on February 13, 1996, and closed on June 12, 1996. A total of 750 patients had samples drawn for Screen 1 plasma HIV RNA. Of these, 329 were randomized into the study. According to sponsor, early during the study, Site 38 was withdrawn from participation due to GCP violations, including irregularities in source document record keeping and other inconsistencies in patient accrual. The eight patients of Site 38 are not included in the number of patients randomized.

Twenty-one patients did not receive therapy. Therefore, only 308 patients received therapy. All 308 patients are eligible for safety analysis.

Three-hundred eight patients were randomized and treated as follows: 101 patients (33%) in the nelfinavir 750 mg + d4T group, 98 patients (32%) in the nelfinavir 500 mg + d4T group, and 109 patients (35%) in the d4T alone group. While 308 patients were treated and appear in disposition and safety analyses, only 307 patients are eligible for efficacy analysis. Patient 17-07 (nelfinavir 500 mg + d4T) was discontinued from the from the study shortly after entry due to protocol violation (elevated baseline amylase and creatinine levels).

Of the 307 patients eligible for analysis, 34 patients (11%) discontinued the study.

Eight patients requested to be withdrawn from the study, seven patients did not comply with therapy, five patients were lost to follow-up and five others discontinued for other reasons. Four patients discontinued therapy due to Adverse Events. Three of these patients had diarrhea and one had a skin rash.

TABLE 17
PATIENTS WHO DISCONTINUED

Reason	Nelfinavir 750 mg+d4T	Nelfinavir 500 mg+d4T	d4T alone	Total
Patient Request	4	0	4	8
Non Compliance	3	2	2	7
Lost to follow up	0	3	2	5
Other	2	1	2	5
Diarrhea	2	0	1	3
Protocol Violation	0	2	0	2
Intercurrent Illness	0	0	1	1
Overdose	0	1	0	1
Skin Rash	1	0	0	1
Progression of Disease	1	0	0	1
TOTAL	13	9	12	34

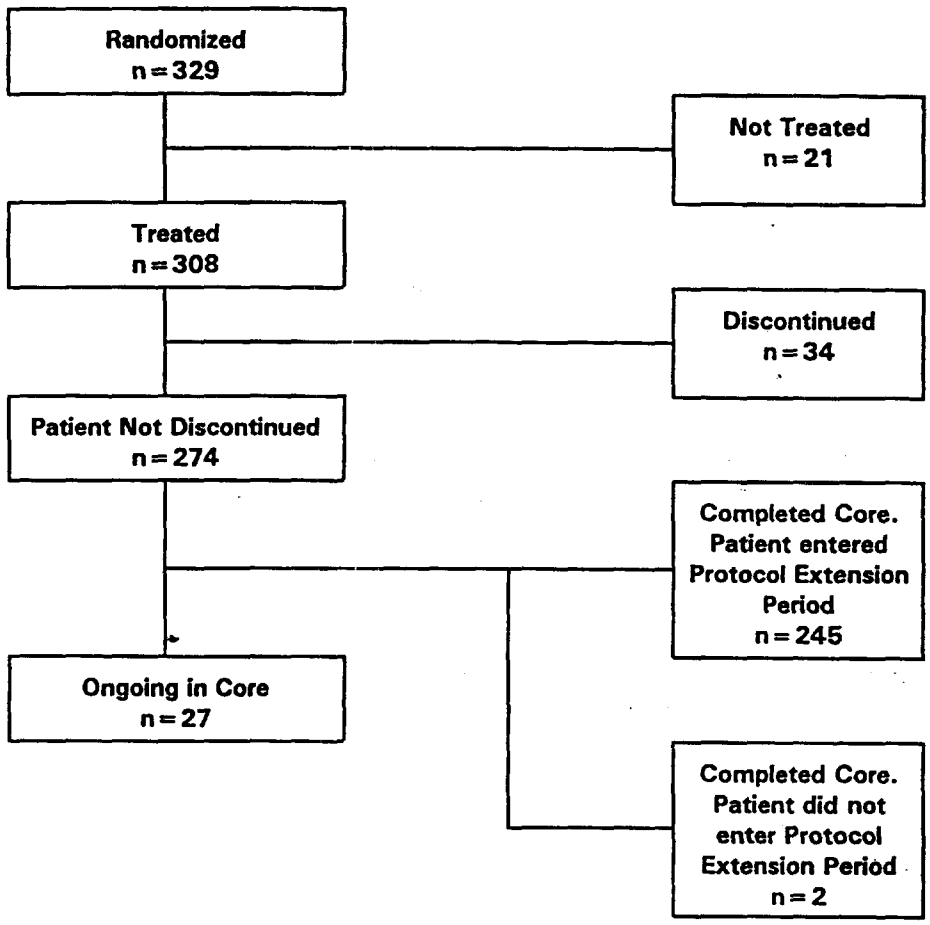
Treatment randomization was stratified to provide a balance of treatment group assignments within each of the four pre-defined patient strata at each investigational site. Table 18 presents the number of patients in each stratum by treatment group at baseline.

TABLE 18
PATIENT DISTRIBUTION BY STRATA AND TREATMENT GROUP

	Patients with < 6 months prior ZDV therapy and		Patients with ≥ 6 months prior ZDV therapy and	
	CD4 count of 50-300 cells/mm^3 N=50 (%)	CD4 count > 300 cells/mm^3 N=50 (%)	CD4 count of 50-300 cells/mm^3 N=50 (%)	CD4 count > 300 cells/mm^3 N=50 (%)
Nelfinavir 750 mg+d4T	18 (36)	21 (35)	43 (31)	19 (31)
Nelfinavir 500 mg+d4T	13 (26)	21 (35)	43 (31)	21 (34)
d4T alone	19 (38)	18 (30)	51 (37)	21 (34)

At the cutoff date, 27 patients (11%) were ongoing and 247 patients had completed the 24-week study. Figure 6 presents the patient disposition flowchart.

Figure 6. Patient Disposition Flow Chart



13.2.4 EFFICACY EVALUATION

13.2.4.1 DEMOGRAPHIC AND OTHER BASELINE CHARACTERISTICS

Demographic data of patients in study 506 is presented in Table 19.

TABLE 19
DEMOGRAPHIC CHARACTERISTICS AND BASELINE CD4 CELL COUNTS

Characteristics	Nelfinavir 750mg+d4T n=101 (%)	Nelfinavir 500mg+d4T n=98 (%)	d4T Alone n=109 (%)	TOTAL n=308 (%)
Gender				
Male	89 (88)	90 (92)	94 (86)	273 (89)
Female	12 (12)	8 (8)	15 (14)	35 (11)
Race				
White	72 (71)	71 (72)	87 (80)	230 (75)
Black	16 (16)	18 (18)	12 (11)	46 (15)
Other	13 (13)	9 (9)	10 (9)	32 (10)
Age (years)				
Mean	36.4	39.2	37.9	37.8
Range	23 - 56	23 - 69	21 - 53	21 - 69
Baseline CD4 cell count (cells/mm^3) - Number of Patients				
$\leq$ 100	13 (13)	13 (13)	20 (18)	46 (15)
> 100 to $\leq$ 300	48 (47)	43 (44)	50 (46)	141 (46)
> 300	40 (40)	42 (43)	39 (36)	121 (39)
Baseline Plasma HIV RNA (Log_{10} copies/mL)				
Mean	4.9	4.9	4.8	4.9
Standard Deviation	0.48	0.46	0.49	0.47
Time since HIV Diagnosis (months)				
Mean	59.2	57.1	61.2	59.3
Range	2 - 151	1 - 183	2 - 136	1 - 183

Two-hundred seventy-three patients were male (89%), two-hundred thirty (75%) were white, forty-six (15%) were black, and thirty-two (10%) were of other ethnic group. The age, CD4 cell counts, and Plasma HIV RNA at baseline had a similar distribution in the three study arms.

13.2.4.2 MEASUREMENTS OF TREATMENT COMPLIANCE

Compliance was to be monitored by tablet count at each visit. Some patients were discontinued due to lack of compliance. However, there is no description of compliance results in the analysis of the data.

13.2.4.3 EFFICACY RESULTS AND TABULATIONS OF INDIVIDUAL PATIENT DATA

13.2.4.3.1 ANALYSIS OF EFFICACY

The sponsor is requesting approval of nelfinavir tablets based on changes of the two surrogate markers of HIV infection most commonly used at the present time: Plasma HIV RNA and CD4 cell counts. The sponsor conducted an Intent To Treat (ITT) analysis on changes of these surrogate markers as specified in the protocol. The sponsor also conducted analyses of the data using different criteria:

1. Patients who switched from the nelfinavir placebo group to one of the nelfinavir doses (500 mg TID or 750 mg TID) were analyzed in the group to which they switched. The sponsors rationale for this type of analysis is that the ITT analysis biased the results in favor of the placebo group because patients who switched to nelfinavir showed changes in the surrogate markers that tend to approach those of the patients originally randomized to one of the nelfinavir doses. Therefore, the difference between the groups would tend to disappear. Fortunately, most patients assigned to ZDV + 3TC remained in that arm of the study. As of twenty-four weeks of study, only fourteen patients switched from ZDV + 3TC alone to one of the nelfinavir doses.

2. The sponsor also conducted an analysis with the Last Observation Carried Forward.

Comment: As previously noted, the statistical analysis of the data was done using Intent-To-Treat analysis. The sponsor also conducted two other types of analysis. The protocol specified analysis was performed on data up to the time of the second return to baseline for patients who were treatment failures or up through Week 24 or study discontinuation for patients who were not treatment failures. The other analysis carried the last observation forward. The carry forward analysis incorporates, for patients who fail therapy, the surrogate marker data at confirmation of treatment failure as if that value had been observed at all subsequent time points up through and including Week 24. With either approach, the results of this study arrived to the same conclusion: Nelfinavir appears to be an active antiretroviral drug demonstrated by the changes in CD4 cell counts and HIV RNA over time. Other details of the statistical analysis are discussed in Dr. Michael Elashoff's review of the data.

These different analysis techniques were not expected to yield significant differences. The results presented by the sponsor in all three analysis are very similar. FDA traditionally has requested sponsor to performed ITT analysis of the data. Therefore, this review will only refer to results obtained by the Intent To Treat analysis.

The ITT analysis was performed on data based on original treatment assignment, disregarding treatment changes or switches, and includes all efficacy data obtained through Week 24 or study discontinuation. Some patients in the nelfinavir groups were allowed to change to another RTI due to adverse events or intolerance of d4T.

Comment: According to the sponsor, this type of analysis that does not account for treatment switches, may not show the actual contribution of the investigational agent to the differences observed. HIV RNA and CD4 cell count in the placebo group tend to approach the values obtained in the active drug treatment groups.

During the course of the study, 14 of 101 (14%) nelfinavir 750 mg+d4T, 19 of 97 (19%) nelfinavir 500 mg+d4T, and 47 of 109 (43%) d4T alone patients experienced treatment failure. Table 20 lists patients in the nelfinavir containing regimens who added to or changed their antiretroviral regimen. Of the 47 d4T patients who experienced treatment failure, 40 patients switched to nelfinavir + d4T prior to Week 24, four patients switched after Week 24 and two discontinued study prior to switch. Of the 40 patients who switched to nelfinavir + d4T, 19 patients switched to nelfinavir 750 mg+d4T arm and 21 patients switched to nelfinavir 500 mg+d4T arm.

TABLE 20
ANTIRETROVIRAL TREATMENT CHANGES FOR PATIENTS ON NELFINAVIR PRIOR TO WEEK 24

ID	Rx Failure Time to Failure	Time on Study Before Add/Change	Changed or Added New Antiretroviral	Reason
		Nelfinavir 750 mg + d4T		
	Yes Week 6	10 Weeks	Added 3TC	Treatment failure
	No	16 Weeks	Added ZDV+3TC	Patient's choice
	Yes Week 17	21 Weeks	Changed to ZDV +3TC+Ritonavir	Treatment failure
	Yes Week 8	10 Weeks	Changed to ddI+ ddC+Nevirapine	Treatment failure
	Yes Week 12	23 Weeks	Stop d4T, added ZDV+3TC	Treatment failure
	Yes Week 4	20 Weeks	Stop d4T, added ZDV+3TC	Adverse Event (Peripheral Neuropathy)
		Nelfinavir 500 mg + d4T		
*	No	11 Weeks	Stop d4T	Adverse Event (Peripheral Neuropathy)
	Yes Week 8	16 Weeks	Added 3TC	Treatment failure

*This patient also appears in Table 21 because at Week 21 he was considered a failure. At Week 24 patient started ZDV+3TC

TABLE 21
ANTIRETROVIRAL TREATMENT CHANGES FOR PATIENTS ON NELFINAVIR INITIATED AT OR AFTER WEEK 24

ID	Rx Failure Time to Failure	Time on Study Before Add/Change	Changed or Added New Antiretroviral	Reason
Nelfinavir 750 mg + d4T				
	Yes Week 7	28 Weeks	Added ddI	Adverse Event (Kaposi's Sarcoma)
Nelfinavir 500 mg + d4T				
	No	24 Weeks	Add 3TC	Triple therapy
	Yes Week 16	24 Weeks	Add 3TC	Treatment failure
	No	25 Weeks	Stop d4T, Add 3TC	Adverse Event (Peripheral Neuropathy)
	Yes Week 16	24 Weeks	Change to Ritonavir +ZDV+3TC	Treatment failure
	Yes Week 20	24 Weeks	Add 3TC	Treatment failure
	Yes Week 21	24 Weeks	Add ZDV+3TC	Adverse Event (Peripheral Neuropathy)

Tables 20 and 21 were generated using data from Tables 8, 9 (section 8, volume 23), and Data Listing 11B (section 8, volume 26) of applicant's submission.

13.2.4.3.2 STATISTICAL/ANALYTICAL ISSUES

As previously noted, the statistical analysis of the data was done using the Intent-To-Treat analysis. The sponsor also conducted two other types of analysis. In one of the analysis, the sponsor grouped the placebo patients who failed in the group to which they were switched. The other analysis carried the last observation forward. With either approach, the results of the study arrived to the same conclusion: Nelfinavir appears to be an active antiretroviral drug demonstrated by the changes in CD4 cell counts and HIV RNA over time. Other details of the statistical analysis are discussed in Dr. Michael Elashoff's review of the data.

13.2.4.3.3 EFFICACY RESULTS

The ITT analysis was performed on data based on original treatment assignment, disregarding treatment changes or switches, and includes all available efficacy data obtained through Week 24 or study discontinuation. Data for patients originally assigned to the placebo group (i.e., d4T alone) included data from patient who were switched to nelfinavir + d4T as a result of protocol defined treatment failure. In addition, data for patients assigned to nelfinavir + d4T actually included data for patients who failed on their initially assigned treatment and were allowed to change to another RTI.

More than twice as many d4T alone patients experienced treatment failure as compared to the nelfinavir 750 mg + d4T or nelfinavir 500 mg + d4T groups. By Week 24, a total of 14 (14%) nelfinavir 750 mg + d4T patients, 19 (19%) nelfinavir 500 mg + d4T patients and 47 (43%) d4T alone patients experienced treatment failure. The majority of treatment failure events were determined by CD4 lymphocyte count criteria (i.e., return to calculated baseline of CD4 lymphocyte count as measured at two consecutive timepoints after four weeks of treatment). Of the 47 d4T patients who experienced treatment failure, 40 patients switched to nelfinavir + d4T prior to Week 24, four patients switched after Week 24 and two discontinued study prior to switch.

Figure 7 presents the changes from baseline in mean $\log_{10}$ transformed plasma HIV RNA observed during the 24-Week treatment period, as determined by bDNA, for the three treatment groups. This figure shows the difference between the nelfinavir arms and the d4T alone arm. Over time, the difference appear to decrease as the plasma HIV RNA in the nelfinavir groups rises to approach the values in the d4T arm. The difference between the two nelfinavir arms was not statistically significant.

Figure 7 and 8 were generated by FDA reviewers using electronic data submitted by the applicant. The lower limit of HIV RNA quantification for the generation of these figures was 1,200 copies/mL.

Figure 7:

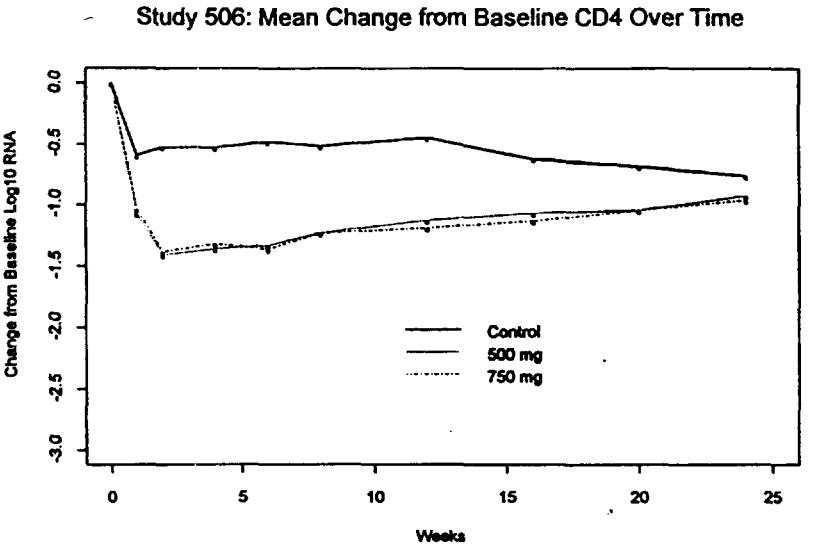
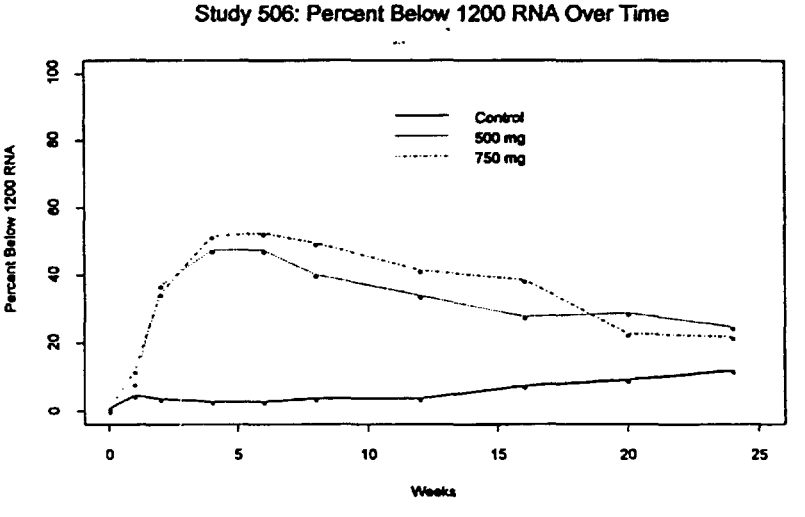


Figure 8 presents the proportion of patients with untransformed plasma HIV RNA values below the limit for which the assay can acceptably estimate viral particles (1,200 estimated viral particles/mL) at each timepoint during the study.

Figure 8:



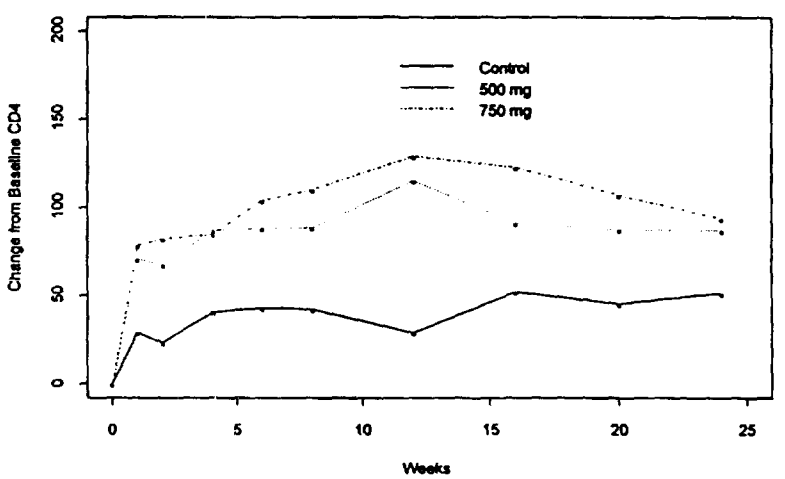
Decreases in estimated plasma HIV RNA to below 1,200 copies/mL were seen in all three treatment groups by Week 4. At this timepoint, 52%, 45% and 3% of patients in the nelfinavir 750 mg + ZDV + 3TC, nelfinavir 500 mg + ZDV + 3TC, and ZDV + 3TC alone groups, respectively, were below the estimated RNA value of 1,200. By Week 12, 33%, 27%, and 4% of patients in nelfinavir 750 mg + ZDV + 3TC, nelfinavir 500 mg + ZDV + 3TC, and ZDV + 3TC alone groups, respectively, were below the 1,200 estimated RNA

value. By Week 16, 26%, 18%, and 5% of patients in nelfinavir 750 mg+ZDV+3TC, nelfinavir 500 mg +ZDV+3TC, and ZDV+3TC alone groups, respectively, were below estimated RNA value of 1,200.

Comment: The percentages in the paragraph above are based on the total number of patients randomized to treatment groups. The sponsor presented data in percentages using as a denominator the total number of patients still in study at the time of the analysis. However, this reviewer considers the applicant approach potentially misleading because the sponsor does not take into account all the patients in the study. All these patients had the same potential to reach estimated HIV RNA values below 1,200 copies/mL. All patients should remain in the denominator because they had the same potential to contribute to the numerator as did the patients who remain in the study.

Figure 9 presents the changes from baseline of CD4 lymphocyte counts observed during the 24-Week treatment period for the three treatment groups. This figure shows the difference between the nelfinavir arms and the d4T alone arm. The differences between nelfinavir 750 mg+d4T and d4T alone and between nelfinavir 500 mg+d4T and d4T alone in mean CD4 lymphocyte counts were statistically significant ($p<0.0001$). The difference between the two nelfinavir containing arms was not statistically significant.

Figure 9: Study 506: Mean Change from Baseline CD4 Over Time



13.2.4.3.4 EFFICACY CONCLUSIONS

Changes on CD4 lymphocyte counts and HIV RNA shortly after starting therapy with nelfinavir in combination with d4T clearly showed activity of this treatment regimen. The comparator regimen of d4T alone had also some antiretroviral activity, although this was more modest than the double combination therapy of nelfinavir + d4T. Forty patients randomized to the d4T alone arm switched to one of the nelfinavir containing arms. This may have contribute to a decrease in the difference between the nelfinavir containing arms and d4T alone arm. The maximum effect on viral suppression was observed between Study Week-2 and Study Week-8. Patients in both nelfinavir containing arms had also greater increases in CD4 lymphocyte counts than patients in the d4T alone arm. These changes in surrogate markers were sustained up to the predetermined cut-off date for this study (Week-24). Although, the Applicant has not demonstrated that these changes in surrogate markers translate into clinical benefits for patients, the changes observed in this study are considered adequate evidence of antiretroviral activity.

13.2.5 SAFETY EVALUATIONS

All patients who received drug and had at least one follow up visit are included in the analysis of safety. Patients were exposed to different dosage levels and different lengths of treatment. A comparison is made for patients receiving the two different nelfinavir doses and those receiving nelfinavir-placebo. Data from patients who discontinued drug due to adverse events were reviewed in an attempt to identify possible risk factors associated with their ADEs. All patients with serious ADEs were also reviewed individually in an effort to recognize possible risk factors associated with the ADEs. No patient died during this study.

13.2.5.1 EXTENT OF EXPOSURE

According to sponsor, mean days on study were similar among treatment groups. Mean days on study treatment for the three treatment groups were 153 (range, 10 to 151), 154 (range, 7 to 177), and 154 days (range, 7 to 185) for the nelfinavir 750 mg + d4T, nelfinavir 500 mg + d4T and the d4T alone groups respectively. Table 22 summarizes nelfinavir exposure by dose and time in this study.

TABLE 22
EXPOSURE TO NELFINAVIR OVERALL

Drug Exposure	Nelfinavir 750 mg+d4T n=101	Nelfinavir 500 mg+d4T n=98
Cumulative dose (g)[1]		
Mean	327.1	219.8
Min	27.0	10.5
Max	437.3	294.0
Average daily dose (mg/day)[2]		
Mean	2,143	1,430
Min	981	805
Max	3,000	1,826

[1] Cumulative dose = (total tablets dispensed-total tablets returned)Xdose strength
[2] Average daily dose = cumulative dose/number of days in study before the time of drug discontinuation

13.2.5.2 ADVERSE EVENTS

13.2.5.2.1 BRIEF SUMMARY OF ADVERSE EVENTS

The most common adverse event observed in this clinical trial was diarrhea. Diarrhea appears to be associated to the use of nelfinavir. Most patients appear to tolerate this adverse event without the need to modify the dose or schedule of drug administration. Some patients successfully used antimotility agents to treat this nelfinavir-associated diarrhea. All other clinical and laboratory adverse events do not appear to be associated with nelfinavir therapy.

13.2.5.2.2 DISPLAY OF ADVERSE EVENTS

The sponsor summarized Adverse Events by investigator's assessment of severity and causality. Unfortunately, causality of Adverse Events is a complex process that goes beyond the perception of individual investigators. Therefore, in this review Adverse Events will be summarized by treatment groups regardless of perceived causality to drug. (Table 23, page 90).

TABLE 23
NUMBER OF PATIENTS WITH ADVERSE EVENTS OF AT LEAST GRADE 2 SEVERITY REPORTED BY ≥ 3% OF PATIENTS DURING THE STUDY

	nelfinavir 750mg +d4T n=101 n (%)	nelfinavir 500mg +d4T n=98 n (%)	d4T alone n=109 n (%)	Total n=308 n (%)
Gastrointestinal System				
Diarrhea	32 (31)	31 (32)	11 (10)	74 (24)
Abdominal Pain	6 (6)	4 (4)	6 (6)	16 (5)
Flatulence	3 (3)	9 (9)	4 (4)	16 (5)
Nausea	3 (3)	6 (6)	2 (2)	11 (4)
Body as a Whole				
Headache	5 (5)	9 (9)	5 (5)	19 (6)
Asthenia	1 (1)	6 (6)	6 (6)	13 (4)
Pain	6 (6)	3 (3)	1 (1)	10 (3)
Fever	3 (3)	9 (9)	4 (4)	16 (5)
Nervous System				
Depression	3 (3)	4 (4)	4 (4)	11 (4)
Hematology				
Leukopenia	2 (2)	3 (3)	7 (7)	12 (4)
Skin and Appendages				
Rash	4 (4)	6 (6)	3 (3)	13 (4)

This table was generated by the FDA Medical Reviewer using data submitted by the applicant.

Table 23, generated by this reviewer using Agouron's data, presents ADEs of at least grade 2 severity reported by ≥ 3% of patients. Table 24 shows the applicant's summary of adverse drug events related to study drug. This table presents ADEs with the same severity criterion but reported by ≥ 2% of patients. This reviewer's table (Table 23) presents the data regardless of relationship to drug where as in Table 24 the applicant presents ADEs perceived to be related to drug.

Comment: The differences between both ways of presenting these data are small.

TABLE 24
STUDY DRUG RELATED, TREATMENT EMERGENT ADVERSE EVENTS OF AT LEAST GRADE 2 SEVERITY REPORTED BY ≥ 2% OF PATIENTS

Body System Adverse Event	nelfinavir 750mg +d4T n=101 n (%)	nelfinavir 500mg +d4T n=98 n (%)	d4T alone n=109 n (%)	Total n=308 n (%)
At Least One Drug Related Adverse Event of at Least Grade 2 Severity	49 (49)	43 (44)	34 (31)	126 (41)
Body as a Whole				
Abdominal Pain	4 (4)	2 (2)	3 (3)	9 (3)
Asthenia	1 (1)	3 (3)	4 (4)	8 (3)
Headache	2 (2)	2 (2)	1 (1)	5 (2)
Digestive System				
Diarrhea	32 (31)	27 (28)	11 (10)	70 (23)
Flatulence	3 (3)	8 (8)	4 (4)	15 (5)
Nausea	2 (2)	3 (3)	1 (1)	6 (2)
Hemic/Lymphatic System				
Leukopenia	0	0	3 (3)	3 (1)
Nervous System				
Insomnia	0	2 (2)	1 (1)	3 (1)
Skin and Appendages				
Pruritus	2 (2)	1 (1)	1 (1)	4 (1)
Rash	3 (3)	4 (4)	0	7 (2)

13.2.5.2.3 ANALYSIS OF ADVERSE EVENTS

As in study 511, the most common ADE observed in study 506 was diarrhea. More patients in the nelfinavir groups had diarrhea than patients in the nelfinavir-placebo group. In this study, this association does not appear to be dose related as it appeared to be in study 511. All other ADEs do not appear to be associated with nelfinavir.

13.2.5.2.4 LISTING OF SERIOUS ADVERSE EVENTS BY PATIENT

Table 25 lists all patients having serious adverse events at the 24-Week evaluation point.

TABLE 25
PATIENTS EXPERIENCING SERIOUS ADVERSE EVENTS

Site and Patient No.	COSTART Term	Severity Grade	Action Taken
	Nelfinavir 750 mg TID + d4T		
	Bacterial infection	3	Therapy required, hospitalization
	Pneumonia	4	Hospitalization (required or prolonged)
	Depression	4	Therapy required, hospitalization
	Pneumonia	2	Therapy required, hospitalization
	Cholelithiasis	3	Hospitalization (required or prolonged)
	Nelfinavir 500 mg + d4T		
	Meningitis	3	Therapy required, hospitalization
	Deep vein thrombophlebitis	4	Therapy required, hospitalization
	Diarrhea	4	Hospitalization (required or prolonged)
	Overdose	4	Study drug stopped
	Depression Dehydration	3 3	Hospitalization (required or prolonged)
	Accidental injury	2	Hospitalization (required or prolonged)
	Accidental injury	3	Hospitalization (required or prolonged)
	d4T alone		
	Chest pain	3	Therapy required, hospitalization
	Asthma	3	Hospitalization (required or prolonged)
	Pneumonia	2	Hospitalization (required or prolonged)
	Accidental injury	3	Therapy required, hospitalization
	Kidney calculus	4	Therapy required, hospitalization

Five patients in each the nelfinavir 750 mg + d4T and the d4T alone had serious adverse events. Seven patients in the nelfinavir 500 mg + d4T had serious adverse events. Patient 37-05 in the nelfinavir 500 mg + d4T ingested 27 nelfinavir tablets ever a period of 24 hours. No patient died during study and at follow-up and no patient discontinued the study due to serious ADE.

13.2.5.3 DEATHS

No patient in this study died as of Week-24.

13.2.5.4 CLINICAL LABORATORY EVALUATION

All the information presented in this section is based on the analysis of data at Week-16 of study.

Hematology

Anemia was an expected ADE; however, only patient 33-03 had a hemoglobin value below 8 g/dL at some point during the study. This patient, assigned to the d4T alone arm, had a hemoglobin of 7.5 g/dL at day 14 of study. This was also the last visit of this patient due to seizure activity.

Neutropenia (absolute neutrophil count less than 750 cell/mm^3). There were 16 patients who had neutropenia. Seven patients were in the nelfinavir 750 mg arm, 3 patients were in the nelfinavir 500 mg arm, and 6 patients were in the d4T alone arm. No patient left the study due to this ADE.

Serum Chemistry

Serum Alanine Aminotransferase (ALT) was greater than five times the upper limit of normal in 26 patients. The upper limit of normal for the purpose of this review is 30 U/L. Twelve patients were in the nelfinavir 750 mg arm, 10 patients were in the nelfinavir 500 mg arm, and four patients were in the d4T alone arm. Although the numbers are small, it appears that increases in ALT are associated to nelfinavir use. However, this finding is only present in this study. Other studies did not show a association between nelfinavir use and ALT increases.

Serum Aspartate Aminotransferase (AST) was greater than five times the upper limit of normal in 12 patients. The upper limit of normal for the purpose of this review is 35 U/L. Three patients were in the nelfinavir 750 mg arm, seven patients were in the nelfinavir 500 mg group, and two patients were in the d4T alone arm.

Serum Gamma-Glutamyl Transferase (GGT) was greater than five times the upper limit of normal in 17 patients. The upper limit of normal for the purpose of this review is 50 U/L. Six patients were in the nelfinavir 750 mg arm, nine patients were in the nelfinavir 500 mg arm, and two patients were in the d4T alone arm.

Serum Lactate Dehydrogenase (LDH). No patient had elevated LDH during this study.

Serum creatinine greater than 1.5 mg/dL was observed in three patients. All three patients were in the d4T alone arm.

13.2.5.5 SAFETY CONCLUSIONS

The overall safety of nelfinavir two dosages in this group of patients do not appear to be different from the group of patients on d4T alone. The only adverse event that is clearly associated to study drug was diarrhea. Most patients tolerated diarrhea with antimotility agents for symptomatic relief.

Of the 329 patients randomized into the study, forty-five did not complete study due to various reasons. Seventeen patients were in the nelfinavir 750 mg arm, 14 were in the nelfinavir 500 mg arm, and 14 were in the d4T alone arm.

No patient died during the study.

The safety profile of nelfinavir in children, pregnant women, elderly patients, and people with abnormalities of drug metabolism or excretion was not tested during this clinical trial. As with most clinical trials, the population that participated in this study was fairly homogenous due to the enrollment and exclusion criteria. Therefore, there are limitations in the extrapolation of these data to the general population. This drug has the potential to be use in combination with drugs other than stavudine. In another study, the sponsor used nelfinavir in combination with ZDV and 3TC. However, the safety profile of nelfinavir in combination with other reverse transcriptase inhibitors

and protease inhibitors have not been tested. Physicians prescribing nelfinavir should be made aware of the limitations of the known safety of nelfinavir. Pharmacovigilance and future clinical trials may contribute to a better understanding of the safety profile of nelfinavir in clinical use.

13.2.6 OVERALL CONCLUSIONS

The effects of nelfinavir in combination with d4T on the surrogate markers of HIV disease (viremia and CD4 cell count) is best summarized in figures 7, 8, and 9 above. Nelfinavir in combination with d4T decreases viremia in most patients. In some patients viremia decreases to levels that are difficult or impossible to measure with accuracy with the current available technology. Low viremia is perceived by sponsors, investigators, and practicing clinicians as the goal of therapy with current drug combinations for HIV infection. Although the clinical meaning of changes in these surrogate markers has not been validated, physicians in clinical practice are using viremia to modify and monitor their patients treatment. Therefore, the results observed in this study are relevant to current medical practice. The safety profile of nelfinavir in combination with d4T was summarized in section 18 of this review. The most common drug associated adverse event was diarrhea. Diarrhea appears to be tolerated by most patients either without treatment or with symptomatic treatment with antimotility agents. Other adverse events, clinical or laboratory, do not appear to be particularly associated to nelfinavir use. Therefore, the potential benefits of nelfinavir in combination with d4T appear to outweigh the risks of using this drug as part of this combination. However, this combination did not caused drops in viremia at the same magnitude as the triple combination of nelfinavir + ZDV + 3TC.

13.3 STUDY NUMBER 3: STUDY 505

"A Phase 2, Randomized, Double-Blind, Placebo-Controlled, Dose-Range Finding Study of Viracept as Monotherapy in HIV Positive Patients."

The objectives of this study were:

1. To evaluate the safety and efficacy of two doses of nelfinavir compared to placebo administered for 24 weeks as monotherapy in 90 antiretroviral-naive (< 6 months) or reverse transcriptase inhibitor-experienced HIV positive patients with CD4 lymphocyte counts $\geq$ 50 cells/mm^3 and quantitative plasma HIV RNA levels $\geq$ 15,000 copies/mL.

2. To obtain long term safety and efficacy data on the two nelfinavir treatment groups.

3. To assess the potential for viral resistance, including changes in genotype, phenotype, and viral sensitivity, in a subset of patients at selected clinical sites.

Randomization was stratified according to duration of previous RTI treatment (< 6 months and ≥ 6 months). Each stratum was to have a pre-defined number of patients. Further balance was achieved by a second stratification with respect to CD4 lymphocyte counts (≥ 50 to ≤ 300, or > 300 cells/mm^3). Randomization then occurred within each of the four strata.

Thirty patients each were to be randomized to receive nelfinavir 500 mg or 750 mg TID or placebo for four weeks (Stage I). After four weeks, all placebo patients started nelfinavir 500 mg or 750 mg TID for 20 weeks on a double blind basis. Patients were to continue the study for a total of 24 weeks (Stage II). Table 26 summarizes the distribution of patients for both Stage I and Stage II.

TABLE 26
DISTRIBUTION OF PATIENTS IN STAGE I AND STAGE II

Stage I: Weeks 1 to 4		
Number of Patients	**Study Drug Dose**	**Total Daily Dose**
30	Nelfinavir 750 mg TID	2,250 mg
30	Nelfinavir 500 mg TID	1,500 mg
30	Placebo	
Stage II: Weeks 5 to 24		
Number of Patients	**Study Drug Dose**	**Total Daily Dose**
45	Nelfinavir 750 mg TID	2,250 mg
45	Nelfinavir 500 mg TID	1,500 mg

13.3.2 RESULTS

Note: The following summary of efficacy and safety results are presented as summarized by the applicant. This reviewer agrees with results as presented by Agouron Pharmaceuticals.

Patient enrollment began on February 15, 1996, and closed on May 30, 1996. A total of 213 patients had samples drawn for Screen 1 plasma HIV RNA. Of these, 97 were randomized into the study. Of the 97 patients randomized into the study, four patients were not treated. Ninety-three patients were randomized, treated, and included in Stage I analysis as follows: 29 patients (31%) in the nelfinavir 750 mg group, 32 patients (34%) in the nelfinavir 500 mg group, and 32 patients (34%) in the placebo group. Of the 32 patients initially randomized to placebo, 15 patients were to receive nelfinavir 750 mg and 17 patients were to receive nelfinavir 500 mg after completion of Week 4 evaluations. While 93 patients were treated and appear in Stage I disposition and safety analyses, only 91 patients were eligible for efficacy analysis. Two patients discontinued shortly after entry due to toxicity. Ninety-one patients were included in Stage II analyses. Of these, 43 patients (47%) were in the nelfinavir 750 mg group and 48 patients (53%) were in the nelfinavir 500 mg group.

The following two tables present the demographic characteristics of patients in Stage I and Stage II of the study.

The mean age of patients was 39 years (range, 20 to 68). The majority of patients were white males (91% Caucasian and 90% male). In all of these characteristics, the three groups were similar. (Table 27, page 98).

TABLE 27
DEMOGRAPHIC AND BASELINE CHARACTERISTICS: STAGE I

Characteristics	Nelfinavir 750mg n=29 (%)	Nelfinavir 500mg n=32 (%)	Placebo n=32 (%)	TOTAL n=93(%)
Gender				
Male	27 (93)	27 (84)	30 (94)	84 (90)
Female	2 (7)	5 (16)	2 (6)	9 (10)
Race				
White	27 (93)	29 (91)	29 (91)	85 (91)
Black	2 (7)	1 (3)	1 (3)	4 (4)
Other	0	2 (6)	2 (6)	4 (4)
Age (years)				
Mean (SD)	41.7 (8.4)	37.3 (10.1)	38.3 (9.4)	39.0 (9.4)
Range	25 - 60	23 - 68	20 - 57	20 - 68
Baseline CD4 cell count				
Mean (SD)	286 (140.9)	261 (152.4)	283 (157.0)	276 (149.4)
Range	55 - 581	32 - 594	44 - 737	32 - 737
Baseline Plasma HIV RNA (Log_{10} copies/mL)				
Mean (SD)	4.95 (0.5)	4.91 (0.6)	4.82 (0.5)	4.89 (0.5)
Range	4.0 - 6.13	3.67 - 6.11	4.05 - 5.62	3.67 - 6.13
Time since HIV Diagnosis (months)				
Mean (SD)	74.7 (41.1)	62.2 (44.9)	57.0 (40.2)	64.3 (42.3)
Range	7 - 146	7 - 159	< 1 - 160	< 1 - 160

Table 28 (page 99) summarizes the demographic characteristics of patients in Stage II of this study. The differences observed were not considered clinically significant.

On Stage I, no patient received other antiretrovirals. During Stage II, nine patients received other antiretrovirals; eight patients (9%) received 3TC, six patients (7%) received ZDV, four patients (4%) received d4T, and one patient (1%) received ddI.

TABLE 28
DEMOGRAPHIC AND BASELINE CHARACTERISTICS: STAGE II

Characteristics	Nelfinavir 750mg n=43 (%)	Nelfinavir 500mg n=48 (%)	TOTAL n=91(%)
Gender			
Male	40 (93)	42 (88)	82 (90)
Female	3 (7)	6 (13)	9 (10)
Race			
White	41 (95)	42 (88)	83 (91)
Black	2 (5)	2 (4)	4 (4)
Other	0	4 (8)	4 (4)
Age (years)			
Mean (SD)	41.7 (8.6)	36.5 (9.7)	39 (9.5)
Range	25 - 60	20 - 68	20 - 68
Baseline CD4 cell count			
Mean (SD)	280 (152.7)	284 (153.4)	282 (152.2)
Range	33 - 659	32 - 715	32 - 715
Baseline Plasma HIV RNA (Log_{10} copies/mL)			
Mean (SD)	4.91 (0.53)	4.83 (0.54)	4.87 (0.53)
Range	3.58 - 6.13	3.67 - 6.11	3.58 - 6.13
Time since HIV Diagnosis (months)			
Mean (SD)	71.6 (40.4)	57.1 (40.8)	64 (41)
Range	4 - 146	3 - 159	3 - 159

13.3.2.1 EFFICACY RESULTS

The Stage I ITT analysis showed statistically significant differences among treatment groups for mean AUCMB for log_{10} transformed plasma HIV RNA values ($p<0.0001$). The difference in the mean AUCMB for log_{10} transformed HIV RNA values between nelfinavir 750 mg and placebo was statistically significant ($p<0.0001$), as was the difference between nelfinavir 500 mg and placebo ($p<0.0001$). The difference between nelfinavir 750 mg and nelfinavir 500 mg was not significant (See Table 29, page 100).

TABLE 29
MEAN AUCMB FOR Log_{10} TRANSFORMED PLASMA HIV RNA FOR STAGE I

Treatment Group	Stage I			
	Mean AUCMB (log_{10} copies/mL)	p-value		
		Overall[a]	Between Treatments[b]	
Nelfinavir 750mg	-1.019	<0.0001	750mg vs. placebo	<0.0001
Nelfinavir 500mg	-0.842		500mg vs. placebo	<0.0001
Placebo	-0.026		750mg vs. 500mg	0.1023

[a] p-value derived from an ANOVA model, with factors for treatment and site
[b] p-value derived from contrasts in the ANOVA model for pairwise comparisons

The ITT analysis of Stage II results showed a statistically significant difference (p=0.0410) between treatment groups for mean AUCMB for log_{10} transformed plasma HIV RNA values. Nelfinavir 750 mg produced a greater reduction in plasma HIV RNA compared to nelfinavir 500 mg. See Table 30 below).

TABLE 30
MEAN AUCMB FOR Log_{10} TRANSFORMED PLASMA HIV RNA FOR STAGE II

Treatment Group	Stage II	
	Mean AUCMB (log_{10} copies/mL)	Overall p-value[a]
Nelfinavir 750mg	-0.925	0.0410
Nelfinavir 500mg	-0.673	

[a] p-value derived from an ANOVA model, with a fixed factor for treatment

Comment: This statistically significant difference on Mean AUCMB log_{10} plasma HIV RNA in favor of nelfinavir 750 mg over nelfinavir 500 mg was not observed when nelfinavir was administered in combination with nucleoside analog agents (Studies 511 and 506). In study 511 the difference between these two doses was in the proportion of patients below 1,200 estimated HIV RNA copies/mL. This difference was only observed in a subgroup of patients considered to have advanced HIV disease (patients with CD4 $\leq$ 300 cells/mL and HIV RNA > 100,000 copies/mL). Although the clinical significance of these findings are difficult to assess due to the limitations of this study (monotherapy, large attrition [> 50% in both arms]), it appears most appropriate to recommend nelfinavir 750 mg TID for clinical use pending further data.

The Stage I analysis (ITT) showed statistically significant differences among treatment groups in the mean AUCMB for CD4 lymphocyte count (p=0.0001). The difference in the mean AUCMB for CD4 lymphocyte counts between nelfinavir 750 mg and placebo were statistically significant (p=0.0001), as was the difference between nelfinavir 500 mg and placebo (p=0.0015). The difference between nelfinavir 750 mg and nelfinavir 500 mg was not significant (See Table 31 below).

TABLE 31
MEAN AUCMB FOR CD4 LYMPHOCYTE COUNT FOR STAGE I

	Stage I			
	Mean AUCMB (cells/mm^3)	p-value		
Treatment Group		Overall[a]	Between Treatments[b]	
Nelfinavir 750mg	+68	0.0001	750mg vs. placebo	0.0001
Nelfinavir 500mg	+55		500mg vs. placebo	0.0015
Placebo	+3		750mg vs. 500mg	0.3003

[a] p-value derived from an ANOVA model, with factors for treatment and site
[b] p-value derived from contrasts in the ANOVA model for pairwise comparisons

The ITT analysis for Stage II showed that nelfinavir 750 mg produced a statistically significant greater increase in CD4 lymphocyte count compared to nelfinavir 500 mg. This analysis included patients who experienced improvement in immune function after adding RTIs to nelfinavir (See Table 32 below).

TABLE 32
MEAN AUCMB FOR CD4 LYMPHOCYTE COUNT FOR STAGE II

	Stage II	
Treatment Group	Mean AUCMB (Cells/mm^3)	Overall p-value[a]
Nelfinavir 750mg	+93	0.0227
Nelfinavir 500mg	+59	

[a] p-value derived from an ANOVA model, with a fixed factor for treatment

Comment: This difference in Mean CD4 AUCMB is congruent with the findings presented on Table 30 of this review. This difference in favor of nelfinavir 750 mg TID also supports the clinical use of this dose.

13.3.2.2 SAFETY RESULTS

In the Stage I analysis, the proportion of patients experiencing at least one study drug related, treatment emergent adverse event of at least grade 2 severity did not differ among the treatment groups (see Table 33 below). There were no statistically significant differences among treatment groups with regard to the incidence of a particular drug related adverse event of at least grade 2 severity. Diarrhea was the most common adverse event reported. Six of 29 patients (21%) in the nelfinavir 750 mg group had diarrhea, four of 32 patients (13%) in the nelfinavir 500 mg group had diarrhea, and five of 32 patients (16%) in the placebo group had diarrhea.

TABLE 33
STUDY DRUG RELATED, TREATMENT EMERGENT ADVERSE EVENTS OF AT LEAST GRADE 2 SEVERITY REPORTED BY ≥2% OF PATIENTS: STAGE I

Body System Adverse Event	Treatment Group Nelfinavir 750mg n = 32 n (%)	Treatment Group Nelfinavir 500mg n = 32 n (%)	Treatment Group Placebo n = 32 n (%)	Total n = 93 n (%)
Body as a whole				
Asthenia	1 (3)	1 (3)	2 (6)	4 (4)
Digestive System				
Diarrhea	6 (21)	4 (13)	5 (16)	15 (16)
Vomiting	1 (3)	1 (3)	1 (3)	3 (3)
Nausea	1 (3)	0	1 (3)	2 (2)
Nervous System				
Depression	2 (7)	0	0	2 (2)

In Stage II, there were no statistically significant differences between treatment groups with regard to the incidence of a particular drug related, treatment emergent adverse event of at least grade 2 severity. Diarrhea, the most common adverse event, was reported by 10 of 43 patients (23%) in the nelfinavir 750 mg group and six of 48 patients (13%) in the nelfinavir 500 mg group (see Table 34, page 103).

TABLE 34
STUDY DRUG RELATED, TREATMENT EMERGENT ADVERSE EVENTS OF AT LEAST GRADE 2 SEVERITY REPORTED BY≥2% OF PATIENTS: STAGE II

Body System Adverse Event	Treatment Group Nelfinavir 750mg n = 43 n (%)	Treatment Group Nelfinavir 500mg n = 48 n (%)	Total n = 91 n (%)
Body as a whole			
Asthenia	1 (2)	1 (2)	2 (2)
Headache	1 (2)	1 (2)	2 (2)
Digestive System			
Diarrhea	10 (23)	6 (13)	16 (18)
Flatulence	3 (7)	0	3 (3)
Nausea	3 (7)	0	3 (3)
Vomiting	1 (2)	1 (2)	2 (2)
Nervous System			
Depression	3 (7)	0	3 (3)
Skin/Appendages			
Rash	1 (2)	2 (4)	3 (3)
Special Senses			
Eye Disorder	2 (5)	0	2 (2)

No deaths were reported during study.

13.3.3 CONCLUSIONS

Nelfinavir administered as monotherapy at 750 mg and 500 mg TID was effective in reducing HIV RNA and increasing CD4 lymphocyte counts over 12 to 16 weeks of treatment. The overall safety profile was similar across all three treatment groups. Diarrhea was the most common adverse events found. Diarrhea was observed in all three groups; however, patients receiving nelfinavir 750 mg had a higher incidence of diarrhea than patients in the other 2 groups.

13.4 STUDY NUMBERS 4 AND 5: STUDY 524 AND 546 (PEDIATRIC STUDIES)

These studies were studies in pediatric patients under 13 years of age. Study 524 was a pharmacokinetic study of nelfinavir single and multiple doses. The pharmacokinetic results of this study are discussed by Biopharmaceutics reviewer Dr. Kellie Reynolds on her written review of the submission. The objective of this study was to find a dose that would provide a pediatric drug exposure (AUC and C_{MAX}) similar to the drug exposure in adults. The applicant, with the FDA Biopharmaceutics reviewer concurrence, found that a dose of 20 to 30 mg/kg/dose administered TID was comparable to adult exposures of 750 mg TID.

The sponsor submitted data on 63 pediatric patients; forty-seven pediatric patients from Study 524 and 16 pediatric patients from study 546.

13.4.1 RESULTS OF STUDY 524

Twenty-one pediatric patients entered the single-dose pharmacokinetic phase of this study. Forty-seven children entered the multiple dose phase of this study. Of these 47 patients, two patients (9%) discontinued at their request. Tables 35 and 36 summarize the demographic characteristics of patients in the two phases of this study.

TABLE 35
DEMOGRAPHIC AND BASELINE CHARACTERISTICS OF PEDIATRIC PATIENTS
SINGLE-DOSE PHASE

	Group I 7 to 13 years n = 6	Group II 2 to < 7 years n = 11	Group III 3 months to < 2 years n = 4	TOTAL n = 21
Age in years				
Mean ± SD	9.7 ± 1.5	5.1 ± 1.6	1.1 ± 0.3	5.7 ± 3.3
Range	8.2 - 12.3	2.5 - 7.1	0.9 - 1.5	0.9 - 12.3
Gender (%)				
Male	5 (83.3)	5 (45.5)	2 (50)	12 (57.1)
Female	1 (16.7)	6 (54.5)	2 (50)	9 (42.9)
Race (%)				
White	1 (16.7)	4 (36.4)	1 (25)	6 (28.6)
Black	2 (33.3)	2 (18.2)	2 (50)	6 (28.6)
Other	3 (50)	5 (45.4)	1 (25)	9 (42.8)
Presence of Symptomatic HIV infection (%)				
Yes	5 (83.3)	11 (100)	3 (75)	19 (90.5)
No	1 (16.7)	0	1 (25)	2 (9.5)
Time in months since diagnosis of HIV infection (%)				
Mean ± SD	29.7 ± 22.1	28.4 ± 17.7	8.4 ± 6.4	25 ± 19.6
Range	6.2 - 65.4	3.7 - 51.8	1.9 - 17.1	1.9 - 65.4
Baseline Log_{10} HIV RNA				
Mean ± SD	3.71 ± 0.39	4.5 ± 0.74[1]	4.65 ± 0[2]	4.23 ± 0.71
Range	3.17 - 4.38	3.03 - 5.38	4.65 - 4.65	3.03 - 5.38
Baseline CD4 lymphocyte count (cells/mm^3)				
Mean ± SD	423.73 ± 299.01	797.38 ± 473.9[3]	1006 ± 720.97	723.37 ± 511.61
Range	12 - 909	59 - 1501	260 - 1890	12 - 1890

[1] There were data only for 10 patients.
[2] There were data only for one patient.
[3] There were data only for 9 patients.

TABLE 36
DEMOGRAPHIC AND BASELINE CHARACTERISTICS OF PEDIATRIC PATIENTS
MULTIPLE-DOSE PHASE

	Group I 7 to 13 years n = 16	Group II 2 to < 7 years n = 22	Group III 3 months to < 2 years n = 9	TOTAL n = 47
Age in years				
Mean ± SD	8.9 ± 1.5	4.8 ± 1.5	1.2 ± 0.4	5.5 ± 3.1
Range	7.1 - 12.3	2.3 - 7.1	0.8 - 1.8	0.8 - 12.3
Gender (%)				
Male	12 (75)	13 (59.1)	6 (66.7)	31 (66)
Female	4 (25)	9 (40.9)	3 (33.3)	16 (34)
Race (%)				
White	4 (25.0)	5 (22.7)	2 (22.2)	11 (23.4)
Black	5 (31.3)	7 (31.8)	4 (44.4)	16 (34.0)
Other	7 (43.7)	10 (45.5)	3 (33.3)	20 (42.6)
Presence of Symptomatic HIV infection (%)				
Yes	12 (75)	20 (90.9)	7 (77.8)	39 (83)
No	4 (25)	2 (9.1)	2 (22.2)	8 (17)
Time in months since diagnosis of HIV infection (%)				
Mean ± SD	40.5 ± 22[1]	32.9 ± 15.2[2]	9.4 ± 5.6	30.3 ± 19.6
Range	6.2 - 74.4	3.7 - 52.7	1.9 - 17.1	1.9 - 74.4
Baseline Log_{10} HIV RNA				
Mean ± SD	4.13 ± 0.69	4.59 ± 0.69	4.89 ± 0.82[3]	4.48 ± 0.75
Range	3.22 - 5.33	3.43 - 5.46	3.64 - 5.80	3.22 - 5.8
Baseline CD4 lymphocyte count (cells/mm^3)				
Mean ± SD	492.71 ± 715.94	583.64 ± 420.95	1496 ± 1295	727.55 ± 827.38
Range	0 - 2873	44 - 1501	177 - 4692	0 - 4692

[1] There were data only for 13 patients.

[2] There were data for only 21 patients.

[3] There were data for only 8 patients.

All treatment adverse events observed during the single-dose phase are presented in Table 37 (below). Few adverse events were observed during this period. Only one patient in each age group experienced diarrhea. Table 33 contains all treatment adverse events observed during the multiple dose phase of the study. In both adults and children the most common adverse event was diarrhea (23% in both groups). In this small number of pediatric patients, the incidence of diarrhea is not different than that observed in adults.

No patients discontinued therapy due to adverse events and no deaths have been reported. It should be noted that the protocol was designed as a 42-day pharmacokinetic study with a long-term extension phase. Many of the patients have now completed the initial observation period and have entered the extension phase.

The mean number of days of drug exposure for these 47 patients was 37.8 days, the range was 1 to 104 days. All patients received 20 mg/kg/dose TID as a tablet or as a powder. However, some of these patients received few doses of 10 mg/kg/dose and 30 mg/kg/dose as part of the single and multiple dose pharmacokinetic study.

TABLE 37
SUMMARY OF TREATMENT EMERGENT ADVERSE EVENTS: SINGLE-DOSE STUDY

COSTART Term	Group I 7 to 13 years n = 6 Patients (%)	Group II 2 to < 7 years n = 11 Patients (%)	Group III 3 months to < 2 years n = 4 Patients (%)	TOTAL n = 21 Patients (%)
Body as a whole				
Fever	0	2 (18.2)	0	2 (9.5)
Headache	0	1 (9.1)	0	1 (4.8)
Infection	0	1 (9.1)	0	1 (4.8)
Back Pain	0	1 (9.1)	0	1 (4.8)
Pharyngitis	1 (16.7)	2 (18.2)	0	3 (14.3)
Gastrointestinal System				
Diarrhea	1 (16.7)	0	0	1 (4.8)
Vomiting	0	1 (9.1)	1 (25)	2 (9.5)
Hemic and Lymphatic				
Anemia	0	1 (9.1)	0	1 (4.8)
Lymphadenopathy	0	0	1 (25)	1 (4.8)
Respiratory				
Increased cough	0	1 (9.1)	0	1 (4.8)
Pharyngitis	0	1 (9.1)	0	1 (4.8)
Pneumonia	0	1 (9.1)	0	1 (4.8)
Rhinitis	0	1 (9.1)	0	1 (4.8)
Sinusitis	1 (16.7)	0	0	1 (4.8)
Skin and Skin Structure				
Contact Dermatitis	1 (16.7)	0	0	1 (4.8)
Rash	0	0	1 (25)	1 (4.8)
Sweat (excessive)	0	0	1 (25)	1 (4.8)

TABLE 38
SUMMARY OF TREATMENT EMERGENT ADVERSE EVENTS: MULTIPLE-DOSE STUDY

COSTART Term	Group I 7 to 13 years n = 16 Patients (%)	Group II 2 to < 7 years n = 22 Patients (%)	Group III 3 months to < 2 years n = 9 Patients (%)	TOTAL n = 47 Patients (%)
Body as a whole				
Chills	1(6.3)	0	0	1 (2.1)
Fever	0	6 (27.3)	1 (11.1)	7 (14.9)
Flu Syndrome	1	0	0	1 (2.1)
Headache	0	2 (9.1)	0	2(4.3)
Infection	0	2 (9.1)	0	2(4.3)
Accidental Injury	0	1 (4.5)	1 (11.1)	2(4.3)
Abdominal Pain	1(6.3)	2 (9.1)	0	3 (6.4)
Pharyngitis	2 (12.5)	4 (18.2)	0	6 (12.8)
Cardiovascular				
Cardiovascular Disease	0	2 (9.1)	0	2(4.3)
Gastrointestinal System				
Constipation	1 (6.3)	0	0	1 (2.1)
Diarrhea	4 (25.0)	7 (31.8)	0	11 (23.4)
Flatulence	1 (6.3)	0	0	1 (2.1)
Hepatomegaly	0	0	1 (11.1)	1 (2.1)
Oral moniliasis	1 (6.3)	1 (4.5)	0	2(4.3)
Nausea	0	1 (4.5)	0	1 (2.1)
Aphthous Stomatitis	1 (6.3)	0	0	1 (2.1)
Vomiting	2 (12.5)	2 (9.1)	0	4 (8.5)
Endocrinology				
Cushing Syndrome	1 (6.3)	0	0	1 (2.1)

COSTART Term	Group I 7 to 13 years n = 16 Patients (%)	Group II 2 to < 7 years n = 22 Patients (%)	Group III 3 months to < 2 years n = 9 Patients (%)	TOTAL n = 47 Patients (%)
Hemic and Lymphatic				
Anemia	0	2 (9.1)	0	2(4.3)
Leukopenia	1 (6.3)	1 (4.5)	1 (11.1)	3 (6.4)
Lymphadenopathy	2 (12.5)	1 (4.5)	1 (11.1)	4 (8.5)
Metabolic and Nutritional				
Hyponatremia	1 (6.3)	0	0	1 (2.1)
Nervous				
Hyperkinesia	0	1 (4.5)	0	1 (2.1)
Somnolence	0	0	1 (11.1)	1 (2.1)
Thinking abnormal	0	1 (4.5)	0	1 (2.1)
Respiratory				
Asthma	1 (6.3)	2 (9.1)	0	3 (6.4)
Bronchiolitis	0	0	1 (11.1)	1 (2.1)
Increased cough	0	2 (9.1)	1 (11.1)	3 (6.4)
Epistaxis	0	2 (9.1)	0	2(4.3)
Hyperventilation	0	1 (4.5)	0	1 (2.1)
Pharyngitis	0	1 (4.5)	1 (11.1)	2(4.3)
Pneumonia	1 (6.3)	1 (4.5)	0	2(4.3)
Rhinitis	3 (18.8)	2 (9.1)	2 (22.2)	7 (14.9)
Sinusitis	0	1 (4.5)	0	1 (2.1)
Skin and Skin Structure				
Dermatitis	1 (6.3)	0	0	1 (2.1)
Eczema	0	0	1 (11.1)	1 (2.1)
Herpes simplex	1 (6.3)	0	0	1 (2.1)

COSTART Term	Group I 7 to 13 years n = 16 Patients (%)	Group II 2 to < 7 years n = 22 Patients (%)	Group III 3 months to < 2 years n = 9 Patients (%)	TOTAL n = 47 Patients (%)
Rash	1 (6.3)	5 (22.7)	1 (11.1)	7 (14.9)
Skin Discoloration	1 (6.3)	0	0	1 (2.1)
Dry skin	1 (6.3)	1 (4.5)	0	2 (4.3)
Urticaria	1 (6.3)	0	0	1 (2.1)

No drug related clinical adverse events of grade 2 or greater severity were observed during the single-dose phase. One grade 2 diarrhea and one grade 2 rash were the only two non-mild adverse events observed in the multiple dose phase. One patient had two serious adverse events during the single-dose phase: Fever and pneumonia. These adverse events were not considered causally related to study drug. During the multiple-dose phase there were 3 patients who developed the following serious adverse events: Infection, lymphadenopathy, and hyperventilation. None of these adverse events were considered causally related to study drug.

Laboratory Adverse Events during the single-dose phase of the study: Four patients in total had laboratory adverse events. Only one patient had a grade 2 severity hematological adverse event. His hemoglobin decreased from 10.6 g/dL to 9.6 g/dL 24 hours after his nelfinavir dose. His hemoglobin value returned to normal spontaneously and one month later enrolled in the multiple-dose phase of the study. Three patients had serum chemistry abnormalities of grade 2 or greater severity. Two patients had hyperkalemia. Both patients' potassium levels returned to normal spontaneously and two months later enrolled in the multiple-dose phase of the study. One patient had a grade 3 elevation in his serum amylase. His serum amylase level also returned to normal spontaneously and two months later he also enrolled in the multiple-dose phase of the study.

Laboratory Adverse Events during the multiple-dose phase of the study: Fourteen patients in total had laboratory adverse events. Nine patients had hematological laboratory adverse events of grade 2 or greater severity. Three had neutropenia, five had anemia, and one had thrombocytopenia. No patient discontinued study due to these adverse events. Five patients had serum chemistry abnormalities of grade 2 or greater severity. One patient had hypocalcemia, one patient had hyponatremia, one patient had hypokalemia, and two patients had an increased serum amylase value. No patient discontinued due to serum chemistry abnormalities.

13.4.2 STUDY 546: PEDIATRIC EXPANDED ACCESS PROGRAM

Study 546 is "An Open-Label Study to Evaluate Viracept Treatment of HIV Infected Children Who Could Benefit From a Protease Inhibitor Based on Clinical or Immunologic Status." This has been called the Pediatric Expanded Access Study. This study was allowed to proceed in order to provided an opportunity to clinicians caring for HIV infected pediatric patients access to the only protease inhibitor with appropriate dosing data in children and a

suitable formulation for patients who cannot swallow tablets. This study also provides the sponsor with an opportunity to collect safety data in pediatric patients.

The applicant did not provide safety or efficacy data on the patients that have been enrolled in this study. The applicant provided only the following information: "Sixteen children have been enrolled into the study since it was initiated in January 1997. Of these 16 children, 10 have received oral powder, 5 have received tablets and one child has been supplied both formulations. Although the follow-up for these children has been short, there have been no serious adverse events reported to date."

13.4.3 CONCLUSIONS

The pediatric data available thus far is very limited. However, there is an urgent need for drugs of this class for the pediatric population. Children have less treatment options than adults due to lack of formulations suitable for pediatric patients. Even when formulations suitable for pediatric patients may be available, appropriate dosing information is lacking. Study 524 included data on the single and multiple dose pharmacokinetics of nelfinavir. These data were reviewed and discussed by Dr. Kellie Reynolds in her review of these NDAs. From these pharmacokinetic data, it was concluded that 20 - 30 mg/kg/dose are the pediatric doses that will provide a similar drug exposure to that observed in adults using the 750 mg TID dose. Assuming that the HIV disease process in children is similar to the HIV disease process in adults, it is inferred that nelfinavir is also going to be active in the treatment of HIV infection in children as it has been demonstrated in adequate and well controlled clinical trials in adults. The safety profile observed in children and summarized in this section of this NDA review appears to be similar to the safety profile observed in adults. The most common adverse event observed in both populations (adult and children) was diarrhea. In both populations 23% of patients developed diarrhea. However, the pediatric data is based only in 47 patients who participated in the pharmacokinetic study. Antiviral activity in the pediatric population has not been analyzed.

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13.5 STUDY NUMBER 6: STUDY 503

This was "A Pilot Phase II, Open-Label, Dose Range-Finding Study of AG1343 in HIV Positive Patients." This study was conducted between June 21, 1995 and December 13, 1995. The objectives of this study were: 1) To evaluate the safety of different doses and regimens of AG1343 (nelfinavir mesylate) for 28 days as monotherapy in HIV positive patients; 2) to evaluate the clinical activity of different doses and regimens of nelfinavir using virologic and immunologic markers of HIV disease progression; and 3) to determine the pharmacokinetics of nelfinavir at different doses and regimens.

This study was designed to be an open-label, dose range-finding study in HIV positive patients with baseline CD4 lymphocyte counts $\geq$ 200 cells/mm^3 and plasma HIV RNA levels $\geq$ 20,000 copies/mL. A total of 55 patients were to be enrolled in this study; at least 40 patients were to receive nelfinavir two times daily (BID) and at least 15 patients were to receive nelfinavir three times daily (TID). Initially, patients were to be randomized to treatment with nelfinavir for 28 days at one of three BID dose regimens: 500 mg BID, 600 mg BID, or 750 mg BID (1000, 1200, or 1500 mg/day). Enrollment of patients in the TID dose groups was started after all patients enrolled in the BID dose groups completed the 28 day study. At least 15 patients were to be randomized to treatment with nelfinavir at one of three TID dose regimens: 500 mg TID, 750 mg TID, or 1000 mg TID (1500, 2250, or 3000 mg/day).

13.5.1 RESULTS

Sixty-five patients were enrolled in and completed this study. Thirty five patients received BID regimens and 30 patients received TID regimens. The majority of patients were white (61 patients; 94). The mean age of the patients was 38.6 years, with range of 24 to 58 years. The mean time since HIV diagnosis was 73.1 months, with a range of 2 to 176 months. Median decreases from baseline in plasma HIV RNA were seen in all six dose groups at all timepoints (Days 4 through 28). A median decrease from baseline greater than one $\log_{10}$ in plasma HIV RNA was seen in all three TID dose groups. The percentage of patients who experienced a decrease in untransformed plasma HIV RNA to below 500 copies/mL was greater in the TID dose groups compared to the BID dose groups (15/30 TID patients [50%] compared to 9/35 BID patients [26%]). Median increases from baseline in CD4 lymphocyte counts were seen in all six dose groups at both Days 14 and 28. Twenty-nine patients (45%) reported at least one $\geq$ grade 2 (moderate or severe) adverse event. The most frequent occurring $\geq$ grade 2 adverse events were diarrhea (12 patients; 19%) and asthenia (3 patients; 5%). No deaths, serious adverse events, grade 4 (very severe) adverse events occurred during the study. No patients withdrew from the study because of an adverse event.

Comment: The analysis presented here is the applicant's analysis. This reviewer agrees in general with the results of this study. However, no modifications were made to the HIV RNA levels reported by the applicant.

13.5.2 CONCLUSIONS

Nelfinavir mesylate demonstrated antiretroviral activity and was well tolerated as monotherapy in doses of 500 mg BID, 600 mg BID, 750 mg BID, 500 mg TID, 750 mg TID, and 1000 mg TID. Nelfinavir 1000 mg TID did not appear to provide a significant advantage over 750 mg TID to reduce plasma HIV RNA levels and this dose produced a higher incidence of $\geq$ grade 2 diarrhea (50%). After this study, the Applicant decided to continue development of the following treatment regimens: nelfinavir 750 mg TID and nelfinavir 500 mg TID.

13.6 STUDY NUMBER 7: STUDY 509

This study was "A Phase II Pilot Study of Viracept, Zidovudine, and Lamivudine in Antiretroviral Naive HIV Infected Subjects." This study started on February 8, 1996. The Objectives of this study were: 1) To evaluate the safety and efficacy of the three drug combination ZDV, 3TC and nelfinavir, administered to antiretroviral naive HIV infected patients with plasma HIV RNA > 10,000 copies/mL; 2) To assess the feasibility of viral knockout with a potent therapeutic regimen. If study subjects became both culture and plasma HIV RNA negative, then lymph node biopsy was to be performed to assess the significance of the antiretroviral response; 3) To characterize the immunologic effects of suppression of viral load by lymphocyte phenotype studies including markers for memory, activation, and cell cycling.

This was an open label, single site phase II, pilot study investigating the safety and virologic activity of ZDV 200 mg TID, 3TC 150 mg BID, and nelfinavir 750 mg TID in 12 antiretroviral naive subjects with at least 10,000 HIV RNA copies/mL plasma. Patients are to be treated for one year. Patients were monitored weekly for four weeks, biweekly through 16 weeks, then monthly.

13.6.1 RESULTS

This study is ongoing. The Applicant submitted an interim analysis with a data cut-off of August 9, 1996. Twelve HIV-infected patients with no prior history of antiretroviral therapy were enrolled in this trial. This included 11 males and 1 female patient. Mean calculated baseline plasma HIV RNA was 4.85 $\log_{10}$ copies/mL (range 4.2 - 5.8) and mean CD4 lymphocyte count was 259 cells/μL (range 26 - 504). Eight of the 11 patients who continued on treatment had HIV RNA below the detectable limits at week 6 and all had HIV RNA levels below the threshold of detection (500 copies/mL) by week 12, which continued through month 10 of study. Incremental effects on CD4 lymphocyte counts were observed with a mean increase in CD4 lymphocytes of 90 cells/μL at week 12 and 130 cells/μL at week 24. One patient had dosing discontinued due to mild diarrhea and cramping abdominal pain which was attributed to nelfinavir. This patient also experienced an episode of grade 3 CPK elevation which was attributed to nucleoside analog therapy. Ten of twelve patients experienced at least one treatment emergent adverse event of grade 2 severity that was attributed by the investigator to nelfinavir. These included abdominal pain (1), asthenia (3), diarrhea (5), nausea (5), and vomiting (2). No serious or grade 3 or greater adverse events were observed.

13.6.2 CONCLUSIONS

This pilot study demonstrated that the combination of nelfinavir + zidovudine + lamivudine was well tolerated by most patients and displayed antiretroviral activity with suppression of HIV RNA below detectable levels in the majority of these antiretroviral naive patients for at least 40 weeks of treatment.

13.7 STUDY NUMBER 8: STUDY 510

This was "A Phase I/II Pilot Study of Viracept in Combination with Stavudine (d4T) versus Stavudine (d4T) Alone in HIV Positive Patients." This study was conducted between November 16, 1995 and March 22, 1996. The objectives of this study were: 1) To evaluate the safety and antiretroviral activity of nelfinavir administered in combination with d4T for eight weeks in d4T-naive HIV positive patients with CD4 lymphocyte counts $\geq$ 200 cells/mm^3 and plasma HIV RNA $\geq$ 15,000 copies/mL. The efficacy of nelfinavir at 500 mg, 750 mg and 1000 mg TID administered in combination with d4T was to be compared to d4T alone, using virologic and immunologic markers of HIV disease progression; 2) To determine any potential pharmacokinetic interactions between nelfinavir and d4T in HIV positive patients.

This pilot, four-arm, open-label study was designed to evaluate the safety and efficacy of three different doses of nelfinavir administered in combination with d4T versus d4T alone in cohorts of at least five d4T-naive HIV positive patients. Patients were randomized to one of four treatments: d4T plus either 500 mg, 750 mg, or 1000 mg nelfinavir TID or d4T alone. Patients were to receive treatment with study drugs for eight weeks.

13.7.1 RESULTS

Thirty-nine patients were randomized to treatment: 11 in the nelfinavir 500 mg + d4T group; 10 in each of the nelfinavir 750 mg + d4T and nelfinavir 1000 mg + d4T groups; and eight in the d4T group. Thirty-two patients completed the study. Seven patients discontinued the study; reasons included non-compliance (3 patients), patient request (2 patients), increased liver enzymes at baseline (one patient), and splenomegaly (one patient).

Most patients (94.9) were male, and the majority were white (71.8%). Mean age was 37.1 years, ranging from 21 to 57 years. The majority (63.6%) of patients in all treatment groups had received prior HIV treatment; the most

common treatment was ZDV. Mean duration of treatment was 41 months (nelfinavir 500 mg+d4T, 35 months (nelfinavir 750 mg+d4T), 24 months (nelfinavir 1000 mg+d4T) and 23 months (d4T). The median baseline log_{10} transformed plasma HIV RNA ranged from 4.752 to 4.837 log_{10} copies/mL in the combination nelfinavir groups and was 4.355 log_{10} copies/mL in the d4T group. The median baseline CD4 lymphocyte counts ranged from 321 to 341 cells/mm^3 in the combination nelfinavir groups and was 359 cells/mm^3 in the d4T group. Mean duration of treatment (nelfinavir and/or d4T) was comparable among treatment groups, ranging from 49.1 days in nelfinavir 1000 mg+d4T group to 54.6 days in the d4T group. Table 39 summarized median and median change from baseline for HIV RNA and CD4 lymphocyte counts at the end of the study (Day 56).

TABLE 39
MEDIAN AND MEDIAN CHANGE FROM BASELINE FOR PRIMARY EFFICACY VARIABLES AT DAY 56

	Treatment Group							
	500 mg + d4T		750 mg + d4T		1000 mg + d4T		d4T	
Variable *	Median	Median Change from Baseline	Median	Median Change from Baseline	Median	Median Change from Baseline	Median	Median Change from Baseline
Log_{10} transformed plasma HIV RNA (log_{10} copies/mL)	2.88	-1.59	2.70	-1.86	2.70	-2.04	3.77	-0.52
CD4 lymphocyte count (cells/mm^3)	444	+100	483	+137	447	+126	553	+64

At day 56, median decreases in log_{10} transformed plasma HIV RNA were 3-fold to 4-fold greater in the nelfinavir groups than in the d4T group. Median decreases in log_{10} transformed plasma HIV RNA were generally comparable among the nelfinavir groups throughout the study. No patients in the d4T group had plasma HIV RNA levels below the limit of detection (<500 copies/mL) at Day 56. In the nelfinavir groups, up to 86% of the patients had undetectable levels of plasma HIV RNA.

The proportion of patients experiencing at least one treatment emergent adverse event of $\geq$ grade 2 severity and considered by the investigator to be at least possibly related to nelfinavir was similar across the nelfinavir groups (4, 4, and 5 patients in the 500, 750, and 1000 mg nelfinavir groups, respectively). The most common nelfinavir related $\geq$ grade 2 adverse event was diarrhea; the incidence of diarrhea was similar across the three nelfinavir groups. Three patients (7.7%) were discontinued from study medication due to a treatment emergent adverse event (elevated serum liver enzymes, hyperglycemia, and splenomegaly); hyperglycemia was classified as a serious adverse event.

13.7.2 CONCLUSIONS

Nelfinavir was well tolerated when given for eight weeks in combination with d4T. All three doses given in combination (500, 750, and 1000 mg TID nelfinavir) produced reductions in plasma HIV RNA levels at 56 days. Greater increases in CD4 lymphocyte cell counts were also observed at 56 days with the combination regimen compared to d4T alone.

14 DISCUSSION AND OVERALL CONCLUSIONS

A total of 815 patients who received treatment with study medication and had follow up efficacy data available during the six clinical trials conducted with nelfinavir comprise the efficacy population for this NDA. A total of 699 patients were treated in double blind trials and 116 patients were treated in open label trials.

In the three open label studies, 65 patients were included in Study 503, a 28 day dose range-finding monotherapy study, 12 patients were included in Study 509, a one year pilot triple combination treatment study with ZDV + 3TC, and 39 patients were included in Study 510, a 56-day pilot study to evaluate the efficacy of three doses of nelfinavir administered in combination with d4T versus d4T alone.

Overall, median decreases from baseline in plasma HIV RNA and median increases from baseline in CD4 lymphocytes were seen at all timepoints in each open label study. Over time, median decreases in plasma HIV RNA were three-fold to four-fold greater with nelfinavir combination therapy than with monotherapy. Median increases from baseline in CD4 lymphocyte counts were three-fold to four-fold greater in the nelfinavir groups than with d4T monotherapy; maximum antiretroviral activity was observed with nelfinavir + ZDV + 3TC.

Results from the open label studies formed the rationale for the selection of nelfinavir doses to be evaluated in larger controlled trials. In study 503, nelfinavir had antiviral activity and was well tolerated at doses of 500 mg BID, 600 mg BID, 750 mg BID, 500 mg TID, 750 mg TID, and 1000 mg TID. Nelfinavir 1000 mg TID did not appear to provide a significant advantage over 750 mg TID in the reduction of plasma HIV RNA and this dose produced a higher incidence of grade 2 or greater diarrhea. The safety profile and antiretroviral activity of both the 500 mg TID and 750 mg TID dose groups in the open label study of nelfinavir in combination with d4T (Study 510) provided further support for the Applicant's decision to evaluate both doses of nelfinavir as monotherapy and in combination with RTIs in controlled trials.

Of the 699 patients evaluated in the three double blind controlled trials, 93 patients were included in the second part of Study 505 (also known as Study 505, Stage II), a double blind monotherapy trial to evaluate 500 mg TID versus 750 mg TID nelfinavir, 308 patients were included in Study 506, a double blind comparative trial of a double combination d4T + nelfinavir 500 mg TID or 750 mg TID versus d4T alone, and 298 patients were included in a double blind comparative trial of triple combination ZDV + 3TC + 500 mg TID or 750 mg TID nelfinavir versus ZDV + 3TC alone.

Results from pooled analyses of the AUCMB from both plasma HIV RNA and CD4 lymphocyte counts across the three double blind, controlled studies showed that the presence of either nelfinavir 500 mg TID or 750 mg TID in double combination with d4T or in triple combination with ZDV + 3TC, produced significantly greater reductions in plasma HIV RNA ($p = 0.0001$). There were significantly greater increases in CD4 lymphocyte count for nelfinavir + d4T compared to d4T alone ($p = 0.0001$) and for nelfinavir + ZDV + 3TC compared to ZDV + 3TC alone ($p < 0.049$).

Given the current trend in clinical practice to use multiple drugs in the treatment of HIV infection, the most relevant clinical trial done by the Applicant is Study 511. This study compared two doses of nelfinavir, 500 mg and 750 mg TID, in combination with ZDV + 3TC to ZDV + 3TC alone. Two-hundred ninety-eight patients participated in this study. In Study 506, the Applicant compared the double combination of nelfinavir 500 mg TID or 750 mg TID + d4T to d4T alone. Three-hundred eight patients participated in this study.

15 LABELING

Several discussions and suggestions regarding the proposed labeling from the applicant were made throughout the NDA review. The final draft labeling submitted on March 14, 1997, adequately addresses concerns raised by all NDA reviewers.

16 PHASE IV COMMITMENTS

The applicant submitted a list of phase 4 commitments on March 13, 1997. The applicant's commitments are as follows:

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17 REGULATORY RECOMMENDATIONS

The Applicant has accepted the FDA modifications to the VIRACEPT package insert. The Applicant has also committed to do studies outlined in section 16 of this review as part of clinical confirmatory studies under the accelerated approval regulations. This reviewer recommends both NDAs (Nelfinavir Tablets and Nelfinavir Oral Powder) for approval under CFR 314.510.

Samuel D. Maldonado, M.D.
Medical officer / DAVDP
HFD-530 FDA

Statistical Review and Evaluation

NDA#:	20,779
APPLICANT:	Agouron
NAME OF DRUG:	Viracept® (nelfinavir)
INDICATION:	Treatment of HIV infection
DOCUMENTS REVIEWED:	NDA volumes, supplemental submissions
MEDICAL INPUT:	Sam Maldonado, M.D.

1. Summary

Two 24 week studies were submitted by the applicant for this NDA. Both studies were randomized, double-blind studies conducted at multiple centers. Study 506 used d4T, d4T+500mg nelfinavir, and d4T+750mg nelfinavir as the three arms of the trial. Study 511 used AZT+3TC, AZT+3TC+500mg nelfinavir, and AZT+3TC+750mg nelfinavir as the three arms of the trial. An additional study (Study 505) comparing nelfinavir to placebo over 4 weeks was submitted but not is not discussed in this review (see medical review for details). The applicant found that over the 24 week period, both nelfinavir arms in 506 and 511 had significantly greater mean increases from baseline CD4 and significantly greater mean decreases from baseline RNA compared to the respective control arms. In addition, in both trials, the applicant found that the nelfinavir arms had a higher proportion of subjects below the "limit of detectio. compared to the control arms. This limit of detection was initially reported as This value was also used in the mean change in RNA analysis as a substitute for any values that were reported as below the limit of detection. Subsequently, the applicant reported that the limit of detection could be reduced to and presented the mean change from baseline RNA analysis and the proportion below the limit of detection analysis using

This review focuses both on the reported efficacy results and on the validation of the HIV-RNA assay. Based upon the validation material the applicant provided initially and additional validation material submitted during this review, was selected as the "lower limit of reliable quantification" for the assay. Here, lower limit of reliable quantification is used to denote the a value above which the assay can distinguish a positive value from background noise, and above which the assay is reasonably linear and accurate. Values reported below in the analyses of RNA data conducted for this review. It is important to point out that this is not a formal review of the assay or its "lower limit", but is a limited assessment of the assay for the purposes of this review. The effect of using was to reduce the estimated RNA treatment effect of nelfinavir. The overall conclusion of nelfinavir's efficacy on CD4 and RNA was unchanged.

2. Study AG1343-506: A Phase III Randomized, Double-Blind, Placebo-Controlled Study of Viracept in Combination with Stavudine (d4T) Versus Stavudine Alone in HIV Positive Patients.

2.1 Study Design

Study 506 was initiated in February, 1996. This was a 3 arm study, with each arm receiving d4T plus either 750 mg nelfinavir, 500 mg nelfinavir, or placebo. It was planned to enroll 240 subjects, 80 in each arm. Randomization was to be stratified by duration of prior AZT therapy (<6 months or >6 months), CD4 count (50-300 or >300) and study center. Treatments were assigned using a latin square based on patient sequence and stratum. To be eligible for the study, subjects had to be d4T-naïve, with >50 CD4 and >15000 HIV-RNA. The protocol defined treatment failure as a return to baseline CD4 or RNA on two consecutive study visits after four weeks on study. In the event of treatment failure, subjects could have their treatment regimen changed. In the d4T arm, this meant being randomized to add either 500 mg nelfinavir or 750mg nelfinavir. In the nelfinavir arms, treatment changes were to be determined on a case by case basis.

2.2 Endpoints

The primary endpoints for this study were HIV-RNA and CD4 counts. These were to be assessed at Day 0 and Weeks 1, 2, 4, 6, 8, 12, 16, 20, and 24.

2.3 Planned Analysis

Baseline CD4 and RNA values were to be calculated by averaging the Day 0 value with the most recent previous value. The values used to determine study eligibility were not used to calculate baselines. The AUCMB (area under the curve minus baseline) was to be used as the summary measure for both RNA and CD4. The applicant defined the primary efficacy analysis as follows. For subjects who discontinued prior to 24 weeks, only RNA and CD4 values up through 1 week after treatment discontinuation would be used. For subjects who met the definition of treatment failure, only CD4 and RNA values up through the time of treatment failure would be used. All available data would be used for all other subjects. The applicant also indicted that an analysis would be performed using all data for all subjects (the ITT analysis). Pairwise comparisons for each nelfinavir arm vs. control would be performed with an alpha level of .025. RNA values were to be transformed by taking the log 10 of all values, and values below the limit of detection were to be set at The AUCMB was to be calculated by the trapezoidal method (linear interpolation).

3. Study AG1343-511: A Phase III Randomized, Double-Blind, Placebo-Controlled Study of Viracept in Combination with Zidovudine (AZT) + Lamivudine (3TC) Versus AZT + 3TC Alone in HIV Positive Patients with <1 Month or No Prior Antiretroviral Treatment.

3.1 Study Design

Study 511 was initiated in February, 1996. This was a 3 arm study, with each arm receiving d4T plus either 750 mg nelfinavir, 500 mg nelfinavir, or placebo. It was planned to enroll 210 subjects, 70 in each arm. Randomization was to be stratified by center and CD4 count (<100, 100-300, >300). Treatments were assigned using a latin square based on patient sequence and stratum. To be eligible for the study, subjects had to have received AZT for less than 1 month, with >15000 HIV-RNA. The protocol defined

treatment failure as a return to baseline CD4 or RNA on two consecutive study visits after four weeks on study. In the event of treatment failure, subjects could have their treatment regimen changed. In the AZT+3TC arm, this meant being randomized to add either 500 mg nelfinavir or 750mg nelfinavir. In the nelfinavir arms, treatment changes were to be determined on a case by case basis.

3.2 Endpoints

The primary endpoints for this study were HIV-RNA and CD4 counts. These were to be assessed at Day 0 and Weeks 1, 2, 4, 6, 8, 12, 16, 20, and 24.

3.3 Planned Analysis

Baseline CD4 and RNA values were to be calculated by averaging the Day 0 value with the most recent previous value. The values used to determine study eligibility were not used to calculate baselines. The AUCMB (area under the curve minus baseline) was to be used as the summary measure for both RNA and CD4. The applicant defined the primary efficacy analysis as follows. For subjects who discontinued prior to 24 weeks, only RNA and CD4 values up through 1 week after treatment discontinuation would be used. For subjects who met the definition of treatment failure, only CD4 and RNA values up through the time of treatment failure would be used. All available data would be used for all other subjects. The applicant also indicted that an analysis would be performed using all data for all subjects (the ITT analysis). Pairwise comparisons for each nelfinavir arm vs control would be performed with an alpha level of .025. RNA values were to be transformed by taking the log 10 of all values, and values below the limit of detection were to be set at The AUCMB was to be calculated by the trapezoidal method (linear interpolation).

4. Applicant Analysis

This section summarizes the applicant's analyses of both studies.

4.1 Enrollment

A total of 329 subjects were randomized into Study 506. Of these, 21 subjects were considered not treated since they were not dispensed study drug and had no follow-up data, primarily due to a withdrawal of consent. One subject was discontinued early in the study due to study entry criteria violations. This left 307 subjects for the efficacy analyses. The enrollment by arm was 109 for d4T, 97 for d4T+500mg nelfinavir, and 101 for d4T+750mg nelfinavir. The enrollment in the 4 strata formed by baseline CD4 and duration of AZT use was 48 (<6 months AZT, 50-300 CD4), 59 (<6 months AZT, >300 CD4), 139 (>6 months AZT, 50-300 CD4), and 61 (>6 months AZT, >300 CD4). Treatment assignment was balanced across strata.

A total of 316 subjects were randomized into Study 511. Of these, 19 subjects were considered not treated since they were not dispensed study drug or had no follow-up data, primarily due to a withdrawal of consent. This left 297 subjects for the efficacy analyses. The enrollment by arm was 101 for AZT+3TC, 97 for AZT+3TC+500mg nelfinavir, and 99 for AZT+3TC+750mg nelfinavir. The enrollment in the 3 strata formed by baseline CD4 was 62 (<100 CD4), 97 (100-300 CD4), and 138 (>300 CD4). Treatment assignment was balanced across strata.

4.2 Demographics

In Study 506, the mean baseline RNA was 4.86 log copies (73,000 copies) and the mean baseline CD4 count was 279. Subjects were primarily male (89%) and Caucasian (75%). No differences across treatment arms were seen in any baseline variable.

In Study 511, the mean baseline RNA was 4.86 log copies (73,000 copies) and the mean baseline CD4 count was 288. Subjects were primarily male (89%) and Caucasian (78%). No differences across treatment arms was seen in any baseline variable.

4.3 Patient Disposition

Table 1 summarizes the patient disposition for the two studies. Some patients both discontinued and were treatment failures. Treatment failures remained in the study.

Table 1:Patient Disposition

Study	Randomized	No study drug	No follow-up data	ITT population	Discontinued, last follow-up week: <=8	<=16	<24	Treatment Failures
506	329	20	2	307	22	9	4	80
511	316	16	3	297	24	12	15	41

In Study 506, 35 subjects discontinued the study prior to 24 weeks. Follow-up data was not collected after discontinuation. The discontinuation rate was similar in each arm, with 13 subjects in the d4T arm, 9 subjects in the d4T+500mg nelfinavir, and 13 subjects in the d4T+750mg nelfinavir arm discontinuing. Subjects discontinued at a roughly constant rate over the 24 weeks of the study. Of the 35 subjects who discontinued, 6 were lost to follow-up, the others discontinued for reasons including patient request, toxicity, and non-compliance. A total of 80 (47, 19, and 14 respectively) subjects met the treatment failure criteria. Of the 47 subjects on d4T alone who were classified as treatment failures, 41 had nelfinavir added to their treatment regimen. Some subjects had their treatment regimen altered for reasons other than treatment failure (3, 2, and 6 respectively), primarily by substituting AZT for d4T.

In Study 511, 51 subjects discontinued the study prior to 24 weeks. Follow-up data was not collected after discontinuation. The discontinuation rate was similar in each arm, with 17 subjects in the AZT+3TC arm, 18 subjects in the AZT+3TC+500mg nelfinavir, and 16 subjects in the AZT+3TC+750mg nelfinavir arm discontinuing. The rate of discontinuation was fairly constant over the 24 weeks of the study. Of the 51 subjects who discontinued, 7 were lost to follow-up, the others discontinued for reasons including patient request, toxicity, and non-compliance. A total of 41 (14, 12, and 15 respectively) subjects met the treatment failure criteria, however, none of these subjects had their treatment changed as a result. The reason for this was that most of these subjects met the CD4 treatment failure criteria while their RNA levels were still well below baseline and it was felt that treatment had not in fact failed. Some subjects did have their treatment regimen altered (12, 8, and 6 respectively), primarily by substituting d4T for AZT due to side effects associated with AZT.

4.4 Primary Endpoints

Table 2 shows the results of the applicant's intent-to-treat analyses for the two studies. The AUCMB was calculated using all available data and employing linear interpolation between the observed values (trapezoidal rule). Values were not extrapolated for subjects who discontinued, that is, a subject's AUCMB was calculated over just the period of observation for that subject. RNA values below 500 copies were set to 500 copies. The treatment effect and p-value columns show the comparison of the

nelfinavir-containing arms to the control arms. The p-value was calculated by comparing the least-squares means in an ANOVA with treatment and center as factors.

Table 2: Applicant Analysis of Studies 506 and 511

Study	Endpoint	Treatment	Mean AUCMB	Treatment Effect	p-value
506	HIV-RNA	d4T	-.57	NA	NA
		d4T+500mg N	-1.21	-.64	.0001
		d4T+750mg N	-1.29	-.72	.0001
	CD4	d4T	42	NA	NA
		d4T+500mg N	92	50	.0001
		d4T+750mg N	96	54	.0001
511	HIV-RNA	AZT+3TC	-1.39	NA	NA
		AZT+3TC+500mg N	-1.78	-.39	.0001
		AZT+3TC+750mg N	-1.80	-.41	.0001
	CD4	AZT+3TC	83	NA	NA
		AZT+3TC+500mg N	108	25	.050
		AZT+3TC+750mg N	109	26	.029

The protocol-specified analyses were very similar and are not tabulated here.

The applicant examined the number of subjects with RNA less than . In Study 506, at 24 weeks 2 subjects on d4T, 14 subjects on d4T+500mg nelfinavir, and 12 subjects on d4T+750mg nelfinavir were below 500 RNA copies. In Study 511, at 24 weeks 18 subjects on AZT+3TC, 52 subjects on AZT+3TC+500mg nelfinavir, and 68 subjects on AZT+3TC+750mg nelfinavir were below 500 RNA copies.

For Study 511, the applicant also reported mean AUCMB values using 100 copies as the lower limit instead of 500 copies. The mean AUCMBs were -1.54 for AZT+3TC, -2.09 for AZT+3TC+500mg nelfinavir, and -2.15 for AZT+3TC+750mg nelfinavir.

4.5 Conclusions

In both studies, the applicant concluded that the addition of either 500mg nelfinavir or 750mg nelfinavir resulted in significant greater increases in CD4 and significantly greater decreases in RNA over the control arms.

5. Reviewer Comments

5.1 500mg vs. 750mg

An issue that arose during the review process was the choice of the nelfinavir dose. No apparent differences were seen in mean changes in CD4 or RNA between 500mg and 750mg in either Study 506 or Study 511. The proportion of subjects below was greater for the 750mg dose (69% vs 54%, p=.04). Subgroup analyses provided by the applicant of the proportion below as well as the AUCMB analysis of RNA, suggested that subjects with simultaneously high baseline RNA and low baseline CD4 values might receive additional benefit from the 750 dose as compared to the 500 dose.

5.2 Intent-to-Treat

The applicant found little difference between the protocol analyses and the ITT analyses for either endpoint in either study. The only arm where a sizable number of subjects changed treatment as a result of reaching the treatment failure criteria was the d4T arm in Study 506 (41 subjects). The mean AUCMB for RNA was -.50 log copies in the protocol analysis and -.57 log copies in the ITT analysis. The mean AUCMB for CD4 was 38 in the protocol analysis and 42 log copies in the ITT analysis. Thus, the effect of treatment changes on the analyses was small.

5.3 Discontinuation

Subjects who discontinued had their AUCMB calculated up through their last available follow-up. It seems likely that subjects' RNA values increased towards baseline after discontinuation. Thus, the AUCMB estimates for each treatment arm are likely exaggerations of the true effect of each treatment on RNA. An estimate of the degree of exaggeration can be made by the following procedure. Say 20% of subjects discontinued on one arm, and all discontinuations were at 12 weeks. After discontinuation, the RNA values returned to baseline within a few weeks. Then, the AUCMB for these subjects out to 24 weeks would be about one-half that of subjects who did not discontinue. Thus, the overall effect of having all the data would be to reduce the mean AUCMB by 10%, that is, the AUCMB actually observed would be overstated by a little more than 10%. In the applicant's analysis of the RNA AUCMBs, the AZT+3TC AUCMB would be high by about .15 log copies, and the nelfinavir AUCMBs would be high by about .2 log copies. Thus, under the scenario described here, discontinuation likely did not benefit the nelfinavir arms in the treatment comparisons. Since equal numbers of subjects discontinued on each arm, and since the time distribution of discontinuation was similar, it is reasonable to conclude that the comparison of the nelfinavir arms to the control arm was not unduly affected by discontinuation.

5.4 Gender, Age, Race, Baseline, Center

Using the ITT data, analyses were performed by the reviewer to asses possible treatment effect differences by race, gender, age, baseline CD4, baseline RNA, and center. With the exception of the effect of low baseline CD4 and high baseline RNA on the difference between the 500mg arm and the 750mg arm in Study 511, no evidence was seen to indicate a treatment interaction with any of the factors.

5.5 Stratification

The applicant submitted permutation-based analyses that stratified by the factors that were used to stratify the randomization. These analyses showed similar results to the ANOVA analyses. However, due to the unusual nature of the randomization scheme, it is difficult to assess the appropriateness of the these analyses.

5.6 HIV-RNA

The applicant supplied validation reports for the Chiron RNA assay. Of specific interest was the accuracy and linearity of the assay across the range of RNA values, and the lower limit of the assay. This was not meant to serve as a formal review of the assay or its lower limit, but was a determination, specific to the two studies reviewed, about how the RNA values should best be used to compare the treatment arms. In the applicant's AUCMB analysis of RNA, values below were set to copies. As will be described below it may be more appropriate to substitute for values

The applicant submitted data to address the question of the assay's linearity and sensitivity. A tissue culture stock was assayed to estimate the amount of RNA present. It was determined to contain 7,922,000 copies/ml. A total of 24 dilution series were made to approximate 100,000 copies/ml, 33,000 copies/ml, etc. down to 15 copies/ml. Some assay values were classified as inconsistent when the individual determinations that made up that value had greater than 35% CV. Other assay values were classified as below the limit of detection, while not explicitly stated in the submission. It appears that 100 copies/ml was used as the value for this lower limit in this experiment

In the submission, the applicant fit a regression model allowing for a different slope and intercept for each of the 24 dilution series. Values that were below the limit of detection or inconsistent (CV>35%) were excluded from the analysis. Log-transformed values were used in the analysis. The applicant concluded from their analysis that the assay was linear between 400 and 100,000 copies.

The applicant also ran a negative standard in each dilution series. The mean and upper 95% confidence value of this standard was computed, as well as the mean at each expected quantification. Cube-root transformed values were used in this analysis. The point at which the 95% upper confidence value intersected a linear interpolation between the mean quantifications. The applicant reported that the lower limit, as defined by this method, was 337 or 380 copies, depending on the type of negative standard used. The applicant concluded that 400 copies/ml was a conservative estimate of the lower limit.

Table 3 is a summary of the reviewer analysis of the assay data described above.

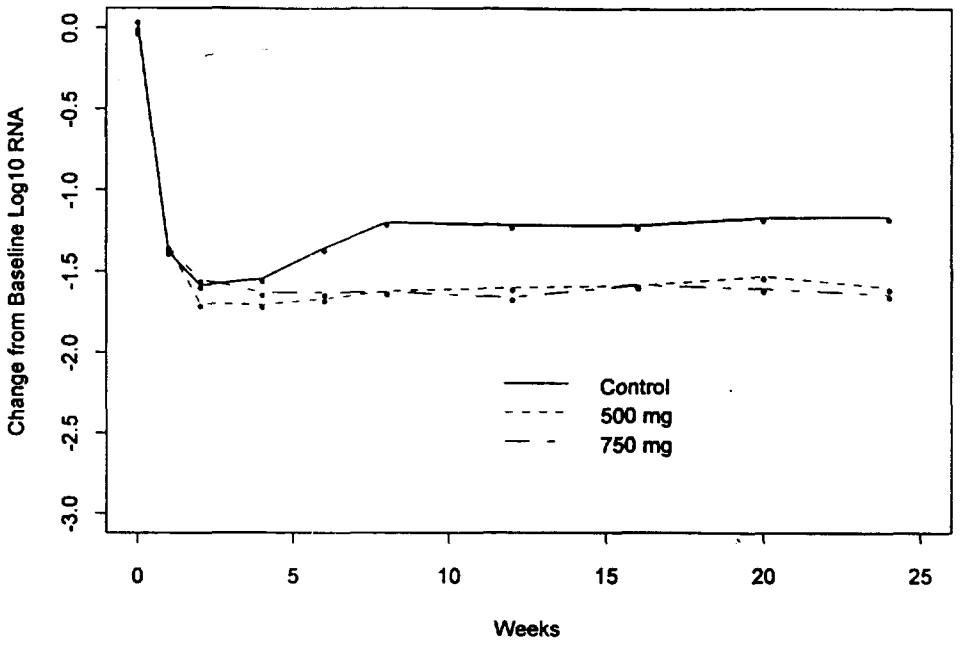
Table 3: Summary of Data Supplied on 3/4/97

	Expected quantification (copies/ml)						
# Samples	100,000	33,000	11,000	3,700	1,200	400	140
Above Expected	24	23	19	18	11	4	3
Below Expected	0	0	4	6	10	18	18
Mean (using 100 for values <100)	129,000	45,300	13,200	4,230	1,200	310	135
Mean (using 400 for values <100)	129,000	45,300	13,200	4,230	1,210	450	405
	Rate of undetectability and/or inconsistency						
	0%	4%	4%	0%	12.5%	33.3%	79%

As the first two lines of the table indicate, below 1,200 copies the assay tended to under-report the number of copies. Above 1,200 copies the assay tended to over-report the number of copies. The percentage of values reported as undetectable or inconsistent rose rapidly below 1,200 copies. The observed error rate at expected copy numbers below 1,200 seems unacceptably high for achieving reliable quantification of RNA levels (see Microbiology review).

The mean quantification of the assay is dependent on the value used for observations below a designated lower limit. For example, at an expected 400 copies/ml, if 100 copies/ml was used in place of observation below the limit, the mean quantification was 310 copies, while if 400

Study 511: Mean Change from Baseline Log10 RNA Over Time



5.7 Conclusions

The addition of nelfinavir significantly improved both CD4 and RNA compared to the control arms in both Study 506 and Study 511. Based upon the validation information provided by the applicant, RNA values below 1,200 copies were set to 1,200 copies in the analysis of the RNA data. While this produced an estimate of the treatment effect that was smaller than the applicant's, the conclusions were unchanged.

Michael Elashoff, Ph.D.
Mathematical Statistician

Concur: Dr. Flyer EF 3/19/97
cc:
Archival NDA# 50,708
HFD-530/Ms. Struble
HFD-530/Dr. Maldonado
HFD-530/Dr. Gitterman
HFD-530/Dr. Chikami (via Teamlinks)
HFD-530/Dr. Freeman (via Teamlinks)
HFD-104/Ms. Sage (via Teamlinks)
HFD-725/Dr. Elashoff
HFD-725/Dr. Flyer
HFD-725/Dr. Harkins
HFD-725/Ms. Shores
This review contains 9 pages

CLINICAL PHARMACOLOGY/BIOPHARMACEUTICS REVIEW

NDA: 20-778 and 20-779
DRUG: Nelfinavir mesylate
(VIRACEPT®)
250 mg tablets
Oral powder (50 mg/g)
APPLICANT: Agouron Pharmaceuticals
TYPE: 1P (NME)

REVIEWER: Kellie Schoolar Reynolds, Pharm.D.
SUBMISSION DATES: 12-23-96, 01-16-97, 02-20-97
DRAFT REVIEW: 02-25-97, 03-07-97

FINAL REVIEW

BACKGROUND: Nelfinavir mesylate is an inhibitor of HIV-1 protease. Three other HIV-1 protease inhibitors have been approved (saquinavir, ritonavir, and indinavir). Inhibition of HIV protease renders the enzyme unable to process the gag-pol polyprotein precursor, which leads to production of non-infectious immature HIV particles. The applicant submitted the NDAs for accelerated approval of nelfinavir mesylate tablets and oral powder to the FDA on December 23, 1996. The primary basis for a claim of efficacy are the results of Studies 505 (nelfinavir monotherapy vs. placebo), 506 (nelfinavir + d4T vs. d4T), and 511 (nelfinavir + ZDV/3TC vs. ZDV/3TC). The FDA review considers Study 511 to be the most important and Study 505 contributes very little. It is not likely that a protease inhibitor will be used as monotherapy.

SYNOPSIS:
Pharmacokinetics: Absorption: Following oral administration of a single dose (250 mg-1000 mg) of nelfinavir with food, nelfinavir was slowly absorbed with peak concentrations occurring between 3 and 5 hours. There appeared to be a disproportional increase in AUC_∞ and Cmax with dose. Oral clearance decreased and elimination half-life increased when the dose was increased from 250 mg to 750 mg. Pharmacokinetics did not appear to change between the 750 mg and 1000 mg doses. These data suggest that the single dose pharmacokinetics of nelfinavir are non-linear over the dose range of 250 mg to 750 mg.

Steady-state pharmacokinetic data are available for HIV positive patients who received nelfinavir q8hrs (with food) in Studies 503, 509, and 510. The data are summarized in the table below. The applicant also investigated q12hr dosing regimens, but abandoned them because the resulting trough nelfinavir concentrations were considered too low.

Nelfinavir Steady State Pharmacokinetic Parameters (HIV positive patients)
Arithmetic mean ± SD (Range)

Parameter	500 mg tid (n = 19)	750 mg tid (n = 30)	1000 mg tid (n = 15)
AUC_τ (μg*hr/mL)	17.23 ± 5.41 (5.44-27.43)	18.36 ± 7.38 (4.33-33.0)	25.90 ± 9.52 (14.85-44.08)
Cmax (μg/mL)	3.00 ± 0.84 (1.02-4.76)	3.27 ± 1.17 (0.71-5.39)	4.48 ± 1.65 (2.41-7.58)
Tmax (hr)	3.58 ± 1.37 (1.50-5.00)	2.97 ± 1.12 (1.00-6.00)	2.70 ± 0.75 (1.50-4.00)
CL/F (L/hr)	33.50 ± 17.09 (18.23-91.90)	50.14 ± 30.14 (22.7-172.96)	44.02 ± 16.35 (22.69-67.34)

Oral clearance of nelfinavir was relatively constant across this dose range in HIV positive patients. The steady-state AUC values overlapped a great deal between the three different dose groups.

In several healthy volunteer studies, it was observed that the daily trough concentrations increased to a maximum around the second day of dosing and thereafter decreased toward steady-state approximately 5-7 days after initiation of treatment. At the 500 and 750 mg tid dose levels (Studies 520 and 521), the average trough concentrations on the sixth day of treatment were about 55% of the trough concentrations on the second day of treatment. This pattern is suggestive of modest autoinduction of clearance during multiple dosing. For patients receiving 750 mg tid in Study 503, higher plasma ratios of the metabolite M8 relative to nelfinavir on Day 28 versus Day 1 were observed, further suggesting that autoinduction occurs. Very little accumulation of nelfinavir was evident at steady-state relative to following a single dose.

The applicant was unable to formulate nelfinavir as a solution for the determination of relative bioavailability. An oral powder has been developed for pediatric patients who are unable to take tablets. The relative bioavailability of the powder to the tablet was not determined prior to the first study in pediatric patients, because the formulation was developed just prior to study initiation. The formulations were not compared in adults because the bulk of the powder makes clinical use impractical. A cross-over comparison of the tablet and oral powder was performed in children aged 7 to 13 years participating in the pediatric study (Study 524). Based on preliminary data in six children, dose-normalized AUC_{0-8} for the oral powder was $115 \pm 37\%$ of that for the standard tablet formulation; dose normalized Cmax for the oral powder was $92 \pm 22\%$ relative to the tablets. There was no trend for increased or decreased concentrations with the oral powder.

The proposed commercial formulation was used in clinical trials, so a pivotal bioequivalence study was not needed.

Administration of nelfinavir with food prolongs and increases absorption. Due to the increase in bioavailability (2-3 fold) when administered with food, nelfinavir was administered with food during the clinical trials; it is recommended that patients take nelfinavir with a meal or snack. The effect of different meals on the pharmacokinetics of nelfinavir was not investigated.

Distribution: Nelfinavir is highly protein bound (>99%) to serum proteins. This binding appears to be dose dependent at high concentrations. The binding does not appear to be dose dependent at clinically relevant nelfinavir concentrations. Nelfinavir was also extensively (97-99%) bound to human serum albumin and alpha-1-acid glycoprotein. The extensive binding of nelfinavir to serum proteins complicates interpretation of plasma drug concentrations in relation to in vitro antiviral ED_{50} and ED_{95} estimates.

In vitro data indicate that nelfinavir is mostly confined to the blood plasma, with very little sequestration into the red blood cells. The in vivo blood to plasma ratio for nelfinavir (determined in the radiolabeled nelfinavir ADME study) was approximately 0.7, consistent with in vitro data. These data indicate that nelfinavir is mostly confined to the blood plasma with very little sequestration into the blood cells.

Metabolism: Nelfinavir is extensively converted to many oxidative metabolites. Pathways of oxidative microsomal metabolism can be described as follows: 1) hydroxylation of the perhydroisoquinolone moiety; 2) hydroxylation of the benzamide ring; 3) sulfur oxidation; 4) hydroxylation of the thiophenyl ring; and 5) hydroxylation of the *t*-butylamide group. Using

human liver microsomes, cDNA-expressed cytochrome P450 isoforms, and specific chemical inhibitors of cytochrome P450 isoforms, it was determined that the major enzyme metabolizing nelfinavir is CYP3A4. Other isoforms appear to be involved: CYP2C19>CYP2D6>CYP2C9. CYP3A4 appears to be responsible for approximately 50% of nelfinavir metabolism. Some glucuronidation may also occur.

Results of the ADME study indicated unchanged nelfinavir comprised the approximately 80% of radioactivity in plasma. The predominant circulating metabolite was the hydroxy-*t*-butylamide, M8 (12-14%). In vitro antiviral activity tests indicated that M8 was of comparable potency to nelfinavir in acute HIV-1 infection models, suggesting that M8 may contribute to antiviral effects of nelfinavir in patients. The 3"-methoxy-4"-hydroxynelfinavir metabolite, M1, comprised 2 to 5% of radioactivity in plasma. Similar amounts of these metabolites were observed in the plasma of patients in Study 503 who received nelfinavir 750 mg tid for 28 days.

Effect on Metabolism: In vitro studies indicate that nelfinavir inhibits CYP3A4 at clinically relevant concentrations (Ki=4.8 μM). Nelfinavir also inhibited CYP2C19, CYP2D6, and CYP1A2, but the Ki values were much greater than the Cmax values measured for nelfinavir in clinical studies. Nelfinavir did not inhibit CYP2C9 or CYP2E1. The metabolite M8 was also observed to inhibit CYP3A4 (Ki=4.35 μM).

Elimination: Over a period of seven days, the median radiochemical recovery after a single dose of [^{14}C]nelfinavir was 89%, with 87% recovered in feces and 1.6% recovered in urine. Intact nelfinavir accounted for 22% of recovery in feces, nearly 20 different oxidative metabolites were detected in feces.

Special Populations: The pharmacokinetics of nelfinavir were not investigated in patients with hepatic or renal impairment. No difference in nelfinavir pharmacokinetics based on gender has been observed, but this is still under review. Pharmacokinetic differences due to race have not been evaluated. The pharmacokinetics of nelfinavir have not been evaluated in subjects>65 years of age.

A pediatric study is ongoing. Oral clearance of nelfinavir appears to be more rapid in pediatric (<13 years of age) patients relative to adults. A dose of 20-30 mg/kg tid is being evaluated; this is approximately 2-3 times the adult dose (750 mg tid) based on body weight. A target plasma AUC of 50% to 200% of the median concentrations observed in adults following a single 750-mg dose was used to determine the optimal dose for pediatric patients. The steady state pharmacokinetics are compared between pediatric patients and adults in the following table:

Pediatric Multiple Dose Pharmacokinetics Versus Adults (Arithmetic Mean ± SD)

Age	N	Dose (tid)	AUC_{0-8} (μg*hr/mL)	Cmax (μg/mL)	Tmax (hr)	Cmin (μg/mL)	CL/F (L/hr)	CL/F (L/hr/kg)
7-13 yr	6	20 mg/kg	16.7 ± 12.5	2.69 ± 1.63	3.3 ± 1.6	1.74 ± 1.42	67.0 ± 41.8	2.08 ± 1.41
2-7 yr	8	20 mg/kg	20.2 ± 8.2	4.03 ± 1.33	3.8 ± 2.0	1.68 ± 1.08	22.3 ± 8.8	1.25 ± 0.55
Adults	19	500 mg	17.4 ± 5.7	2.85 ± 0.80	3.6 ± 1.3	1.50 ± 0.76	33.4 ± 17.2	0.48 ± 0.25
	30	750 mg	18.5 ± 7.6	3.16 ± 1.21	3.0 ± 1.1	1.50 ± 0.82	50.1 ± 29.8	0.72 ± 0.43

Drug Interactions: A number of drug interaction studies have been performed. There does not appear to be an interaction between nelfinavir and either lamivudine (3TC) or stavudine (d4T). Nelfinavir decreases zidovudine (ZDV) AUC by approximately 35%; the clinical significance of this interaction is under review. The pharmacokinetics of nelfinavir do not appear to be altered when nelfinavir is administered one hour after a dose of didanosine (ddI). It is recommended that ddI be administered on an empty stomach; therefore, nelfinavir should be administered (with food) one hour after or two hours before ddI.

Nelfinavir (750 mg q8hr) inhibits the metabolism of terfenadine (60 mg) to the pharmacologically active metabolite terfenadine carboxylate. Because accumulation of unchanged terfenadine has been associated with potentially lethal ventricular arrhythmias, the inhibition of terfenadine metabolism and modest prolongation of QTC intervals after concomitant administration of terfenadine and nelfinavir are clinically significant. Nelfinavir should not be administered concurrently with terfenadine. The results of this study are consistent with inhibition of CYP3A4; thus, nelfinavir should also not be coadministered with astemizole, cisapride, triazolam, or midazolam.

When nelfinavir (500 mg q8hr) was coadministered with ketoconazole (400 mg qd), nelfinavir AUC was increased 35% (95% CI: 21-49%), Cmax was increased 25% (95% CI: 8-44%), and elimination half-life was prolonged by greater than 100%. These changes were not clinically significant. Ketoconazole, at its usual clinical doses (up to 400 mg per day), is noted for its ability to inhibit metabolism via CYP3A4. For example, concomitant administration of ketoconazole increases the AUC of saquinavir by 3-fold and increases the AUC of indinavir by 68%. The more modest inhibitory effect of ketoconazole on nelfinavir indicates that pathways other than CYP3A4 probably contribute to the elimination of nelfinavir. These results suggest that selective inhibitors of CYP3A4 of similar or lesser potency compared to ketoconazole, such as macrolide antibiotics and other azole antifungals, are not likely to have a clinically significant impact on nelfinavir pharmacokinetics.

Coadministration of rifampin (600 mg qd) decreased nelfinavir (750 mg q8hr) AUC by 82% (95% CI: 77-86%), consistent with rifampin's capacity as an inducer of CYP3A4. The interaction between nelfinavir and rifampin is clinically significant. The magnitude of the interaction indicates that patients receiving rifampin in combination with nelfinavir would experience a substantial lowering of nelfinavir plasma concentrations, potentially resulting in a decrease in or loss of nelfinavir efficacy. Nelfinavir and rifampin should not be administered together.

Coadministration of rifabutin (300 mg qd) and nelfinavir (750 mg q8hr) decreased nelfinavir AUC by 32% (95% CI: 10-48%) and increased rifabutin AUC by 220% (95% CI: 151-276%). These results are consistent with induction of CYP3A4 by rifabutin and inhibition of CYP3A4 by nelfinavir. These pharmacokinetic changes are similar to those observed when indinavir was coadministered with rifabutin. The indinavir approved label recommends that the rifabutin dose be reduced by 50% when coadministered with indinavir. The same adjustment is recommended in the nelfinavir label. The lower rifabutin dose may cause less induction of nelfinavir metabolism. The effect of this dose adjustment should be confirmed with a clinical drug interaction study.

Nelfinavir (750 mg q8hr) decreased ethinyl estradiol AUC by 47% (95% CI: 41-63%) and norethindrone AUC by 18% (95% CI: 12-27%). Subjects received oral contraceptive tablets containing 35 μg ethinyl estradiol and 0.4 mg norethindrone. The effects of nelfinavir on ethinyl estradiol and norethindrone are consistent with metabolic induction. For ethinyl estradiol, routes of elimination include oxidation by CYP3A and direct conjugation with glucuronic acid. The inductive effect of nelfinavir in this case may involve glucuronidation and/or oxidation pathways other than CYP3A. A similar effect on the concentrations of ethinyl estradiol was observed when coadministered with ritonavir. The extent of decrease in ethinyl estradiol concentrations, combined with the smaller decrease in norethindrone concentrations, indicates that alternate or additional contraceptive measures should be used during nelfinavir treatment.

Coadministration of nelfinavir and saquinavir resulted in a small increase in nelfinavir AUC and a 4-fold increase in saquinavir AUC. Saquinavir has very low oral bioavailability (approximately 4% when administered with food) due to extensive intestinal first-pass metabolism via CYP3A. The results of this study are consistent with inhibition of intestinal first pass metabolism. Drug interactions that increase saquinavir concentrations to the degree observed in this study are considered beneficial; a dose adjustment is not needed.

Administration of indinavir (800 mg q8hr) inhibited the metabolism of nelfinavir (single 750 mg dose); nelfinavir AUC increased by 83% (95% CI: 34-150%). Administration of nelfinavir (750 mg q8hr) inhibited the metabolism of indinavir (single 800 mg dose); indinavir AUC increased by 51% (95% CI: 25-83%). Both indinavir and nelfinavir are substrates for and inhibitors of CYP3A; the mutual inhibitory effects of these drugs likely involve inhibition of CYP3A. The magnitude and clinical significance of the interaction between indinavir and nelfinavir requires further investigation in patients receiving chronic multiple dosing of this combination. The results of this study provide guidance for dose selection in future clinical trials. Although the applicant states that the results suggest that a modest reduction of daily dose and/or frequency may be possible for one or both drugs in this combination, it appears most appropriate to reduce the dose of nelfinavir.

Administration of ritonavir (500 mg q12hr x 3 doses) inhibited the metabolism of nelfinavir (single 750 mg dose); nelfinavir AUC increased by 152% (95% CI: 86-242%). Administration of nelfinavir (750 mg q8hr x 5 doses) modestly inhibited metabolism of ritonavir (single 500 mg dose). Nelfinavir is a substrate for multiple human cytochrome P450 enzymes, including CYP3A, CYP2D6, CYP2C19, and CYP2C9. Ritonavir has been reported to be a clinical inhibitor of the same enzymes. The magnitude and clinical significance of the pharmacokinetic interaction between nelfinavir and ritonavir requires further investigation in patients during chronic multiple dosing of the combination. Results of this study will provide guidance for dose selection in future combination trials.

PK/PD: The applicant did not analyze potential correlations between pharmacokinetic parameters and surrogate markers of efficacy. The superiority of viral RNA rather than CD4 cell count as a surrogate marker emerged during the time nelfinavir was being developed. The endpoint that investigators and patients now seek is viral RNA below the limit of detection. Validation of the detection limit for different viral RNA assays is ongoing.

The applicant did not pursue the 1000 mg tid dose regimen because of increased incidence and severity of diarrhea. No other adverse events were consistently present in clinical

trials. Patients were able to tolerate the diarrhea experienced at 500 mg tid and 750 mg tid by using antidiarrheal medications. Although the 500 mg tid and 750 mg tid dose regimens appeared to have similar efficacy, the 750 mg tid dose regimen was selected based on a subset analysis. The applicant will continue to investigate both doses.

Dissolution: The following dissolution method had been proposed by the applicant for the

Due to the timing of the development of the pediatric formulation, a dissolution method has not yet been developed. The development of a dissolution method for the oral powder is ongoing and will build upon the work completed on the tablet dissolution method.

RECOMMENDATION: The pharmacokinetic studies provided in section 6 of NDAs 20778 and 20779 (Nelfinavir mesylate tablets and oral powder) submitted to the Division of Antiviral Drug Products to fulfill Section 320 of the Code of Federal Regulations (21 CFR) provide an understanding of the pharmacokinetics of nelfinavir. The information on the pharmacokinetics of nelfinavir provided is adequate to support approval.

PHASE IV: The following are proposed as Phase IV commitments:

Kellie Schoolar Reynolds, Pharm.D.
Reviewer, Antiviral Drugs Section, DPEIII
Office of Clinical Pharmacology and Biopharmaceutics

Concurrence: Janice B. Jenkins, Ph.D.
Acting Team Leader, Antiviral Drugs Section, DPEIII
Office of Clinical Pharmacology and Biopharmaceutics

cc: HFD-530 /NDA/20778 and 20779
/MO/SMaldonado
/CSO/KStruble
/Biopharm/KReynolds
/TLBiopharm/JJenkins
HFD-880 /DPEIII

wpd6.1, c:\ag1343\nda\draft2.wpd, 02-25-97, 03-07-97

TABLE OF CONTENTS

I. CHEMISTRY

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

-Molecular Formula- $C_{32}H_{45}N_3O_4S \bullet CH_4O_3S$

-Molecular Weight- 663.90 (567.79 as free base)

-Solubility- Nelfinavir mesylate is slightly soluble in aqueous solutions at pH≤4 and insoluble in aqueous solutions at pH>4. The approximate solubilities of nelfinavir mesylate in organic solvents were determined at room temperature:

SOLVENT	SOLUBILITY (mL/g)	USP DEFINITION

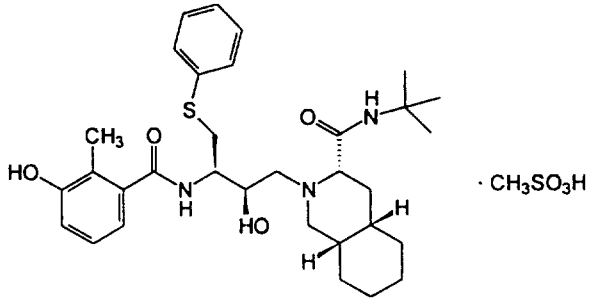
-Partition Coefficient- log $P_{octanol/water} = 4.07 \pm 0.2$

log D @pH 7.4 = 4.02

-pKa- $pKa_1 = 6.00 \pm 0.10$

$pKa_2 = 11.06 \pm 0.10$

-Structure-



II. FORMULATIONS

VIRACEPT Tablets, 250 mg

Ingredient	Theoretical Quantity (mg/Tablet)	% (w/w)
Nelfinavir Mesylate, anhydrous, solvent free		
Calcium Silicate		
Crospovidone, NF		
Magnesium Stearate, NF		
FD&C Blue #2		
FD&C Blue #2 Aluminum Lake		
Purified Water, USP		
Total Target Weight/Tablet		

*equal to 250 mg of nelfinavir mesylate as the free base

VIRACEPT Oral Powder, 50 mg/g

Ingredient	Unit Formula (mg/g)	Unit Formula % (w/w)
Nelfinavir Mesylate (anhydrous, solvent free)		
Microcrystalline Cellulose, NF		
Maltodextrin, NF		
Dibasic Potassium Phosphate, USP		
Crospovidone, NF		
Hydroxypropyl Methylcellulose 2910, USP		
Aspartame, NF		
Sucrose Palmitate		
Natural and Artificial Flavor		
Purified Water, USP		
Total		

III. INDICATION (per label)
VIRACEPT is indicated for the treatment of HIV infection in adults and children when antiretroviral therapy is warranted.

IV. DOSAGE AND ADMINISTRATION (per label)
Adults: The recommended dose is 750 mg (three 250 mg tablets) three times daily. VIRACEPT should be taken with a meal or light snack. Antiviral activity is enhanced and the development of viral resistance is reduced when VIRACEPT is administered in combination with other antiretroviral agents. Therefore, it is recommended that VIRACEPT should be used in combination with other antiretroviral agents.

Pediatric Patients (2-13 years): The recommended oral dose of VIRACEPT for pediatric patients 2 to 13 years of age is 20-30 mg/kg per dose, three times daily with food. For children unable to take tablets, VIRACEPT Oral Powder may be administered. The oral powder may be mixed with water, milk, formula, soy formula, soy milk, or dietary supplements. The recommended use period for storage of the product in these media is 6 hours. Dosing media not recommended include any acidic food or juice (e.g., orange juice, apple juice or apple sauce) because the combination may result in a bitter taste, The recommended pediatric dose of VIRACEPT to be administered three times daily is in the following table:

Body Weight		Number of 1 gram scoops	Number of level Teaspoons	Number of Tablets
Kg	Lbs			
7.5 to <10	16 to <22	4	1	---
10 to <12	22 to <26	5	1 1/4	---
12 to <15	26 to <33	6	1 ½	---
15 to <20	33 to <44	8	2	2
20 to <30	44 to <66	---	3	2
30 to <40	66 to <88	---	4	3
>40	>88	---	4	3

V. PHARMACOKINETICS

RADIOLABELED NELFINAVIR ADME

The absorption, disposition, metabolism, and excretion of nelfinavir and its metabolites in four healthy male volunteers were investigated following the administration of a single oral 750 mg dose of [^{14}C]-nelfinavir. Each 750 mg dose of [^{14}C]-nelfinavir was administered as ten capsules, each containing 75 mg (free base weight) of nelfinavir mesylate labeled with 10 μCi of ^{14}C. The dose was administered 10 minutes after eating. Serial blood samples and complete collection of urine and feces were performed for seven days after dosing.

Plasma concentrations of nelfinavir were measured
detection. Total radioactivity in plasma, red blood cells, urine, and feces was measured by liquid scintillation counting (LSC). Plasma, urine, and fecal extracts were analyzed for nelfinavir and metabolites by HPLC and detected by a radiochemical detector, followed by an electrospray triple quadrupole mass spectrometer. Structures of metabolites were elucidated by two types of MS/MS experiments: daughter ion mass spectrum and multiple reaction monitoring (MRM) detection. Authentic standards were available for four metabolites: M1, M8, M10, and M11. Many hydroxylated metabolites were assigned with partial structures due to the limitations of mass spectrometry. Semi-quantitative assessment of nelfinavir and metabolites was performed for (1) plasma samples from each subject, pooled between 2.5 and 4 hours and between 6 and 8 hours, (2) urine samples containing greater than 0.1% of the total dose, and (3) fecal homogenate samples containing greater than 5% of the total dose.

Plasma pharmacokinetic parameters following single 750 mg dose of [^{14}C]-nelfinavir (n = 4)

PARAMETER	UNCHANGED NELFINAVIR	TOTAL RADIOACTIVITY
AUC$_\infty$ (μg*hr/mL)	31.11 ± 12.84	38.84 ± 17.65
Cmax (μg/mL)	3.66 ± 1.22	4.52 ± 1.78
Tmax (hr)	3.38 ± 0.75	3.38 ± 0.75
T½ (hr)	3.53 ± 0.50	4.55 ± 0.76
AUC ratio (unchanged nelfinavir: total radioactivity)	0.81 ± 0.09	
Cmax ratio (unchanged nelfinavir: total radioactivity)	0.82 ± 0.11	

Recovery of radioactivity in urine and feces over 7 days

SOURCE	Sub #1	Sub #2	Sub #3	Sub #4	Mean ± SD (Median)
Feces	52.08%	88.32%	90.69%	85.39%	79.12 ± 18.17 (86.86)
Urine	1.66%	2.19%	0.90%	1.57%	1.58 ± 0.53 (1.62)
Total	53.72%	90.51%	91.59%	86.96%	80.70 ± 18.09 (88.74)

The plasma concentration vs. time profiles for nelfinavir and total radioactivity were similar, with unchanged nelfinavir comprising approximately 80% of radioactivity in plasma. At 36 hours post-dose and later, plasma concentrations of radioactivity and nelfinavir were not measurable.

Relative Amounts of Nelfinavir and Metabolites in Plasma (% total drug related material)

Compound	2.5 to 4 hour sample (n=4)	6 to 8 hour sample (n=4)
Nelfinavir	86±3	82±5
M1	3±0	4±1
M8	12±3	14±5

As indicated in the table above, the predominant circulating metabolite was the hydroxy-*t*-butylamide, M8. In vitro antiviral activity tests indicated that M8 was of comparable potency to nelfinavir in acute HIV-1 infection models, suggesting that M8 may contribute to antiviral effects of nelfinavir in patients. The 3"-methoxy-4"-hydroxynelfinavir metabolite, M1, were 2 to 5% of nelfinavir concentrations. It is notable that this metabolite was observed to co-elute with nelfinavir b also by the standard . Due to the low concentrations of the metabolite present, it is unlikely that M1 significantly confounds pharmacokinetic assessments of nelfinavir b methods. Two nelfinavir *S*-oxides (M10 and M11) were also detected in trace amounts.

Urinary excretion represented only a small portion of the total dose (approximately 1.6%). The major component in urine was unchanged drug (~70%), followed by two metabolites M1 (~10%) and M8 (~20%). The glucuronide conjugate and two *S*-oxides were also detected in trace amounts in urine.

A majority of the radioactivity was excreted into feces, primarily as oxidative metabolites. Intact nelfinavir accounted for 22% of fecal radioactivity. The metabolite profile in feces indicates that nelfinavir undergoes extensive oxidative metabolism in vivo. Nearly 20 different oxidative metabolites were detected in feces, with major sites of hydroxylation including the *t*-butylamide group, the benzamide ring, and the perhydroisoquinoline moiety. Secondary metabolites involving two of these pathways were also abundant. Also, nelfinavir *S*-oxides and hydroxy-thiophenyl metabolites were found as minor metabolites. Very little phase II metabolites were detected in human feces; the glucuronide conjugates and 3"-methyl-catechol derivatives may have undergone microbial metabolism in the intestinal tract.

Total recovery of radioactivity in feces plus urine was 87-91% of the administered dose for 3 of the 4 subjects in this study. Total recovery was considerably less for Subject #1, with approximately 54% of the dose recovered from feces plus urine. The plasma and urinary profiles were not substantially different for Subject #1 as compared to the other 3 subjects. Subject #1 was nauseated at 6 hours after drug administration and vomited at 10.75 and 11.75 hours after receiving the dose. The long interval of time between dosing and vomiting and the observed concentrations in plasma make it unlikely that one-third to one-half of the dose was regurgitated prior to absorption. The applicant states that due to the probable excretion of nelfinavir-related products into bile post-absorption, it is likely that a significant portion of radioactivity excreted into bile was lost in the emesis for Subject #1. Alternatively, a fecal sample may have inadvertently been lost and not reported by Subject #1 (the sponsor states that this is not likely). Based on the cumulative recovery of radioactivity in feces for each subject, it appears that recovery from Subject #1 was similar to recovery from the other subjects through 48 hours. For Subject #1, very little radioactivity was recovered in the feces after 48 hours; for the other subjects, recovery continued through 72 or 96 hours.

Date: November 11, 1996

Company: MOVA Pharmaceutical Corporation

Facility: Caguas, Puerto Rico

Dear Sirs:

I hereby certify that the manufacturing facility noted above is:

1. in compliance with all state and federal environmental laws;
2. in compliance with, or on an enforceable schedule to be in compliance with all emission requirements set forth in all permits; and
3. approval and the subsequent increase in production at this facility is not expected to affect compliance with current emission requirements of compliance with environmental laws.

Furthermore, this manufacturing facility and the surroundings are not on sites of any historic or archeological significance per the Department of Natural Resources or other state agencies.

Signature: J. M. García

Name (printed): José M. García

Title: Vice-President of Operations and Engineering

Agouron Pharmaceuticals, Inc.

MATERIAL SAFETY DATA SHEET

October 10, 1996

PRODUCT: Nelfinavir mesylate
SYNONYMS: AG1343
DESCRIPTION: HIV-PRO COMPOUND
MOLECULAR FORMULA: $C_{33}H_{49}N_3O_7S_2$
CHEMICAL NAME (CA Index Name): N-(1,1-dimethylethyl)decahydro-2-[2-hydroxy-3-[(3-hydroxy-2-methylbenzoyl)amino]-4-(phenylthio)butyl]-3-isoquinolinecarboxamide, [3S-[2(2S*,3S*), 3.α.,4a.β.,8a.β.]], monomethanesulfonate (salt)
CAS #: 159989-65-8

-----PHYSICAL DATA-----

MELTING POINT: 130-200°C
MOLECULAR WEIGHT: 663.90
VAPOR PRESSURE: : NOT APPLICABLE
SPECIFIC GRAVITY: NOT APPLICABLE
VAPOR DENSITY: : NOT APPLICABLE
SOLUBILITY IN WATER: LOW
APPEARANCE AND ODOR: WHITE TO OFF-WHITE, ODORLESS POWDER

----TOXICITY HAZARD DATA-----

ACUTE DATA

ORAL - MOUSE:	LD_{50}: > 500 mg/kg	No Observed Effect Level (NOEL) ≥ 500 mg/kg
ORAL - RAT:	LD_{50}: > 500 mg/kg	No Observed Adverse Effects Level (NOAEL) > 500 mg/kg
INHALATION - RAT: (Whole Body; 4 Hour Exposure)	LD_{50}: ≅ 3.7 mg/L	No Observed Effect Level (NOEL) < 2.1 mg/L
DERMAL - RAT:	LD_{50}: > 2,000 mg/kg	No Observed Effect Level (NOEL) 2,000 mg/kg

DERMAL SENSITIZATION (Buehler) - GUINEA PIG: Non-Sensitizer

SUBCHRONIC DATA

ORAL -RAT	13 weeks - NOAEL 1000 mg/kg/day	No target organ evident.
ORAL - MONKEY	13 weeks - NOAEL 800 mg/kg/day	No target organ evident.

IRRITATION DATA

DERMAL - RABBIT (0.5g):	SLIGHTLY IRRITATING
EYE - RABBIT (21mg):	MILDLY IRRITATING

GENOTOXICITY

Negative: *In vitro* Ames Test including E. coli.
Negative: *In vitro* human PBL chromosome aberration
Negative: *In vivo* rat micronucleus
Negative: *In vitro* mouse TK forward mutation

REPRODUCTIVE TOXICITY

Fertility & Early Embryonic Development- RAT: No Observed Adverse Effects Level (NOAEL) 1,000 mg/kg/day (Parental Toxicity/Reproductive Performance)

Development (Maternal/Fetal) - RAT:	No Observed Adverse Effects Level (NOAEL) 1.000 mg/kg/day
Development (Maternal) - RABBIT:	No Observed Adverse Effects Level (NOAEL) 800 mg/kg/day
Development (Fetal) - RABBIT:	No Observed Adverse Effects Level (NOAEL) 1.000 mg/kg/day
Pre/Post Natal Development - RAT: (Maternal/Neonatal)	No Observed Adverse Effects Level (NOAEL) 1.000 mg/kg/day

-----HEALTH HAZARD DATA-----

ACUTE EFFECTS OF EXPOSURE:
SKIN CONTACT: SLIGHTLY IRRITATING.
EYE CONTACT: MILDLY IRRITATING.
INHALATION: NONE OBSERVED.
INGESTION: NONE OBSERVED.

EFFECTS OF EXPOSURE:
SKIN CONTACT: NON-SENSITIZING.

CHRONIC EFFECTS OF EXPOSURE: NOT ESTABLISHED.

CARCINOGENIC PROPERTIES: NOT ESTABLISHED.

EMERGENCY AND FIRST AID PROCEDURES:

SKIN OR EYE CONTACT: WASH WITH COPIOUS AMOUNTS OF WATER FOR AT LEAST 15 MINUTES; REMOVE CONTAMINATED CLOTHING.
INHALATION: REMOVE TO FRESH AIR, CALL A PHYSICIAN IF NECESSARY.
INGESTION: RINSE MOUTH; DO NOT INDUCE VOMITING; DRINK COPIOUS AMOUNTS OF WATER TO FLUSH THE SYSTEM AND CALL OR TRANSPORT TO A PHYSICIAN.

-----FIRE AND EXPLOSION HAZARD DATA-----

FLASH POINT: NOT APPLICABLE (>300°F)
EXTINGUISHING MEDIA: WATER SPRAY, CO_2, DRY CHEMICAL, FOAM, HALONS.
SPECIAL PROCEDURES/UNUSUAL FIRE HAZARDS: NOT ESTABLISHED.

-----REACTIVITY DATA-----

STABILITY: STABLE AT ROOM TEMPERATURE.
INCOMPATIBILITIES: STRONG ACIDS, STRONG BASES, STRONG OXIDIZING AGENTS.
HAZARDOUS DECOMPOSITION PRODUCTS: NOT ESTABLISHED.

-----SPILL OR LEAK PROCEDURES-----

SPILL PROCEDURE: WEAR GLOVES, LAB COAT, AND DUST MASK.
SWEEP UP AND STORE IN BAG FOR APPROPRIATE DISPOSAL.
AVOID RAISING AND BREATHING DUST.

-----SPECIAL PROTECTION INFORMATION-----

HANDLING INFORMATION: WEAR GLOVES, LAB COAT, AND DUST MASK WHEN HANDLING.
WASH THOROUGHLY AFTER HANDLING.
AVOID INHALATION AND CONTACT WITH SKIN, EYES, AND CLOTHING.
KEEP TIGHTLY CLOSED; STORE IN A COOL, DRY PLACE.

WASTE DISPOSAL METHOD: STORE IN CLEARLY LABELED CONTAINERS UNTIL ABLE TO GIVE TO APPROVED AGENT FOR DISPOSAL IN ACCORDANCE WITH ALL FEDERAL, STATE, AND LOCAL REGULATIONS.

APPENDIX B

References

B1. Hazardous Substances Data Bank. 1992. MIROMEDEX Inc.

B2. Linsley, Jr., R.K., Kohler, M.A., and Paulhus J.L.H. 1975. Hydrology for Engineers - Second Edition. McGraw-Hill Book Company.

B3. Lyman, Warren J. Reehl, W.F., and Rosenblatt, D. 1990. Handbook of Chemical Property Estimation Methods. American Chemical Society, Washington, DC.

B4. Metcalf & Eddy, Inc. 1979. Wastewater Engineering: Treatment, Disposal, Reuse. Revised by G. Tchobanoglous. New York: McGraw-Hill Book Company.

B5. Pharmaceutical Manufacturers Association (PMA). 1991. Interim Guidance to the Pharmaceutical Industry for Environmental Assessment Compliance Requirements for the FDA. PMA Washington, D.C. *Reference not included.

B6. U.S. Environmental Protection Agency (USEPA). 1979. Water-Related Environmental Fate of 129 Priority Pollutants. Prepared by M.A. Callahan, M.W. Slimak, N.W. Gabel, I.P. May, C.F. Fowler et al., for the Office of Water Planning and Standards, U.S. Environmental Protection Agency, Washington, D.C., EPA-440/4-79-029ab.

B7. U.S. Environmental Protection Agency (USEPA). 1987. Expert Systems Questionnaire. Survey Concerning Biodegradation. Prepared by B. Gregg, N.W. Gabel, and S.E. Campbell (Versar, Inc.) for Office of Toxic Substances, Exposure Evaluation Division, U.S. Environmental Protection Agency, Washington, D.C., EPA Contract No. 68-02-4254.

B8. U.S. Food and Drug Administration (USFDA). 1987. Environmental Assessment Technical Assistance Handbook. Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, Washington, D.C.

B9. U.S. Food and Drug Administration. 1995. Guidance for the Industry for the Submission of an Environmental Assessment in Human Drug Applications and Supplements. Center for Drug Evaluation Research, (CDER), FDA, Washington, D.C. *Reference not included.

B10. Velagaleti, R. 1996. Behavior of Pharmaceutical Drugs (Human and Animal Health) in the Environment. Drug Information Association Journal (In Press).

ENVIRONMENTAL ASSESSMENT

AND

FINDING OF NO SIGNIFICANT IMPACT

FOR

Viracept™
(Nelfinavir Mesylate)
Oral Powder
NDA Number 20-778

Agouron Pharmaceuticals, Inc.

FOOD AND DRUG ADMINISTRATION

CENTER FOR DRUG EVALUATION AND RESEARCH

Division of Anti-Viral Drug Products
(HFD-530)

FINDING OF NO SIGNIFICANT IMPACT

NDA 20-778

Viracept

(Nelfinavir Mesylate)

Oral Powder

The National Environmental Policy Act of 1969 (NEPA) requires all Federal agencies to assess the environmental impact of their actions. FDA is required under NEPA to consider the environmental impact of approving certain drug product applications as an integral part of its regulatory process.

The Food and Drug Administration, Center for Drug Evaluation and Research, has carefully considered the potential environmental impact of this action and has concluded that this action will not have a significant effect on the quality of the human environment and that an environmental impact statement therefore will not be prepared.

In support of their new drug application for Viracept, Agouron Pharmaceuticals, Inc., has prepared an environmental assessment (attached) in accordance with [21 CFR 25.31a(a)] which evaluates the potential environmental impact of the manufacture, use and disposal of the product. The maximum expected environmental concentration is at a level that normally relieves the applicant from completing format items 7, 8, 9, 10, 11, and 15 in accordance with the Tier 0 approach specified in the *Guidance for Industry for the submission of an Environmental Assessment in Human Drug Applications and Supplements*.

Nelfinavir Mesylate is a chemically synthesized drug which is administered as a tablet in the medical treatment of Human Immunodeficiency Virus (HIV) disease syndrome. The drug substance will be manufactured by Ganes Chemicals, Inc., U.S.A.; Fuji Chemical Industry Co., Ltd., Japan; Yonezawa Hamari Chemicals, Ltd., Japan; and Niro, Inc., USA. The oral powder will be manufactured and packaged by MOVA Pharmaceuticals Corporation, Puerto Rico. The finished drug product will be used in hospitals, clinics and by patients in their homes.

Nelfinavir Mesylate may enter the environment from excretion by patients, from disposal of pharmaceutical waste and from emissions from manufacturing sites.

Disposal of the drug may result from out of specification lots, discarding of unused or expired product, and user disposal of empty or partly used product and packaging. Drug substance and

product that fail specification, pass expiration period, or are returned from the field are destroyed by high temperature incineration or by land filling in approved and regulated facilities. At U.S. hospitals and clinics, empty or partially empty packages will be disposed according to hospital/clinic regulations. From home use, empty or partially empty containers will typically be disposed of by a community's solid waste management system which may include landfills, incineration and recycling, while minimal quantities of unused drug may be disposed of in the sewer system.

The Center for Drug Evaluation and Research has concluded that the product can be manufactured, used and disposed of without any expected adverse environmental effects. Precautions taken at the sites of manufacture of the bulk product and its final formulation are expected to minimize occupational exposures and environmental release. Adverse effects are not anticipated upon endangered or threatened species or upon property listed in or eligible for listing in the National Register of Historic Places.

2/3/97
Date

PREPARED BY
Carl J. Berninger, Ph.D.
Environmental Scientist
Environmental Assessment Team
Center for Drug Evaluation and Research

2/3/97
Date

CONCURRED
Nancy B. Sager
Team Leader
Environmental Assessment Team
Center for Drug Evaluation and Research

Attachments: Environmental Assessment (FOI copy)

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HFD-530

Kimberly Struble CSO/PM
Original to NDA 20-778, through Kimberly Struble CSO/PM
Division File for NDA 20-778

HFD-205

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Docket File
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NON-CONFIDENTIAL
(N0420-778)

Environmental Assessment of Nelfinavir Mesylate (VIRACEPT®) Oral Powder
[Freedom of Information (FOI) Document]

Agouron Pharmaceuticals, Inc.
10350 North Torrey Pines Road
La Jolla, CA 92037-1020

The FOI, Environmental Assessment (EA) document being submitted by Agouron Pharmaceuticals, Inc. on this product is a non-confidential document and has an appendix I which contains the full confidential EA for review by FDA and is not for public disclosure. Confidential information from the FOI, EA document has been deleted and blocked out.

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1. DATE

December 13, 1996

2. NAME OF APPLICANT/PETITIONER

Agouron Pharmaceuticals, Inc.

3. ADDRESS

10350 North Torrey Pines Road

La Jolla, CA 92037-1020

4. DESCRIPTION OF THE PROPOSED ACTION

4.1. REQUESTED APPROVAL

Agouron Pharmaceuticals, Inc. has filed a New Drug Application (NDA) pursuant to Section 505(b) of the Federal Food, Drug, and Cosmetic Act to synthesize and manufacture the drug substance nelfinavir mesylate (formerly known as AG1343) and manufacture the drug product, nelfinavir mesylate (VIRACEPT®) Oral Powder, 50 mg/g of nelfinavir mesylate as the free base for the treatment of patients suffering from Human Immunodeficiency Virus (HIV) disease syndrome. The oral powder is packaged in HDPE bottles. VIRACEPT® is administered orally for patients with HIV.

The forecasted quantity of the drug substance, nelfinavir mesylate, that will be required to manufacture the VIRACEPT® Oral Powder for the first five years of production (from 1997 to 2001) is included in the volumes presented in the VIRACEPT Tablet NDA 20-779 (see Appendix C). An Environmental Assessment is submitted here pursuant to 21 CFR §25.31a (a), "Environmental Assessment for Proposed Approvals of FDA-Regulated Products - Format 1" (21 CFR, Chapter 1, April 1, 1993).

The format of this Environmental Assessment (EA) report is arranged as required in 21 CFR 25.31a (a) (1993) and Guidance for Industry for the Submission of an Environmental Assessment in Human Drug Applications and Supplements by the Center for Drug Evaluation and Research (CDER) of Food and Drug Administration (CDER, FDA, 1995). According to FDA, CDER (1995) guidelines, a full EA report is required if the Expected Introduction Concentration (EIC) of the drug in the Publicly Owned Wastewater Treatment Plant (POWTP) is equal to or greater than one part per billion. A full EA [corresponding to Sections 1-15 of FDA, CDER (1995) guideline document] is presented for nelfinavir mesylate in NDA 20-779 VIRACEPT Tablets (see Appendix C).

4.2. NEED FOR ACTION

Nelfinavir mesylate (also referred to as AG1343) is a novel non-peptide inhibitor of Human Immunodeficiency Virus-1 (HIV-1). The activity of HIV protease is central to viral infection because of its critical function in the processing of viral p55 gag polyprotein to structural proteins and enzymes. Therefore, inhibitors of this enzyme should have the potential to inhibit replication of HIV. Nelfinavir mesylate is a potent inhibitor of this enzyme and has demonstrated anti-HIV activity in a number of *in vitro*

test systems. It has also been shown to significantly reduce plasma viral RNA in patients receiving either monotherapy or combination therapy with reverse transcriptase inhibitors. Elevations in CD4 cell counts have also been observed in patients receiving nelfinavir mesylate.

4.3. PRODUCTION LOCATIONS

The new drug substance, nelfinavir mesylate, is the mono-methanesulfonic acid salt of (3S,4aS,8aS)-N-*tert*-butyl-2-[(2R,3R)-3-(3,2-cresolamido)-2-hydroxy-4-(phenylthio)-butyl]-decahydro-3-isoquinolinecarboxamide (AG1346).

For a description of the manufacture and manufacturers of nelfinavir mesylate, please refer to NDA 20-779 VIRACEPT Tablets, Section 4.1, Part III - Environmental Assessment Report.

4.3.1. Manufacture of Nelfinavir Mesylate Oral Powder

The drug product, nelfinavir mesylate (VIRACEPT®) oral powder will be formulated and packaged at the following location:

MOVA Pharmaceuticals Corporation
Gialla Blanca Industrial Park
State Road #1, A Street
Kilometer 34.3
Caguas, Puerto Rico 00725

A schematic and manufacturing process for the manufacture of nelfinavir mesylate oral powder is provided in confidential Appendix I under Attachment I. The drug product will be packaged at this location.

4.4. ENVIRONMENTAL SETTING OF PRODUCTION LOCATIONS

For a brief description of the environments at and adjacent to the manufacturing facilities is provided in NDA 20-779 VIRACEPT Tablets, please refer to the Environmental Assessment Report in Section 4.1.

4.5. LOCATIONS OF USE

Please refer to NDA 20-779 VIRACEPT Tablets, Section 4.1, Part III - Environmental Assessment Report.

4.6. DISPOSAL SITES

The disposal of nelfinavir mesylate will be the same for the VIRACEPT Oral Powder as presented in NDA 20-779 for VIRACEPT Tablets.

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5. IDENTIFICATION OF CHEMICAL SUBSTANCES THAT ARE THE SUBJECT OF THE PROPOSED ACTION

5.1. NELFINAVIR MESYLATE

The information on the drug substance nelfinavir mesyalte is the same as provided for VIRACEPT Tablets in NDA 20-779.

5.2. EXCIPIENTS

The excipients of the drug product, VIRACEPT Oral Powder, are presented in Table 5-1. The majority of the excipients are naturally occurring and are expected to be metabolized and eliminated from the body of patients along with the drug substance and released into the domestic sewage where they are likely to be further degraded. The impact of these excipient related emissions into the environment is discussed under Section 6.

VIRACEPT Oral Powder, 50 mg/g

PRODUCT COMPONENTS

Ingredient
Nelfinavir Mesylate
Microcrystalline Cellulose
Maltodextrin
Dibasic Potassium Phosphate
Crospovidone
Hydroxypropyl Methylcellulose 2910
Aspartame
Sucrose Palmitate
Natural and Artificial Flavor
Purified Water

5.3. IMPURITIES AND DEGRADANTS

Please refer to NDA 20-779 VIRACEPT Tablets for this discussion.

6. INTRODUCTION OF SUBSTANCES INTO THE ENVIRONMENT

For a discussion of the bulk drug intermediates and the drug substance, please refer to NDA 20-779 VIRACEPT Tablets. The potential emissions and their controls for VIRACEPT Oral Powder as manufactured and packaged by MOVA Pharmaceuticals Corporation, Caguas, Puerto Rico are described in this section.

6.1. PREPARATION OF VIRACEPT ORAL POWDER AND PACKAGING AT MOVA PHARMACEUTICAL CORPORATION, CAGUAS, PUERTO RICO

The manufacturing and packaging of the drug product, VIRACEPT® (nelfinavir mesylate) Oral Powder is conducted at MOVA Pharmaceutical Corporation, Puerto Rico. The manufacturing steps for nelfinavir mesyalte oral powder are provided in confidential Appendix I. Information on the manufacturing facility is provided in NDA 20-779 VIRACEPT Tablets, Section 4, Part III.

6.1.1. Substances Expected to be Emitted

Atmospheric

Manufacture of nelfinavir mesylate oral powder involves no alcohol in granulating or coating steps (Table 5-1). Thus, emission of organic volatiles will be non-existent. The drug substance is stable. A total volatile content of approximately 1% for this compound was observed at 120 °C, suggesting that the compound is anhydrous and solvent free (see NDA 20-779, Section 4, Part I.A. - Physical and Chemical Characteristics). Based on this data, the volatile atmospheric emissions are unlikely during manufacture or packaging. Emissions of organics are negligible for the ink used at the label printing stage or packaging. These minor emissions are not subject to any control devices.

Aqueous Waste

The processing areas and equipment are thoroughly hand-scraped prior to washdown. As a result, very little product is sewered. At the manufacturing plant, washdown water is treated onsite in the wastewater treatment plant for primary and secondary treatment, after which the plant effluents are discharged into the Puerto Rico Aqueduct and Sewer Authority (PRASA).

Solid Waste

The emissions will be controlled by a filter with an efficiency of 90%. Filters will be disposed of as solid waste. Approximately [redacted] of product waste per batch (batch size of [redacted]) will be generated from the manufacturing and packaging process and will be disposed of as a solid waste at the finished good incineration facility stated in Section 6.1.3.

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6.1.2. Controls Exercised

Process aqueous wastes and wastes from cleaning of equipment will be discharged to an onsite treatment facility that consists of primary and secondary treatment systems that include solids removal, pH adjustment, biological treatment and filtration. The effluent from the onsite wastewater treatment plant is discharged to the PRASA's Regional Wastewater Treatment Plant. Air vent filters, floor and equipment sweeping, and protective clothing worn by operators are disposed of as solid wastes.

6.1.3. Citation of and Statement of Compliance with Applicable Emission Requirements

The wastes generated during the manufacturing activities are packaged according to the Department of Transportation regulations and accumulated in areas designated for those purposes. The hazardous waste is disposed of using Ochoa Environmental Services, EPA ID number PRD090128562. The non-hazardous waste is disposed of in an industrial non-hazardous waste landfill in Penuelas, PROTECO. The finished goods are disposed of in a non-hazardous waste incineration facility, Commercial Incineration Corporation, located in Penuelas. The washwaters are pumped to a wastewater treatment plant for primary and secondary treatment. The effluent discharge is then sent to PRASA's regional treatment plant for further treatment.

The Environmental Quality Board (EQB) issued the Permit for the Operations of the Emission Sources, PFE-LC-13-0895-1881-I-II-0, on January 30, 1996. This permit will expire October 17, 2000.

MOVA is a large quantity generator and is inspected yearly by the EQB. Federal regulations do not apply because MOVA is a minor source. The EPA provided the identification number for the generation of hazardous waste as PRD-174-050-377 on April, 1988.

PRASA is the state agency that issued the permit for the industrial discharge of wastewaters. MOVA's permit was issued in December 24, 1994 and will expire on December 23, 1996. MOVA is in the process of renewal for this permit number GDA-88-602-010. The stormwater discharge permit is PRR-00-A-134 and was issued by EPA in October, 1992. MOVA has a general permit.

A statement of General Environmental compliance by the facility manager of MOVA Pharmaceutical Inc. is provided in NDA 20-779 VIRACEPT Tablets, Section 4, Part III - Environmental Assessment.

6.1.4. Effect of Approval on Compliance with Current Emission Requirements

The drug product manufacture and packaging at MOVA, will be within the limits of current permits and emission requirements of those permits.

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6.2. OCCUPATIONAL SAFETY

There are no special precautions required due to the oral powder product. Please refer to NDA 20-779 VIRACEPT Tablets for a full description.

6.3. EXPECTED INTRODUCTION CONCENTRATIONS FROM USE

The VIRACEPT Oral Powder will not change the path or amount of materials introduced into the environment. Please refer to NDA 20-779 for VIRACEPT Tablets for a full description.

6.4. EXPECTED INTRODUCTION FROM DISPOSAL

Drug product manufacture at MOVA Pharmaceuticals Corporation is expected to release [redacted] of the product during the manufacturing and packaging process. These wastes are disposed of at an incineration facility for the finished product as stated in Section 6.1.3. Very little waste is released into the process sewers from equipment washes. Disposal of residues of unused drug product (empty or partially empty packages) after human use will be at homes, hospitals or clinics and this process is not expected to contribute to the exposure of the drug to the environment to any significant extent. Rejected, expired or returned drug product which are expected to be insignificant are sent to Agouron Pharmaceuticals, Inc. where they are disposed of as pharmaceutical waste limiting any environmental releases. Thus, the exposure of the drug to the environment is limited through the disposal process. The excipients used in the drug product are naturally occurring and many of them are easily biodegradable. Hence no environmental impact is anticipated. EIC estimations due to disposal are not made for drug substance, drug product or its excipients, for the reasons stated above.

7. FATE OF EMITTED SUBSTANCES IN THE ENVIRONMENT

The environmental fate and transport of nelfinavir mesylate is analyzed based on the physical and chemical properties and known mammalian metabolism of the compound. Processes affecting transport between air, water, and soil and processes affecting structural degradation of the compound are also discussed. The physical and chemical properties such as vapor pressure, melting temperature, water solubility, octanol/water partition coefficient, dissociation constant as well as environmentally relevant processes such a biodegradation, hydrolysis, photolysis, oxidation, volatilization, sorption, bioaccumulation, and bioconcentration will be relevant for the prediction of environmental behavior of nelfinavir mesylate.

8. ENVIRONMENTAL EFFECTS OF RELEASED SUBSTANCES

Please refer to NDA 20-779 VIRACEPT Tablets, Section 4, Part III - Environmental Assessment.

9. USE OF RESOURCES AND ENERGY

Manufacture of the drug substance and the drug product or the packaging of the drug product at the respective facilities will not require large commitment of resources, and would be scheduled to fit with the current operations of the facility. Based on the discussions in the previous sections and in NDA 20-779 VIRACEPT Tablets, Section 4, Part III - Environmental Assessment, the residues, if any, from manufacture of nelfinavir mesylate and

its products would result in concentration levels that would have no effect on threatened or endangered species.

The manufacturing facility for the oral powder is not near any sites of historical or archaeological significance.

10. MITIGATION MEASURES

No potential adverse environmental impacts have been identified. Therefore, no mitigation measures are planned.

11. ALTERNATIVES TO THE PROPOSED ACTION

Environmental processes such a biodegradation and photodegradation in the POWTP and surface water are likely to deplete nelfinavir mesylate released both from human use and from manufacturing effluents. No environmental impact is anticipated since the resides are likely to be depleted significantly. Because no adverse environmental impact is expected, alternatives to the proposed action are not being considered. If nelfinavir mesylate is not manufactured, patients with HIV may not have an alternative drug available for treatment.

12. PREPARER AND CERTIFICATION

The undersigned certifies the information presented is true, accurate, and complete for preparation of the Environmental Assessment Report in accordance with 21 CFR 25.31(a).

The undersigned further certifies that this EA document contains confidential information and was prepared for FDA review and not for public disclosure.

Signature Michael A Adam Date: 12/13/96
Michael A. Adam, Ph.D.

Title: Director, Regulatory Affairs

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

NDA #: 20-779
20-778

REVIEWER : Shukal Bala and
Lauren Iacono-Connors

CORRESPONDENCE DATE: 12-20-96
CDER RECEIPT DATE: 12-23-96; 12-26-96
REVIEW ASSIGN DATE: 12-30-96; 02-24-97
REVIEW COMPLETE DATE: 02-14-97

SPONSOR: Agouron Pharmaceuticals, Inc
10350 North Torrey Pines Road
La Jolla, CA 92037

SUBMISSION REVIEWED: Original

DRUG CATEGORY: Anti-viral/protease inhibitor

INDICATION: Treatment of adult and pediatric patients with HIV

DOSAGE FORM: Tablets for oral administration; powder for oral administration

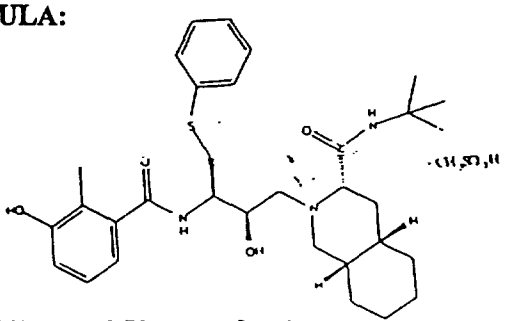
PRODUCT NAMES:

a. **PROPRIETARY:** Viracept

b. **NONPROPRIETARY:** Nelfinavir mesylate, AG 1343

c. **CHEMICAL:** [3S-[2(2S*,3S*,3R*]]-N-(1,1-dimethylethyl)decahydro-2[2-hydroxy-3-[(3-hydroxy-2-methylbenzoyl)amino-4-(phenylthio)butyl-3-isoquinolinecarboxamide, monomethane sulfonate (salt)

STRUCTURAL FORMULA:



Molecular weight: 663.9 (567.79 as the free base)
Empirical formula: $C_{32}H_{45}N_3O_4S \cdot CH_4O_3S$

SUPPORTING DOCUMENTS: IND #
DMI

BACKGROUND:

The subject of this NDA is nelfinavir mesylate (AG 1343), a protease inhibitor with activity against HIV-1. Like other protease inhibitors, nelfinavir interferes with the activity of the HIV-encoded protease enzyme which mediates cleavage of the *gag-pol* polyprotein precursor to form essential virus proteins. The result of this inhibitory activity is the production of immature, noninfectious virus particles. The antiviral activity of nelfinavir, like other protease inhibitors, is reversible; the drug must be continually present in order to mediate its antiviral effects. This fact, coupled with high degree of natural diversity observed in the protease gene sequence and the low fidelity of the HIV reverse transcriptase indicates the potential for development of drug resistance

Biology of HIV

HIV, the etiological agent for acquired immunodeficiency syndrome is a retrovirus that infects primarily CD4 lymphocytes and cells of monocyte/macrophage lineage. A diagrammatic representation of the life cycle of HIV is shown in Figure 1 (Ref: HIV and the pathogenesis of AIDS by J A Levy, 1994).

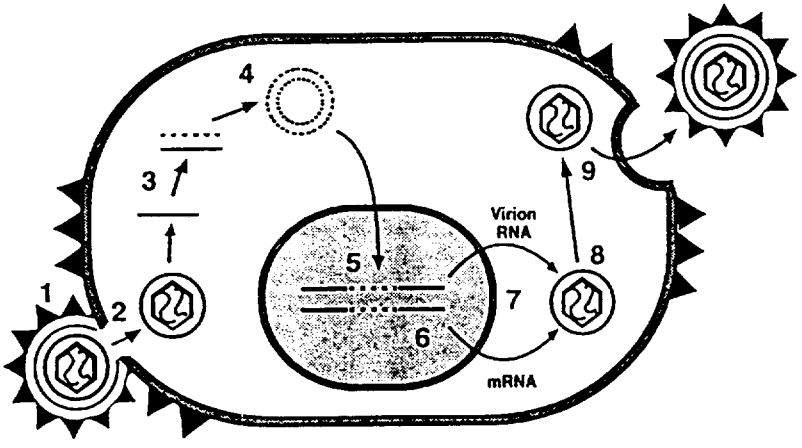


FIGURE 1. The HIV infection cycle. The steps are as follows: 1, attachment; 2, uncoating; 3, reverse transcription; 4, circularization; 5, integration; 6, transcription; 7, translation; 8, core particle assembly; 9, final assembly and budding. In steps 3 to 5, some viral core proteins are associated with the viral genome (——, RNA, - - - -, DNA). Double-stranded circular forms can be found both covalently and noncovalently bound. The latter are the forms that integrate into the cell chromosome. Antiviral therapies can be directed against each step and can potentially interrupt virus replication and spread. Figure courtesy of H. Kessler.

The RNA of the virus replicates through a DNA intermediate which is synthesized by a reverse transcriptase (RT). A DNA copy of the viral RNA integrates into the human cellular DNA forming the provirus. Transcription of the proviral DNA and translation of the viral transcripts by the combined action of the host cell and viral encoded products result in the production of the progeny virion. HIV, in the course of its replication cycle, produces polycistronic mRNAs whose long *gag* and *pol* polyprotein products are specifically cleaved to generate the individual functional proteins found in the infectious virus (Figure 2, Ref: HIV and the pathogenesis of AIDS by J A Levy, 1994). This specific proteolytic cleavage is brought about by the HIV-encoded proteolytic enzyme, the **protease.**

The HIV protease is a 99 amino acid peptide which is classified as an aspartyl enzyme by virtue of fact that it contains the signature catalytic site sequence Asp-Thr-Gly which is directly involved in the catalytic cleavage of the *gag* and *pol* polyprotein precursors. The protease dimerizes to form the active enzyme. The active protease processes the *gag* polyprotein precursor (p55) to generate the mature *gag* proteins (p24, p9 and p7) which constitute the viral core structure. The protease also processes the *gag-pol* fusion polyprotein p160$^{gag\text{-}pol}$, to produce the viral enzymes (reverse transcriptase, ribonuclease H and integrase), all of which are essential for HIV replication. The processing of this polyprotein precursor requires at least 8 peptidolytic cleavages (Table 1 - Ref.: Bugelski, P. *et al.*, J Leukocyte Biology, 1994, 56: 374).

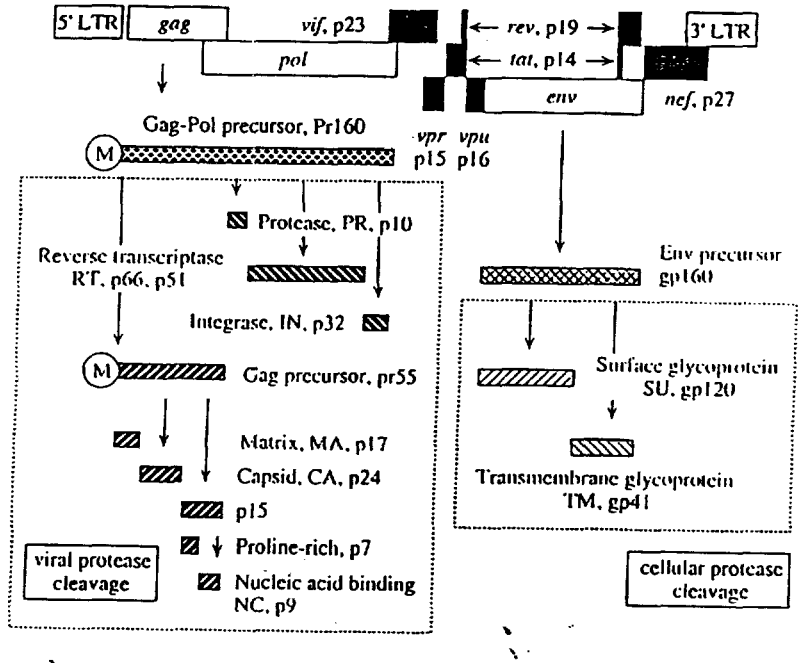


FIGURE 2. Processing of viral proteins. Some HIV-1 proteins, which are translated from ten distinct viral transcripts, are further processed by viral and cellular proteases. From nine translated proteins, which include Tev (not diagrammed), sixteen viral proteins are made. They form the virion structure, direct viral enzymatic activities, and serve regulatory and accessory functions. The Gag-Pol precursor of 160 kDa is processed by the viral (aspartyl) protease into seven proteins, which include four Gag proteins (MA, p17; CA, p24; Proline-rich, p7; NC, p9), protease (P, p10), reverse transcriptase/RNase (RT, p66, p51), and integrase (IN, p32). The Env precursor (gp160) is processed by a cellular protease into the surface glycoprotein (SU, gp120) and the transmembrane glycoprotein (TM, gp41). Viral regulatory and accessory proteins—which include Tat (p14), Tev (p20), Rev (p19), Nef (p27), Vif (p23), Vpr (p15), and Vpu (p16)—are not processed. (M), myristoylated. Figure courtesy of M. Peterlin.

TABLE 1. Proteolytic Cleavage Products of p160 by HIV Protease

Cleavage number	Cleavage "product"	Function of product	Location in mature virion[b]
–	p17	Matrix protein	Innerleaflet of envelope
1	p24	Capsid protein	Viral core
2	X	Unknown	Unknown
3	p7	Nucleocapsid	Inside viral core
4	p6	Contributes to budding	Unknown
5	PR	Protease	Inside viral core
6	RT	Reverse Transcriptase	Inside viral core
7	RH	RNAse H	Inside viral core
8	IN	Integrase	Inside viral core

[a]Adapted from Debouck [10].
[b]Adapted from Gelderblom et al. [69].

SUMMARY:

Effect of nelfinavir on the growth of HIV *in vitro*

Studies to measure the *in vitro* activity of nelfinavir against HIV were conducted using different strains of the virus and a variety of cell types including T-cell lines (acute and chronic infection), macrophages and fresh peripheral blood mononuclear cells (Table 2). The T-cell lines were infected with the virus at a multiplicity of infection (m.o.i.) varying from 0.01 to 0.03; PHA blast cells were infected at an m.o.i. of 0.001; macrophages were infected at an m.o.i. of 0.1. Cells were incubated with the drug for periods varying from 6 to 14 days. The antiviral activity was measured by cell survival, inhibition of reverse transcriptase (RT) or a p24 reduction assay. AZT, ddC and Ro 31-8959 were used as positive controls. The results indicate that the ED_{50} (50% effective dose) values for nelfinavir varied from 9 - 60 nM (i.e., 5.98 - 39.84 ng/ml). An AZT resistant strain of HIV-1 (G910-6) and HIV-2 ROD were also susceptible to nelfinavir with an ED_{50} value of 60 nM (39.84 ng/ml). Nelfinavir was generally cytotoxic only at much higher concentrations. For example, the TC_{50} values against CEM-SS and MT-2 cells were 28 uM (i.e., 18.59 ug/ml) and 23 uM (i.e., 15.27 ug/ml) respectively. For most virus/cell combinations the therapeutic index (TI) was > 500 fold when determined on the basis of ED_{50} values.

The sponsor has also determined the ED_{95} values for those experiments where such values could be determined (23 of the 37 experiments conducted). Some of the values (14) which did not fall in the linear part of the curve were excluded from analysis. Overall, the results of the studies conducted in different laboratories using different strains of viruses and different cell lines indicate that the ED_{95} values for nelfinavir vary from 7.4 nM to 195.8 nM with a mean of 109.21 ± 46.21 nM. The TI decreased accordingly (range 43 to 46) when the ED_{95} (95% effective dose) values were used in place of the ED_{50}.

Table 2

In Vitro Antiviral Activity of AG1343 in Acute and Chronic HIV Infection Models

Virus Strain	Host Cell Type	Assay Endpoint[a]	ED_{50} (nM) AG1343	Ro 31-8959	AZT	ddC
HIV-1 Ba-L	Macrophage	RT	23	17	ND	ND
HIV-1 RoJo	PBMC[b]	RT	10	9	140	ND
HIV-1 IIIB A17 (pyridinone-resistant)	MT-2	CPE	30	ND	30	ND
HII2-1 HIV-1 (AZT-sensitive)	MT-2	CPE	10	20	30	580
G910-6 HIV-1 (AZT-resistant)	MT-2	CPE	60	30	>149,000	900
HIV-1 IIIB (chronic infection)	CEM-SS	RT	40	13	9,000	ND
HIV-2 ROD	CEM-SS	RT	9	2	3	11

[a] RT = reverse transcriptase; CPE = cytopathic effect.
[b] PBMC = peripheral blood mononuclear cells.

It should be noted that of the above 23 *in vitro* dose response experiments, 19 were conducted at the Southern Research Institute and the remaining 4 at other sites (Roche, Abbot, Bayer and ADARC). The studies conducted at Southern Research Institute were performed using 5 different strains of viruses and 2 different cell lines (Table 3). For the label, the sponsor has calculated 2 different means from the studies conducted using HIV-1-RF and the HIV-1IIID strain of virus in CEM-SS and MT-2 cells respectively. These 2 mean values (120.5 and 133.4 nM) in combination with 3 other values obtained from studies conducted in the same laboratory (Southern Research Institute) were used to calculate another mean (114 nM). The 2nd mean along with data from 4 other experiments (conducted at different sites) were then used to calculate a 3rd mean. On the basis of these calculations it is stated in the label that the EC_{95} (95% effective concentration) of nelfinavir ranged from 7 to 120 nM with a mean of 58.3 nM. The sponsor has stated that "this rationale was designed to avoid the introduction of any biases which could result when one value is unfairly weighted as could come when one lab performs the assay a significantly greater number of times than any other laboratory." However, it should be borne in mind that these assays were performed using different strains of viruses, cell lines and end points (CPE, RT or p24) for measurement of antiviral activity. Also, whether any alterations were made in the protocol used at different sites to measure *in vitro* activity of nelfinavir is not known. A range should be based on individual values and not on the basis of metaanalysis as proposed by the sponsor especially when variations in the experimental designs exist.

Table 3

LAB	SRI ED95 (nM)				
Virus/Host	RF/CEM-SS	IIIB/MT-2	G9106/MT-2	H112/MT-2	A17/MT-2
	120.5	135.5	135.5	45.2	135.5
	90.4	135.5			
	90.4	135.5			
	150.6	135.5			
	120.5	135.5			
	135.5	150.6			
	90.4	105.4			
	90.4				
	195.8				
Mean ED95 (nM)	120.5	133.4	135.5	45.2	135.5
SRI Mean ED95 (nM)	114.0				
	Laboratory	n	ED95 (nM)		
	SRI	19	114		
	Roche	1	7.4		
	Abbott	1	15.1		
	Bayer	1	120		
	ADARC	1	35		
		Mean	58.3		

Antiviral activity of metabolites of nelfinavir

The antiviral activity of the metabolites (M 1, 8, 10 and 11) of nelfinavir was determined in CEM-SS cells infected with HIV-1 RF or MT-2 cells infected with HIV-1 IIIB. Results in Tables 4 and 5 indicate that AG1402 (M8) showed antiviral activity comparable to the parent compound AG1343. However, the other metabolites tested were less active (AG1365 [M1] and AG1361B [M11]) or not active (AG1361B [M10]), although the TC_{50} values were either similar to or higher than the parent compound.

Table 4

Antiviral Activity and Cytotoxicity Evaluations of AG1343 and Metabolites of AG1343 in an Acute Infection of CEM-SS cells with HIV-1 RF [a]

Compound	ED50 (nM)	ED95 (nM)	TC50 (μM)	Therapeutic index [b]
AG1365 (M1)	150.8	1675.0	26.1	173
AG1402 (M8)	34.2	154.1	96.6	2825
AG1361A (M11)	1301.4	ND	51.4	40
AG1361B (M10)	>32,700	> 32,700	32.7	<1
AG1343	30.1	120.5	28.9	960
AZT	52.3	543.1	>374.5	>7161
ddC	94.70	142.0	37.69	398

[a] Antiviral efficacy was determined by measuring MTT reduction 6 days after infection. Results are from one experiment or represent the mean of two experiments (AZT); ND = Not determined.

[b] Therapeutic index = Cytotoxicity (TC_{50}) ÷ Antiviral activity (ED_{50}).

Table 5

Antiviral Activity and Cytotoxicity Evaluations of AG1343 and Metabolites of AG1343 in an Acute Infection of MT-2 cells with HIV-1 IIIB [a]

Compound	ED50 (nM)	ED95 (nM)	TC50 (μM)	Therapeutic index [b]
AG1365 (M1)	653.3	ND	13.3	20
AG1402 (M8)	85.6	ND	92.6	1082
AG1361A (M11)	4554.8	ND	54.8	12
AG1361B (M10)	>28,300	ND	28.3	<1
AG1343	60.2	195.8	11.1	184
AZT	430.7	ND	109.4	254
ddC	5924	ND	176.3	30

[a] Antiviral efficacy was determined by measuring MTT reduction 7 days after infection. Results are from one experiment or represent the mean of two experiments (AG1343, AZT); ND = Not determined.

[b] Therapeutic index = Cytotoxicity (TC_{50}) ÷ Antiviral activity (ED_{50}).

Effect of protein binding of nelfinavir on its antiviral activity

Nelfinavir was found to be highly protein bound. The sponsor has tested the antiviral activity of the drug in the presence of different concentrations of fetal calf serum or human serum. CEM-SS cells infected with HIV-1 RF (for 4 hours) were exposed to nelfinavir for 6 days and the antiviral activity measured by the cell protection assay. Although ED_{50} values increased in the presence of higher concentrations of fetal calf serum the TC_{50} concentrations were also increased. The results indicate that fetal bovine serum did not significantly alter the therapeutic index. Increasing concentrations of human serum did not alter either the ED_{50} or TC_{50} values.

In another experiment, MT-4 cells infected with the HIV-1 NL4.3 strain of virus were exposed to the drug for 4 days in the presence of different concentrations of α1-acid glycoprotein. The antiviral activity was measured by the p24 reduction assay. The results in Table 6 indicate a significant increase in the ED_{50} and ED_{90} values. However, the TC_{50} values observed for this experiment were not provided in the submission, so it is not possible to determine if the therapeutic index was also affected. Nevertheless, the plasma concentration (Cmax) of nelfinavir in patients with HIV has been shown to be in the range of 9 to 12 ug/ml, which exceeds the highest ED_{95} value (800 nM i.e., 0.5 ug/ml) generated *in vitro* (shown in Table 6). These studies suggest that binding of nelfinavir to glycoprotein *in vitro* may alter its antiviral activity, but that concentrations achieved *in vivo* are still likely to be sufficient to mediate a significant antiviral effect. However, the relevance of this finding to the *in vivo* situation is not known at this point in time.

Table 6

Effect of α1-acid Glycoprotein (AAG) on Antiviral Efficacy of Protease Inhibitors[a]

Compound	[AAG], mg/ml	ED_{50} (nM)	ED_{95} (nM)
AG1343	0	8	35
	0.2	40 (5x)	180 (3x)
	2.0	200(25x)	800(23x)
SC-52151	0	35	75
(Searle)	0.2	80 (2x)	350 (5x)
	2.0	2000 (57x)	9000 (120x)
ABT-538	0	60	350
(Abbott)	0.2	180 (3x)	380
	2.0	900 (15x)	1900 (5x)

[a] The antiviral efficacy of each compound was assayed against a HIV-1 NL4.3 infection of MT-4 cells using p24 as an endpoint. Culture medium was adjusted to contain the indicate concentrations of AAG. Values in parenthesis represent fold change in ED_{50} or ED_{95} values of AAG-treated versus untreated cultures.

In vitro activity of nelfinavir against other microorganisms

The *in vitro* activity of nelfinavir was measured against *Candida albicans*, gram-positive, gram negative and anaerobic bacteria. Results in Table 7 indicate that nelfinavir exhibits no activity against *Candida albicans* or gram-positive and gram-negative bacteria. Nelfinavir inhibited the growth of *Corynebacterium acnes* and *Helicobacter pylori* at a concentration of 30 and 100 ug/ml respectively.

Table 7

Summary of Antimicrobial Screen Test Results for Nelfinavir Mesylate

Organism	Source/Strain	Description	Nelfinavir MIC [a] (µg/mL)	Positive Control	MIC of Positive Control (µg/mL)
Mycobacterium ranae	ATCC 110	Gram positive	>100	Gentamycin	0.3
Staphylococcus aureus	ATCC 6538 P	Gram positive	>100	Gentamycin	0.1
Streptococcus faecalis	ATCC 10541	Gram positive	> 100	Gentamycin	30
Bacillus subtilis	ATCC 43223	Gram positive	>100	Gentamycin	0.03
Corynebacterium minutissimum	ATCC 23347	Gram positive	>100	Gentamycin	0.10
Escherichia coli	ATCC 10536	Gram negative	>100	Gentamycin	0.3
Proteus vulgaris	A 9539	Gram negative	>100	Gentamycin	0.3
Klebsiella pneumoniae	A 9977	Gram negative	>100	Gentamycin	0.1
Pseudomonas aeruginosa	ATCC 9027	Gram negative	>100	Gentamycin	0.3
Corynebacterium acnes	ATCC 6919	Anaerobe	30	Ampicillin	0.10
Helicobacter pylori	ATCC 43504	Anaerobe	100	Ampicillin	0.03
Clostridium sporogenes	ATCC 7955	Anaerobe	>100	Ampicillin	1.0
Bacteroides fragilis	ATCC 23745	Anaerobe	>100	Ampicillin	1.0
Candida albicans	ATCC 10231	Fungus	>100	Amphotericin B	0.03

[a] MIC: Minimum Inhibitory Concentration

Resistance

(a) *In vitro* antiviral activity of nelfinavir against a ZDV-resistant clinical isolate, and a pyridinone-resistant laboratory isolate

MT-2 cells were acutely infected with either a ZDV-sensitive (HIV1-HII2-1), or a ZDV-resistant (HIV1-G910-6) clinical isolate, or a pyridinone-resistant (HIV1-IIIBA17) laboratory derived isolate. HIV1-IIIBA17 possesses a resistant phenotype *in vitro* to non-nucleoside reverse transcriptase inhibitors (NNRTIs), including pyridinone derivatives, BI-RG-587 and TIBO compounds (Nunberg *et al.*, 1991. J.Virol 65:4887-4892). Using the MTT/CPE reduction assay (Alley et al., 1988. Cancer Res. 48:589-601) ED_{50} values were determined for nelfinavir and ZDV (Table 2, page 5).

The data provided demonstrate that for the clinical specimens tested under these assay conditions the ZDV-resistant clinical isolate (HIV1-G910-6) and the pyridinone-resistant laboratory isolate (HIV1-IIIBA17) retained sensitivity to nelfinavir *in vitro*. Since these antiviral agents have divergent mechanisms of action this result is not surprising. However, the data provided were generated with only one ZDV-resistant clinical isolate. Thus, it is an extremely limited treatment of the subject. If nelfinavir is to be approved for use in combination with or subsequent to therapy with ZDV, or other reverse transcriptase inhibitors (RTIs), or selected non-nucleoside (NN) RTIs the sponsor should consider a more thorough analysis of RTI-resistant HIV-1 clinical isolates for susceptibility to nelfinavir.

(b) Development and analysis of nelfinavir-resistant HIV-1 variants *in vitro*

An HIV-1 laboratory strain ($HIV\text{-}1_{NL4\text{-}3}$) was propagated in MT-4 cells in the presence of nelfinavir at subinhibitory concentrations (that which produced p24 antigen levels of ≤ 10 ng/mL), and ranged in a stepwise fashion from 0.002 μM up to 1.6 μM. A change in HIV-1 nelfinavir-susceptibility was first noted in the passage 22 (p22) virus population. Nelfinavir concentration at p22 was up to 0.300 μM. By p28 the nelfinavir concentration was up to 1.6 μM. A standard p24 HIV-antigen production analysis was performed on the p22 and p28 virus populations. The ED_{90} value of these virus populations had increased 7-fold and 30-fold in p22 and p28, respectively (Table 8). These data demonstrate that it is biologically possible for $HIV\text{-}1_{NL4\text{-}3}$ to develop a measurable degree of nelfinavir resistance. The clinical ramifications of these data are not predictable at this time.

Table 8

Serial Passage of HIV-1 NL4.3 in the Presence of AG1343[a]

Virus	Concentration (μM)	ED_{90} (μM)
HIV-1 NL4.3		0.080
p1	0.001	0.080
p22	0.300	0.560 (7x)
p28	1.600	2.400 (30x)

[a] Wild-type HIV-1 NL4.3 was serially passaged in the presence of increasing concentrations of AG1343 as indicated. Values in parenthesis (resistance levels) represent the relative difference in ED_{90} between virus isolated following serial passage for indicated times and wild-type HIV-1 NL4.3 as determined by susceptibility assay.

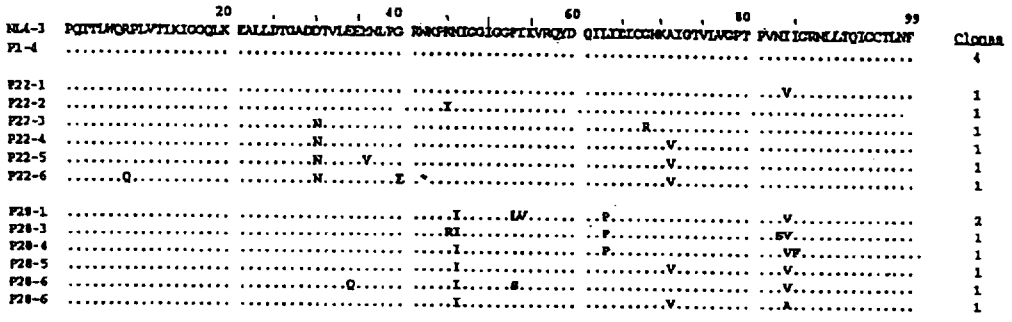
Genotypic analysis of the protease (PR) genes of representative virus clones from p22 (6 clones) revealed certain specific nucleotide substitution mutations which should alter the deduced amino acid sequence at positions R8Q (1 of 6 clones), M36V (1 of 6 clones), G40E (1 of 6 clones), K45I (1 of 6 clones), G68R (1 of 6 clones), I84V (1 of 6 clones), D30N (4 of 6 clones), and A71V (3 of 6 clones) (Figure 3). By p28 the amino acid position 30 mutation was no longer detectable, but a double amino acid mutation at positions M46I/I84V,A was detected in all p28 clones tested (6/6), and an L63P change (3 of 6 clones), a A71V change (2 of 6 clones) and E35Q (1 of 6 clones) were also observed. Thus, the amino acid position D30N mutation appears early and may be interpreted as one of the "dominant" early changes in the *in vitro* system. However, the D30N mutation reverts back to wild type (D30) just 6 passages later and is replaced by amino acid changes at positions M46I/I84V/L63P/A71V/E35Q which emerge later under nelfinavir selection. These data demonstrate that HIV-1_{NL4-3} variant populations (p22 and p28) contain amino acid mutations in the PR gene sequence which may or may not contribute to the phenotypic decrease in nelfinavir susceptibility measured *in vitro*.

Figure 3

Genotypic Analysis of the Protease Gene from AG1343-resistant Virus

Deduced amino acid sequences of proteases from clones of HIV-1 NL4.3 isolated following 1, 22 and 28 passages (p1, p22, p28). Dashed lines represent homologous sequences.

Deduced amino acid sequences of proteases obtained for p22-1343, and p28-1343 variant viruses.



To address the question of whether a causative relationship exists between some of these genetic mutations and the phenotypically expressed nelfinavir resistance *in vitro* site-directed mutagenesis was used to construct HIV-1_{NL4-3} variants containing selected single, double and triple mutations in the PR gene. These virus variants should only contain the mutations shown in Table 9 against a parent HIV-1_{NL4-3} genetic background. Susceptibility testing was performed on these virus constructs using the p24 inhibition assay in acutely infected MT-4 cells. Of the single mutations evaluated only positions I84V and L90M produced a shift in nelfinavir susceptibility (ED_{90}); 5-fold. The double

mutants R8Q/M46I, R8K/M46I, and M46I/I84V produced a 5-fold decrease in nelfinavir susceptibility. The other mutations analyzed (Table 9) either failed to result in susceptibility changes or were not measurable due to impaired growth kinetics in cell culture. No change in nelfinavir susceptibility was observed for virus constructs containing single mutations at positions M46I, L63P, and A71V. Since amino acid position 30 appears to be a relevant point mutation in the p22 variant and since the position 30 mutation was shown to be a predominant mutation site in HIV-1 clinical isolates from patients on nelfinavir therapy (described below) it is not clear why that point mutation was not assessed in these studies. An HIV-$1_{NL4.3}$ D30N variant assessed for nelfinavir susceptibility changes *in vitro* would demonstrate a causative relationship between changes in nelfinavir susceptibility and the D30N point mutation, albeit *in vitro*.

These data demonstrate that certain point mutations in the HIV-1 PR can cause a shift in nelfinavir susceptibility and that these changes may be involved, the degree of which is unknown, in the changes in susceptibility observed in the p22 and p28 "nelfinavir-resistant" isolates selected *in vitro*.

Table 9

Summary of Antiviral Susceptibilities of Mutant HIV-1 NL4.3 to AG1343[a]

Recombinant HIV-1 Mutants	Resistance Level
10F	2
32I	1
46I	1
46L	1
46F	1
48V	3
63P	1
71V	1
82A	1
82F	2
84V	5
90M	5
8Q + 46I	5
8K + 46I	5
8K + 82I	4
10F + 48V	3
10F + 84V	3
32I + 82A	4
46F + 82A	1
46I + 84V	5
8Q + 32I	NV
8Q + 46F	NV
8Q + 82A	NV
8K + 82A	NV
8Q + 32I + 82A	NV
8Q + 46F + 82A	NV
8K + 32I + 82A	NV
8K + 32I + 82I	NV
8K + 48V + 82A	NV

[a] Resistance levels represent the relative difference in ED_{90} between mutant HIV-1 NL4.3 strains and wild-type HIV-1 NL4.3 as determined by susceptibility assay; *NV* = nonviable.

Protease gene from HIV-1 NL4.3 strain was modified by site-directed mutagenesis to contain the indicated substitutions.

In a separate study *in vitro* site-directed mutagenesis was used to construct additional HIV-$1_{NL4.3}$ variants containing mutations in the PR gene which would produce variants with single amino acid substitutions at positions D30N or N88D, or a double mutant D30N/N88D (Table 10). Susceptibility testing was performed on these virus constructs using the p24 inhibition assay in acutely infected MT-4 cells. Susceptibility testing was also performed as above using a series of alternate PRIs (Table 10). The p22 and p28 HIV-$1_{NL4.3}$ variants, as well as the parent HIV-$1_{NL4.3}$, were included in the *in vitro* susceptibility analysis. Similar to previous *in vitro* susceptibility analyses the nelfinavir ED_{90} values for p22 and p28 variants were 9-fold and 38-fold less susceptible to nelfinavir than the parent construct, respectively. The sponsor provided methodologic evidence and data which described a 3-fold change in *in vitro* susceptibility to be approximately two standard deviations above the mean-fold changes (ratio between the mean ED_{90} and the lowest value obtained for that data set) observed for a set (n=18) of HIV-$1_{NL4.3}$ samples (data not shown). The mean-fold change of nelfinavir ED_{90} values, *in vitro*, for HIV-$1_{NL4.3}$ was 1.2 ± 0.2. The derivation of significance of fold-changes as described here is adequate for these analyses only. The p22 virus population retained full susceptibility *in vitro* to the alternate protease inhibitors (PRIs) tested here with the exception of DMP450 (3-fold shift in susceptibility). The p28 virus population appeared to express some degree of cross-resistance (4- to 11-fold) *in vitro* compared to baseline for those PRIs tested here with the exception of U-103017 and 141W94 (not done), Table 10. The D30N variant and the D30N/N88D variant each expressed a loss in nelfinavir-susceptibility *in vitro* of approximately 9-fold while that of the N88D variant retained full susceptibility *in vitro*. For all other PRIs tested here (Table 10) the D30N variant, the N88D variant, and the D30N/N88D variant retained a susceptibility value *in vitro* which was not different from the parent HIV-$1_{NL4.3}$ value. The clinical significance of these data is unclear at this time.

Table 10

Susceptibility of HIV-1 Variants to AG1343 and Other Protease Inhibitors

Compound	HIV-1 NL4.3[b]	ED_{90} (μM) (Fold-change)[a] HIV-1 NL4.3 Variants[b]				
		Passage 22	Passage 28	D30N	N88D	D30N/N88D
AG1343	0.022	0.2(9)	0.83 (38)	0.19 (9)	<0.008 (<3)	0.18 (8X)
ritonavir	0.08	0.045 (<2)	0.9 (11)	<0.008 (<10)	<0.008 (<10)	<0.008 (<10)
indinavir	0.08	0.08 (1)	0.78 (10)	<0.008 (<10)	<0.008 (<10)	<0.008 (<10)
saquinavir	0.03	0.01 (<3)	0.16 (5)	<0.008 (<3)	0.03 (1)	<0.008 (<3)
DMP 450	0.25	0.74 (3)	2.0 (8)	ND	ND	ND
U-103017	5.0	1.1 (<5)	5.0 (1)	ND	ND	ND
S-338	0.008	0.003 (<3)	0.033 (4)	ND	0.01 (1)	ND
SC-52151	0.04	0.025 (<2)	0.19 (5)	<0.008 (<1)	ND	ND
141W94	0.17	ND	ND	0.02 (<9)	0.02 (<9)	0.036 (<5)

[a] Values in parentheses (resistance levels) were calculated by comparing the ED_{90} of wild-type HIV-1 NL4.3 and variant HIV-1 NL4.3 strains as determined in susceptibility assays in MT-4 cells described in Methods. Values are derived from one experiment or represent the mean of duplicate experiments. Variant HIV-1 NL4.3 strains and wild-type HIV-1 NL4.3 were analyzed in parallel for sensitivity to all inhibitors tested.

[b] Virus strains: HIV-1 NL4.3 represent wild-type HIV-1; passage 22 and passage 28 variants are HIV-1 NL4.3 strains isolated following serial passage (p) for the indicated number of times; D30N, N88D, D30N/N88D represent recombinant HIV-1 NL4.3 strains containing HIV protease genes which were modified by oligo-mediated site-directed mutagenesis to contain the indicated substitutions.

Limited, only done once on some cases

Certain HIV-$1_{NL4.3}$ point mutation variants, previously reported to have a significant shift in alternate PRI selection drug susceptibility (Table 11), were tested *in vitro* for their susceptibility to nelfinavir. Variant p22 (selected with A-84538), which contain amino acid changes at positions V82F/I84V/M46I/L63P/A71V, had a shift in nelfinavir susceptibility of 20-fold (ED_{90}) *in vitro*. Variant p19 (selected with A-77003), which contains amino acid changes at positions R8K/M46I, had a shift in nelfinavir susceptibility of 15-fold (ED_{90}) *in vitro*. Although these data suggest cross-PRI decreases in susceptibility from other PRIs to nelfinavir, the clinical ramifications of these limited observations are unknown.

Table 11

Summary of Antiviral Susceptibilities of Mutant HIV-1 NL4.3 to AG1343[a]

HIV-1 NL4.3 Variants[b]	Resistance Level
P19 (A-77003) (8K/46I)	6
P34 (A-77003) (8K/46I/63P 71V/90M)	15
P22 (A-84538) (82F/84V/46I/63P/71V)	20
P13 (MP-134) (10F/84V)	2
P14 (MP167) (48V)	5

[a] Resistance level represents the relative difference in ED_{90} between mutant HIV-1 NL4.3 strains and wild-type HIV-1 NL4.3 as determined by susceptibility assay.

[b] Represent HIV-1 NL4.3 strains serial passaged (p) for the indicated number of times in the presence of indicated drugs. Mutations in protease genome indicated in parenthesis.

(c) Phenotypic susceptibility and genotypic sequence analysis of HIV isolates from patients during therapy with nelfinavir

HIV PR gene sequences were monitored during nelfinavir therapy for a sample of 58 trial participants, AG1343-503 (n=52) and AG1343-510 (n=6), Table 12, with evaluable plasma specimens from both baseline and ongoing therapy time points; 3 to 52 weeks (Table 13). The ongoing median and mean time of analysis for all 58 patients was 13 and 18 weeks, respectively. The sponsor has identified virus PR deduced amino acid substitutions observed during nelfinavir therapy. Predominant PR gene point mutations which result in changes in the deduced amino acid sequence are those which are observed in >10% of patients. Observed genotypic changes are those which were detected at some time point after initiation of nelfinavir therapy when compared to baseline (Table 14). Observed genotypic changes in patient specimens that later reverted back to baseline sequence during nelfinavir therapy are not considered in the "after therapy maintained" column in Table 14.

Table 12

Summary information for clinical protocols and patient selection

Protocol Number	Protocol Title	Investigators	Number of patients
AG1343-503	A Pilot Phase II, Open-Label, Dose Range Finding Study of AG1343 in HIV Positive Patients.		
AG1343-510	A Phase I/II Pilot Study of VIRACEPT (AG134 in Combination with Stavudine (d4T) Versus Stavudine (d4T) Alone i HIV Positive Patients		

Table 13

Summary information for number of patients and time of genotypic analysis by dose regimen[a]

Nelfinavir Dose Regimen[b]	n	Median time (weeks)	Mean time (weeks)	Range (weeks)
500 mg BID	9	4	13	3-52
600 mg BID	8	15	18	4-48
750 mg BID	12	14	20	4-52
500 mg TID	9	30	24	5-35
750 mg TID	8[c]	17	23	8-49
1000 mg TID	6	13	16	12-31
500 mg TID + d4T	3	14	12	8-14
750 mg TID + d4T	1	12	12	NA
1000 mg TID + d4T	2	10	10	8,12

[a]The HIV protease gene was obtained by RT-PCR of vRNA obtained from matched pairs of patient plasma samples at baseline and various times after nelfinavir therapy as indicated (in weeks). NA = not applicable.

[b]Doses of nelfinavir (mg) were given twice a day (BID) or three times a day (TID) alone (protocol AG1343- 503) or with d4T (protocol AG1343-510) as described in Methods.

[c]Includes two patients, 2-2 PKS and 3-32 RLH who initiated nelfinavir therapy with 750 mg BID and 1000 mg TID, respectively, and who were subsequently switched to 750 mg TID.

Table 14

Quantitation of genotypic changes in HIV protease obtained from plasma vRNA from patients treated with nelfinavir[a]

HIV protease amino acid residue	Number of occurrences: Baseline	After Therapy	After therapy (maintained)
P9	-	1	1
L10	8	2	1
T12	12	2	2
I13	13	4	4
K14	3	-	-
I15	13	-	-
G16	2	1	1
G17	1	-	-
Q18	1	-	-
L19	5	-	-
K20	2	4	3
– D30	-	25	25–
L33	2	-	-
E34	-	1	1
– E35	12	6	6–
–M36	15	8	8–
N37	15	5	5
P39	2	1	0
G40	-	2	2
R41	10	-	-
K43	1	-	-
P44	1	-	-
–M46	-	8	7–
I47	1	-	-
G48	1	-	-
G52	-	1	0
I54	-	1	1
R57	5	4	3
Q58	-	1	1
D60	2	2	2
Q61	1	-	-
I62	14	5	5
L63	41	9	5
I64	11	4	3
E65	1	-	-
C67	2	-	-
P79	3	2	2
K70	2	-	-
–A71	4	10	9–
I72	8	2	1
G73	-	1	1
T74	-	2	2
V75	-	1	1
–V77	15	8	7–
V82	-	2	1
–N88	-	11	11–
L89	2	-	-
L90	-	3	3
T91	1	1	0
Q92	2	-	-
I93	11	4	4

As would be expected the baseline HIV PR gene sequences from patient isolates were highly variable. When previous studies of baseline PR sequences were compared to the HIV-1 clade B prototype sequence approximately 37% and 50% of the nucleotide sequence and deduced amino acid sequence were found to be naturally polymorphic, respectively. The PR gene sequence analysis was successfully performed in these studies by sequencing nested RT-PCR amplification products derived from plasma viral RNA specimens from 55 patients before (baseline) and at various times during nelfinavir therapy (Table 15). A degree of PR gene polymorphism, observed in virus isolates from patients prior to initiation of nelfinavir therapy, was found at approximately 37 of 99 amino acid positions (data not shown). The sponsor identified 14 amino acid variable positions in the baseline PR gene sequence of virus isolates from these patients; defined for this study as that found in >10% of patients evaluated (≥6 occurrences/55 baseline sequences) (Table 14). The baseline PR polymorphic amino acid positions were 10, 12, 13, 15, 35, 36, 37, 41, 62, 63, 64, 72, 77, and 93 (Table 14). Once nelfinavir therapy was introduced to patients their HIV-1 populations, evaluated as a whole, developed 7 specific PR amino acid sequence mutations which are thought to be directly related to the presence of nelfinavir; 30, 35, 36, 46, 71, 77, and 88 (Table 14). The predominant genotypic change was at amino acid position D30N with 25/55 of the patient population maintaining that change. These changes are also identified as those which occur in >10% of the patient population and were maintained in later time points tested (Figure 4). Although the sponsor has conducted a detailed analysis of the percentage of patients with changes on a per amino acid, per patient basis, the significance of a 10% cut-off for an amino acid position change is unclear.

Table 15

Dose and plasma sample modifications for patients from Protocols AG1343-503 and AG1343-510

AG1343 protocol	Patient[a]	Dose (initial/modification)[b]	Plasma sample modification[c]

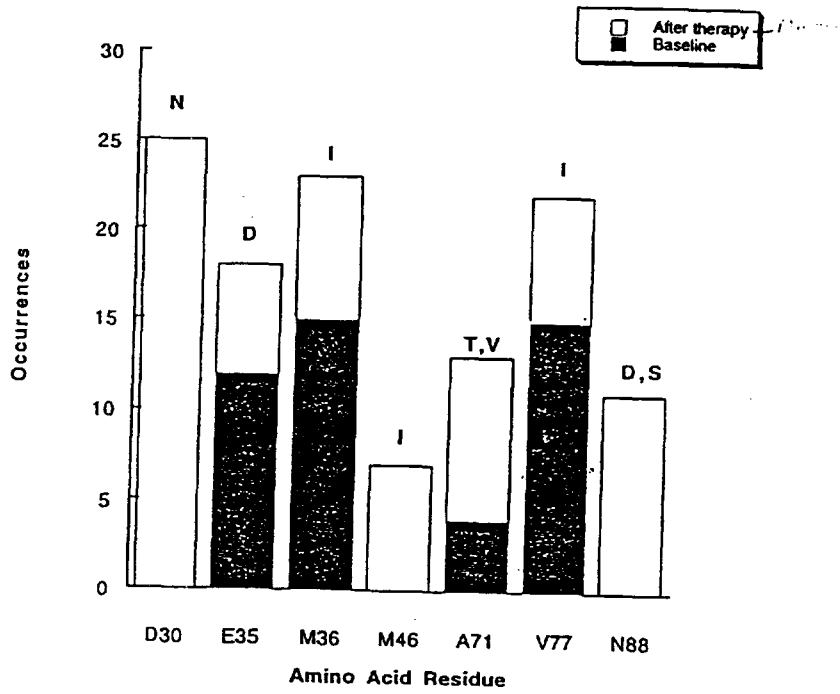
[a] Protocol site-patient number-patient initials.
[b] Doses were adjusted for 2 patients as indicated. NC = no dose change.
[c] Plasma samples from 58 patients were subjected to RT-PCR amplification to obtain HIV protease gene regions for sequence analysis. Samples from 3 patients (2-25 CGR, 4-4 MHG and 5-11 LMT) were not obtained or were indeterminant and were not included in further analyses as indicated.

n = 58 - 3 = 55

Sponsor's baseline genotypic analysis, for evaluable baseline + Trial Specimens!

Figure 4

Predominant genotypic changes in plasma vRNA from patients treated with nelfinavir



Sequence analysis was performed on HIV protease genes obtained from matched plasma samples from patients at baseline and after nelfinavir therapy. Mutations were defined as predominant if they were observed in the HIV protease after nelfinavir therapy in >10% patients (≥ 6 changes/55 baseline sequences). The corresponding number of occurrences of substitutions for samples derived at baseline are indicated.

The predominant and secondary mutations associated with nelfinavir therapy in these clinical specimens are different from those mutations observed in clinical specimens associated with alternate PRI therapy; ritonavir, indinavir, and saquinavir. However, it is unclear how these nelfinavir experienced patients would respond to subsequent alternate PRI therapy.

To examine whether additional PR gene mutations selectively occur in D30N-positive HIV populations during nelfinavir therapy an analysis of D30N-subsequent PR mutations was performed on 16/25 D30N-positive participants (Table 16). The results of this analysis are shown in Table 17. These data suggest that the nelfinavir-associated PR mutation at amino acid position D30N is stable and can be followed by one or more of the secondary nelfinavir-associated mutations in >50% of D30N variants; 35, 36, 46, 63, 71, 77, and 88. Although the position 63 amino acid change was not found in >10% of the total patient population during nelfinavir therapy, it appears to be associated with the D30N mutation in this selected patient population.

Table 16

Sequential genotypic analysis of HIV protease from patients[a]

AG1343 Protocol Number	Patient	Time of follow-up (weeks)	Figure number

Table 17

Association of D30N substitution with other predominant substitutions[a]

Amino Acid Residue	Incidence of D30N	
	Baseline[b]	After therapy[c]
35	8/12 (67%)	3/6 (50%)
36	6/15 (40%)	7/8 (88%)
46	Not observed	5/8 (63%)
63	8/41 (20%)	5/9 (56%)
71	1/4 (25%)	8/10 (80%)
77	6/15 (40%)	4/8 (50%)
88	Not observed	8/11 (73%)

[a]Substitutions were defined as predominant if they were observed in >10% patients (≥ 6 changes/55 baseline sequences).

[b]Values represent the ratio of the number of times substitutions were observed at baseline and were later associated with the D30N substitution to the number of times substitutions were observed at baseline and were later not associated with the D30N substitution.

[c]Values represent the ratio of the number of times substitutions were observed only after therapy and were associated with the D30N substitution to the number of times substitutions were observed only after therapy and were not associated with the D30N substitution.

Selected patients treated with nelfinavir have been shown to develop HIV-1 isolates (derived by PBMC cocultivation *in vitro*) with reduced susceptibility to the drug; ranging from a ≥5- to 93-fold decrease in EC_{90} from matched pair baseline values (Table 18). An HIV-1 isolate was considered by the sponsor to be "phenotypically resistant" if ≥5-fold change in nelfinavir susceptibility (EC_{90}) was observed *in vitro* compared to baseline. The sponsor was queried on the method by which a 5-fold cut-off for significant change in susceptibility was derived for these data interpretation. The sponsor provided methodology evidence and data which described a 5-fold change in *in vitro* susceptibility to be approximately two standard deviations above the mean-fold changes (ratio between the mean EC_{90} and the lowest value obtained for that data set) observed for a set (n=10) of HIV-1 isolates. The mean-fold change of nelfinavir EC_{90} values, *in vitro*, for clinical isolates evaluated in PBMCs was 2.7 ± 1.3. The derivation of significance of fold-changes as described here is adequate for these analyses only. Assay variability should be assessed for all subsequent data sets which may employ a different method for nelfinavir susceptibility analysis or be performed by a different laboratory.

Table 18

Phenotypic analysis of HIV variants from patients treated with nelfinavir

A. Phenotypically sensitive isolates

Patient ID	EC_{90} (μM)		Time after therapy (weeks)	Fold-Change	Genotypic changes
	Baseline	After Therapy			

Matched pair isolates from nineteen patients, pre-treatment and during nelfinavir therapy, were analyzed for changes in susceptibility to nelfinavir *in vitro*. Ten patients were shown to contain virus isolate populations which retain full nelfinavir susceptibility *in vitro* while 9 other patients were shown to possess virus populations which had a measurable shift in nelfinavir susceptibility *in vitro* (Table 18 A/B). The sponsor argues that if the D30N mutation is present in a patients' virus population, assumed >10%, then the isolate populations will possess a measurable degree of shift in nelfinavir susceptibility (≥5-fold) (EC_{90}) *in vitro* compared to baseline. In addition, the sponsor wishes to conclude that the D30N mutation is a genetic marker for nelfinavir-resistance in patients. However, no data defining or validating a clinical resistance parameter was provided. The EC_{90} *in vitro* assay value cut-off for what is a statistically significant change in nelfinavir susceptibility was demonstrated to be ≥5-fold changes from a matched-pair baseline value. However, it is difficult to state with confidence that the presence of a D30N mutation causes a "phenotypically resistant" virus population which will result in some clinical impact on that patient.

The limited data provided here do suggest that the D30N mutation, once detectable, is stable and may premise further genetic changes. It is not clear, however, if those subsequent changes are relevant to nelfinavir-resistance development A thorough analysis of the relationship between genetic changes detected in clinical isolates to phenotypic changes in nelfinavir-susceptibility *in vitro* will be needed to determine the contribution, if any, certain genetic changes have on nelfinavir susceptibility changes as measured *in vitro*. Although these genotypic and phenotypic susceptibility data suggest that genetic-based resistance development is biologically possible the clinical relevance of these changes has not been thoroughly studied, therefore, has not been established.

Certain HIV isolates (n=5) generated during nelfinavir therapy which were found to possess decreased sensitivity to the treatment drug *in vitro* (5-fold to 93-fold) were evaluated *in vitro* for their susceptibility changes to other PRIs compared to matched pair baseline isolates; including ritonavir, indinavir, saquinavir, and 141W94 (Table 19). These 5 isolates did not express a decrease in *in vitro* susceptibility compared to matched pair baseline values for any of the PRIs tested here. These cross-resistance data suggest, as has been previously discussed by the sponsor, that there is no detectable overlap of phenotypic resistance development, measured *in vitro*, between the treatment drug (nelfinavir) and other PRIs for which a patient is naive. The potential for HIV cross-resistance between PRIs has not been fully explored clinically. Therefore, it is unknown what effect nelfinavir therapy will have on the activity of concordantly or subsequently administered PRIs *in vivo*. The impact of PRI-resistance development, in the target HIV population, on the clinical progression of AIDS in PRI recipients has not been thoroughly explored, therefore the clinical impact on patients is unknown.

Table 19

Susceptibility Of Nelfinavir-Resistant HIV Variants To Other Protease Inhibitors[a]

A. Laboratory A[b]

		EC_{90} (nM) (Fold-Change)				
Patient	Week	Nelfinavir	Ritonavir	Indinavir	Saquinavir	141W94

(d) Phenotypic susceptibility and genotypic sequence analysis of HIV isolates from patients with prior protease inhibitor experience.

In an effort to characterize nelfinavir susceptibility of clinical isolates obtained from patients with prior alternate protease inhibitor therapy the sponsor acquired and analyzed 23 virus isolates. These isolates represent 20 patients who were determined to be PRI "treatment failures" during saquinavir, ritonavir, or indinavir therapy. For a detailed description of specimen history, and characterization, methodology and interpretation please refer to IN The sponsor's goal is to demonstrate that a cross-resistance risk to nelfinavir is low in patients who have failed the specified PRI therapy.

These 20 patients were defined as "clinical failures", however, upon closer inspection it was determined that these individuals were actually HIV RNA plasma viremia failures. Although the agency, regulated industry, and academia are currently working towards defining treatment failures using the HIV RNA surrogate marker a clinically validated definition has not yet been derived from existing data. Therefore, for the purposes of this review the "treatment failure" definition used here will not be considered for data interpretation. The sponsor has assessed all clinical specimens for both phenotypic susceptibility to the treatment drug and to nelfinavir, for which the patients are naive.

In order to determine significant fold-changes from baseline patients normally have matched-pair baseline isolates (pre-treatment) assessed phenotypically at the same time the correlating treatment isolate is assessed. In this data set (20 patients) 14 have both a baseline and treatment isolate. A mean ED_{90} (ref: M. Markowitz: 0.056 μM, n=23) or median ED_{90} (ref: D. Kuritzkes: 0.0200 μM, n=7) was used to estimate nelfinavir baseline susceptibility values for missing baseline isolates. The validation of a method for estimating baseline isolate susceptibility values for nelfinavir or any other PRI has not been established. The limited data submitted for this purpose is not adequate. Therefore, only treatment specimens (one set per patient) with matched-pair baseline isolates (n=14) will be considered for susceptibility assessments.

Seven matched-pair clinical isolates are from saquinavir studies conducted by Merigan et al., the other seven specimen sets are from ritonavir studies conducted by Markowitz et al. The sponsor intends to address the question of nelfinavir cross-resistance in specimens with alternate PRI clinical experience. Currently, the clinical specimen data sets available to the FDA on PRI resistance/cross-resistance profiles suggest that if a PRI-experienced clinical specimen is fully susceptible to the treatment drug, *in vitro*, then that clinical specimen will also possess baseline susceptibility to alternate PRIs *in vitro*. Therefore, at this time, if a clinical specimen has not developed a significant change in susceptibility to the treatment drug, as measured *in vitro*, it is expected to possess baseline susceptibility, *in vitro*, to alternate antiretrovirals of the same drug class. If compelling, scientifically generated data becomes available which argues otherwise then the DAVDP will reassess its position on these types of data. Of the 14 matched-pair clinical isolates only 11 were appropriately analyzed for susceptibility changes *in vitro* to both the original treatment drug and to nelfinavir. Of these 11 clinical isolate sets 4 had saquinavir experience and 7 had ritonavir experience. When each set was analyzed for susceptibility changes *in vitro* to their treatment drugs the 4 clinical isolates with saquinavir experience showed essentially no change in susceptibility to saquinavir, therefore will not be considered for nelfinavir cross-resistance analysis. As a result of the following "criteria" (see below), only 7 of the 23 clinical specimens presented in this study will be evaluable for a nelfinavir cross-resistance phenotype.

1. Each patient is considered a biological entity for the purpose of phenotypic/genotypic assessments, *in vitro*, of treatment drug resistance development in clinical isolates.

2. In order for a clinical specimen to be considered for cross-resistance susceptibility analysis, *in vitro*, to nelfinavir it must first demonstrate a significant change in susceptibility *in vitro* to the treatment drug.

3. Each treatment clinical isolate must be accompanied by a matched-pair baseline clinical isolate and be assessed concordantly with that clinical isolate. Susceptibility breakpoint estimates are not acceptable at this time.

Seven HIV clinical isolates generated during ritonavir (ref: Markowitz) therapy which were found to possess decreased sensitivity to the treatment drug (ritonavir) *in vitro* (8- to 60-fold) were evaluated *in vitro* for their susceptibility changes to nelfinavir (Table 20). Six of the seven isolates

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expressed a measurable shift in nelfinavir susceptibility (5- to 40-fold) *in vitro*. Of those six isolates five possessed point mutations at either amino acid position 82, or multiple changes at positions 82/84, or 82/90. None possessed the D30N nelfinavir-associated point mutation (Table 21). The potential for HIV cross-resistance between PRIs has not been fully explored. It is unknown what effect alternate PRI therapy will have on the activity of subsequently administered nelfinavir.

(e) Analysis of the incidence of nelfinavir-associated genotypic changes *in vivo*

To analyze the clinical incidence of nelfinavir-associated genotypic changes, defined here as the frequency of occurrence of the D30N mutation in clinical isolates from randomly selected patients in Phase II/III nelfinavir trials, plasma HIV-1 RNA samples were derived from 142 patients and their PR gene sequences determined. After 12-16 weeks of nelfinavir monotherapy (AG1343-505), or 16 weeks of nelfinavir in combination with zidovudine and 3TC (AG1343-511) 64 and 78 trial participants from -505 and -511, respectively, were randomly selected for PR gene sequence analysis of their HIV-1 isolate populations. Mutations which represented ≥10% of an entire virus population were measurable.

Of 64 patients on nelfinavir monotherapy for 3 to 4 months at 500 mg TID or 750 mg TID, the incidence of patients with the D30N mutation was 56% (Table 22). Of the 49 patients on nelfinavir therapy (500 mg TID or 750 mg TID) in combination with zidovudine/3TC for 4 months, the incidence of patients with the D30N mutation was <10% (Table 22). Of the 29 patients in the nelfinavir placebo arm in combination with zidovudine/3TC the incidence of patients with the D30N mutation was 0% (Table 22). The proportion of patients in this analysis who were PCR negative, thus assumed D30N negative, are shown in Table 22. The proportion of patients undergoing

nelfinavir monotherapy or nelfinavir placebo with zidovudine/3TC combination therapy who were PCR negative was approximately 15%, while the proportion of the nelfinavir combination study participants was approximately 57%.

Table 22

Incidence Of Nelfinavir-Associated Genotypic Change At 16 Weeks[a]

Treatment Arm	Study	Incidence of nelfinavir-associated mutations (%)	PCR negative (%)
nelfinavir, 500 mg TID	AG1343-505	18/32 (56)	5/32 (16)
nelfinavir, 750 mg TID	AG1343-505	18/32 (56)	4/32 (13)
nelfinavir, (500 mg TID)+ AZT+3TC	AG1343-511	2/22 (9)	11/22 (50)
nelfinavir, (750 mg TID)+ AZT+3TC	AG1343-511	1/27 (4)	17/27 (63)
AZT+ 3TC	AG1343-511	0/29 (0)	4/29 (14)

[a]Sequence analysis was performed on HIV protease genes obtained from plasma samples derived from 142 randomly selected patients after antiretroviral therapy in Protocols AG1343-505 and 511. The proportion of patients containing nelfinavir-associated genotypic changes, defined as the occurrence of a D30N substitution within HIV protease, for all the patients analyzed are indicated. Likewise the proportion of patients from which HIV protease genes could not be PCR amplified for all the patients analyzed are indicated (Appendices 1,2).

Drug combination studies with reverse transcriptase and protease inhibitors

The sponsor has conducted *in vitro* studies to investigate the antiviral activity of nelfinavir in combination with reverse transcriptase inhibitors (which include AZT, ddI, ddC, d4T and 3TC) and protease inhibitors, including saquinavir, ritonavir and indinavir. CEM-SS cells were infected with HIV-1 RF strain (acute infection model) at an m.o.i. of 0.09. Antiviral activity was measured by cell protection assay after 6 days of exposure to different concentrations of the drugs (which included nelfinavir and a nucleoside analog). The results indicate that the combination of nelfinavir with AZT, ddC or 3TC showed synergistic antiviral activity. A triple combination of nelfinavir with 3TC and AZT was also shown to exhibit synergistic antiviral activity *in vitro*. A combination of nelfinavir with ddI or d4T was, however, additive.

The combination of nelfinavir with indinavir was shown to be antagonistic in one experiment. Combinations of nelfinavir with ritonavir or saquinavir gave variable results from one experiment to another, ranging from additive with regions of antagonism to synergism. However, the relevance of these *in vitro* findings to the *in vivo* situation is not known at this point in time.

Mechanism of Action

The mechanism by which nelfinavir exhibits activity against HIV was tested by studying the effect of nelfinavir on (a) the activity of purified protease enzyme derived from HIV, using a synthetic peptide as a substrate, and (b) the conversion of p55 to p24 protein in the supernatant of HIV infected CEM-SS cells.

(a) Effect of nelfinavir on the enzyme activity *in vitro*

The sponsor has tested the activity of nelfinavir against the purified recombinant protease isolated from the HIV IIIB strain of virus. The activity of the protease enzyme was measured using synthetic peptide as a substrate at a concentration of 200 uM. The results in Table 23 indicate that 11 different batches of nelfinavir showed K_i values varying from 0.82 to 3.2 nM with mean of 1.71 (1.13 ng/ml). The standard inhibitor used for comparison was Ro 31-8959 with a K_i of 0.99 nM. Pepstatin A (a general inhibitor of aspartic proteases) exhibited a K_i value of 3150 nM. The binding of nelfinavir to the protease enzyme was shown to be competitive.

An attempt was also made to study the effect of nelfinavir on mammalian (human and rat) aspartic proteinases. The results indicate a negligible inhibitory effect of nelfinavir on the catalytic activity of human pepsin and gastricin enzymes. Human cathepsin D and E and rat cathepsin D enzymes were inhibited at very high concentrations [K_i = 435 nM (i.e., 288.84 ug/ml), 74,000 nM (i.e., 49136 ng/ml) and 450 nM (i.e., 298.8 ng/ml) respectively]. These results indicate that nelfinavir is > 250 fold more potent against the protease enzymes derived from HIV as compared to those derived from mammalian cells.

Table 23

The Inhibition of HIV Protease by Differing Synthetic Batches of AG1343

AG1343 Batch #	$K_{i,app}$ ± SD (nM)	Expt. Date	Enzyme Preparation Date
SRIII 285-1-93	2.00 ± 0.34	12-21-93	12-1-93
KRW-632-32	1.70 ± 0.65	4-25-94	3-2-94
AT 575 311-3-112	3.20 ± 0.28	7-13-94	5-1-94
AT 575 311-1-95	1.32 ± 0.65	10-9-94	7-16-94
AT 575 311-2-109	1.92 ± 0.74	10-9-94	7-16-94
KRW-632-46	1.78 ± 0.71	10-9-94	7-16-94
4K0001Z	1.95 ± 0.53	10-9-94	7-16-94
5A0004Z	1.29 ± 0.60	3-9-95	7-16-94
5A0004Z-R1	0.82 ± 0.63	3-9-95	7-16-94
5A0017Z	1.18 ± 0.77	3-10-95	7-16-94
P102-220-20	1.67 ± 0.96	3-10-95	7-16-94

Comments:

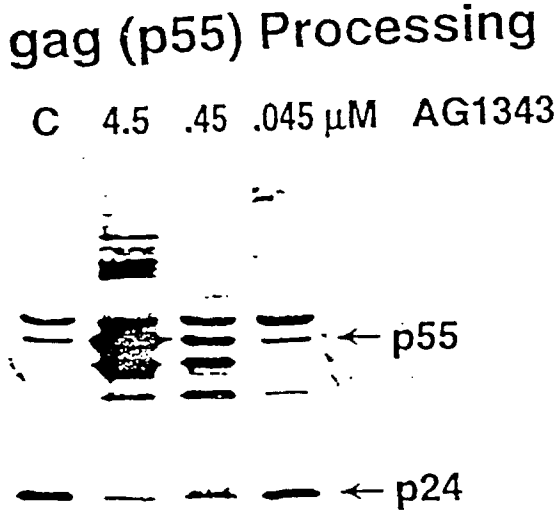
The *in vitro* enzyme activity studies should be interpreted with caution. It should be noted that the inhibition of enzyme activity was measured using a fixed concentration of the synthetic peptide (about 10-12 amino acids long) at high salt concentrations and low pH (pH 5.6). These conditions are not a representation of the physiological conditions which exist *in vivo* for inhibition and cleavage. The total length of HIV polyprotein precursors subject to processing by the viral protease *in vivo* is approximately 1500 amino acids. The protease enzyme recognizes about 10 to 12 different sequences as processing sites on the polyproteins and cleaves these sites at different rates. Furthermore, the situation does not take into account the tertiary structure of the protein. Nevertheless, the assays are useful for routine evaluation of potential protease inhibitors.

(b) Effect of nelfinavir on p55

Nelfinavir was shown to inhibit the conversion of p55 to p24. These studies were conducted using CEM-SS cells infected with the HIV-IIIB strain of virus. Virus infected cells were incubated with different concentrations of the drug for 3 days. The virions in the supernatant were harvested by ultracentrifugation and solubilized with SDS. The solubilized virions were analyzed by SDS-PAGE and western blot using monoclonal antibodies to p55 and p24 as probes. The results shown in Figure 5 show a dose dependent decrease in the p24 protein and an increase in the p55 protein. These studies suggest that nelfinavir inhibits the HIV protease enzyme which is responsible for converting p55 to p24 protein.

Figure 5

Inhibition of gag (p55) Processing by AG1343



Chronically infected HIV-1 IIIB/CEM-SS were treated with varying concentrations of AG1343 as indicated, or medium alone (c) for 3 days. Virions were purified from culture supernatant by ultracentrifugation, solubilized in SDS and the polyproteins separated by SDS-PAGE. Polyproteins were analyzed by Western blot analysis using a monoclonal antibody which recognizes p55 and p24.

The antiviral activity of nelfinavir appears to be **reversible**. CEM-SS cells chronically infected with HIV-1 IIIB were exposed to different concentrations of the drug (7.5 uM i.e., 4.98 ug/ml and 4 uM i.e., 2.66 ug/ml) and incubated for 72 hours. Supernatants were harvested, ultracentrifuged and the virions resuspended in PBS. Virions were then incubated at 37°C for up to 40 hours. An aliquot of the samples was collected at different time points to test for the presence of p55 and p24 proteins by western blot. The level of p24 antigen produced was also measured by ELISA. Ro 31-8959 (a known protease inhibitor) was used for comparison. By western blot no p24 protein was detectable when exposed to 7.5 uM of nelfinavir (Figure 6). However, a decrease in p55 protein and an increase in some intermediary proteins was observed at 36 hours as compared to the 0 hour drug free supernatant. When exposed to 4 uM of nelfinavir, p24 bands were identified as early as 3 hours of incubation in drug free medium as compared to 0 hour time point (Figure 7). An increase in p24 antigens was observed by ELISA (Figures 8 and 9), thereby indicating partial reversibility of the antiviral activity of the drug. The reversibility was shown to occur earlier with the 4 uM treated group as compared to the cells treated with the higher concentration (7.5 uM). It should be noted that the drug concentrations used in this study were much higher (> 4000-fold) than the ED_{50} values determined in the *in vitro* antiviral studies. Therefore, there is a possibility that the reversibility may occur more rapidly and to a higher degree in the presence of lower concentrations of the drug than used in this *in vitro* study. The clinical relevance of these observations is not known. The plasma concentration (Cmax) of nelfinavir in patients with HIV has been shown to be in the range of 9 to 12 ug/ml which is almost 2-fold higher than the concentration used in this study. Therefore, maintenance of this drug concentration should be sufficient to suppress the conversion of p55 to p24 protein which is important for the production of infectious virion (unless drug resistance develops). However, the short half-life (2 to 3 hours) may be an important consideration if treatment is interrupted or discontinued.

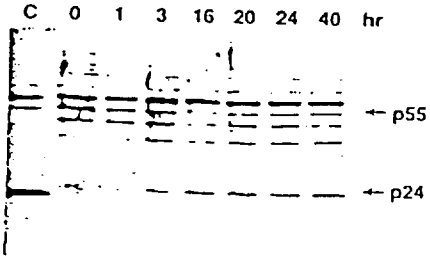
Figure 6

Experiment 1: Reversibility of the Inhibition of HIV-1 p55 (gag) Processing by AG1343

Chronically infected HIV-1 IIIB/CEM-SS were treated with 7.5 μM AG1343 or medium alone (C) for 3 days. Virions were removed from drug by ultracentrifugation, resuspended in PBS, and incubated at 37° C. Samples were removed at indicated times (hr), solubilized and analyzed for p24/p55 by SDS-PAGE and western blot.

Figure 7

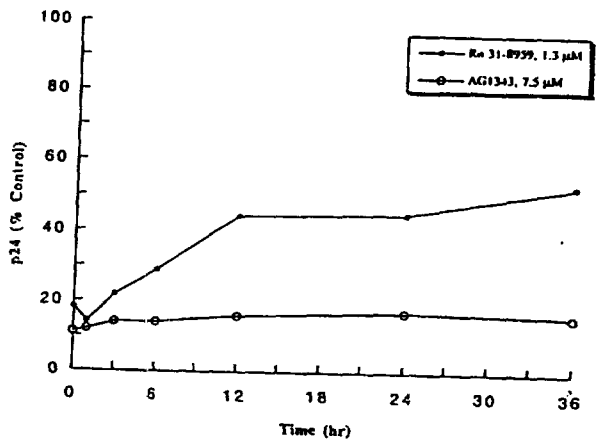
Experiment 3: Reversibility of the Inhibition of HIV-1 p55 (gag) Processing by AG1343



Chronically infected HIV-1 IIIB/CEM-SS were treated with 4.0 μM AG1343 or medium alone (C) for 3 days. Virions were removed from drug by ultracentrifugation, resuspended in PBS and incubated at 37° C. Samples were removed at the indicated times (hr), solubilized and analyzed for p24/p55 by SDS-PAGE and western blot.

Figure 8

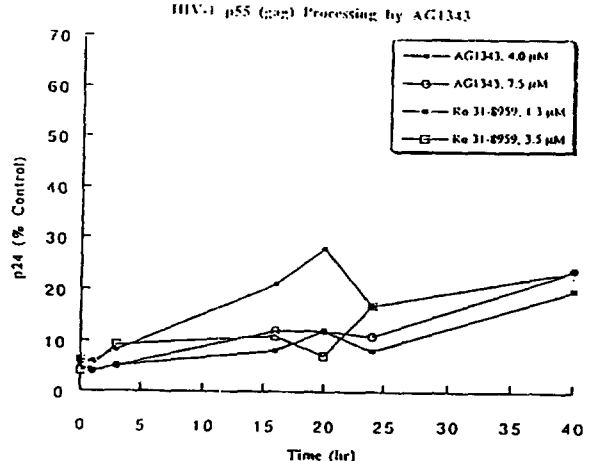
Experiment 1: Reversibility of the Inhibition of HIV-1 p55 (gag) Processing by AG1343



Chronically infected HIV-1 IIIB/CEM-SS were treated with AG1343, Ro 31-8959 or medium alone for 3 days. Virions were removed from drug by ultracentrifugation, resuspended in PBS, and incubated at 37° C. Samples were removed at indicated times after incubation and analyzed for p24 by ELISA.

Figure 9

Experiment 3: Reversibility of the Inhibition of HIV-1 p55 (gag) Processing by AG1343



Chronically infected HIV-1 IIIB/CEM-SS cells were treated with AG1343, Ro 31-8959 or medium alone for 3 days. Virions were removed from drug by ultracentrifugation, resuspended in PBS, and incubated at 37° C. Samples were removed at indicated times after incubation and analyzed for p24 by ELISA

Measurement of Viral RNA in the Clinical Trial

The sponsor seeks the approval of nelfinavir based on reduction of viral RNA and an increase in the CD4 cell number in treated patients.
(Todd *et al.*, 1995, J AIDS and Human Retrovirology 10 {Suppl2}: S35; Kern *et al.*, 1996, J Clin Microbiology 34: 3196) was used for the measurement of plasma viremia in the clinical trials conducted to support the efficacy of nelfinavir. This assay has also been referred to as the enhanced sensitivity It should be noted that the bDNA assay is not an FDA approved test. The assay is similar to the original version of the assay except that it has an enhanced amplification signal. The kit was submitted for FDA review in 1996, but did not receive marketing approval. The validation data for the enhanced sensitivity assay submitted to this NDA is inadequate as highlighted below.

The ES bDNA method is a sandwich nucleic acid hybridization assay designed to quantitate cell-free HIV-1 RNA in human plasma using recombinant bacteriophage as a standard (a single-stranded DNA construct which encodes the *gag-pol* products - Ref.: Collins *et al.*, 1995, Analytical Biochemistry 226: 120). The data submitted by the sponsor suggests that the assay is linear between 3000 (dilution 1:512) to 1.6 x 10^6 RNA copies per ml (Figure 10). However, the limited data provided by the sponsor are not sufficient to evaluate the reliability of the numbers below 3000 RNA copies/ml.

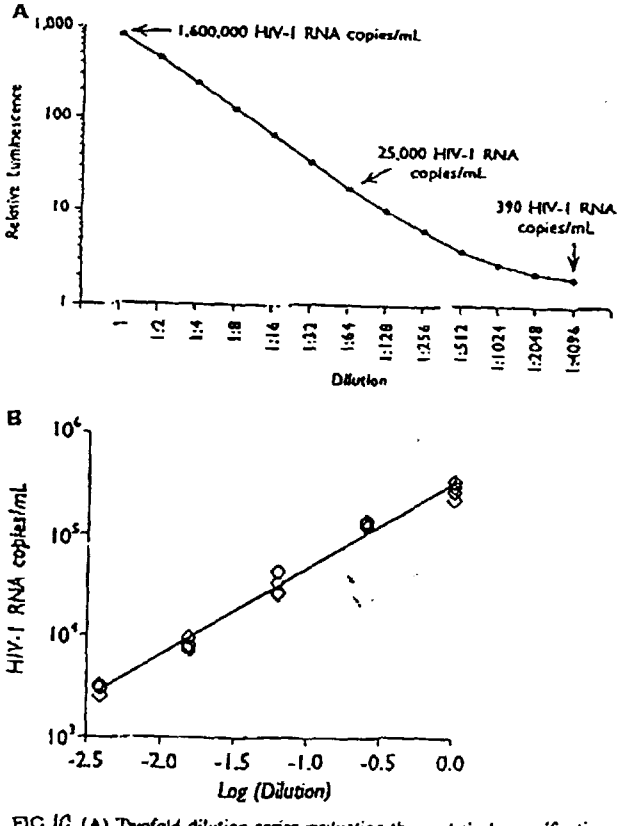


FIG. 10 (A) Twofold dilution series evaluating the analytical quantification limit of the ES bDNA assay. (B) Fourfold dilution series evaluating the linearity of the ES bDNA assay.

The specificity of the ES HIV bDNA assay and the effect of possible interfering substances on viral measurements was investigated by Todd *et al.*, 1995 (J AIDS and Human Retrovirology 10 {Suppl2}: S35) and Kern *et al.*, 1996 (J Clin Microbiology 34: 3196). The studies suggest that the assay was shown to be specific for HIV-1 since no reaction was observed against bacteria, fungi and some of the viruses which include CMV, HAV, HBV (DNA positive plasma) and HCV (RNA positive plasma). The drugs most commonly used for the treatment of AIDS associated diseases which include zidovudine, acyclovir, gancyclovir, fluconazole, trimethoprim/sulfamethoxazole, dapsone, rifabutin, foscarnet, or clarithromycin did not alter the sensitivity of the bDNA assay. Similarly, bilirubin, hemoglobin or lipids did not significantly alter the measurement of HIV RNA by the Plasma samples showed higher copy numbers as compared to the serum samples. Also, plasma samples with EDTA as the anticoagulant exhibited higher copy numbers as compared to heparin or ACD.

It should be noted that the measurement of plasma viremia is based on a standard curve which was generated using bacteriophage recombinant DNA material. The bDNA assay is capable of measuring both DNA and RNA. However, in plasma or serum HIV RNA is the predominant form of virus genetic material. It should also be borne in mind that a bDNA negative sample may not necessarily mean that a patient is free of HIV infection. The virus may be present intracellularly in different tissue compartments such as the lymph nodes, which are difficult to accurately sample and the virus content of which may differ substantially from virus levels in blood. In addition, the lower limit of detection indicates the minimum level of virus that can be accurately detected. Measurements below this "floor" may appear to be negative when they are not, i.e., virus is present in concentrations that are too low to reliably amplify and detect.

THE LABEL PROPOSED BY THE SPONSOR

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CONCLUSIONS

The *in vitro* studies indicate that nelfinavir exhibits antiviral activity against HIV-1. These studies were conducted using various laboratory strains of the virus and clinical isolates infecting T-cell lines, macrophages or peripheral blood mononuclear cells. The ED_{50} value varied from 9 - 60 nM (i.e., 5.98 - 39.84 ng/ml) and the therapeutic index was calculated to be > 500 fold. The ED_{95} values varied from 7.4 to 195.8 nM with a mean of 109.21 (± 46.3) nM (72.52 ng/ml). These values are based on the actual observations and not on the basis of 'mean of mean' values as proposed by the sponsor. For accurate representation of the data, appropriate changes should be made in the label. A metabolite of nelfinavir (M8) was also shown to exhibit comparable antiviral activity, however, other metabolites of nelfinavir (M1, 10 and 11) were not active against HIV. The presence of increasing concentrations of fetal bovine serum or human serum did not significantly alter the therapeutic index for this drug. However, the presence of increasing concentrations of α1-acid glycoprotein increased the ED_{50} values, thereby indicating a decrease in antiviral activity.

Nelfinavir inhibits the HIV protease enzyme activity *in vitro* but had no significant effect on the activity of mammalian aspartase class of enzymes. Nelfinavir inhibits the conversion of p55 protein to p24 which is an important step in the production of infectious virions. However, an electron micrograph showing the morpphologic effects of nelfinavir on virion maturation was not done. The effect of nelfinavir on the production of p24 from p55 is reversible and depends upon the concentration of the drug.

Nelfinavir, like other protease inhibitors has been shown to induce resistance. HIV-1 isolates with reduced susceptibility to nelfinavir (7- to 30-fold) have been selected *in vitro*. Genotypic analysis of these isolates showed the variable presence of mutations in the HIV protease gene at amino acid positions 8, 30, 35, 36, 40, 45, 46, 63, 68, 71, and 84. The presence of one or more of these mutations varied depending upon the length of time the virus population was pressured with nelfinavir. These data showed that the development of nelfinavir-resistant HIV-1 isolates is biologically possible. Site-directed mutagenesis studies demonstrated that point mutations at positions I84V or L90M could each decrease nelfinavir-susceptibility of an HIV-1 clone by 5-fold. Double mutants R8Q/M46I, R8K/M46I, and M46I/I84V also produced a 5-fold decrease in nelfinavir susceptibility *in vitro*. In a separate study point mutations at positions D30N, N88D, or the double mutation D30N/N88D were tested *in vitro* for nelfinavir susceptibility changes compared to a parent construct. The D30N variant and the D30N/N88D variant each expressed a loss in nelfinavir susceptibility of approximately 9-fold *in vitro*. The clinical significance of these data remains unclear.

In order to address the question of nelfinavir resistance development *in vivo* 55 nelfinavir trial participants with evaluable viral isolates from baseline, were monitored during therapy for PR gene mutations. When nelfinavir therapy was introduced to patients their HIV-1 populations developed one or more of the following 7 specific PR amino acid mutations at positions 30, 35, 36, 46, 71, 77, and 88. The most frequent mutation site found in *in vivo* specimens appears to be at PR amino acid position 30, which was different from what was seen *in vitro*. Phenotypic susceptibility changes

were evaluated in 19 of the above 55 participants. Nine of 19 patients had isolates with reduced susceptibility to nelfinavir ranging from 5-fold to 93-fold compared to matched pair baseline values. Evaluation of the 9 "resistant" isolates revealed that the PR amino acid position 30 mutation was present in all specimens as either a single or one of multiple PR mutations. With the limited data available to date there are no exceptions to this observation. HIV-1 isolates obtained from 5 patients which showed a decrease in nelfinavir susceptibility *in vitro* did not demonstrate a concordant decrease in susceptibility to indinavir, ritonavir, saquinavir, or 141W94 *in vitro* when compared to matched baseline isolates. The potential for cross-resistance between PRIs has not been fully explored. Therefore, it is unknown what effect nelfinavir therapy will have on the activity of concordantly or subsequently administered PRIs.

Seven HIV clinical isolates generated during ritonavir therapy which were found to possess decreased sensitivity to the treatment drug (ritonavir) *in vitro* (8- to 60-fold) were evaluated *in vitro* for their susceptibility changes to nelfinavir . Six of the seven isolates expressed a measurable shift in nelfinavir susceptibility (5- to 40-fold) *in vitro*. Of those six isolates five possessed point mutations at either amino acid position 82, or multiple changes at positions 82/84, or 82/90. None possessed the D30N nelfinavir-associated point mutation. The potential for HIV cross-resistance between PRIs has not been fully explored. It is unknown what effect alternate PRI therapy will have on the activity of subsequently administered nelfinavir.

Drug combination studies were conducted with a variety of approved antiretroviral drugs. *In vitro*, nelfinavir exhibits enhanced antiviral activity when given at appropriate concentrations in combination with the nucleoside analogs AZT, ddC or 3TC . A triple combination of the drug with AZT and 3TC also exhibited synergistic antiviral activity *in vitro*. Additive effects were observed in combination with ddI or d4T.

Drug combination studies with other protease inhibitors gave variable results. For example, combinations with ritonavir or saquinavir showed additive effects with regions of synergism and antagonism. A combination with indinavir was, however, antagonistic. The clinical relevance of these *in vitro* findings is not known at this point in time.

The sponsor seeks the approval of nelfinavir based on a decrease in viral RNA as measured by assay and an increase in the CD4 cell number. It should be noted that tl This assay is a more advanced form of the which was submitted for FDA review in 1996, but not approved for marketing. The limited data provided by the sponsor suggests linearity of the standard curve for the ultrasensitive assay is between 3000 to 1.6 x 10^6 RNA copies/ml. Insufficient validation data were provided to the FDA to support the claim that the lower limit of accurate detection is 500 RNA copies/ml. This issue is dealt with in more detail in the statistical and medical review of this NDA.

Based on the studies reviewed, the changes made in the label are as follows (some of the proposed changes to the sponsor's version of the label are struck out and the recommended changes are underlined):

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Purged

RECOMMENDATIONS:

This NDA supplement is approvable with respect to microbiology pending an acceptable version of the label. The sponsor should also consider conducting the following phase IV studies:

Shukal Bala

Shukal Bala
Microbiologist, HFD-530

Lauren Iacono-Connors
Microbiologist, HFD-530

CONCURRENCES:

HFD-530/Deputy Dir. Signature 3/13/97 Date
HFD-530/SMicro Signature 3/4/97 Date 3/4/97
CC:
HFD-530/Original NDA 20-778 and 20-779
HFD-530/Division File
HFD-530/MO
HFD-530/Pharm
HFD-530/Chem
HFD-530/SMicro
HFD-530/Review Micro
HFD-530/CSO/KStruble

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

NDA #: 20-779

REVIEWER	: Shukal Bala
CORRESPONDENCE DATE	: 01-30-97
CDER RECEIPT DATE	: 01-31-97
REVIEW ASSIGN DATE	: 02-11-97
REVIEW COMPLETE DATE	: 02-14-97

SPONSOR: Agouron Pharmaceuticals, Inc
10350 North Torrey Pines Road
La Jolla, CA 92037

SUBMISSION REVIEWED: N-BI

DRUG CATEGORY: Anti-viral/protease inhibitor

INDICATION: Treatment of adult patients with HIV

DOSAGE FORM: Tablets for oral administration

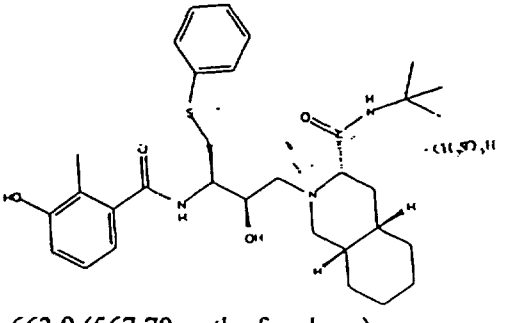
PRODUCT NAMES:

a. **PROPRIETARY:** Viracept

b. **NONPROPRIETARY:** Nelfinavir mesylate, AG 1343

c. **CHEMICAL:** [3S-[2(2S*,3S*,3R*]]-N-(1,1-dimethylethyl)decahydro-2[2-hydroxy-3-[(3-hydroxy-2-methylbenzoyl)amino-4-(phenylthio)butyl-3-isoquinolinecarboxamide, monomethane sulfonate (salt)

STRUCTURAL FORMULA:



Molecular weight: 663.9 (567.79 as the free base)
Empirical formula: $C_{32}H_{45}N_3O_4S \cdot CH_4O_3S$

SUPPORTING DOCUMENTS:

BACKGROUND:

The sponsor has submitted data from selected microbiology studies in support of NDA 20-779. The content of these studies are discussed in the NDA review dated 2/14/97.

CONCLUSIONS

The studies submitted by the sponsor were in support of the NDA 20-779 (for details see microbiology review dated 2/14/97). No further action is indicated at this point in time with respect to this ammendment.

Shukal Bala

Shukal Bala
Microbiologist, HFD-530

CONCURRENCES:

HFD-530/Deputy Dir. Signature 3/13/97 Date
HFD-530/SMicro Signature 3/5/97 Date
CC:
HFD-530/Original NDA 20-779
HFD-530/Division File
HFD-530/MO
HFD-530/Pharm
HFD-530/Chem
HFD-530/SMicro
HFD-530/Review Micro
HFD-530/CSO/KStruble

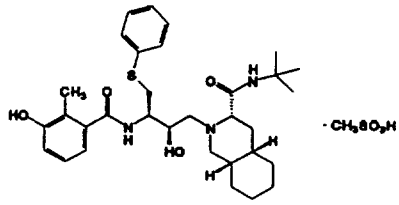
PHARMACOLOGIST'S REVIEW

NDA #: 20-779

DATE SUBMITTED: Dec. 26, 1996
DATE ASSIGNED: Dec. 30, 1996
DATE REVIEW COMPLETED: March 6, 1997
HFD-530

SPONSOR: Agouron Pharmaceuticals, Inc.
10350 North Torrey Pines Road
La Jolla, CA 92037-1020

DRUG: Nelfinavir mesylate (AG1343; VIRACEPT®)
CHEMICAL NAME: [3*S*-[2(2*S**,3*S**,3α,4αβ,8αβ]]-*N*-(1,1-dimethylethyl) decahydro-2-[2-hydroxy-3-[(3-hydroxy-2-methyl benzoyl)amino-4-(phenylthio)butyl]-3-isoquinoline carboxamide, monomethanesulfonate (salt)
MOLECULAR FORMULA: $C_{32}H_{45}N_3O_4S \cdot CH_4O_3S$
MOLECULAR WEIGHT: 663.90 (salt)
567.79 (free base; AG1346)
CAS REGISTRY NUMBER: 159989-65-8 (AG1343)
159989-64-7 (AG1346)
CHEMICAL STRUCTURE:



RELATED DOCUMENTS:

PROPOSED USE: Treatment of HIV infection

BACKGROUND

Nelfinavir is a protease inhibitor which has demonstrated significant *in vitro* and clinical activity against HIV. Clinical studies have shown the drug to be relatively safe, especially compared to previously marketed protease inhibitors. In addition, nelfinavir appears to produce a different pattern of viral resistance compared to other protease imhibitors, which could indicate that it might be useful in unique combination therapies. Like other protease inhibitors, nelfinavir is extensively metabolized to numerous products, although potential for toxic interaction with other drugs appears to be relatively low.

The sponsor has pursued an aggressive strategy in the development of this drug. The original request for a Pre-IND consult was made to DAVDP on Nov. 7, 1994. The original IND was submitted on June 7, 1995. All of the nonclinical pharmacology, pharmacokinetic, and toxicology studies were submitted under the IND and no new studies were submitted with the NDA. Therefore, no original reviews will be needed. A listing of all previously submitted studies will be included in this review and the IND reviews will be appended.

NONCLINICAL PHARMACOKINETICS

Pharmacokinetic Studies

1. Determination of plasma levels of nelfinavir mesylate in dogs associated with an intravenous safety pharmacology study.
   Study # JT-H8-137; study dated 12-4-96.
2. The effect of AZT on oral bioavailability of AG1343 in the rat.
   Study # PH-0406-095; study dated 5-24-95.
3. The effect of antacids on oral bioavailability of AG1343 in the rat.
   Study # PH-0407-095; study dated 5-22-95.
4. *In vitro* equilibrium dialysis studies of AG1343 serum protein binding: the effect of concomitant therapeutic agents on AG1343 free fraction.
   Study # PH-1001-095; study dated 12-20-95.
5. The effect of concomitant administration of AZT, d4T and acyclovir on oral bioavailability of AG1343 in the rat.
   Study # PH-1101-095; study dated 12-18-95.
6. Mechanism based studies to evaluate potential drug-drug interactions with AG1343 and warfarin, erythromycin, quinidine and ibuprofen.
   Study # PH-1102-095; study dated 12-21-95.
7. Studies on the potential for acute drug-drug interaction with nelfinavir mesylate and 17α-ethynyl estradiol.
   Study # PH-0201-096; study dated 8-2-96.
8. Studies on the potential for sub-chronic drug-drug interactions with nelfinavir mesylate and 17α-ethynyl estradiol.
   Study # PH-0202-096; study dated 8-2-96.
9. The effects of concomitant administration of ketoconazole and sulfamethoxazole on nelfinavir mesylate pharmacokinetics in the rat.
   Study # PH-0603-096; study dated 8-2-96.

10. The effect of concomitant administration of saquinavir and indinavir on nelfinavir mesylate pharmacokinetics in the rat.
    Study # PH-0701-096; study dated 8-2-96.
11. Studies on the potential for acute drug-drug interactions with nelfinavir mesylate and dapsone.
    Study # PH-1102-096; study dated 11-14-96.
12. The effect of saquinavir on nelfinavir *in vitro* serum protein binding.
    Study # PH-1103-096; study dated 11-22-96.
13. Re-evaluation of drug-drug interactions with nelfinavir mesylate and warfarin, erythromycin, ketoconazole, ibuprofen and sulfamethoxazole

    Study # PH-1106-096; study dated 12-4-96.
14. The effect of magnesium hydroxide on oral bioavailability of nelfinavir mesylate in the rat.
    Study # PH-1108-096; study dated 12-4-96.
15. Validation of the analytical procedure for the determination of AG1343 in monkey plasma

    Study # 1245/7-1010; study dated 12-14-94.
16. Cross validation of the analytical procedure for the determination of AG1343 in monkey plasma and the extended human range

    Study # 1245/20; study dated 5-1-96.
17. Validation of the analytical procedure for the detection of AG1343 in rat plasma with

    Study # 1245/21; study dated 7-15-96.
18. Validation of the analytical procedure for the determination of AG1343 in rat plasma

    Study # 1245/29; study dated 7-3-96.
19. *In vitro* metabolism of nelfinavir mesylate by human liver microsomes and c-DNA expressed cytochrome P450 isozymes.
    Study # PH-0203-096; study dated 8-6-96.
20. Liver microsome metabolism in rats treated daily with oral nelfinavir mesylate for 13 weeks.
    Study # PH-0401-096; study dated 8-2-96.
21. Liver microsome metabolism in cynomolgus monkeys treated daily with oral nelfinavir mesylate for 13 weeks.
    Study # PH-0402-096; study dated 8-2-96.

22. Toxicokinetics of AG1343 in a 26 week oral chronic toxicity study in the monkey.
    Study # PH-0801-096; study dated 10-29-96.
23. Toxicokinetics of AG1343 in a 26 week oral chronic toxicity study in the rat.
    Study # PH-0805-096; study dated 10-29-96.
24. Pharmacokinetic studies on AG1343 absorption, distribution and excretion in rats.
    Study # JT-H8-127; study dated 12-17-96.
25. Method determinations of AG1343 in dosing formulations and plasma.
    Study # 165052; study dated 9-24-96.
26. Absorption and excretion of AG1343 in rats after single administration.
    Study # JT-H7-68; study dated 9-95.
27. Pharmacokinetics study on [^{14}C]AG1343 in monkeys after single administration.
    Study # JT-H7-110; study dated 2-29-96.
28. Metabolite composition of AG1343 in rats after multiple oral administration.
    Study # JT-H7-112; study dated 6-96.
29. Metabolite profiles of AG1343 in plasma of humans, rats and monkeys.
    Study # JT-H8-81; study dated 11-96.
30. The analysis of AG1343 plasma levels in rats receiving 14 day and 28 day repeat dose oral treatment with AG1343.
    Study # PH-0901-094; study dated 3-1-95.
31. The analysis of AG1343 plasma levels in monkeys receiving 28 day repeat dose oral treatment with AG1343.
    Study # PH-0902-094; study dated 3-1-95.
32. Pharmacokinetics and oral bioavailability of AG1343 in cynomolgus monkeys and marmosets.
    Study # PH-0903-094; study dated 3-2-95.
33. Pharmacokinetics and oral bioavailability of AG1343 in the rat.
    Study # PH-1008-094; study dated 5-31-95.
34. Pharmacokinetics and oral bioavailability of AG1343 in the dog.
    Study # PH-1009-094; study dated 3-1-95.
35. Fifteen day repeat dose oral administration of AG1343 to rats.
    Study # PH-0401-095; study dated 3-26-95.
36. LCMS analysis of nelfinavir mesylate metabolites in rat bile and plasma.
    Study # PH-0902-096; study dated 11-14-96.

37. Pharmacokinetics and oral bioavailability of nelfinavir mesylate in rats: the effect of gender and comparison of

    Study # PH-1107-096; study dated 12-4-96.
38. Determination of the site-specific intestinal permeability and bioavailability in rats and dogs for Agouron protease inhibitors.
    Study # TSRL; study dated 1-3-96.
39. Repeat dose bioavailability study of nelfinavir mesylate in dogs.
    Study # -165060; study dated 10-22-96.
40. *In vitro* protein binding studies with AG1343 using the

    Study # PH-0408-095; study dated 5-24-95.
41. Equilibrium dialysis studies of AG1343 in rats and humans.
    Study # UA-01-095; study not dated.
42. Pharmacokinetic studies on AG1343 repeated dosing in rats.
    Study # JT-H8-128; study dated 12-17-96.
43. Radiotracer tissue distribution study of AG1343 in the rat.
    Study # PH-0904-094; study dated 3-1-95.
44. Distribution of nelfinavir mesylate to rat brain and spleen.
    Study # PH-0602-096; study dated 7-29-96.
45. Documentation of ^{14}C-AG1343 lots employed in preclinical studies and a human mass balance study.
    Study # PH-1109-096; study not dated.
46. Investigation into hepatic cytochrome P450 parameters in the rat following repeat dosing for 5 days.
    Study # 1245/27-1050; study dated 2-1-96.
47. Inhibition of cytochrome P4502D6-catalyzed bufuralol 1'-hydroxylase activity by AG1343.
    Study # 951010A; study dated 2-16-96.
48. Inhibition of cytochrome P4503A4-catalyzed testosterone 6β-hydroxylase activity by the test substance AG1343.
    Study # 951010C; study dated 8-15-96.
49. *In vitro* metabolism of AG1343 by rat and human liver microsomes.
    Study # PH-1011-094; study dated 5-16-95.

50. The inhibition of cytochrome P4503A4 catalyzed testosterone 6β-hydroxylase activity by nelfinavir mesylate: reanalysis and redetermination of the K_i value.
    Study # PH-0403-096; study not dated.
51. Identification of cytochrome P450 isoforms inhibited by nelfinavir mesylate.
    Study # PH-0702-096; study dated 8-20-96.
52. Mechanism of CYP3A4 inhibition by nelfinavir mesylate.
    Study # PH-0703-096; study dated 11-19-96.
53. Incubation of nelfinavir mesylate with human liver microsomes in the presence of specific inhibitors of cytochrome P450.
    Study # PH-0706-096; study dated 8-8-96.
54. Assessment of the inhibitory potency of the nelfinavir metabolites M1, M3 and M8 towards cytochrome P4503A4 (CYP3A4).
    Study # PH-1012-096; study dated 12-3-96.
55. Inhibition of CYP3A4 by ritonavir, indinavir and saquinavir: determination of K_i values with testosterone as the probe substrate.
    Study # PH-1105-096; study dated 12-4-96.
56. Identification and synthesis of nelfinavir metabolites.
    Study # CD-001; study not dated.
57. Metabolite composition of AG1343 in rats after single administration.
    Study # JT-H7-70; study dated 9-95.
58. Isolation and characterization of metabolites of AG1343 in rat bile.
    Study # JT-H7-72; study dated 11-95.
59. Metabolite composition of AG1343 in monkeys after single administration.
    Study # JT-H7-111; study dated 6-96.
60. Western blot analysis of male rat liver cytochrome P450 levels following 15-day repeat-dose oral administration of AG1343.
    Study # PH-0402-095; study dated 5-22-95.
61. Western blot analysis of rat liver cytochrome P450 levels following 5-day repeat-dose oral administration of AG1343.
    Study # PH-0403-095; study dated 5-18-95.
62. Identification of nelfinavir metabolites in rat and pooled human liver microsomes by LC-MS/MS.
    Study # PH-1101-096; study dated 11-20-96.

Summary of Nonclinical Pharmacokinetic Studies

The sponsor conducted extensive nonclinical pharmacokinetic studies. These studies were all submitted under IND and were reviewed previously. No additional review of these studies is needed here. However, several general observations should be made. Oral bioavailability was highly variable and dependent upon both species and vehicle. Nelfinavir was demonstrated to be extensively metabolized and the pattern of biotransformation was species dependent. Systemic exposure decreased significantly with repeated dosing in rats and the reason for this was never clearly established by the sponsor. Systemic exposure was also limited at higher doses in cynomolgus monkeys, apparently due to saturation of oral absorption at higher doses. This also appeared to be true for rabbits and limited systemic exposure compromised the validity of reproductive toxicology/teratology studies conducted in this species. Nelfinavir metabolism appears to occur primarily via the hepatic CYP3A4 pathway. Inhibiton of this enzyme by nelfinavir appeared to be relatively modest and comparable to another protease inhibitor (saquinavir). Metabolism via other pathways occurs, but does not appear to be significant. One nelfinavir metabolite, designated M8 and chemically characterized as nelfinavir hydroxy-*t*-butylamide, appears to be relatively unique to humans, accounting for ~ 10% of parent drug plasma concentration (although amounts of up to 48% of parent drug were observed in clinical trials). The sponsor has agreed to conduct Phase IV studies to determine the

NONCLINICAL PHARMACOLOGY

Pharmacodynamic Safety Studies

1. General pharmacology of AG1343, a new potent inhibitor of HIV-1 protease.
   Study # JT-H8-137; study dated 12-96.
2. The *in vitro* effect of nelfinavir mesylate on human renin activity.
   Study # PH-1112-096; study dated 12-5-96.
3. A cardiovascular and respiratory study of AG1343.
   Study # SBL 20-21; study dated 12-12-94.

Summary of Pharmacodynamic Safety Studies

Nelfinavir was demonstrated to be relatively devoid of significant adverse pharmacological activity and no extensive

discussion is needed here.

NONCLINICAL TOXICOLOGY

Toxicity Studies

1. Single dose oral (gavage administration) sub-chronic toxicity study in the rat.
   Study # 1245/1-1050; study dated 10-4-94.
2. Single dose oral (gavage administration) sub-chronic toxicity study in the mouse.
   Study # 1245/2-1050; study dated 11-10-94.
3. Investigative tolerance studies in mice with AG1343.
   Study # PH-1010-094; study dated 3-1-95.
4. Twenty-eight day oral (gavage administration) sub-chronic toxicity study in the rat.
   Study # 1245/3-1050; study dated 11-30-94.
5. Tolerance study in the cynomolgus monkey.
   Study # 1245/4-1050; study dated 11-30-94.
6. Twenty-eight day oral (gavage administration) sub-chronic toxicity study in the monkey.
   Study # 1245/5-1050; study dated 11-30-94.
7. Fourteen day oral (gavage administration) sub-chronic toxicity study in the rat.
   Study # 1245/6-1050; study dated 11-29-94.
8. Twenty-six week oral (gavage administration) chronic toxicity study in the rat with a 13 week interim kill and a 4 week treatment free period.
   Study # 1245/15; study dated 1-96.
9. Twenty-six week oral (gavage administration) chronic toxicity study in the monkey with a 13 week interim kill and a 4 week treatment free period.
   Study # 1245/16; study dated 9-25-95.
10. Maximum tolerated dose (MTD) followed by a fixed dose (oral administration) toxicity study in the monkey.
    Study # 1245/22; study dated 6-4-96.
11. Twenty-eight day oral (gavage administration) range-finding toxicity study in the rat.
    Study # 1245/23-1050; study dated 7-1-96.
12. Acute dermal toxicity of nelfinavir mesylate in albino rats.
    Study # -165056; study dated 8-29-96.
13. Primary dermal irritation study of nelfinavir mesylate in albino rabbits.
    Study # -165057; study dated 8-29-96.

14. Primary eye irritation study of nelfinavir mesylate in albino rabbits.
    Study # -165058; study dated 8-29-96.
15. Skin sensitization study of nelfinavir mesylate in albino guinea pigs.
    Study # -165059; study dated 8-29-96.
16. Acute inhalation study of nelfinavir mesylate in albino rats.
    Study # -165061; study dated 10-4-96.
17. A study of fertility and early embryonic development to implantation of AG1343 in rats.
    Study # -165053; study dated 9-5-96.
18. A dose range-finding developmental toxicity study of AG1343 in rats.
    Study # -165048; study dated 8-7-96.
19. A developmental toxicology study of AG1343 in rats.
    Study # -165049; study dated 1-25-96.
20. A dose range-finding developmental toxicity study of AG1343 in rabbits.
    Study # -165050; study dated 8-23-96.
21. A developmental toxicology study of AG1343 in rabbits.
    Study # -165051; study dated 1-25-96.
22. Study of the effects of AG1343 on pre- and postnatal development, including maternal function in the rat.
    Study # -165054; study dated 9-24-96.
23. Induction of chromosome aberrations in cultured human peripheral blood lymphocytes.
    Study # 1245/9-1052; study dated 12-1-94.
24. Reverse mutation in histidine-requiring strains of *Salmonella typhimurium* and tryptophan-requiring strains of *Escherichia coli*.
    Study # 1245/10-1020; study dated 11-28-94.
25. Mutagenicity test on nelfinavir mesylate in the L5178Y TK+/- mouse lymphoma forward mutation assay with a confirmatory assay.
    Study # CHV 17312-0-431R; study dated 7-29-96.
26. Mutagenicity test on nelfinavir mesylate in an *in vivo* rat micronucleus assay.
    Study # CHV 17312-0-454CO; study dated 7-29-96.

Summary of Nonclinical Toxicology Studies

The most remarkable observation made in nonclinical toxicology studies was the relative lack of toxicity due to nelfinavir. Although oral treatment failed to result in significant systemic exposure, especially in cynomolgus monkeys, nelfinavir appears to be a relatively non-toxic compound.

Oral bioavailability appeared to be adequate in rats, but systemic exposure decreased markedly in repeat-dose studies. The reason fo this was never clearly established by the sponsor, although induction of hepatic metabolism appeared to play a role. No specific pattern of enzyme induction was ever demonstrated in the rat, however. Thyroid hypertrophy and occassional increases in relative liver weights were the only significant adverse effect observed in rat studies.

In cynomolgus monkeys, the only significant adverse effect observed in repeat-dose studies was chronic diarrhea at very high doses. This effect was associated with failure to grow and death in some animals. Treatment with neomycin reversed this effect and was taken by the sponsor to indicate that death in high-dose group monkeys was due to stress and resulting stress-induced alterations in gut flora. This remains an unproven hypothesis. However, it should be pointed out that systemic exposure did not increase proportionately with dose in higher-dose group animals. Thus, gut exposure to the drug was (probably) much higher than systemic exposure in animals that died on study. Increased systemic exposure was not associated with increased risk of death. In addition, no histopathologic effect were observed in monkeys that died on study. No adverse effects were observed in monkeys at doses comparable to human therapeutic doses or at levels of systemic exposure comparable to those observed in clincial trials.

No significant evidence of either reproductive toxicity or teratogenic potential were observed in nonclinical studies. However, as discussed above, rats did not demonstrate significnat toxicity due to nelfinavir at any dose tested. In addition, systemic exposure following oral treatment was unacceptably low in rabbits.

PROPOSED LABEL

The proposed label, as amended in consultation with DAVDP, is acceptable.

PHASE IV COMMITMENTS

EVALUATION AND CONCLUSION

The application for marketing is complete and should be approved.

Kenneth L. Hastings, Dr.P.H.

concurrences:
HFD-530/ADDir/GChikami Gary K Chikami 3/20/97
HFD-530/TL/JFarrelly 3/17/97
KENNETH L. HASTINGS/Pharm/3-6-97

disk:
HFD-530/JFarrelly

cc:
HFD-530 Original NDA
HFD-530 Division File
HFD-340
HFD-530/CSO/KStruble
HFD-530/Pharm/KHastings
HFD-530/MO/SMaldonado
HFD-530/Chem/PLiu

Figure 15. Nelfinavir Metabolite Profiles in Human Feces

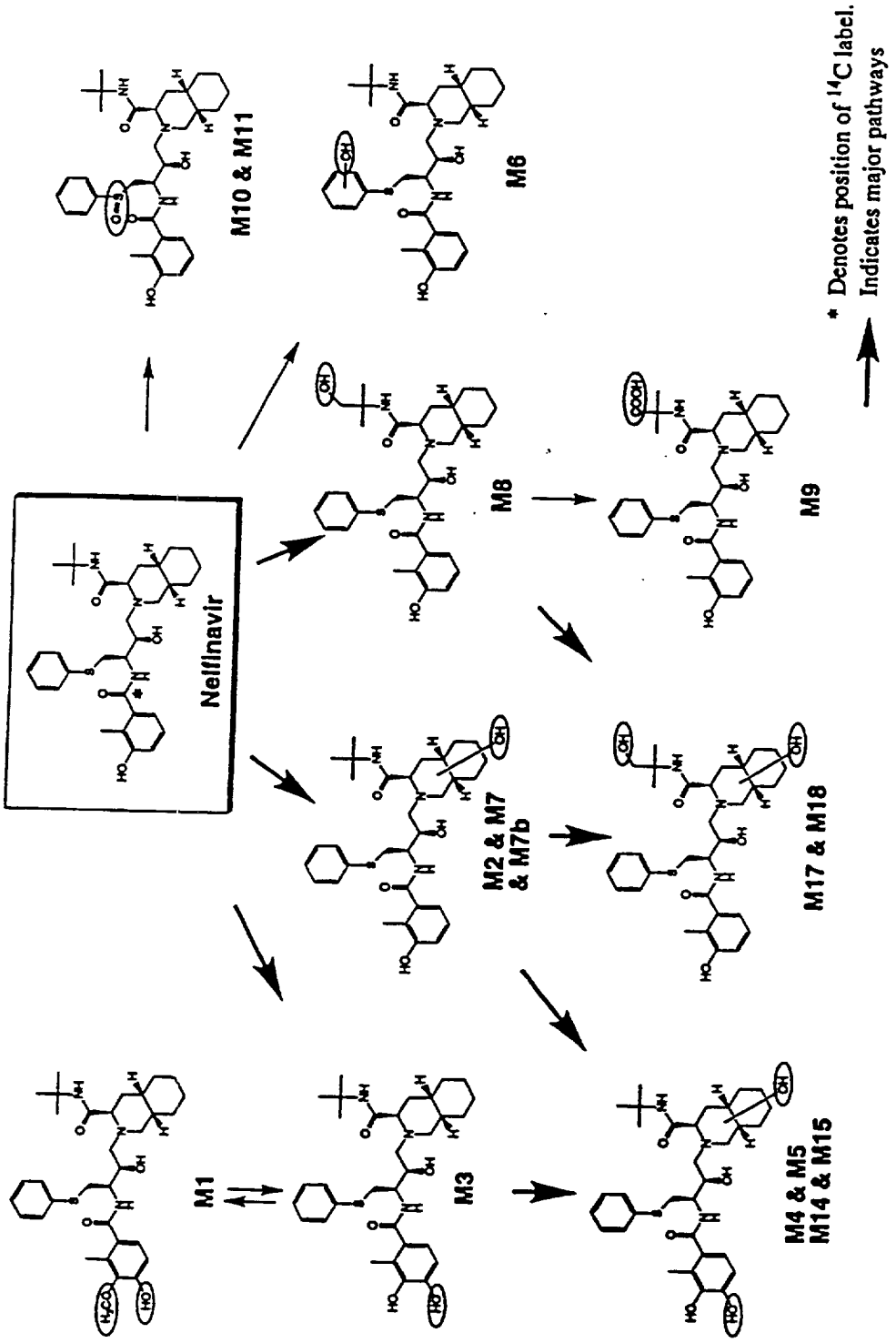
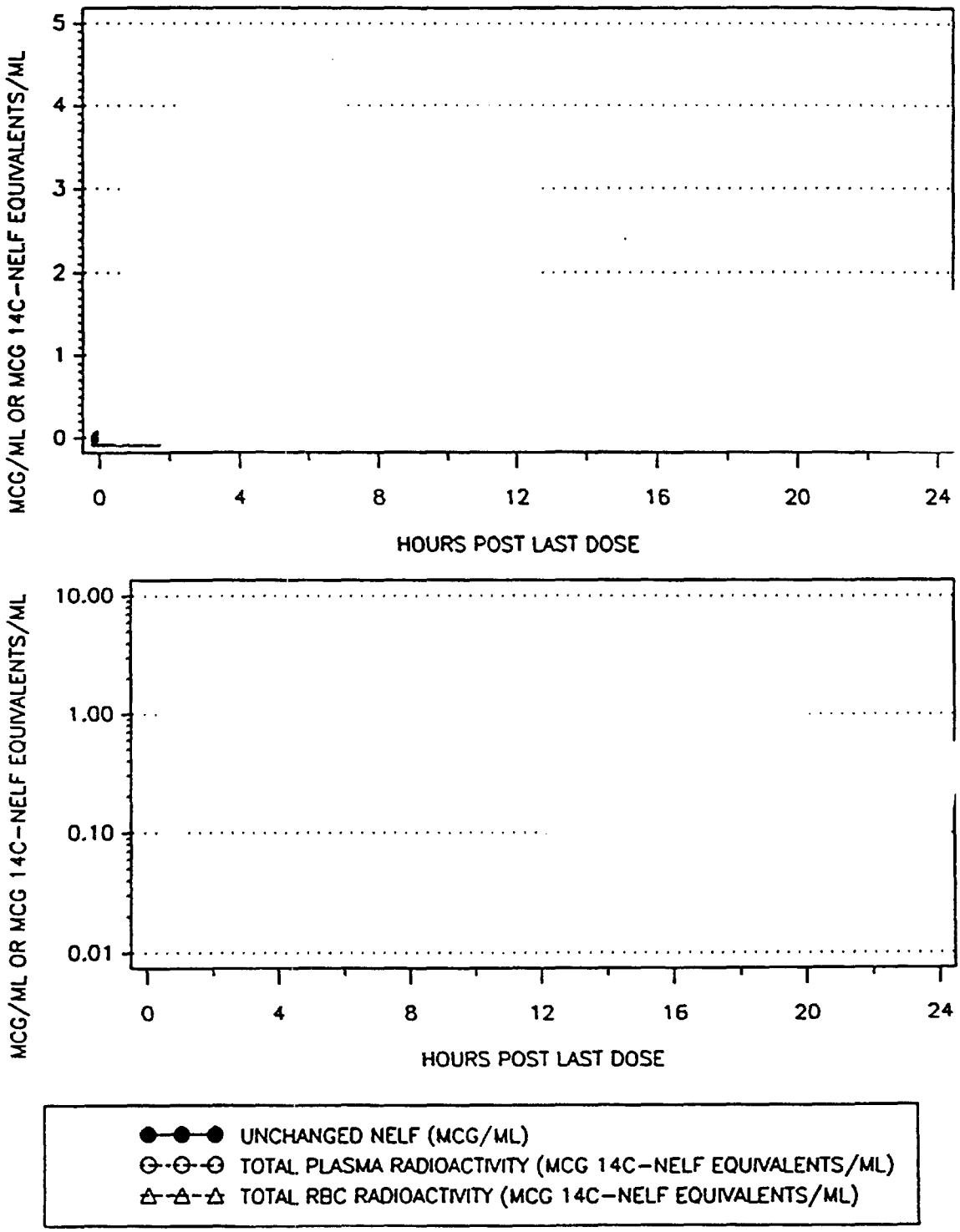


FIGURE 2
AGOURON PHARMACEUTICALS PROTOCOL AG1343-527

MEDIAN CONCENTRATIONS OF PLASMA NELFINAVIR (NELF), TOTAL RADIOACTIVITY, RED BLOOD CELL RADIOACTIVITY

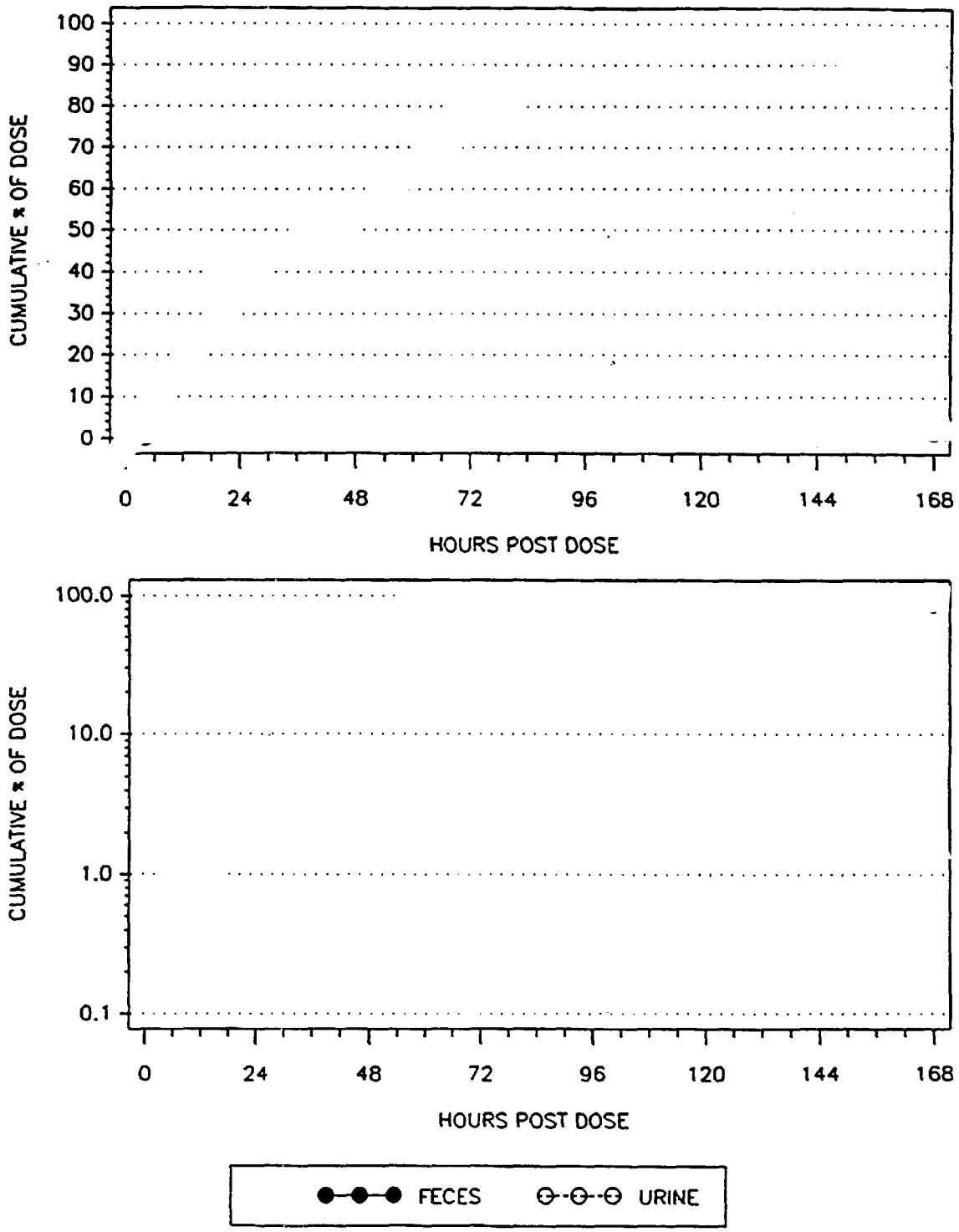


(N=4)

~~161056~~
12C

FIGURE 3
AGOURON PHARMACEUTICALS PROTOCOL AG1343-527

MEDIAN CUMULATIVE PERCENT OF ADMINISTERED RADIOACTIVITY IN URINE AND FECES



(N=4)

The relative proportions of nelfinavir, M8, and M1 in plasma were further evaluated using a quantitative to reassay plasma samples from ten patients in Study 503 who received nelfinavir mesylate 750 mg tid for 28 days.

of Plasma Samples: Nelfinavir 750 mg tid (n = 10)
Arithmetic Mean ± SD

Day	Species	AUC_{0-8} (μg*hr/mL)	Relative %
Day 1	Nelfinavir	13.24 ± 5.96	78 ± 11%
	M8	3.86 ± 4.16	18 ± 11%
	M1	0.61 ± 0.32	3 ± 1%
Day 8	Nelfinavir	13.87 ± 5.80	72 ± 8%
	M8	4.98 ± 2.70	25 ± 9%
	M1	0.64 ± 0.37	3 ± 1%

The relative amounts of nelfinavir, M8, and M1 in plasma on Days 1 and 8 of this clinical study were similar to the amounts determined in the single dose ADME study. As previously mentioned, M8 may contribute to the antiviral effects observed in patients.

ABSORPTION AND PHARMACOKINETICS

Single dose pharmacokinetics

Study JT-H8-82 was a placebo controlled, dose escalation study that examined the pharmacokinetics and dose-proportionality of nelfinavir after single-dose administration. Healthy male volunteers were assigned to one of four treatment groups. Eight subjects were assigned to each group; six subjects in each group received active drug and two received placebo. Nelfinavir was administered as 250 mg tablets, 30 minutes after a standardized breakfast (514 kcal, 18.1 g protein, 16.8 g fat, 73.4 g carbohydrate). Blood and urine were both collected over the 24 hours following dose administration.

Mean ± SD Pharmacokinetic Parameters Following Single Doses of Nelfinavir (Fed)

Parameter	250 mg (n = 6)	500 mg (n = 6)	750 mg (n = 6)	1000 mg (n = 6)
AUC_∞ (μg*hr/mL)	3.1 ± 1.2	16.3 ± 6.5	47.9 ± 10.7	65.5 ± 24.9
Cmax (μg/mL)	0.6 ± 0.2	1.9 ± 0.6	4.9 ± 0.7	6.7 ± 2.3
Tmax (hr)	3.3 ± 0.8	3.5 ± 0.5	4.7 ± 1.0	4.5 ± 1.2
T½ (hr)	2.1 ± 0.1	3.4 ± 0.9	4.8 ± 0.9	4.1 ± 0.8
CL/F (L/hr)	90.0 ± 31.7	37.4 ± 22.2	16.4 ± 4.0	18.8 ± 12.1
V_{darea}/F (L)	275 ± 94	168 ± 67	110 ± 18	104 ± 56
% excreted unchanged in urine	0.04 ± 0.03	0.11 ± 0.06	0.18 ± 0.13	0.16 ± 0.06

Following oral administration of a single dose of nelfinavir with food, nelfinavir was slowly absorbed with peak concentrations occurring between 3 and 5 hours. There was a disproportional increase in AUC_∞ and Cmax with dose. Oral clearance decreased and elimination half-life increased when the dose increased from 250 mg to 750 mg. Pharmacokinetics did not appear to change between the 750 mg and 1000 mg doses.

These data suggest that the pharmacokinetics of nelfinavir are non-linear over the dose range of 250 mg to 750 mg. Although the cumulative urinary excretion of unchanged drug increased with increasing dose, the highest mean excretion was 0.18 ± 10.13% of the dose.

For comparison, the pharmacokinetic parameters following the first dose of a multiple dose study in HIV infected patients (Study 503, described below) are presented in the following table. Note that the AUC values are AUC_{0-8}, not AUC_{∞}.

Mean ± SD Pharmacokinetic Parameters Following Single Doses of Nelfinavir (Fed)

Parameter	500 mg (n = 10)	750 mg (n = 10)	1000 mg (n = 10)
AUC_{0-8} (μg*hr/mL)	11.6 ± 5.7	16.1 ± 7.0	20.1 ± 6.7
Cmax (μg/mL)	2.19 ± 0.92	3.3 ± 1.5	3.78 ± 1.08

Multiple Dose Pharmacokinetics

The multiple dose pharmacokinetics of nelfinavir were evaluated in Study 503, a pilot, open-label, dose range-finding study. The pharmacokinetic objective of this study was to determine the pharmacokinetics of nelfinavir at different doses and regimens administered to HIV positive patients. In addition, the antiretroviral activity of each dose level and regimen was evaluated. Patients included in this study were HIV positive, over 12 years of age, had a pre-study HIV RNA level ≥ 20,000 copies/mL, and a CD_4 lymphocyte count ≥ 200 cells/mm^3. The following regimens were evaluated in this parallel study:

500 mg nelfinavir bid x 28 days
600 mg nelfinavir bid x 28 days
750 mg nelfinavir bid x 28 days
500 mg nelfinavir tid x 28 days
750 mg nelfinavir tid x 28 days
1000 mg nelfinavir tid x 28 days

All nelfinavir doses were administered with food. Nelfinavir was administered as 200 mg or 250 mg tablets. Patients did not receive other antiretroviral agents during this study. Blood samples for nelfinavir pharmacokinetic profiles were collected over a dosing interval on Day 0 (1st dose) and Day 28. Trough concentrations were collected prior to the morning dose on Days 7, 14, and 21.

Day 28 Nelfinavir Pharmacokinetic Parameters (Arithmetic Mean ± SD)

Parameter	500 mg bid (n = 9)	600 mg bid (n = 10)	750 mg bid (n = 15)	500 mg tid (n = 10)	750 mg tid (n = 10)	1000 mg tid (n = 9)
AUC_T (μg*h/mL)	17.49 ± 6.47	22.84 ± 7.91	25.63 ± 10.64	15.86 ± 5.51	16.30 ± 7.71	29.67 ± 9.31
Cmax (μg/mL)	2.53 ± 0.76	2.92 ± 0.71	3.52 ± 1.30	3.90 ± 1.43	2.93 ± 1.29	5.11 ± 1.63
Tmax (hr)	2.6 ± 1.5	3.9 ± 1.7	3.6 ± 1.6	2.7 ± 0.8	2.8 ± 1.0	2.9 ± 0.8
T½ (hr)	3.6 ± 1.1	4.1 ± 1.5	4.2 ± 2.2	3.8 ± 0.9	4.0 ± 0.6	4.2 ± 1.3
CL/F (L/hr)	31.9 ± 10.3	26.2 ± 6.0	33.4 ± 11.8	30.7 ± 4.8	36.4 ± 3.7	40.5 ± 14.6
Cavg (μg/mL	1.46 ± 0.54	1.90 ± 0.66	2.14 ± 0.89	1.98 ± 0.69	2.04 ± 0.96	3.71 ± 1.16
R	1.54 ± 0.65	1.90 ± 0.63	1.53 ± 1.28	1.53 ± 0.68	1.12 ± 0.67	1.50 ± 0.49

Cavg = AUC/dosing interval
R = AUC_T(Day 28)/AUC_T(Day 0)

Based on visual inspection of the nelfinavir concentration vs. time profiles, it is apparent that in many patients, oral absorption continued up to 8 hours. This made it impossible to obtain reasonable estimates of terminal elimination rate for the Day 0 profiles. On Day 28, additional concentrations were obtained after the dosing interval for some patients and the elimination rate was determined.

Nelfinavir trough concentrations by visit

Day	500 mg bid (n=9)	600 mg bid (n=10)	750 mg bid (n=15)	500 mg tid (n=10)	750 mg tid (n=10)	1000 mg tid (n=9)
4	1.37±0.69	2.16±0.88	1.87±1.32	NA	NA	3.40±2.12
7	1.18±0.61	1.83±1.01	1.74±1.46	1.40±0.50	1.83±0.92	2.46±1.12
14	1.09±0.79	1.89±1.37	1.36±1.21	1.89±0.78	1.69±0.62	2.18±1.28
21	1.01±0.78	1.60±1.22	1.54±0.93	1.72±0.63	2.02±1.28	2.79±2.30

In several healthy volunteer studies, it was observed that the daily trough concentrations increased to a maximum around the second day of dosing and thereafter decreased toward steady-state approximately 5-7 days after initiation of treatment. At the 500 and 750 mg tid dose levels (Studies 520 and 521), the average trough concentrations on the sixth day of treatment were about 55% of the trough concentrations on the second day of treatment. This pattern is suggestive of modest autoinduction of clearance during multiple dosing. For patients receiving 750 mg tid in Study 503, the higher plasma ratios of the metabolite M8 relative to nelfinavir on Day 28 versus Day 1 further suggest that autoinduction occurs.

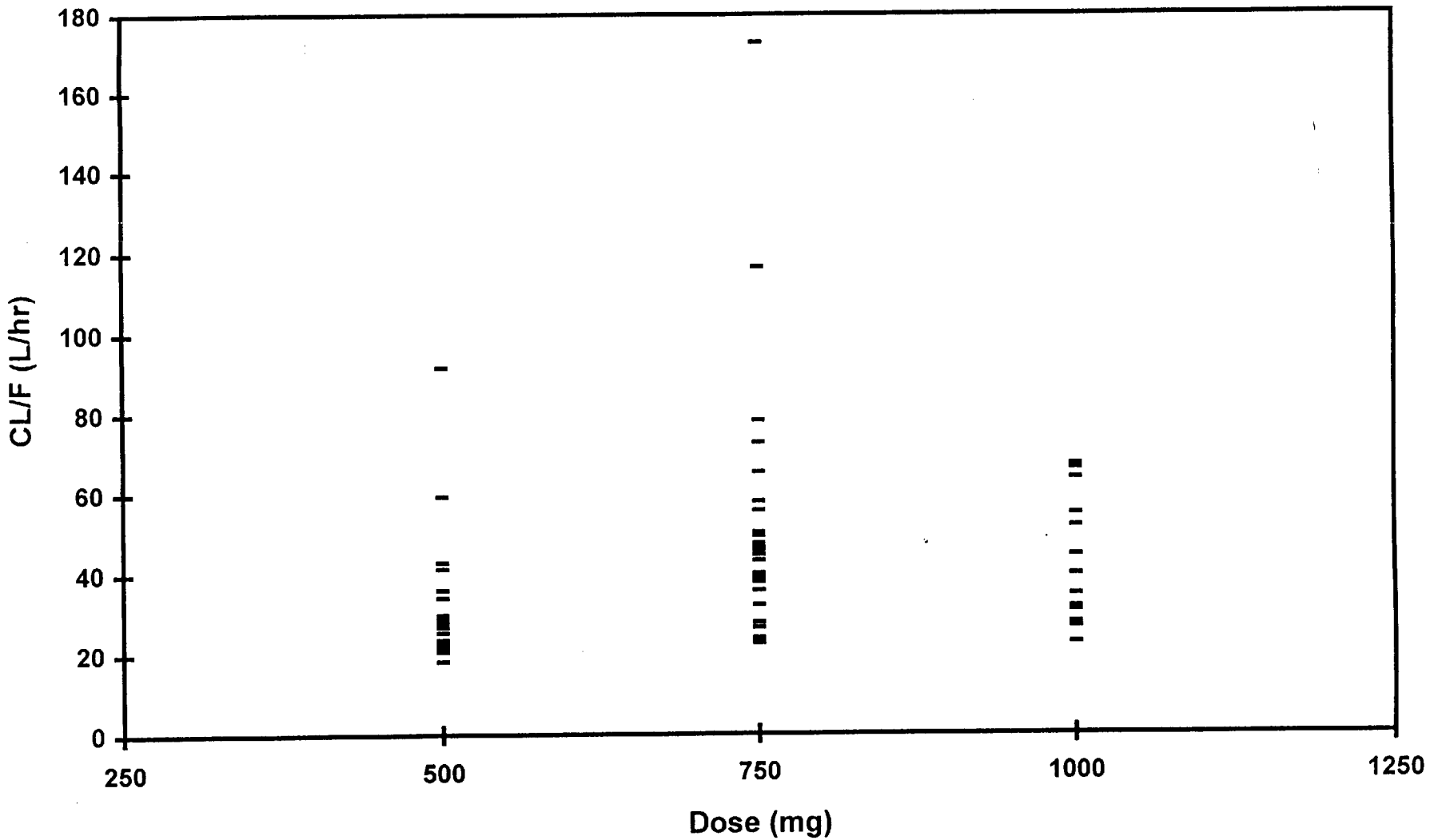
Steady-state pharmacokinetic data are available for HIV positive patients who received nelfinavir q8hrs (with food) in Studies 503, 509, and 510. The data are summarized in the following table.

Nelfinavir Steady State Pharmacokinetic Parameters (HIV positive patients)
Arithmetic mean ± SD (Range)

Parameter	500 mg tid (n=19)	750 mg tid (n=30)	1000 mg tid (n=15)
AUCτ (μg*hr/mL)	17.23±5.41 (5.44-27.43)	18.36±7.38 (4.33-33.0)	25.90±9.52 (14.85-44.08)
Cmax (μg/mL)	3.00±0.84 (1.02-4.76)	3.27±1.17 (0.71-5.39)	4.48±1.65 (2.41-7.58)
Tmax (hr)	3.58±1.37 (1.50-5.00)	2.97±1.12 (1.00-6.00)	2.70±0.75 (1.50-4.00)
CL/F (L/hr)	33.50±17.09 (18.23-91.90)	50.14±30.14 (22.7-172.96)	44.02±16.35 (22.69-67.34)

Oral clearance of nelfinavir was relatively constant across this dose range in HIV positive patients.

Nelfinavir CL/F vs. Dose (t.i.d. dosing)



Nelfinavir AUC at Steady-State (t.i.d. dosing)

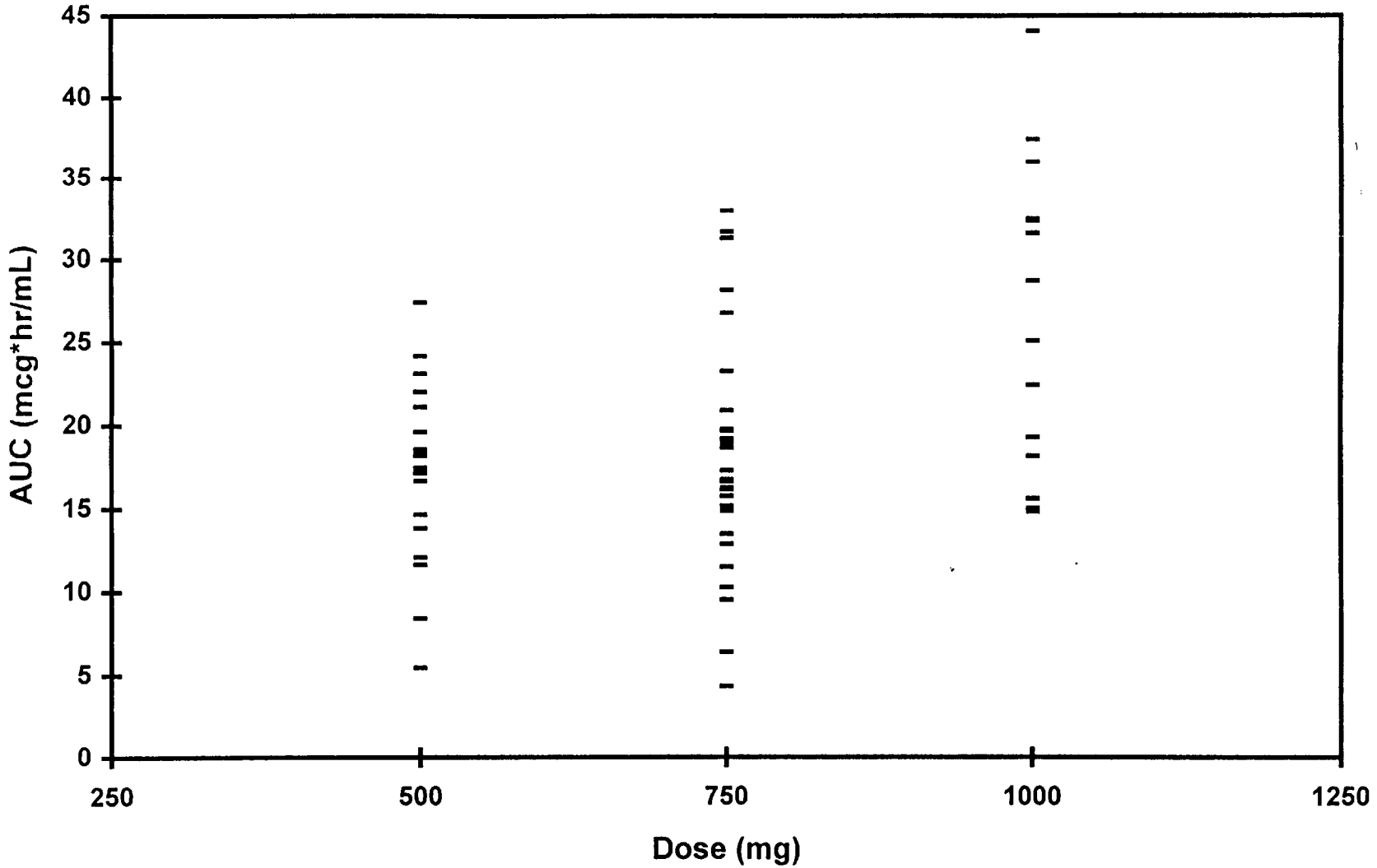


Figure 5
Protocol AG1343 – 503

Median Nelfinavir Plasma Concentration versus Time

Visit = Day 0 Dosing = TID

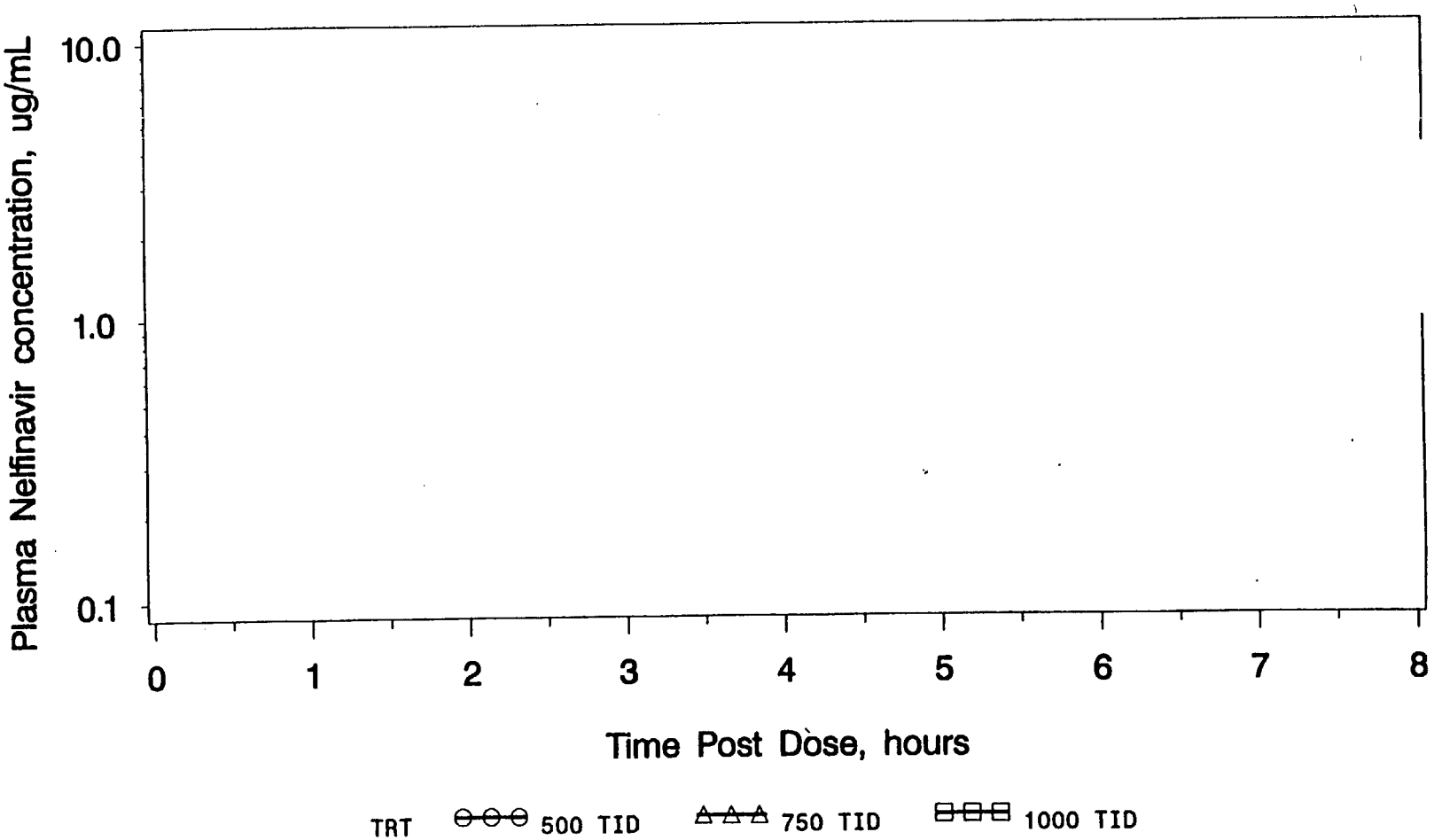
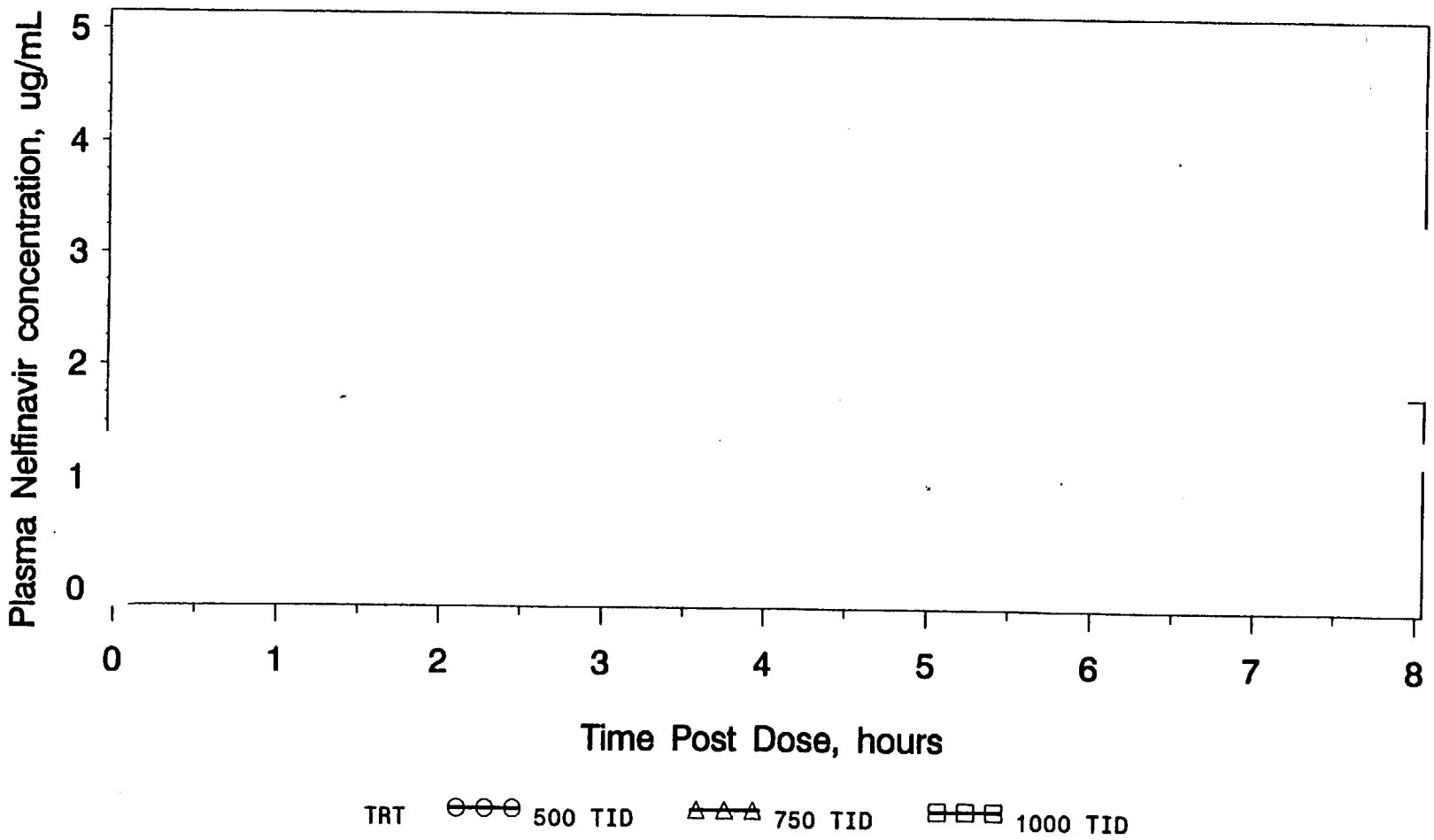


Figure 6
Protocol AG1343 – 503

Median Nelfinavir Plasma Concentration versus Time

Visit = Day 0 Dosing = TID



PSI

Figure 7
Protocol AG1343 – 503

Median Nelfinavir Plasma Concentration versus Time

Visit = Day 28 Dosing = TID

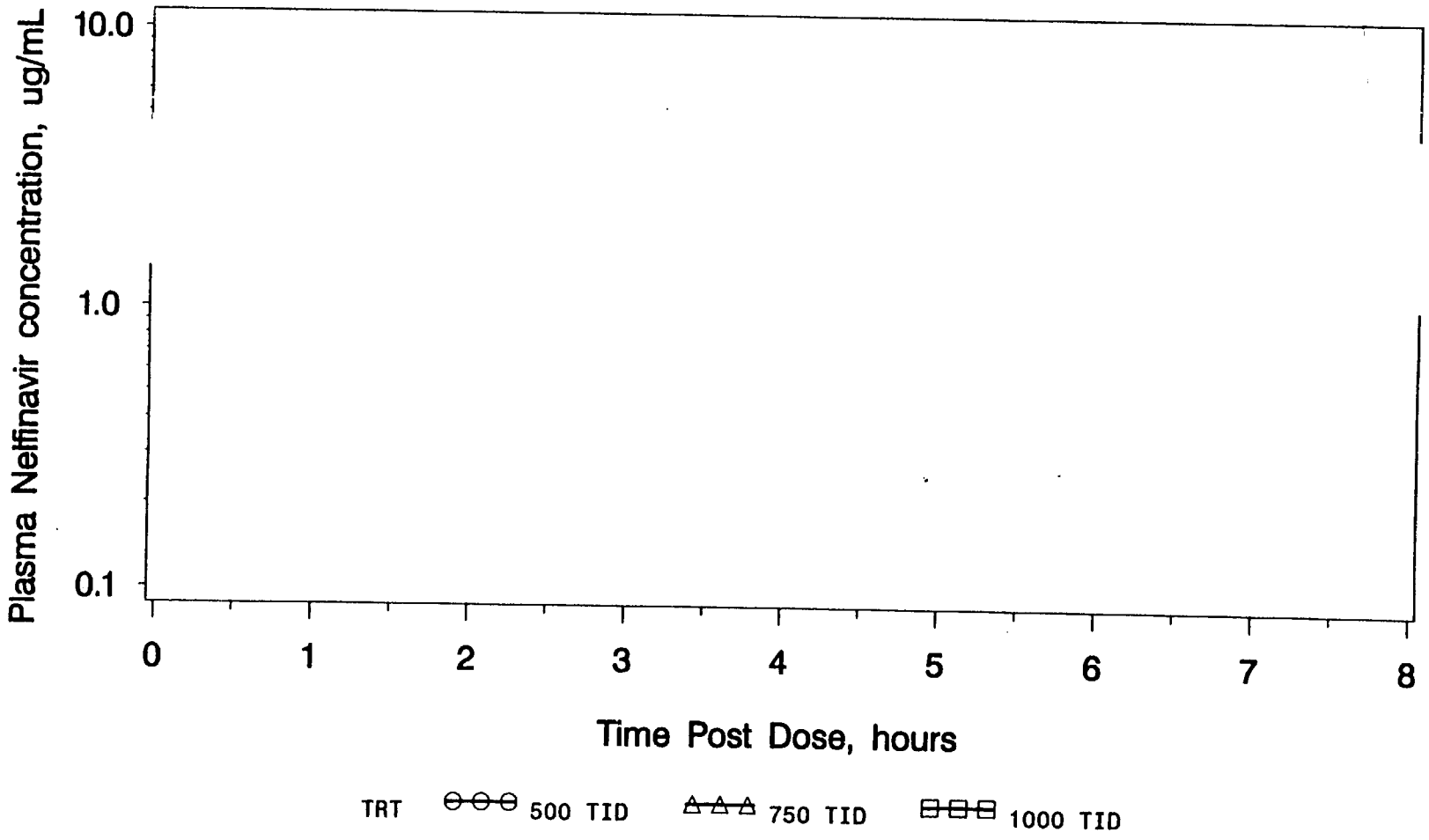
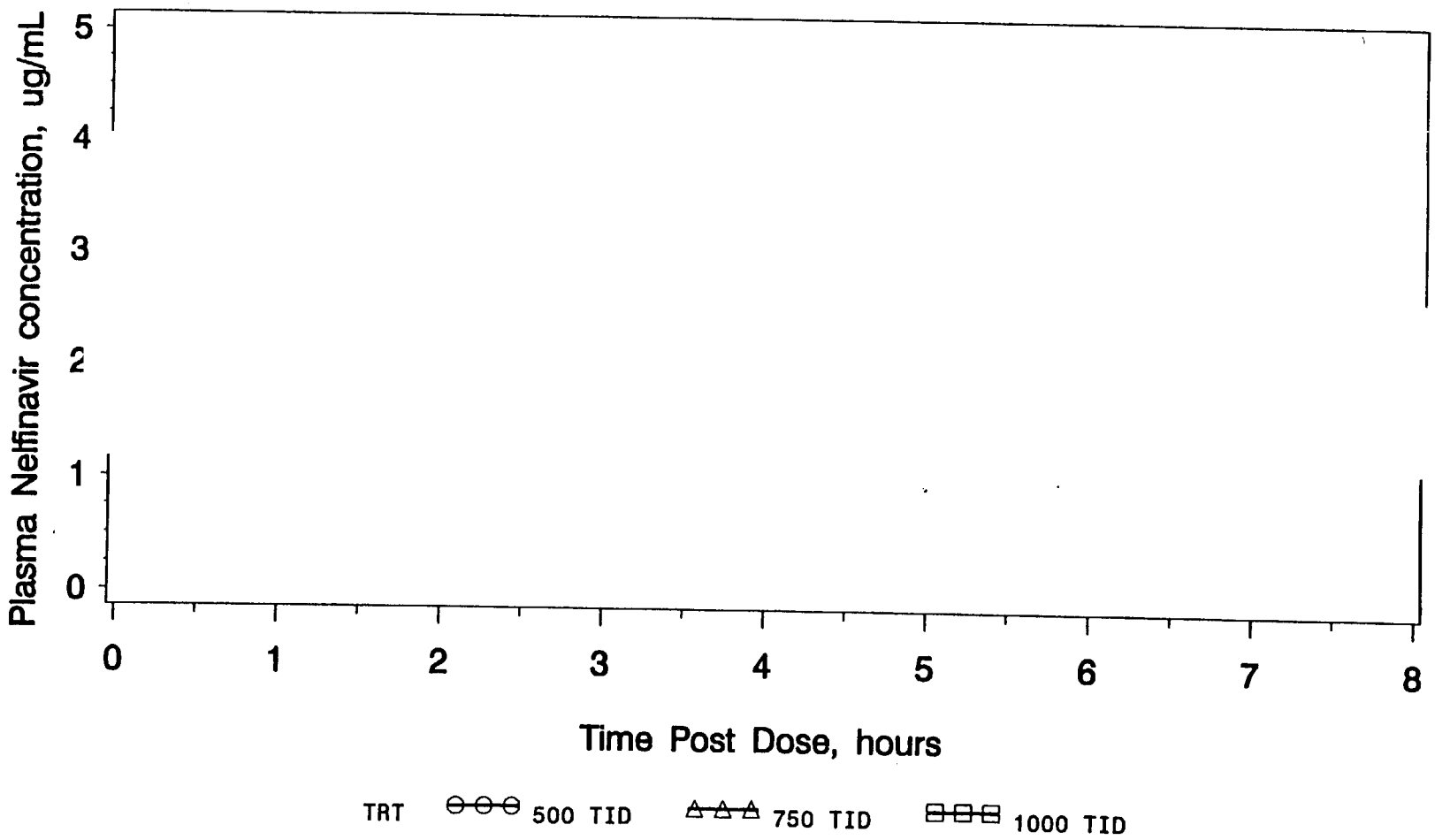


Figure 8
Protocol AG1343 – 503

Median Nelfinavir Plasma Concentration versus Time

Visit = Day 28 Dosing = TID



At both the 500 mg tid and 750 mg tid dose levels, healthy volunteers had higher Cmax and AUC values than observed in HIV+ patients. The mean AUC values from studies in which healthy volunteers received 750 mg tid ranged from 21.0 to 23.1 μg*hr/mL (Studies 521, 523, 540). The applicant states that the modestly higher plasma concentrations for the healthy volunteers may be due to the more rigorously timed administration of drug in studies that were performed entirely within a clinic. In the studies in HIV+ patients, doses were ingested on a less strict schedule on an outpatient basis, with the last dose before pharmacokinetic assessment occurring the evening before entering the clinic. Thus, patients may have taken their most recent dose more than 8 hours before the pharmacokinetic evaluation. The small differences in pharmacokinetics observed between patients and healthy subjects should not alter the interpretation of pharmacokinetic studies that were performed in healthy subjects.

Relative Bioavailability

The applicant was not able to formulate nelfinavir as a solution for the determination of relative bioavailability. A powder formulation has been developed for pediatric patients who are not able to take the tablets. The relative bioavailability of the powder to the tablet was not determined prior to the first study in pediatric patients, because the formulation was not developed until just prior to study initiation. The formulations were compared in adults because the bulk of the powder makes clinical use impractical.

A cross-over comparison of the tablet and oral powder formulations was performed in children of ages 7 to 13 years who participated in the pediatric study (Study 524). Single dose of each formulation were administered in the fed state at a dose level of 10 or 20 mg/kg with a washout period between doses of at least 2 days. Based on preliminary data in six children, the dose-normalized plasma AUC_{0-8} for the oral powder was 115 ± 37% of that for the standard tablet formulation; the dose normalized Cmax for the oral powder formulation was 92 ± 22% relative to the tablets. There was no trend for increased or decreased concentrations with the oral powder.

Food Effect

The effect of food on the pharmacokinetics of nelfinavir was evaluated in 6 healthy male volunteers in Study JT-H8-82. Each subject received a single 500 mg (2 x 250 mg) dose of nelfinavir 30 minutes following a standardized breakfast (514 kcal, 18.1 g protein, 16.8 g fat. 73.4 g carbohydrates) and fasted (no food for 11 hours before and 4.5 hours after dose). Blood and urine were both collected over the 24 hours following dose administration.

Mean ± SD Pharmacokinetic Parameters Following Single 500 mg Doses of Nelfinavir (Fed vs. Fasted)

Parameter	500 mg Fed (n = 6)	500 mg Fasted (n = 6)
AUC_∞ (μg*hr/mL)	16.3 ± 6.5	6.6 ± 5.3
Cmax (μg/mL)	1.9 ± 0.6	0.97 ± 0.60
Tmax (hr)	3.5 ± 0.5	2.2 ± 0.8
T½ (hr)	3.4 ± 0.9	3.4 ± 0.8

When nelfinavir was administered within 30 minutes following a meal, AUC was increased by 250 ± 166% (range; 37% to 448%) and Cmax was increased by 153 ± 133% (range: 27% to 378%), relative to the fasted treatment. When nelfinavir was administered with food, variability (%CV) was decreased from 80.5% to 39.5% for AUC and from 61.1% to 33.8% for Cmax. Subjects with lower AUC and Cmax values for the fasted treatment experienced greater relative increases in AUC and Cmax when nelfinavir was administered with food. Tmax did not change for one subject and occurred 1-2 hours later for the other 5 subjects. Elimination half-life was not influenced by food.

The effect of food was also evaluated in Study 501 (Europe). Four healthy male volunteers received 400 mg (4 x 100 mg) nelfinavir mesylate capsules (342 mg nelfinavir mesylate) fasted and 10 minutes following a standardized breakfast (cereal, low-fat milk, toast with butter). Another four subjects received the same treatments, but the nelfinavir mesylate

dose was 800 mg (8 x 100 mg), equivalent to 684 mg nelfinavir free base. In the fed state, AUC was increased 86 ± 23% for the 400 mg group and 387 ± 340% in the 800 mg group. In this study, Tmax occurred 1.5 to 3 hours later for the fed treatment. These results are qualitatively similar to the results of Study JT-H8-82.

Administration of nelfinavir with food appears to prolong and increase absorption. Due to the increase in bioavailability when administered with food, nelfinavir was administered with food during the clinical trials and it is recommended that patients take nelfinavir with a meal or snack. The effect of different meals on the pharmacokinetics of nelfinavir was not investigated.

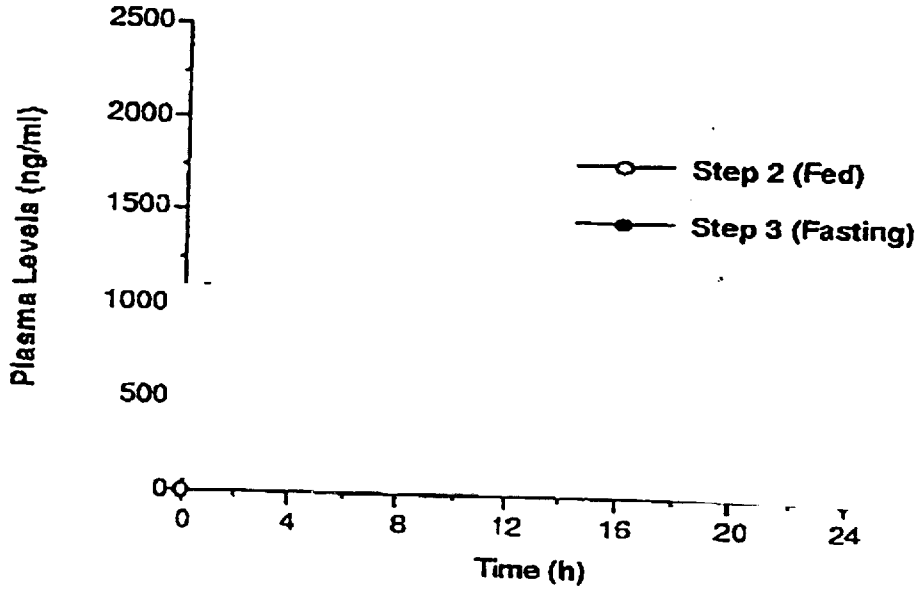


Fig.3 Plasma Concentration of Unchanged Nelfinavir after Single Oral Administration of 500 mg Nelfinavir to Healthy Volunteers on Food

Data represent the mean + S.D. values, n=6.

DISTRIBUTION

Plasma/Serum Protein Binding

The in vitro protein binding of nelfinavir in human serum and purified human serum proteins was determined using equilibrium dialysis. The human blood samples for these studies were collected from seven adult males.

Effect of Nelfinavir Concentration on Human Serum Protein Binding

Nelfinavir concentration (μg/mL)	% Unbound (n=4)	% Recovery from equilibrium dialysis
1.2	0.75±0.21	92.8±3.1
10.2	0.93±0.09	92.7±1.9
25.1	1.34±0.27	95.1±7.6

Nelfinavir is highly protein bound (>99%) to serum proteins. This binding appears to be dose dependent at high concentrations. The binding does not appear to be dose dependent at clinically relevant nelfinavir concentrations. Nelfinavir was also extensively (97-99%) bound to human serum albumin. The extensive binding of nelfinavir to serum proteins complicates interpretation of plasma drug concentrations in relation to in vitro antiviral ED_{50} and ED_{95} estimates.

Red Blood Cell Partitioning

Blood to plasma ratio experiments were determined in glass test tubes with gentle shaking in a 37°C water bath. For these experiments whole blood specimens (EDTA anticoagulant) were spiked with [^{14}C]-nelfinavir as a tracer. The nelfinavir concentration was 5 μg/mL. The blood to plasma ratio (n=6) was 0.76±0.048; the hematocrit was 0.41±0.02. These data indicate that nelfinavir is mostly confined to the blood plasma with very little sequestration into the blood cells.

Following administration of [^{14}C]-nelfinavir to healthy volunteers (Study 527), the total radioactivity in plasma (consisting primarily of unchanged nelfinavir) exceeded red blood cell radioactivity by 3- to 5- fold at all timepoints postdose when radioactivity was measurable. These results indicate that the in vivo blood to plasma ratio for nelfinavir is approximately 0.7, consistent with in vitro data.

METABOLISM

Identification of Metabolites

<u>In vitro</u>

In vitro metabolism of nelfinavir was studied in rat and pooled human liver microsomes. Metabolite profiles were found to be very similar between the two species. Pathways of oxidative microsomal metabolism in both species can be described as follows: 1) hydroxylation of the perhydroisoquinolone moiety; 2) hydroxylation of the benzamide ring; 3) sulfur oxidation; 4) hydroxylation of the thiophenyl ring; and 5) hydroxylation of the *t*-butylamide group. The relative importance of each pathway could be ranked in both rat

and human liver microsomes as 1≈2>3>4>5. It should be noted that significant *S*-oxidation also occurred in heat-inactivated microsomes, probably by air oxidation. The profiles of microsomal metabolites resembled those found in feces of each species, except that mono rather than dihydroxylated metabolites were more abundant in microsomes than in feces. M1 (3"-methoxy-4"-hydroxynelfinavir), found in rat and human plasma and feces, was not formed in liver microsomal incubations because microsomes lack the enzyme catechol-*o*-methyl transferase (COMT) that catalyzes the methylation of M3 to M1.

In vivo: rats

In vivo metabolism of nelfinavir mesylate was studied in male Sprague-Dawley rats using LC-MS/MS. In one study, 3 rats each were administered an IV dose of [^{14}C] nelfinavir mesylate (25 mg/kg) via a jugular vein cannula or an oral dose of [^{14}C] nelfinavir mesylate (50 mg/kg). Blood samples were collected at 2 hr and 6 hr. Feces were collected from 0-8 hr, 8-24 hr, and 24-48 hr. In another study, bile duct cannulated rats received an IV dose of [^{14}C] nelfinavir mesylate (50 mg/kg) via the jugular vein cannula. Bile samples were collected at 1 hr intervals up to 8 hr, and from 8-24 hr.

Following IV or oral administration of nelfinavir mesylate, the major component in plasma was the parent drug (75-88%), followed by M1 (12-25%). Other metabolites, including M8, M10, and M11, were detected in trace amounts. Rat feces contained some unchanged drug (~17%), and the remaining radioactivity was accounted for by more than 20 oxidative metabolites. Important pathways of nelfinavir metabolism included hydroxylation on the benzamide ring, the perhydroisoquinoline moiety, and the thiophenyl ring. Secondary metabolites involving two of these processes were also abundant. Two nelfinavir S-oxides were found as minor metabolites. In rat bile, nelfinavir and its metabolites were found mainly as glucuronide conjugates, as well as some unconjugated polar metabolites. In feces, however, only the unconjugated forms were found, presumably due to microbial hydrolysis in the intestinal tract.

In vivo: humans: See Radiolabeled Nelfinavir ADME Section (Study 527). The metabolites that were observed in the human ADME study were also observed following administration of [^{14}C] nelfinavir mesylate to rats.

Enzymes Metabolizing Nelfinavir:

Nelfinavir metabolism was investigated in vitro using human liver microsomes and cDNA-expressed cytochrome P450 isoforms. The results were confirmed by incubating nelfinavir with isozyme selective chemical inhibitors in human liver microsomes.

The typical human liver microsome incubation mixture consisted of liver microsomes (2.5 mg protein/mL), 5 mM magnesium chloride, and 1 mM NADPH in 10 mM potassium phosphate buffer (pH 7.4) in a final volume of 0.250 mL. One set of human liver microsome incubation mixtures contained 1 mM UDP-glucuronic acid in addition to the 1 mM NADPH. Control incubation mixtures did not contain NADPH. Nelfinavir was added to the mixture at a final concentration of 10 μg/mL. The percent nelfinavir metabolism results are summarized for individual male, individual female, and pooled male and female human liver microsome mixtures incubated with nelfinavir under various conditions in the following table.

Percent Nelfinavir Metabolism (Mean ± SD, Range)

Microsome source	-NADPH, -UDPGA	+NADPH, -UDPGA	-NADPH, +UDPGA	+NADPH, +UDPGA
Female (n=5)	5.91 ± 4.93 (0.24-12.43)	89.85 ± 15.45 (62.48-99.02)	12.06 ± 8.23 (4.25-22.04)	88.10 ± 13.91 (63.44-97.41)
Male (n=5)	1.25 ± 0.93 (0.00-2.30)	48.58 ± 25.42 (31.36-79.86)	15.39 ± 12.51 (3.84-34.97)	53.94 ± 19.44 (33.64-79.76)
3 pooled mixtures (5M/5F, 15M/17F, 7M)	6.63 ± 3.64 (3.16-10.42)	61.94 ± 18.67 (43.03-80.36)	17.66 ± 15.01 (4.31-33.91)	51.30 ± 9.83 (42.28-61.78)

These results indicate that nelfinavir was well metabolized by a NADPH-dependent enzyme. There may have also been some metabolism contribution from the UDP-glucuronyl transferase enzyme; however, the contribution appeared to be small. The microsomes from females showed a statistically significantly higher rate of metabolism than those from males. The rate of metabolism in the presence of NADPH (+/-UDPGA) was also significantly higher for females when corrected for protein content. A non-significant difference between males and females was noted when corrected for cytochrome P450 content. The specific cytochrome P450 isoform activities (1A2, 2A6, 3A, 4A, 2C, 2D, 2E) were determined for each microsomal mixture. CYP3A activity was significantly higher for females ($p < 0.05$) and CYP4A activity was significantly higher for males ($p < 0.05$). Thus, CYP3A may be predominantly involved in the metabolism of nelfinavir by human liver microsomes. Confirmation of the likely involvement of CYP3A in the metabolism of nelfinavir was obtained when the rate of nelfinavir was correlated with each specific cytochrome P450 isoform activity determined in this study. Only CYP3A showed a correlation above $r^2 = 0.5$ (result: $r^2 = 0.622$) of activity with metabolism rate.

The involvement of CYP3A and the apparent minor involvement of other isoforms in the metabolism was further confirmed with studies using **cDNA-derived human cytochrome P450 isoforms** in microsomes prepared from a human lymphoblastoid cell line. The typical incubation with microsomes prepared consisted of a cDNA-expressed human cytochrome P450 isoform in microsome, 3.3 mM magnesium chloride, and a NADPH generating system in 10 mM potassium phosphate buffer (pH 7.4) in a final volume of 0.5 mL. Nelfinavir was added to the mixture at a final concentration of 10 μg/mL.

Percent Nelfinavir Metabolism (Average of triplicate incubations)

Cytochrome P450 Isoform	-NADPH (t=3 hrs)	+NADPH (t=1 hr)	+NADPH (t=3 hrs)
Control Microsomes	2.08	0.47	4.13
CYP1A1	7.16	4.79	5.59
CYP1A2	2.14	1.44	6.37
CYP2A6	2.58	2.47	4.52
CYP3A4	5.94	57.71	71.72
CYP2B6	4.83	ND	3.64
CYP2C8	5.34	0.62	2.13
CYP2C9-cys	4.35	4.09	8.24
CYP2C9-arg	6.92	13.89	24.17
CYP2C19	4.24	12.99	26.78
CYP2D6	1.31	11.45	22.58
CYP2E1	5.16	6.46	7.41

Nelfinavir metabolism rate in the presence of NADPH
per microsomal protein content and per cytochrome P450 content

Cytochrome P450 Isoform	Rate determined at 1 hour		Rate determined at 3 hours	
	ng/mg protein/min	pg/nmole P450/min	ng/mg protein/min	pg/nmole P450/min
Control Microsomes	ND	N/A	0.51	N/A
CYP1A1	0.43	12.8	0.67	19.5
CYP1A2	0.16	1.6	0.82	8.3
CYP2A6	1.13	7.9	0.71	5.0
CYP3A4	35.45	788	14.69	327
CYP2B6	ND	ND	0.67	5.3
CYP2C8	0.073	7.3	0.40	39.3
CYP2C9-cys	2.55	160	1.71	107
CYP2C9-arg	8.80	144	5.11	83.6
CYP2C19	7.27	404	5.00	277
CYP2D6	6.51	203	4.27	134
CYP2E1	2.87	22.1	1.07	8.2

Based on the extent of nelfinavir metabolism (per nmol P450) during a one hour incubation, nelfinavir was metabolized primarily by the CYP3A4 isoform. Other isoforms involved were CYP2C19>CYP2D6>CYP2C9. These isoforms had different rates of metabolism over time (1 hr vs. 3 hrs). Rates may change over time due to depletion of NADPH, inactivation/degradation of the isoform, possible competition by metabolites, or other enzyme kinetic issues.

In another study, nelfinavir mesylate was incubated with a mixed pool of human liver microsomes (pooled from 15 males and 17 females) in the presence of **specific chemical inhibitors of cytochrome P450 isoforms.** The typical incubation mixture consisted of pooled human liver microsomes (2.5 mg protein/mL), 2 mM NADPH, and the specific cytochrome P450 inhibitors in 100 mM potassium phosphate buffer (pH 7.4) in a final volume of 0.500 mL. Inhibitor concentrations were chosen to be specific for each cytochrome P450 isoform and in the final incubation mixture were as follows: 100 μM troleandomycin (CYP3A4), 25 μM furafylline (CYP1A2), 50 μM diethyldithiocarbamate (CYP2E1), 5 μM quinidine (CYP2D6), 5 μM sulfaphenazole (CYP2C9), and 250 μM S-mephenytoin (CYP2C19). Reactions were initiated by the addition of nelfinavir mesylate at a final concentration of 10 μg/mL.

Inhibition of Nelfinavir Metabolism by Pooled Human Liver Microsomes in the Presence of Specific Cytochrome P450 Inhibitors

Incubation Conditions	%Nelfinavir Metabolism	% Inhibition Relative to Control Incubations
microsomes, -NADPH	9.42	----
microsomes, +NADPH	50.10±7.34	0
microsomes, +NADPH +troleandomycin (CYP3A4)	24.27±6.99	52
microsomes, +NADPH +furafylline (CYP1A2)	43.68	13
microsomes, +NADPH +diethyldithiocarbamate (CYP2E1)	37.94±4.55	24
microsomes, +NADPH +quinidine (CYP2D6)	39.47±9.18	21
microsomes, +NADPH +sulfaphenazole (CYP2C9)	41.06±10.60	18
microsomes, +NADPH +S-mephenytoin (CYP2C19)	33.75±11.20	33
microsomes, +NADPH +all above inhibitors	12.37±1.54	75

None of the specific inhibitors were able to inhibit nelfinavir metabolism completely. The results suggest that although the CYP3A4 isoform may be the predominant contributor to NADPH-dependent nelfinavir metabolism, other isoforms are also involved and the inhibition of one or more of the isoforms in vivo may not completely block NADPH-dependent nelfinavir metabolism.

Metabolite Activity:

The applicant determined the antiviral activity of several nelfinavir metabolites in cell protection assays utilizing HIV strains RF and IIIB. Antiviral activity comparable to that observed for nelfinavir was detected for the metabolite M8. Five- to 10-fold and 40 to 80-fold reductions in antiviral activity relative to nelfinavir were determined for the metabolites M1 and M11, respectively. No (or insignificant) antiviral activity was detected for M10.

Effect of Nelfinavir on Metabolism: Preclinical Results

In vitro studies with human liver microsomes and various P450 probe substrates were conducted to evaluate the ability of nelfinavir mesylate to inhibit the activity of specific cytochrome P450 isoforms. Ten individual human liver microsomes with P450 content greater than 0.3 nmol/mg were pooled together based on equivalent milligrams of protein. The probe substrates used and their concentrations, as well as the concentrations of nelfinavir, are summarized in the following table.

Isoform	Probe substrate (concentration)	Nelfinavir concentration (screening)	Nelfinavir concentrations (Ki determination)
CYP3A4	testosterone (40, 80, and 200 μM)	0.35 to 10 μM	0.35, 1, 2.5, 4.5, 7, 10 μM
CYP2C19	S-mephenytoin (40, 80, and 200 μM)	1 to 40 μM	1, 10, 25, 45, 70, 100 μM
CYP2D6	dextromethorphan (4, 8, and 20 μM)	1 to 40 μM	1, 2.5, 5, 10, 20, 40 μM
CYP2C9	tolbutamide (40, 80, and 200 μM)	10 μM	not determined
CYP1A2	phenacetin (40, 80, and 200 μM)	1 to 100 μM	1, 10, 25, 45, 70, 100 μM
CYP2E1	chlorzoxazone (20, 60, and 120 μM)	1.5, 5, 15 μM	not determined

Preliminary inhibition screening experiments showed that nelfinavir inhibited CYP3A4, CYP2C19, CYP2D6, and CYP1A2 activity in human liver microsomes, but did not inhibit either CYP2C9 or CYP2E1 activity.

Summary of Ki values for Nelfinavir Mesylate Inhibition of Various Cytochromes P450

Probe substrate	Isoform	Nelfinavir Ki (μM)
testosterone	CYP3A4	4.8
S-mephenytoin	CYP2C19	68
dextromethorphan	CYP2D6	126
phenacetin	CYP1A2	190
tolbutamide	CYP2C9	n.d.
chlorzoxazone	CYP2E1	n.d.

The in vitro Ki values, with the exception of CYP3A4, are much greater than the Cmax measured for nelfinavir in human plasma at therapeutic doses. The fact that nelfinavir is highly protein bound should also be considered.

Inhibitory Potency of Several Nelfinavir Metabolites:
Because M1 and M8 were present in human plasma and M3 is obligatory in the formation of M1, it was of interest to the applicant to investigate whether these metabolites could inhibit CYP3A4 activity in human liver microsomes. Testosterone (40, 80, 200 μM) was used as the CYP3A4 probe substrate. In the concentration range of 0.1 to 1.0 μM, M1 was found to not inhibit 6β-hydroxylase activity in human liver microsomes. M3 at concentrations of 0.5 to 5 μM was not inhibitory. In contrast, M8 (1 to 25 μM) was observed to inhibit CYP3A4 activity, with a Ki of 4.35 μM.

In Vivo Inhibition of CYP3A by Nelfinavir
The in vivo inhibition of CYP3A by nelfinavir was confirmed in drug interaction studies. (See the results of interaction studies with terfenadine and rifabutin.)

SPECIAL POPULATIONS

Hepatic Impairment: The pharmacokinetics of nelfinavir have not been studied in patients with hepatic insufficiency.

Renal Impairment: The pharmacokinetics of nelfinavir have not been studied in patients with renal insufficiency. Less than 2% of nelfinavir is excreted in the urine, so the impact of renal impairment on renal elimination should be minimal.

Gender: A formal study of nelfinavir pharmacokinetics in male vs. female subjects was not performed. The applicant performed a cross-study comparison by gender for trough concentrations in healthy volunteers receiving 750 mg every 8 hours in Studies 521 (11 males, 1 female), 526 (12 females), and 529 (6 males). In those studies, the morning nelfinavir trough concentrations were similar for males (2.42 ± 0.85 μg/mL) and females (2.17 ± 0.88 μg/mL). The applicants states these results indicate that profound differences in pharmacokinetics of nelfinavir between males and females are not likely. However, the relevance of trough concentrations has not been established.

A comparison of the pharmacokinetics (male vs. female) in HIV positive patients participating in Studies 503, 509, and 510 will be made once the demographic data from studies 503 and 510 are received from the applicant.

Race: Pharmacokinetic differences due to race have not been evaluated.

Elderly Patients: The pharmacokinetics of nelfinavir have not been studied in subjects >65 years of age.

Pediatric Patients

Study 524 is an on-going, Phase I, open-label study of pediatric powder and tablet formulations of nelfinavir in pediatric patients infected with HIV-1. This study is designed to allow 16 to 24 patients to be enrolled, with at least four evaluable patients in each of four different age groups:

Group I: 7 to 13 years
Group II: 2 to <7 years
Group III: 3 months to <2 years
Group IV: <3 months.

The study is being conducted in two phases, consisting of a single-dose phase and a multiple dose phase, plus an optional 6 month extension.

The primary objectives of the single-dose phase were to evaluate the single dose pharmacokinetic profile of nelfinavir pediatric powder formulation and to compare the relative bioavailability of the pediatric powder formulation to a standard tablet formulation in Group I children (7 to 13 years of age). The enrollment of the single-dose phase was step-wise, with Groups I and II enrolling first. When an optimal dose of the pediatric formulation was identified for Groups I and II, Group III could enroll; when an optimal dose was found for Group III, Group IV could enroll. Target plasma concentration (AUC) of 50% to 200% of median adult concentrations following a 750-mg single dose of the tablet formulation is used to estimate the optimal dose of the pediatric formulation. The initial dose of nelfinavir was 10 mg/kg; dose escalations proceeded until the optimal dose in an age group was determined. Blood samples were collected for 16 hours following the dose

for analysis of nelfinavir concentrations in plasma. Patients in Group I also participated in a relative bioavailability sub-study of the pediatric formulation versus the standard 250-mg tablet formulation. These patients received two single doses of nelfinavir administered 48 hours to 2 weeks apart. One dose was the pediatric powder formulation and the other was the tablet. The order of administration was not randomized.

The multiple dose phase commenced after successful completion of the single-dose pharmacokinetic phase for each respective group. Some patients entered directly into the multiple dose phase of the study. The primary objectives of the multiple dose phase are (1) to evaluate the safety, tolerability, and pharmacokinetics of nelfinavir administered three times daily in combination with reverse transcriptase inhibitor therapy over a 6-week primary observation period and (2) to acquire experience with the antiviral and immunologic activity and the durability of response over the full treatment period of multiple doses of nelfinavir administered three times daily to HIV-infected children and HIV-infected or exposed infants who are also receiving antiretroviral therapy with reverse transcriptase inhibitors. The single dose determined to be optimal for a group is administered to patients in that group three times daily for a primary observation period of 42 days. The doses can be modified during the multiple-dose phase based on trough plasma concentrations, toxicities, and decreases in plasma HIV RNA. Patients may receive either the pediatric powder or tablet formulations for the multiple-dosing period.

All patients were to receive nelfinavir in combination with antiretroviral nucleoside reverse transcriptase inhibitors. The pediatric powder was taken with milk, formula, pudding, or water; the tablet was taken with a light meal. The pharmacokinetic parameters obtained in the pediatric patients were compared to those obtained in adults in studies 503, 509, and 510. The parameters for adults were recalculated to use only the sampling times used in the pediatric patients.

At the time of the pharmacokinetic data cutoff for the interim report (01-09-97), data were available for patients in Group I (N=6), Group II (N=11), and Group III (N=3). The data for groups I and II consisted of all single-dose assessments (including comparisons of oral powder and tablet formulations in Group I) and the multiple dose evaluation on Day 14. The data for Group III patients included one single-dose assessment for each patient.

Pediatric Single Dose Pharmacokinetics Versus Adults (Arithmetic Mean ± SD)

Age	N	Formulation	Nominal Dose	AUC_{0-8} (μg*hr/mL)	Cmax (μg/mL)	Tmax (hr)
7-13 yr	5	Tablets	10 mg/kg	5.20 ± 2.07	1.31 ± 0.50	3.2 ± 1.1
	6	Tablets	20 mg/kg	8.98 ± 6.15	1.73 ± 1.06	4.3 ± 1.5
2-7 yr	5	Powder	10 mg/kg	5.08 ± 1.92	1.10 ± 0.35	5.6 ± 0.9
	8	Powder	20 mg/kg	16.57 ± 5.81	3.45 ± 1.27	4.8 ± 2.1
	6	Powder	30 mg/kg	19.11 ± 6.01	3.77 ± 1.13	4.0 ± 0.0
Adults	30	Tablets	500 mg	10.62 ± 5.27	2.06 ± 1.04	4.0 ± 1.6
	41	Tablets	750 mg	15.73 ± 6.02	2.87 ± 1.09	4.1 ± 1.5

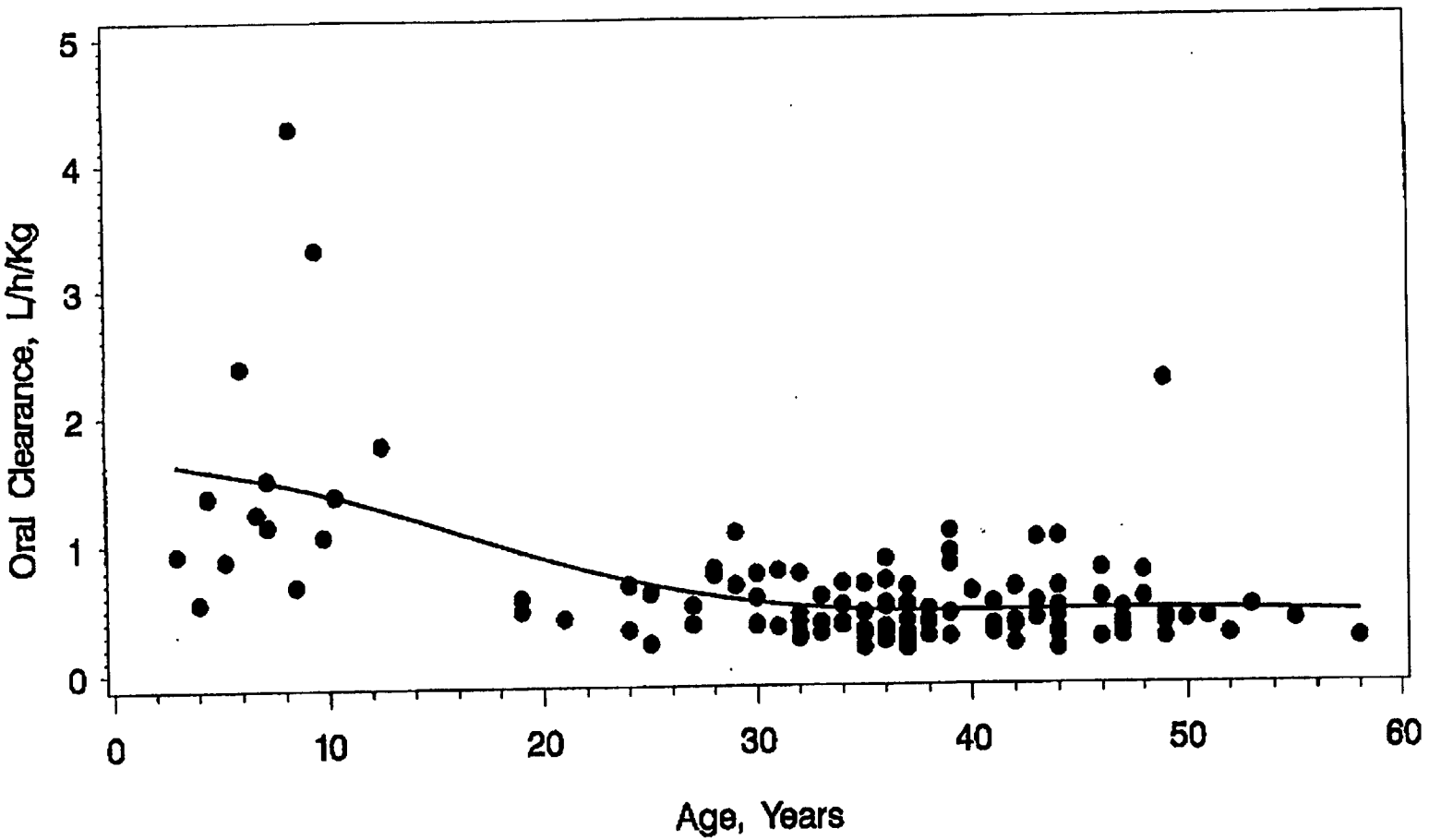
Based on the single-dose data in Groups 1 and 2, a dose of 20 mg/kg tid was selected for initiation of the multiple dose phase of this ongoing study. The multiple dose pharmacokinetic data collected to date are summarized in the following table. Adults and Group 1 received tablets; 7 of 8 Group 2 (age 2-7) patients received the powder and 1 Group 2 patients received tablets.

Pediatric Multiple Dose Pharmacokinetics Versus Adults (Arithmetic Mean ± SD)

Age	N	Dose (tid)	AUC_{0-8} (μg*hr/mL)	Cmax (μg/mL)	Tmax (hr)	Cmin (μg/mL)	CL/F (L/hr)	CL/F (L/hr/kg)
7-13 yr	6	20 mg/kg	16.7 ± 12.5	2.69 ± 1.63	3.3 ± 1.6	1.74 ± 1.42	67.0 ± 41.8	2.08 ± 1.41
2-7 yr	8	20 mg/kg	20.2 ± 8.2	4.03 ± 1.33	3.8 ± 2.0	1.68 ± 1.08	22.3 ± 8.8	1.25 ± 0.55
Adults	19	500 mg	17.4 ± 5.7	2.85 ± 0.80	3.6 ± 1.3	1.50 ± 0.76	33.4 ± 17.2	0.48 ± 0.25
	30	750 mg	18.5 ± 7.6	3.16 ± 1.21	3.0 ± 1.1	1.50 ± 0.82	50.1 ± 29.8	0.72 ± 0.43

These results indicate that after two weeks of treatment with 20 mg/kg tid, nelfinavir plasma concentrations in the Group 1 (age 7-13 yr) and Group 2 (age 2-7 yr) were similar to those in adult patients from Phase II studies who received doses of 500-750 mg tid. Following single or multiple doses of nelfinavir, oral clearance in pediatric patients was approximately double that observed in adults. The safety and efficacy portion of this study is continuing, with patients receiving 20 mg/kg nelfinavir tid.

Weight Adjusted Oral Clearance versus Age
Studies 503, 510, 520, 521, 523, 524



Lines are spline fits to show the trend in the data.

DRUG INTERACTIONS

Reverse Transcriptase Inhibitors

Zidovudine (ZDV) and Lamivudine (3TC):
The objectives of study 509 were to (1) determine the pharmacokinetic parameters for nelfinavir, ZDV, and 3TC on Day 1 and Day 28 of combination treatment and (2) obtain pilot data that would indicate the presence or absence of a drug interaction between nelfinavir and ZDV or 3TC. Twelve antiretroviral naive patients (11 male, 1 female) with plasma HIV-1 RNA values >10,000 copies/mL participated in this open label, single arm study. All patients received the following triple drug therapy:

Nelfinavir 750 mg tid
Zidovudine 200 mg tid
Lamivudine 200 mg bid

The drugs were administered with food. The protocol specified that pharmacokinetic evaluations be performed for all three drugs following the first dose of the day on Day 1 and Day 28. However, pharmacokinetic evaluations were performed in only 6 patients on Day 1 and all 12 patients on Day 28.

This study was not optimally designed to evaluate the pharmacokinetic profile of nelfinavir because the sampling period on Day 1 was only 8 hours. The peak concentrations of nelfinavir did not occur until 4 to 6 hours in some patients, so determination of the elimination rate constant and half-life was not possible. Also, no pharmacokinetic determinations were made when nelfinavir was not coadministered with ZDV/3TC. Within these limitations, nelfinavir pharmacokinetics do not appear to be altered by coadministration of ZDV/3TC; nelfinavir pharmacokinetic parameters were similar to those observed in other studies. The pharmacokinetics of 3TC also do not appear to be altered by coadministration of nelfinavir. The AUC and Cmax for ZDV were decreased by 35% and 46%, respectively, on chronic dosing with nelfinavir (Day 28 vs. Day 1). These results indicate that nelfinavir may induce zidovudine glucuronidation.

Due to the results of Study 510, a formal drug interaction study in healthy volunteers (Study 540) was conducted. Preliminary results of Study 540 have been submitted. The objectives of Study 540 were (1) to determine the effect of multiple doses of nelfinavir on the single dose pharmacokinetics of concurrently administered ZDV and 3TC and (2) to evaluate the multiple dose pharmacokinetics of nelfinavir alone and concurrently with a single dose of ZDV plus 3TC. Eleven healthy volunteers completed this randomized two-way crossover study. Each subject received the following two treatments:

Treatment A: Single dose of 3TC (150 mg) plus ZDV (200 mg). Serial blood samples were collected for 3TC and ZDV. Urine was collected for ZDV and ZDV glucuronide.

Treatment B: Nelfinavir 750 mg q8hr for 10 days. On Days 6 and 10, subjects were given either no additional drug or a single dose of 3TC (150 mg) plus ZDV (200 mg) in a randomized manner. Serial blood samples for nelfinavir were collected on Days 6 and 10; serial blood samples for ZDV and 3TC were collected on Day 6 or 10. Urine was collected for ZDV and ZDV glucuronide on Day 6 or 10.

The following tables summarize the preliminary data that have been submitted by the applicant to date.

Nelfinavir Pharmacokinetic Parameters (Arithmetic Mean ± SD, n = 11)

Parameter	Nelfinavir alone	Nelfinavir + ZDV/3TC	Geometric mean ratio (90% CI)
AUCT (μg*hr/mL)	23.1 ± 7.04	21.8 ± 6.28	0.95 (0.84, 1.07)
Cmax (μg/mL)	3.70 ± 0.88	3.72 ± 1.06	1.00 (0.89, 1.12)
Tmax (hr)	2.91 ± 1.02	3.27 ± 1.42	ND
CL/F (L/hr)	34.7 ± 8.38	37.0 ± 10.0	1.06 (0.94, 1.19)

3TC Pharmacokinetic Parameters (Arithmetic Mean ± SD, n = 11)

Parameter	3TC/ZDV alone	3TC/ZDV + Nelfinavir	Geometric mean ratio (90% CI)
AUC∞ (ng*hr/mL)	5689 ± 926	6231 ± 745	1.10 (1.02, 1.18)
Cmax (ng/mL)	1089 ± 313	1456 ± 534	1.31 (1.09, 1.56)
Tmax (hr)	1.82 ± 0.64	1.64 ± 0.90	ND
CL/F (L/hr)	27.1 ± 5.20	24.4 ± 2.81	0.91 (0.85, 0.98)
T½ (hr)	9.66 ± 2.36	9.22 ± 2.30	ND

ZDV Pharmacokinetic Parameters (Arithmetic Mean ± SD, n = 11)

Parameter	ZDV/3TC alone	ZDV/3TC + Nelfinavir	Geometric mean ratio (90% CI)
AUC∞ (ng*hr/mL)	1222 ± 257	795 ± 129	0.65 (0.60, 0.71)
Cmax (ng/mL)	512 ± 156	353 ± 128	0.69 (0.54, 0.87)
Tmax (hr)	1.55 ± 0.82	1.55 ± 0.65	ND
CL/F (L/hr)	176 ± 35.8	269 ± 41.1	1.55 (1.42, 1.69)
T½ (hr)	1.12 ± 0.17	1.07 ± 0.25	ND

There was no change in nelfinavir pharmacokinetics when coadministered with a single dose of ZDV/3TC. Statistically significant increases in 3TC AUC ($p = 0.0441$) and Cmax ($p = 0.0287$) and a statistically significant decrease in CL/F ($p = 0.0441$) were observed when single dose 3TC/ZDV was coadministered with multiple dose nelfinavir. These changes were not considered clinically significant. As observed in the previous study, there was a statistically significant decrease in ZDV AUC ($p = 0.0001$) and Cmax ($p = 0.0233$) and a statistically significant increase in CL/F ($p = 0.0001$) when ZDV/3TC was coadministered with multiple dose nelfinavir. Urinary data have not been submitted, yet; thus it is not possible to determine whether the amount of ZDV glucuronide excreted increased.

The applicant states that the observed decrease in ZDV concentrations during concomitant nelfinavir administration is not clinically significant because the efficacy of the nelfinavir/ZDV/3TC combination was established in study 511 (pivotal clinical trial). However, the impact of the interaction when nelfinavir and ZDV are administered without 3TC has not been considered. A previous drug interaction study between ZDV and 3TC indicated that 3TC increased ZDV concentrations. The applicant also states that the relationship between ZDV plasma concentrations and efficacy are uncertain because ZDV is a prodrug that is activated intracellularly. The clinical relevance of this interaction is being discussed with the reviewing Medical Officer.

Stavudine

The pharmacokinetic objectives of Study 510 were to evaluate (1) the single and multiple dose pharmacokinetics of nelfinavir at different dose levels and (2) the single and multiple dose pharmacokinetics of stavudine (d4T) alone and when coadministered with nelfinavir. This pilot four-arm open-label study was designed to evaluate the safety and efficacy of three different doses of nelfinavir administered in combination with d4T versus d4T alone in cohorts of at least 5 d4T-naive HIV positive patients. After a two-week washout period during which all HIV antiretroviral treatments and prophylactic therapies for opportunistic infections (except for prophylaxis of *pneumocystis carnii* pneumonia) were to be discontinued, patients were randomized to one of four treatments: d4T plus either 500 mg, 750 mg, or 1000 mg nelfinavir every eight hours (with food) or d4T alone. Patients weighing <60 kg took 30 mg d4T bid and patients weighing >60 kg took 40 mg d4T bid. For combination therapy, d4T was administered with the morning and evening doses of nelfinavir. When administered alone, d4T was administered every 12 hours. All study drugs were administered with food. Blood samples for pharmacokinetic profiles (nelfinavir and d4T) were collected over 8 hours on Day 0 (1st dose) and over 12 hours on Day 56 (only one dose administered on Day 56; 24 hr and 30 hr samples collected if possible).

Nelfinavir Pharmacokinetic Parameters
(Arithmetic Mean ± SD)

Parameter	Day 0			Day 56		
	500 mg tid	750 mg tid	1000 mg tid	500 mg tid	750 mg tid	1000 mg tid
N	10	10	10	9	8	6
AUCT (μg*hr/mL)	12.5±6.3	14.2±4.7	14.2±7.5	18.8±5.2	18.6±6.2	20.2±7.1
Cmax (μg/mL)	2.92±2.32	2.82±1.02	3.07±1.41	3.32±0.81	3.36±1.01	3.54±1.25
Tmax (hr)	3.9±1.7	3.2±1.3	3.4±2.1	3.2±1.3	2.4±0.7	2.4±0.7
CL/F (L/hr)	ND	ND	ND	28.7±8.5	44.1±13.4	53.9±15.3

Visual inspection of nelfinavir concentration vs. time profiles indicated that oral absorption continued for most of the eight hour dosing interval in a majority of patients, so it was not possible to obtain reasonable estimates of the elimination rate constant. No change in mean AUC was observed with increasing dose in this study. The same range of nelfinavir doses was administered in a monotherapy study (503), and AUC values in that study increased in proportion to dose. The rationale for the decreasing oral clearance with increasing dose was not provided. The applicant suggested that the small range of doses and the small sample size may have contributed to this observation.

This study was not designed to assess potential changes in nelfinavir pharmacokinetics due to coadministration of d4T. However, the similarity of oral clearance values in this study to those in other studies suggests that d4T does not significantly alter nelfinavir pharmacokinetics.

Stavudine Day 56 Pharmacokinetic Parameters (Arithmetic Mean ± SD)

Parameter	d4T	d4T +500 mg NFV	d4T +750 mg NFV	d4T +1000 mg NFV
N	7	8	8	6
AUCτ (μg*hr/mL)	1.35±0.31	1.45±0.30	1.57±0.22	1.38±0.36
Cmax (μg/mL)	0.47±0.15	0.52±0.18	0.57±0.25	0.46±0.08
T½ (hr)	1.37±0.21	1.31±0.84	1.41±0.15	1.45±0.23
CL/F (L/hr)	33.5±11.1	31.2±7.3	27.3±4.1	33.5±11.4

The d4T pharmacokinetics observed in this study are consistent with those previously reported. No significant differences in oral clearance were observed between the different treatment arms. Thus, coadministration of nelfinavir does not appear to alter the pharmacokinetics of d4T.

Didanosine

Study 543 was a pilot, single arm, open label study designed to determine the safety and antiviral activity of the triple combination of d4T + ddI + nelfinavir in HIV-infected subjects who are d4T-, ddI-, and protease inhibitor naive and who have ≥10,000 HIV RNA copies/mL. One objective of this study was to assess the effect of the ddI buffered formulation on the pharmacokinetics of nelfinavir in a subset of subjects (first 10 subjects enrolled in the study who agreed to participate in the pharmacokinetics sub-study). Subjects in the pharmacokinetic substudy received nelfinavir 750 mg with a light meal on day -1 and nelfinavir 750 mg with food one hour after a 200 mg dose of ddI on day 1. On each day, plasma samples for nelfinavir pharmacokinetic profile were collected over eight hours.

Nelfinavir Pharmacokinetic Parameters (Arithmetic Mean ± SD, n = 10)

Parameter	Nelfinavir alone	Nelfinavir +ddI	Combination/monotherapy ratio mean ± SD (range)
AUC8 (μg*hr/mL)	15.43±8.00	17.25±7.91	1.17±0.23 (0.86-1.57)
Cmax (μg/mL)	3.22±1.50	3.37±1.22	1.09±0.21 (0.83-1.46)
Tmax (hr)	4.10±0.74	4.50±0.71	*0.40±0.70 (-1.00 to 1.00)

*Difference is reported for Tmax

A summary report was provided for this study sponsored

The actual concentration data have not been provided. Thus, it is not possible to determine whether there was carryover from the Day -1 dose of nelfinavir to Day 1. Nonetheless, it does not appear that the pharmacokinetics of nelfinavir are altered when administered one hour after a dose of ddI. It is recommended that ddI be administered on an empty stomach; therefore, nelfinavir should be administered (with food) one hour after or two hours before ddI.

CYP3A Interaction Studies

Terfenadine:
Twelve healthy male volunteers entered and completed this one sequence (1 x 2) crossover study (Study 519). The objective of this study was to determine the effect of multiple doses of nelfinavir on the pharmacokinetics of the carboxylate metabolite of terfenadine following a single oral dose of terfenadine and to determine if nelfinavir increases plasma concentrations of unchanged terfenadine. The study design was as follows:

Day 1: 60 mg dose of terfenadine administered 10 minutes after completion of a standardized breakfast. Plasma concentrations collected over 72 hours for determination of terfenadine carboxylate and unchanged terfenadine.
Days 6-12: 750 mg (3 x 250 mg) nelfinavir administered every 8 hours, within 10 minutes after eating a light meal or snack.
Day 10: 60 mg dose of terfenadine administered 10 minutes after completion of a standardized breakfast. Plasma concentrations collected over 72 hours for determination of terfenadine carboxylate and unchanged terfenadine.

Plasma concentrations of unchanged terfenadine were below the lower limit of quantitation (<5.00 ng/mL) in all 12 subjects after treatment with terfenadine in the absence of nelfinavir. When terfenadine was administered during nelfinavir treatment, plasma concentrations were at least transiently measurable in all 12 subjects. C_{max} was 10.1 ± 3.3 ng/mL (range: 5.5 to 15.3 ng/mL) and t_{max} ranged from 2 to 6 hours. Due to the low and often transient nature of the plasma concentration (quantifiable concentrations in 1 to 9 samples per subject), no other pharmacokinetic parameters were calculated for unchanged terfenadine. When administered alone to subjects with normal hepatic function, terfenadine is eliminated almost entirely by CYP3A mediated first pass metabolism, in part to the pharmacologically active metabolite terfenadine carboxylate. The results of this study indicate that nelfinavir inhibited terfenadine first-pass metabolism. Another indication of decreased terfenadine first-pass metabolism is the decreased C_{max} and delayed t_{max} observed for the metabolite terfenadine carboxylate in the presence of nelfinavir.

Terfenadine carboxylate pharmacokinetic parameters (Arithmetic mean ± SD, n = 12)

Terfenadine carboxylate parameter	Terfenadine alone	Terfenadine + Nelfinavir	Combination/monotherapy ratio	
			Geometric mean ratio	mean ± SD (range)
AUC (ng*hr/mL)	1153 ± 382	1625 ± 328	1.46	1.50 ± 0.39 (1.39-2.10)
Cmax (ng/mL)	163 ± 46	92 ± 29	0.55	0.57 ± 0.15 (0.37-0.87)
tmax (hr)	2.96 ± 0.78	5.00 ± 1.04	ND	*2.04 ± 1.27 (0.00-3.50)
t½ (hr)	4.43 ± 1.21	27.0 ± 7.24	ND	6.56 ± 2.52 (2.82-10.19)

*For Tmax, difference is reported rather than ratio

The results of this study also indicate that nelfinavir inhibited further metabolism of terfenadine carboxylate. The increases in AUC and t½, the decrease in Cmax, and the delay in Tmax were all statistically significant. It is notable that although the half-life was markedly prolonged, AUC_∞ only increased 50%. It appears that the reduction in the systemic clearance of the metabolite (CL_m) is offset by the increase in the fraction of

terfenadine that is biotransformed to the carboxylate (f_m), resulting in a modest effect on the terfenadine carboxylate AUC_∞. The inhibition of terfenadine clearance by nelfinavir is consistent with in vitro data showing that clinically relevant concentration of nelfinavir inhibit CYP3A.

Average QTC intervals were not statistically different between terfenadine single doses at predose, 24 hr, 36 hr, and 72 hr postdose ($p > 0.05$), but were significantly prolonged by 11 to 16 msec in the presence of nelfinavir at 4 hr, 8 hr, 12 hr, and 48 hr after terfenadine administration ($p < 0.02$).

Because accumulation of unchanged terfenadine has been associated with potentially lethal ventricular arrhythmias, the inhibition of terfenadine metabolism and modest prolongation of QTC intervals after concomitant administration of terfenadine and nelfinavir are clinically significant. Nelfinavir should not be administered concurrently with terfenadine.

Ketoconazole

The objective of study 520 was to determine the effect of multiple doses of ketoconazole on the pharmacokinetics of nelfinavir. Twelve healthy volunteers (8 males, 4 females) completed this open-label, randomized, two-way crossover study. Volunteers received the following two treatments in a randomized order:

Treatment A
- Days 1-5: 500 mg nelfinavir administered every 8 hours
- Day 6: Single 500 mg dose of nelfinavir; serial plasma samples collected over 48 hrs

Treatment B:
- Days 1-5: 500 mg nelfinavir administered every 8 hours plus 400 mg ketoconazole once daily
- Day 6: Single 500 mg dose nelfinavir + 400 mg ketoconazole serial plasma samples for nelfinavir concentration collected over 48 hrs
- Day 7: Single 400 mg dose of ketoconazole (48 hr collection of plasma for nelfinavir concentration continued)

All doses of nelfinavir and ketoconazole were given 10 minutes after eating. Doses of ketoconazole were administered concomitantly with morning doses of nelfinavir. There was a 14 day washout between treatments. In addition to the serial plasma samples collected beginning on Day 6 of each treatment period, single nelfinavir trough samples were collected before the morning dose on Days 1 to 6 of each treatment period.

Nelfinavir Pharmacokinetic Parameters (Arithmetic Mean ± SD, n = 12)

Parameter	Nelfinavir alone	Nelfinavir + Ketoconazole	Combination/monotherapy ratio	
			Geometric mean ratio	mean ± SD (range)
AUC_T (μg*hr/mL)	19.0 ± 5.62	25.3 ± 6.27	1.35	1.36 ± 0.23 (1.10-1.84)
Cmax (μg/mL)	3.40 ± 0.96	4.17 ± 0.77	1.25	1.28 ± 0.27 (0.91-1.79)
Tmax (hr)	3.17 ± 0.94	3.00 ± 0.74	ND	*-0.17 ± 0.58 (-1.0 to +1.0)
CL/F (L/hr)	28.3 ± 7.46	20.9 ± 5.25	0.74	0.75 ± 0.12 (0.54-0.91)
$T_{½}$ (hr)	3.27 ± 1.14	6.86 ± 3.21	ND	2.36 ± 1.34 (0.47-4.77)

*For Tmax, difference is reported rather than ratio

When nelfinavir was coadministered with ketoconazole, nelfinavir AUC, Cmax, and T½ were statistically significantly increased and CL/F was statistically significantly decreased. There was no change in Tmax.

Ketoconazole, at its usual clinical doses (up to 400 mg per day), is noted for its ability to inhibit metabolism via CYP3A4. For example, concomitant administration of ketoconazole increases the AUC of saquinavir by 3-fold and increases the AUC of indinavir by 68%. The more modest inhibitory effect of ketoconazole on nelfinavir (AUC increased by 36%) indicates that pathways other than CYP3A4 probably contribute to the elimination of nelfinavir. These results suggest that selective inhibitors of CYP3A4 of similar or lesser potency compared to ketoconazole, such as macrolide antibiotics and other azole antifungals, are not likely to have a clinically significant impact on nelfinavir pharmacokinetics.

Rifampin

The objective of Study 521 was to determine the effect of multiple doses of rifampin on the pharmacokinetics of nelfinavir following the final dose of a multiple dose regimen. Twelve healthy volunteers (11 males, 1 female) completed this open-label, two-way crossover study. Subjects received the following two treatments in a randomized order:

Treatment A

Days 1-5: 750 mg nelfinavir administered every 8 hrs
Day 6: Single 750 mg dose of nelfinavir; serial plasma samples collected over 48 hrs

Treatment B:

Days 1-5: 750 mg nelfinavir administered every 8 hrs
plus 600 mg rifampin once daily (1 hr prior to morning nelfinavir dose)
Day 6: Single 600 mg dose of rifampin, followed 1 hr later by single 750 mg dose of nelfinavir; serial plasma samples for nelfinavir collected over 48 hrs
Day 7: 600 mg dose of rifampin (48 hr collection of plasma for nelfinavir concentration continued)

Nelfinavir was administered as three 250 mg tablets; rifampin was administered as two 300 mg RIFADIN capsules. All doses of nelfinavir were given 10 minutes after eating, and all doses of rifampin were given without food. There was a 14 day washout between treatments. Nelfinavir trough samples were collected before the morning dose on Days 1 to 6 of each treatment period.

Nelfinavir Pharmacokinetic Parameters (Arithmetic Mean ± SD, n = 12)

Parameter	Nelfinavir alone	Nelfinavir + Rifampin	Combination/monotherapy ratio	
			Geometric mean ratio	mean ± SD (range)
AUCT (μg*hr/mL)	21.0 ± 6.95	3.72 ± 1.15	0.18	0.19 ± 0.07 (0.10-0.36)
Cmax (μg/mL)	3.65 ± 1.04	0.95 ± 0.60	0.24	0.27 ± 0.16 (0.11-0.68)
Tmax (hr)	3.50 ± 0.90	3.33 ± 1.15	ND	*-0.17 ± 1.19 (-3.0 to +2.0)
CL/F (L/hr)	39.1 ± 11.9	219 ± 64.6	5.62	6.00 ± 2.32 (2.81-10.52)
T½ (hr)	2.88 ± 0.84	1.94 ± 1.14	ND	0.69 ± 0.32 (0.37-1.55)

*For Tmax, difference is reported rather than ratio

In the presence of rifampin, nelfinavir plasma concentrations were markedly decreased in all 12 study subjects. Nelfinavir AUC, Cmax, and t½ were statistically significantly decreased and CL/F was statistically significantly increased when nelfinavir was administered with rifampin. Tmax was not significantly altered. As the table above indicates, the change in t½ was more modest than the changes in AUC, Cmax, and CL/F. One possible explanation for this observation is that the interaction with rifampin reflects a large increase in first-pass intestinal metabolism of nelfinavir.

The results observed in this study are consistent with rifampin's ability to induce CYP3A4, an enzyme that contributes to nelfinavir metabolism. It is notable that daily nelfinavir trough concentrations were lower on all days when rifampin was coadministered as compared to when nelfinavir was administered alone. The mean ± SD trough concentrations for both treatments are compared in the following table:

Treatment	Day 2	Day 3	Day 4	Day 5	Day 6
Nelfinavir	3.89 ± 1.62	3.14 ± 1.22	2.59 ± 1.09	2.53 ± 1.29	2.07 ± 0.80
Nelfinavir + Rifampin	1.95 ± 1.16	0.58 ± 0.28	0.29 ± 0.08	0.20 ± 0.07	0.17 ± 0.07

These results suggest that the induction effect of rifampin had occurred to a notable degree after a single dose of rifampin. The results for the ratio of urinary 6β-hydroxycortisol to unchanged cortisol also confirm the inductive effect of rifampin. The baseline ratios did not differ significantly between the two treatments. On both the 3rd and 5th days of treatment, this urinary ratio was significantly higher ($p = 0.0001$) following concomitant treatment with rifampin versus nelfinavir alone.

Day	Nelfinavir	Nelfinavir + Rifampin
0-1	5.8 ± 1.6	5.6 ± 1.8 (n = 11)
3-4	3.7 ± 1.3	7.3 ± 2.6
5-6	4.1 ± 1.0 (n = 11)	11.8 ± 4.1

The interaction between nelfinavir and rifampin is clinically significant. The magnitude of the interaction indicates that patients receiving rifampin in combination with nelfinavir would experience a substantial lowering of nelfinavir plasma concentration, potentially resulting in a decrease or loss of nelfinavir efficacy. Nelfinavir and rifampin should not be administered together.

Rifabutin (Preliminary results)

The objectives of study 523 were to determine (1) the effect of a multiple dose treatment regimen of rifabutin on the pharmacokinetics of a multiple dose treatment regimen of nelfinavir and (2) the effect of a multiple dose treatment regimen of nelfinavir on the pharmacokinetics of a multiple dose treatment regimen of rifabutin. This was an open-label, three-way Latin square design, crossover study in 15 healthy volunteers. Data are available for 10 subjects. Each subject received:

Nelfinavir alone (750 mg q8hrs x 7 days, plus a single dose on day 8)
Rifabutin alone (300 mg daily x 8 days)
Concurrent dosing of nelfinavir and rifabutin at the above doses.

Nelfinavir was administered as three 250 mg tablets. Rifabutin was administered as two 150 mg capsules. There was a 13 or 14 day washout between dosing periods. Trough blood samples were collected on the fourth and sixth day of each dosing period, and serial

blood samples were collected for 24 hours after the last nelfinavir dose and after the seventh rifabutin dose of each dosing period.

Nelfinavir Pharmacokinetic Parameters (Arithmetic Mean ± SD, n = 10)

Parameter	Nelfinavir alone	Nelfinavir + Rifabutin	Geometric mean ratio (90% CI)
AUCT (μg*hr/mL)	22.9 ± 8.5	15.6 ± 5.55	0.68 (0.54, 0.87)
Cmax (μg/mL)	4.10 ± 1.29	3.12 ± 0.95	0.75 (0.63, 0.89)
Tmax (hr)	3.20 ± 0.92	2.90 ± 0.57	ND
T½ (hr)	2.51 ± 0.30	3.26 ± 2.21	ND
CL/F (L/hr)	37.2 ± 14.4	55.2 ± 23.7	1.46 (1.15, 1.86)

The reduction in nelfinavir AUC (p = 0.0319) and Cmax (p = 0.0281) were both statistically significant. The change in pharmacokinetics is consistent with reduced CYP3A induction potency of rifabutin relative to rifampin.

Rifabutin Pharmacokinetic Parameters (Arithmetic Mean ± SD, n = 10)

Parameter	Rifabutin alone	Rifabutin + Nelfinavir	Combination/monotherapy ratio mean ± SD (range)
AUC24 (ng*hr/mL)	4009 ± 1164	12067 ± 2817	3.20 ± 0.95 (1.99-4.69)
Cmax (ng/mL)	404 ± 121	975 ± 245	2.52 ± 0.60 (1.68-3.75)

The large increases in rifabutin AUC and Cmax when coadministered with nelfinavir are consistant with inhibition of rifabutin metabolism by nelfinavir. The pharmacokinetic changes observed in the present study are similar to those observed when indinavir was coadministered with rifabutin. The indinavir approved label recommends that the rifabutin dose be reduced by 50% when coadministered with indinavir. The same adjustment will be recommended in the nelfinavir label. The lower rifabutin dose may cause less induction of nelfinavir metabolism. As with indinavir, the effect of this dose adjustment should be confirmed with a clinical drug interaction study.

Ethinyl Estradiol and Norethindrone (Low Dose Contraceptive)

The objective of Study 526 was to determine the effect of multiple doses of nelfinavir on the pharmacokinetics of 17α-ethinyl estradiol and norethindrone being administered in accordance with a 28-day contraceptive dosing regimen. Twelve healthy female volunteers (age 24-49 years) participated in this open-label two-way crossover study. Subjects were required to have a documented history of tubal ligation or hysterectomy, and at least one intact ovary. All subjects received daily administration of a combination oral contraceptive (OVCON 35® tablets) containing 35 μg ethinyl estradiol (EE) plus 0.4 mg norethindrone (NET) for two full dosing cycles (21 days of active drug per 28-day cycle) and for the first 15 days of a third cycle. During either the second or third cycle, subjects also received nelfinavir 750 mg every 8 hours for 7 days (days 9-15 of cycle). The first monthly cycle with oral contraceptives was intended to regulate subjects' menstrual cycle prior to studying the interaction with nelfinavir in the second and third monthly cycles. Nelfinavir was administered within 10-15 minutes after eating. During the second and third cycles, blood samples were collected over 24 hours following the EE/NET dose on Day 15. Single predose samples were also drawn on Days 1, 9, and 12 of the second and third cycles for

assay of EE and NET. Single predose samples for assay of nelfinavir were collected on Days 14 and 15 of the second and third cycles.

Ethinyl Estradiol Pharmacokinetic Parameters (Arithmetic Mean ± SD, n = 12)

Parameter	EE/NET alone	EE/NET +Nelfinavir	Combination/monotherapy ratio	
			Geometric mean ratio (90% CI)	mean ± SD (range)
AUCT (pg*hr/mL)	974 ± 297	501 ± 105	0.53 (0.48, 0.58)	0.53 ± 0.09 (0.43-0.73)
Cmax (pg/mL)	91 ± 27	64 ± 16	0.72 (0.63, 0.84)	0.74 ± 0.20 (0.47-1.11)
Tmax (hr)	2.5 ± 1.2	1.9 ± 1.2	ND	*-0.58 ± 1.73 (-4.0 to +3.0)
CL/F (L/hr)	38.8 ± 10.8	72.5 ± 13.8	1.90 (1.74, 2.09)	1.93 ± 0.31 (1.37-2.30)
T½ (hr)	15.8 ± 3.3	11.0 ± 1.5	ND	0.72 ± 0.18 (0.45-1.01)

*For Tmax, difference is reported rather than ratio

Norethindrone Pharmacokinetic Parameters (Arithmetic Mean ± SD, n = 12)

Parameter	EE/NET alone	EE/NET +Nelfinavir	Combination/monotherapy ratio	
			Geometric mean ratio (90% CI)	mean ± SD (range)
AUCT (ng*hr/mL)	106 ± 26	85 ± 17	0.82 (0.77, 0.87)	0.82 ± 0.10
Cmax (ng/mL)	11.9 ± 3.8	11.4 ± 2.0	0.98 (0.86, 1.11)	1.01 ± 0.23 (0.66-1.29)
Tmax (hr)	2.1 ± 1.2	1.7 ± 1.1	ND	*-0.42 (-4.0 to +2.0)
CL/F (L/hr)	4.0 ± 1.2	4.9 ± 1.4	1.22 (1.15, 1.30)	1.24 ± 0.14 (1.01-1.41)
T½ (hr)	10.1 ± 2.3	7.2 ± 2.0	ND	0.71 ± 0.08 (0.53-0.83)

*For Tmax, difference is reported rather than ratio

Multiple doses of nelfinavir caused statistically significant decreases in the AUC values of ethinyl estradiol and norethindrone. For both ethinyl estradiol and norethindrone, the terminal elimination half-lives were somewhat shorter when multiple doses of nelfinavir were given concurrently.

The effects of nelfinavir on ethinyl estradiol and norethindrone are consistent with an inductive effect of nelfinavir on the enzymes metabolizing these agents. For ethinyl estradiol, which was affected to a greater extent, routes of elimination include oxidation by CYP3A and direct conjugation with glucuronic acid. Other clinical studies have indicated that nelfinavir is an inhibitor rather than an inducer of CYP3A. Thus, the inductive effect of nelfinavir in this case is more likely to involve glucuronidation and/or oxidation pathways other than CYP3A. A similar effect on the concentrations of ethinyl estradiol was observed when coadministered with ritonavir.

The extent of decrease in ethinyl estradiol concentrations in this study, combined with the smaller decrease in norethindrone concentrations, indicates that alternate or additional contraceptive measures should be used during nelfinavir treatment. It is notable that [illegible] with oral contraceptives was intended to regulate subjects' menstrual cycle prior to studying the interaction with nelfinavir in the second and third monthly cycles. Nelfinavir was administered within 10-15 minutes after eating. During the second and third cycles, blood samples were collected over 24 hours following the EE/NET dose on Day 15. Single predose samples were also drawn on Days 1, 9, and 12 of the second and third cycles for

Protease Inhibitors

Saquinavir

The objectives of Study 538 were to determine the effect of a multiple dose treatment regimen of saquinavir on the pharmacokinetics of nelfinavir administered as a single dose and to determine the effect of a multiple dose treatment regimen of nelfinavir on the pharmacokinetics of saquinavir administered as a single dose. Fourteen HIV-1 infected patients (13 males, 1 female) completed this open-label, randomized, two-way crossover study. Patients received the following two treatments in a randomized order:

Treatment A:

Day 1: 1200 mg dose of saquinavir (Plasma samples collected for 24 hrs.)
Days 2-4: 750 mg nelfinavir tid
Day 5: 750 mg nelfinavir tid plus 1200 mg dose of saquinavir
(Plasma samples for saquinavir collected for 24 hrs.)

Treatment B:

Day 1: 750 mg dose of nelfinavir (Plasma samples collected for 24 hrs.)
Days 2-4: 1200 mg saquinavir tid
Day 5: 1200 mg saquinavir tid plus 750 mg dose of nelfinavir
(Plasma samples for nelfinavir collected for 24 hrs.)

Nelfinavir was administered as three 250 mg tablets; saquinavir was administered as six 200 mg capsules (soft gelatin capsules). Each drug was given within 10 minutes after eating. There was a three to ten day washout between treatments.

Nelfinavir Pharmacokinetic Parameters (Arithmetic Mean ± SD, n = 14)

Parameter	Nelfinavir alone	Nelfinavir + Saquinavir	Combination/monotherapy ratio	
			Geometric mean ratio (90% CI)	mean ± SD (range)
AUC∞ (μg*hr/mL)	33.6 ± 9.0	39.5 ± 9.8	1.18 (1.07, 1.30)	1.20 ± 0.24 (0.82-1.58)
Cmax (μg/mL)	3.39 ± 0.53	3.57 ± 0.92	1.03 (0.93, 1.14)	1.05 ± 0.24 (0.65-1.60)
Tmax (hr)	4.88 ± 2.36	4.28 ± 1.46	ND	*-0.60 ± 2.33 (-6.27 to +2.05)
CL/F (L/hr)	23.9 ± 6.8	20.2 ± 5.5	0.85 (0.77, 0.94)	0.87 ± 0.19 (0.63-1.22)
T½ (hr)	4.53 ± 1.05	4.92 ± 1.33	ND	1.10 ± 0.22 (0.78-1.52)

*For Tmax, difference is reported rather than ratio

Saquinavir Pharmacokinetic Parameters (Arithmetic Mean ± SD, n = 14)

Parameter	Saquinavir alone	Saquinavir + Nelfinavir	Combination/monotherapy ratio	
			Geometric mean ratio (90% CI)	mean ± SD (range)
AUC∞ (ng*hr/mL)	3208 ± 971	16452 ± 7059	4.92 (3.91, 6.21)	5.39 ± 1.99 (1.61-8.12)
Cmax (ng/mL)	996 ± 424	2792 ± 1292	2.79 (2.17, 3.59)	3.14 ± 1.47 (0.90, 5.90)
Tmax (hr)	1.87 ± 0.78	3.04 ± 1.47	ND	*1.17 ± 1.04 (-0.13 to 3.06)
CL/F (L/hr)	408 ± 127	86.1 ± 36.7	0.20 (0.16, 0.26)	0.23 ± 0.15 (0.12-0.62)
T½ (hr)	10.8 ± 3.9	4.82 ± 0.96	ND	0.50 ± 0.21 (0.22-0.91)

*For Tmax, difference is reported rather than ratio

Saquinavir caused a statistically but not clinically significant inhibition of nelfinavir single-dose oral clearance ($p=0.0112$). Results of in vitro studies and the modest inhibitory effect of ketoconazole on the oral clearance of nelfinavir suggest that CYP3A contributes to but does not dominate nelfinavir elimination. In vitro studies have also indicated that saquinavir inhibits CYP3A. The results of the current study are consistent with partial inhibition of CYP3A by saquinavir.

Nelfinavir statistically and clinically significantly decreased the oral clearance of saquinavir ($p=0.0001$). Saquinavir undergoes extensive intestinal first-pass metabolism via CYP3A. The results of this study are consistent with inhibition of intestinal first pass metabolism. The less pronounced effect of nelfinavir versus ritonavir on saquinavir AUC (4-fold versus >20-fold increase) indicates that nelfinavir is less potent than ritonavir as a clinical inhibitor of CYP3A.

The observed terminal elimination half-life of saquinavir was shortened in the presence of nelfinavir (changed from 10.2 to 4.7 hours), which is opposite the expected effect for decreased oral clearance. One possible explanation for this observation is the fact that following administration of saquinavir alone, saquinavir elimination was bi-phasic and following administration with nelfinavir it was mono-phasic. As a result, the saquinavir terminal elimination rate constant was calculated using concentrations from approximately 12 to 24 hours when saquinavir was administered alone and from 6 or 8 to 24 hours when coadministered with nelfinavir. Also, the regression r^2 was greater when saquinavir was administered with nelfinavir ($r^2=0.98\pm0.01$) than when administered alone ($r^2=0.86\pm0.16$).

The bioanalytical results indicate that the predose concentration of nelfinavir was below the limit of quantification for all patients prior to the first dose of nelfinavir and for 10/14 patients prior to the second dose. When patients had received at least one prior dose of saquinavir during the study, predose saquinavir concentrations were measurable in 19/21 cases. The data suggest some degree of carryover from previous doses of saquinavir in this study.

As indicated, the increase in nelfinavir concentrations was modest and was not considered clinically significant. Saquinavir is known to have very low oral bioavailability (approximately 4% when administered with food). Drug interactions that increase saquinavir concentrations to the degree observed in this study are considered beneficial, so a dose adjustment is not warranted.

Indinavir

The objectives of Study 529 were to determine the effect of a multiple dose regimen of indinavir on the single dose pharmacokinetics of nelfinavir and to determine the effect of a multiple dose regimen of nelfinavir on the single dose pharmacokinetics of indinavir. Twelve healthy male volunteers completed this open-label parallel-group study. Subjects were assigned to receive either Treatment A ($n=6$) or Treatment B ($n=6$).

Treatment A:

Day 1: Single 750 mg dose of nelfinavir (Plasma samples collected for 48 hours)
Days 3-9: Indinavir 800 mg q8hr (Trough plasma samples collected Days 8 and 9)
Day 8: Single 750 mg dose of nelfinavir (Plasma samples collected for 48 hours)

Treatment B:

Day 1: Single 800 mg dose of indinavir (Plasma samples collected for 48 hours)
Days 3-9: Nelfinavir 750 q8hr (Trough plasma samples collected Days 8 and 9)
Day 8: Single 800 mg dose of indinavir (Plasma samples collected for 48 hours)

Nelfinavir was administered as 250 mg VIRACEPT tablets; indinavir was administered as 400 mg CRIXIVAN capsules. Nelfinavir was administered within 10 minutes after eating. Indinavir was administered 1 hour before or 2 hours after a meal. To reduce the risk for nephrolithiasis associated with indinavir, subjects were required to drink at least 84 ounces of fluid each day during indinavir treatment.

Nelfinavir Pharmacokinetic Parameters (Arithmetic Mean ± SD, n = 6)

Parameter	Nelfinavir alone	Nelfinavir + Indinavir	Combination/monotherapy ratio	
			Geometric mean ratio (90% CI)	mean ± SD (range)
AUC∞ (μg*hr/mL)	36.9 ± 23.9	66.0 ± 30.6	1.83 (1.42, 2.37)	1.99 ± 0.87 (1.21-3.24)
Cmax (μg/mL)	3.82 ± 0.86	4.96 ± 0.87	1.31 (1.16, 1.48)	1.33 ± 0.28 (0.99-1.74)
Tmax (hr)	4.67 ± 1.86	3.67 ± 1.03	ND	*-1.00 ± 1.26 (-3.00 to 0.00)
CL/F (L/hr)	25.2 ± 9.83	14.1 ± 7.30	0.55 (0.42, 0.70)	0.59 ± 0.24 (0.31-0.83)
T½ (hr)	4.87 ± 1.73	5.70 ± 0.81	ND	1.25 ± 0.31 (0.84-1.64)

*For Tmax, difference is reported rather than ratio

Following multiple doses of indinavir, there were statistically significant increases in nelfinavir AUC (p = 0.0197) and Cmax (p = 0.0268). Nelfinavir terminal T½ values were not significantly different between treatments (p = 0.1298). There was also no significant effect on Tmax. The increase in nelfinavir AUC was considered clinically significant.

Indinavir Pharmacokinetic Parameters (Arithmetic Mean ± SD, n = 6)

Parameter	Indinavir alone	Indinavir + Nelfinavir	Combination/monotherapy ratio	
			Geometric mean ratio (90% CI)	mean ± SD (range)
AUC∞ (μg*hr/mL)	19.1 ± 7.56	27.4 ± 5.02	1.51 (1.29, 1.77)	1.56 ± 0.41 (1.07-2.10)
Cmax (μg/mL)	8.52 ± 2.51	7.82 ± 3.03	0.90 (0.72, 1.13)	0.95 ± 0.29 (0.44-1.25)
Tmax (hr)	1.17 ± 0.41	1.50 ± 0.55	ND	*0.33 ± 0.52 (0.00 to 1.00)
CL/F (L/hr)	47.7 ± 18.9	30.0 ± 5.44	0.66 (0.57, 0.78)	0.59 ± 0.24 (0.31-0.83)
T½ (hr)	2.17 ± 0.60	4.03 ± 2.13	ND	1.25 ± 0.31 (0.84-1.64)

*For Tmax, difference is reported rather than ratio

Plasma indinavir AUC values were statistically significantly higher (p = 0.0138) when indinavir was given concurrently with nelfinavir as compared to indinavir alone. Cmax was not significantly altered (p = 0.542) and there was no change in Tmax. Terminal half-life values for indinavir were not significantly different between treatments (p = 0.0650). The applicant states that the geometric mean ratio (90% CI) for indinavir AUC with and without nelfinavir was 1.51 (1.29-1.77) was within the limits of 0.50-2.00 that Merck & Co. used to define clinical equivalence for indinavir. The nephrolithiasis associated with indinavir is thought to be related to Cmax, which was not increased in this study.

Both indinavir and nelfinavir are substrates for and inhibitors of CYP3A, and it is likely that the mutual pharmacokinetic inhibitory effects of these drugs involve inhibition of this

enzyme. Indinavir AUC values were increased by nelfinavir to a lesser extent in this study (56 ± 41%) than by the CYP3A inhibitor ketoconazole in another study (68 ± 48%). The applicant states that ketoconazole is a more potent inhibitor of CYP3A and that the results of this study suggest partial inhibition of CYP3A by nelfinavir. However, the variability in the degree of increase of indinavir AUC and the overlap of the values between the two studies do not allow an assessment of a difference between inhibition by ketoconazole and by nelfinavir. Also, in the study with ketoconazole, the dose of indinavir was 400 mg, rather than the 800 mg used in this study. Thus, it is not possible to conclude form the results of the current study that nelfinavir inhibits CYP3A to a lesser degree than does ketoconazole.

Nelfinavir AUC values were increased to a greater extent by indinavir in this study (99 ± 87%) than by ketoconazole in another study (36 ± 23%). The applicant states that this difference suggests indinavir may inhibit nelfinavir elimination by pathways other than those mediated by CYP3A, such as other P450 enzymes and/or glucuronyl transferase. In vitro study results submitted with the indinavir NDA (20-685) suggest that indinavir does not inhibit other P450 enzymes. However, in clinical drug interaction studies, indinavir modestly increased plasma concentrations of two other drugs that undergo glucuronidation (ethinyl estradiol and zidovudine). For all patients, the nelfinavir AUC was increased to a greater extent than Cmax, indicating that there was an effect on the systemic clearance of nelfinavir.

As stated by the applicant, the magnitude and clinical significance of the pharmacokinetic interaction between indinavir and nelfinavir requires further investigation in patients receiving chronic multiple dosing of this combination. The results of this study can provide guidance for dose selection in future clinical trials. Although the applicant states that the results suggest that a modest reduction of daily dose and/or frequency may be possible for one or both drugs in this combination, it appears that it would be most appropriate to reduce the dose of nelfinavir.

Ritonavir

The objectives of Study 528 were to determine (1) the effect of a concurrent dosage regimen of ritonavir on the pharmacokinetics of a single dose of nelfinavir and (2) the effect of a concurrent dosage regimen of nelfinavir on the pharmacokinetics of a single dose of ritonavir. This was and open label crossover study. Ten subjects (6 males, 4 females) were included in the pharmacokinetic analyses. One group of healthy volunteers received a single 500 mg dose of ritonavir and serial blood samples were collected for 32 hours. A single 750 mg dose of nelfinavir was then administered concurrently with the second dose of a multiple dosing regimen of ritonavir (500 mg q12hr x 3 doses), and serial blood samples (for nelfinavir) were collected for 32 hours. Following a 17-day washout, a single 750 mg dose of nelfinavir was given and serial blood samples were collected for 32 hours. A single 500 mg dose of ritonavir was then given concurrently with the second dose of a multiple dosing regimen of nelfinavir (750 mg q8hr x 5 doses), and serial blood samples (for ritonavir) were collected for 32 hours. The other group of healthy volunteers received the treatments in the opposite order. Nelfinavir was administered as three 250 mg tablets; ritonavir was administered as five 100 mg capsules. Study drugs were administered within 10 minutes after eating.

Nelfinavir Pharmacokinetic Parameters (Arithmetic Mean ± SD)

Parameter	Nelfinavir alone	Nelfinavir + Ritonavir	Combination/monotherapy ratio	
			Geometric mean ratio (90% CI)	mean ± SD (range)
AUC∞ (μg*hr/mL)	27.6 ± 9.89	70.7 ± 26.4	2.52 (1.96, 3.24)	2.75 ± 1.22 (1.28-4.75)
Cmax (μg/mL)	3.09 ± 0.63	4.46 ± 0.93	1.44 (1.28, 1.63)	1.48 ± 0.41 (1.22-2.61)
Tmax (hr)	3.80 ± 1.03	4.90 ± 2.69	ND	*1.10 ± 2.69 (-1.00 to +8.00)
CL/F (L/hr)	29.6 ± 8.41	12.1 ± 4.80	0.40 (0.31, 0.51)	0.43 ± 0.19 (0.21-0.78)
T½ (hr)	3.40 ± 0.96	8.71 ± 2.50	ND	2.83 ± 1.52 (1.58-6.49)

*For Tmax, difference is reported rather than ratio

The ritonavir trough concentration at the time the nelfinavir single dose was administered was 6.57 ± 2.96 μg/mL and 12 hours later was 6.63 ± 2.53 μg/mL. The label for NORVIR (ritonavir) indicates that the steady-state trough concentration for a 600 mg q12hr regimen in HIV-infected patients was 3.7 ± 2.6 μg/mL. Ritonavir appears to induce its own metabolism over time, this process may take more than one week. Also, ritonavir pharmacokinetics differ between HIV-infected individuals and normal healthy subjects. The applicant states the current study was a pilot drug interaction study, its results can be used to design future studies by not for actual dosing recommendations for the VIRACEPT label.

As indicated in the table above, plasma nelfinavir AUC∞ values were significantly higher ($p = 0.0002$) when nelfinavir was given concurrently with ritonavir as compared to nelfinavir alone. Although the increase in nelfinavir Cmax was statistically significant ($p = 0.0008$), the degree of increase was substantially less than that for AUC∞. The terminal T½ values were significantly longer ($p = 0.0002$) following coadministration of nelfinavir with ritonavir. These results are consistent with inhibition of nelfinavir elimination by ritonavir. Nelfinavir is a substrate for multiple human cytochrom P450 enzymes, including CYP3A, CYP2D6, CYP2C19, and CYP2C9. Ritonavir has been reported to be a clinical inhibitor of the same enzymes.

Ritonavir Pharmacokinetic Parameters (Arithmetic Mean ± SD)

Parameter	Ritonavir alone	Ritonavir + Nelfinavir	Combination/monotherapy ratio	
			Geometric mean ratio (90% CI)	mean ± SD (range)
AUC∞ (μg*hr/mL)	92.7 ± 33.8	101 ± 44.8	1.08 (0.95, 1.23)	1.12 ± 0.30 (0.69-1.65)
Cmax (μg/mL)	10.6 ± 2.79	9.77 ± 2.02	0.93 (0.83, 1.04)	0.96 ± 0.25 (0.67-1.41)
Tmax (hr)	4.00 ± 1.15	3.50 ± 0.53	ND	*-0.50 ± 1.18 (-3.00 to 1.00)
CL/F (L/hr)	6.04 ± 2.06	5.56 ± 1.72	0.92 (0.81, 1.05)	0.95 ± 0.26 (0.61-1.44)
T½ (hr)	3.78 ± 0.81	4.68 ± 1.28	ND	1.23 ± 0.16 (0.99-1.47)

*For Tmax, difference is reported rather than ratio

Nelfinavir trough concentrations at the time of ritonavir coadministration was 2.60 ± 1.01 μg/mL. Ritonavir AUC∞ and Cmax were not significantly altered by coadministration of nelfinavir. The ritonavir terminal T½ values were statistically significantly longer ($p = 0.0006$) when nelfinavir was coadministered. The increased ritonavir terminal T½ is consistent with the fact that ritonavir is a substrate for CYP3A and nelfinavir is an inhibitor of the same enzyme. It has also been demonstrated that CYP2D6 contributes to the metabolism of ritonavir, nelfinavir does not appear to inhibit this enzyme.

The magnitude and clinical significance of the pharmacokinetic interaction between nelfinavir and ritonavir requires further investigation in patients during chronic multiple dosing of both drugs in combination. The results of this study will be used to provide guidance for dose selection in future combination trials.

PHARMACOKINETIC/PHARMACODYNAMIC RELATIONSHIPS

The applicant did not analyze potential correlations between pharmacokinetic parameters and surrogate markers of efficacy. The superiority of viral RNA rather than CD_4 cell count as a surrogate marker emerged during the time nelfinavir was being developed. The endpoint that investigators and patients now seek is viral RNA below the limit of detection. Validation of the detection limit for different viral RNA assays is ongoing.

The applicant did not pursue the 1000 mg tid dose regimen because of increased incidence of diarrhea. No other adverse events were consistently present in clinical trials. Patients were able to tolerate the diarrhea experienced at 500 mg tid and 750 mg tid by using antidiarrheal medications. Although the 500 mg tid and 750 mg tid dose regimens appeared to have similar efficacy, the 750 mg tid dose regimen was selected based on observations at late timepoints.

ANALYTICAL METHODS:

DISSOLUTION

The following dissolution method had been proposed by the applicant for the tablet formulation:

Due to the timing of the development of the pediatric formulation, a dissolution method has not yet been developed. The applicant is currently developing the method. The development of a dissolution method for the oral powder will build upon the work completed on the tablet dissolution method. The applicant has provided the following timeline:

	Completed by:
Method development	4/1/97
Method validation	5/1/97
Specification set and routine testing implemented	6/1/97

Table 8

Drug Product Dissolution Testing

Date Tested	Strength/ Dosage Form	Batch Number	Dissolution Method Media Temperature °C Rotation Speed USP Apparatus	Units Tested	Dissolution (% Dissolved at Specified Times)						
					Time	5 min	10 min	20 min	30 min	45 min	60 min
4/29/96	250-mg Tablet	MNT164V		6	Avg Range %RSD						
10/2/96	250-mg Tablet	MNT299X		6	Avg Range %RSD						
10/8/96	250-mg Tablet	MNT300X		6	Avg Range %RSD						
10/4/96	250-mg Tablet	MNT299X		6	Avg Range %RSD						
10/15/96	250-mg Tablet	MNT300X		6	Avg Range %RSD						

DIVISION OF ANTIVIRAL DRUG PRODUCTS
Review of Chemistry, Manufacturing, and Controls

NDA #: 20-778

CHEMISTRY REVIEW #: 1 **DATE REVIEWED:** 11-Mar-97

SUBMISSION	DOCUMENT DATE	CDER DATE	ASSIGNED DATE
Original	26-Dec-96	26-Dec-96	26-Dec-96
Amendment	10-Dec-96	11-Dec-96	23-Dec-96
Amendment	20-Dec-96	26-Dec-96	10-Jan-97
Amendment	26-Feb-97	27-Feb-97	27-Feb-97
Amendment	04-Mar-97	05-Mar-97	05-Mar-97
Amendment	10-Mar-97	11-Mar-97	11-Mar-97

NAME & ADDRESS OF SPONSOR: Agouron Pharmaceuticals, Inc.
10350 N. Torrey Pines Road
La Jolla, Ca 92037

DRUG PRODUCT NAME

Proprietary:	VIRACEPT®
Nonproprietary:	Nelfinavir mesylate
Code Name/#:	AG 1343 (mesylate salt)
Chem. Type/Ther. Class:	**1 P**

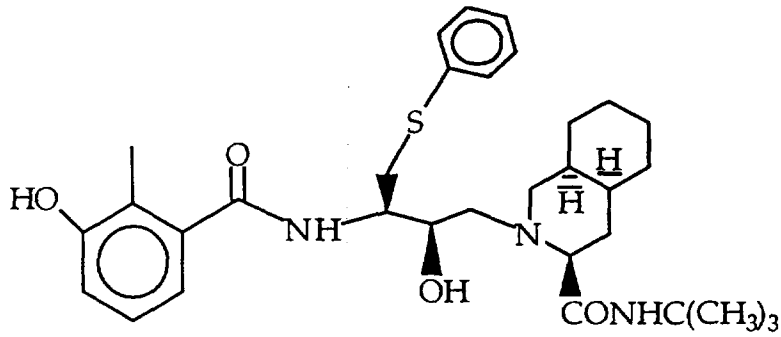
PHARMACOLOGICAL CATEGORY: Antiviral: Anti-HIV

INDICATION: Treatment of HIV infection when antiviral therapy is indicated.

DOSAGE FORM/STRENGTH: Powder, 50 mg/g

ROUTE OF ADMINISTRATION: Oral

CHEMICAL NAME/STRUCTURAL FORMULA:
[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 monomesylate (salt)
$C_{32}H_{45}N_3O_4S.CH_3SO_3H$ (Mol. Wt.: 567.8-free base; 663.9-mesylate salt)



Nelfinavir Mesylate

SUPPORTING DOCUMENTS:

E

RELATED DOCUMENTS:

Chemistry Reviews of INL
Chemistry Review of NDA 20-779
Record of teleconference on 24-Feb-97 (Discussions regarding pre-approval inspections and issues related to CMC of nefinavir mesylate-drug substance and drug product).

CONSULT REVIEWS:

Review of Tradenames (CDER Labeling and Nomenclature Committee, Consult # 674).
Environmental Assessment (C. Berninger/N. Sager, HFD-357).

CONCLUSIONS & RECOMMENDATIONS:

The NDA submission and accompanying amendments provided adequate information on the chemistry, manufacturing and controls for VIRACEPT. The related cGMP and product specific inspections of the manufacturing facilities have been completed and are satisfactory. The Environmental Impact analysis is also acceptable. The NDA, as amended, is therefore recommended for **approval** from a chemistry standpoint.

Paul S. Liu, Review Chemist

Concurrence:
HFD-530/Team Leader 3/4/97

cc:

Orig. NDA 20-778	HFD-530/PLiu	HFD-530/KStruble
HFD-530/Div. File	HFD-530/SMaldonado	
HFD-530/Div. Director	HFD-530/KHastings	
HFD-530/Team Leader	HFD-530/SBala	File: N-20778c.000
HFD-830/Director	HFD-530/KReynolds	

DIVISION OF ANTIVIRAL DRUG PRODUCTS
Review of Chemistry, Manufacturing, and Controls

NDA #: 20-779

CHEMISTRY REVIEW #: 1 **DATE REVIEWED**: 11-Mar-97

SUBMISSION	DOCUMENT DATE	CDER DATE	ASSIGNED DATE
Original	26-Dec-96	26-Dec-96	26-Dec-96
Amendment	10-Dec-96	11-Dec-96	23-Dec-96
Amendment	20-Dec-96	26-Dec-96	10-Jan-97
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Amendment	04-Mar-97	05-Mar-97	05-Mar-97
Amendment	10-Mar-97	11-Mar-97	11-Mar-97

NAME & ADDRESS OF SPONSOR: Agouron Pharmaceuticals, Inc.
10350 N. Torrey Pines Road
La Jolla, Ca 92037

DRUG PRODUCT NAME

Proprietary:	VIRACEPT™
Nonproprietary:	Nelfinavir mesylate
Code Name/#:	AG 1343 (mesylate salt)
Chem. Type/Ther. Class:	1 P

PHARMACOLOGICAL CATEGORY: Antiviral: Anti-HIV

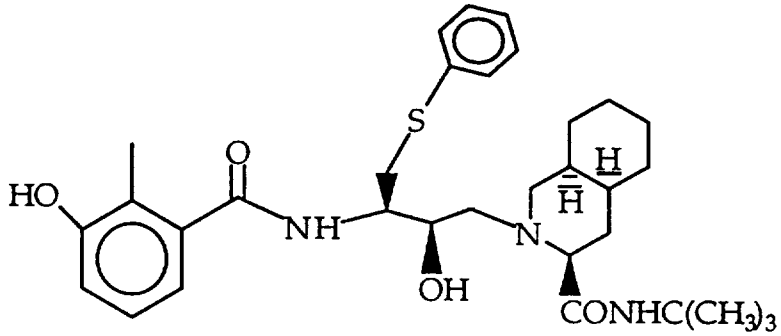
INDICATION: Treatment of HIV infection when antiretroviral therapy is indicated.

DOSAGE FORM/STRENGTH: Tablets, 250 mg

ROUTE OF ADMINISTRATION: Oral

CHEMICAL NAME/STRUCTURAL FORMULA:

[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 monomesylate (salt)
$C_{32}H_{45}N_3O_4S.CH_3SO_3H$ (Mol. Wt.: 567.8-free base; 663.9-mesylate salt)



Nelfinavir Mesylate

SUPPORTING DOCUMENTS:

RELATED DOCUMENTS:

Chemistry Reviews of IND-
Record of teleconference on 24-Feb-97 (Discussions regarding pre-approval inspections and issues related to CMC of nefinavir mesylate-drug substance and drug product).

CONSULT REVIEWS:

Review of Tradenames (CDER Labeling and Nomenclature Committee, Consult # 674).
Environmental Assessment (C. Berninger/N. Sager, HFD-357).

CONCLUSIONS & RECOMMENDATIONS:

The NDA submission and accompanying amendments provided adequate information on the chemistry, manufacturing and controls for VIRACEPT. The related cGMP and product specific inspections of the manufacturing facilities have been completed and are satisfactory. The Environmental Impact analysis is also acceptable. The NDA, as amended, is therefore recommended for **approval** from a chemistry standpoint.

Paul S. Liu, Review Chemist

Concurrence:
HFD-530/Team Leader Miller 3/14/97

cc:
Orig. NDA 20-779
HFD-530/Div. File
HFD-530/Div. Director
HFD-530/Team Leader
HFD-830/Director
HFD-530/PLiu
HFD-530/SMaldonado
HFD-530/KHastings
HFD-530/SBala
HFD-530/KReynolds
HFD-530/KStruble
File: N-20779c.000

ENVIRONMENTAL ASSESSMENT

AND

FINDING OF NO SIGNIFICANT IMPACT

FOR

Viracept™
(Nelfinavir Mesylate)
Tablet Oral 250 mg
NDA Number 20-779

Agouron Pharmaceuticals, Inc.

FOOD AND DRUG ADMINISTRATION

CENTER FOR DRUG EVALUATION AND RESEARCH

Division of Anti-Viral Drug Products
(HFD-530)

FINDING OF NO SIGNIFICANT IMPACT

NDA 20-779

Viracept

(Nelfinavir Mesylate)

Tablet Oral 250 mg

The National Environmental Policy Act of 1969 (NEPA) requires all Federal agencies to assess the environmental impact of their actions. FDA is required under NEPA to consider the environmental impact of approving certain drug product applications as an integral part of its regulatory process.

The Food and Drug Administration, Center for Drug Evaluation and Research, has carefully considered the potential environmental impact of this action and has concluded that this action will not have a significant effect on the quality of the human environment and that an environmental impact statement therefore will not be prepared.

In support of their new drug application for Viracept, Agouron Pharmaceuticals, Inc., has prepared an environmental assessment (attached) in accordance with [21 CFR 25.31a(a)] which evaluates the potential environmental impact of the manufacture, use and disposal of the product. The maximum expected environmental concentration is at a level that normally relieves the applicant from completing format items 7, 8, 9, 10, 11, and 15 in accordance with the Tier 0 approach specified in the *Guidance for Industry for the submission of an Environmental Assessment in Human Drug Applications and Supplements.*

Nelfinavir Mesylate is a chemically synthesized drug which is administered as a tablet in the medical treatment of Human Immunodeficiency Virus (HIV) disease syndrome. The drug substance will be manufactured by

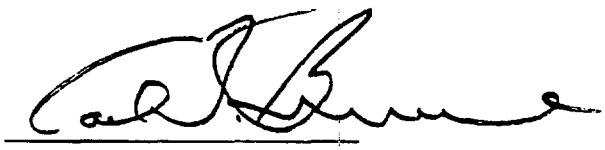
The tablet will be manufactured and packaged by The finished drug product will be used in hospitals, clinics and by patients in their homes.

Nelfinavir Mesylate may enter the environment from excretion by patients, from disposal of pharmaceutical waste or from emissions from manufacturing sites.

Disposal of the drug may result from out of specification lots, discarding of unused or expired product, and user disposal of empty or partly used product and packaging. Drug substance and

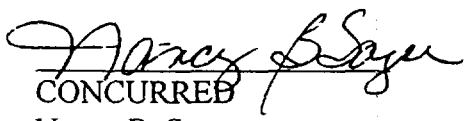
product that fail specification, pass expiration period, or are returned from the field are destroyed by high temperature incineration or by land filling in approved and regulated facilities. At U.S. hospitals and clinics, empty or partially empty packages will be disposed according to hospital/clinic regulations. From home use, empty or partially empty containers will typically be disposed of by a community's solid waste management system which may include landfills, incineration and recycling, while minimal quantities of unused drug may be disposed of in the sewer system.

The Center for Drug Evaluation and Research has concluded that the product can be manufactured, used and disposed of without any expected adverse environmental effects. Precautions taken at the sites of manufacture of the bulk product and its final formulation are expected to minimize occupational exposures and environmental release. Adverse effects are not anticipated upon endangered or threatened species or upon property listed in or eligible for listing in the National Register of Historic Places.



1/29/97
Date

PREPARED BY
Carl J. Berninger, Ph.D.
Environmental Scientist
Environmental Assessment Team
Center for Drug Evaluation and Research



1/29/97
Date

CONCURRED
Nancy B. Sager
Team Leader
Environmental Assessment Team
Center for Drug Evaluation and Research

Attachments: Environmental Assessment (FOI copy)
Material Safety Data Sheet (drug substance)

NON-CONFIDENTIAL

Environmental Assessment of Nelfinavir Mesylate (VIRACEPT™) Tablets
[Freedom of Information (FOI) Document]

Agouron Pharmaceuticals, Inc.
10350 North Torrey Pines Road
La Jolla, CA 92037-1020

The FOI, Environmental Assessment (EA) document being submitted by Agouron Pharmaceuticals, Inc. on this product is a nonconfidential document and has appendices A, B, and C. These are: 1) Non-Confidential, Appendix A containing Material Safety Data Sheets (MSDS); 2) Non-Confidential, Appendix B containing References; and 3) Confidential, Appendix C which is the full EA for review by FDA, and not for public disclosure. Confidential information from the FOI, EA document has been deleted and replaced with asterisks.

TABLE OF CONTENTS

APPENDICES

FIGURES

TABLES

1 DATE

November 25, 1996

2 NAME OF APPLICANT/PETITIONER

Agouron Pharmaceuticals, Inc.

3 ADDRESS

10350 North Torrey Pines Road

La Jolla, CA 92037-1020

4 DESCRIPTION OF THE PROPOSED ACTION

4.1 REQUESTED APPROVAL

Agouron Pharmaceuticals, Inc. has filed a New Drug Application (NDA) pursuant to Section 505(b) of the Federal Food, Drug, and Cosmetic Act to synthesize and manufacture the drug substance nelfinavir mesylate (formerly known as AG1343) and manufacture the drug product, nelfinavir mesylate (VIRACEPT™) tablets (each tablet containing 250 mg of nelfinavir mesylate as the free base) for the treatment of patients suffering from Human Immunedeficiency Virus (HIV) disease syndrome. The tablets are packaged in HDPE bottles. VIRACEPT™ Tablets are administered orally for patients with HIV.

The forecasted quantity of the drug substance, nelfinavir mesylate, that will be required to manufacture the VIRACEPT™ Tablets for the first five years of production (from 1997 to 2001) is presented in confidential Appendix C. An Environmental Assessment is submitted here pursuant to 21 CFR §25.31a (a), "Environmental Assessment for Proposed Approvals of FDA-Regulated Products - Format 1" (21 CFR, Chapter 1, April 1, 1993).

The format of this Environmental Assessment (EA) report is arranged as required in 21 CFR 25.31a(a) (1993) and Guidance for Industry for the Submission of an Environmental Assessment in Human Drug Applications and Supplements by the Center for Drug Evaluation and Research (CDER) of Food and Drug Administration (CDER, FDA, 1995). According to FDA, CDER (1995) guidelines, a full EA report is required if the Expected Introduction Concentration (EIC) of the drug in the Publicly Owned Wastewater Treatment Plant (POWTP) is equal to or greater than one part per billion. A full EA [corresponding to Sections 1-15 of FDA, CDER (1995) guideline document] is presented for nelfinavir mesylate. Supporting documents for the items discussed in this FOI, EA document have been organized as attachments to this document as well as Appendices A (MSDS), B (References), and C (Confidential EA Report).

4.2 NEED FOR ACTION

Nelfinavir mesylate (also known formerly as AG1343) is a novel non-peptide inhibitor of human immunodeficiency virus-1 (HIV-I). The activity of HIV protease is central to viral infection because of its critical function in the processing of viral p55 gag polyprotein to structural proteins and enzymes. Therefore, inhibitors of this enzyme should have the potential to inhibit replication of HIV. Nelfinavir mesylate is a potent inhibitor of this enzyme and has demonstrated anti-HIV activity in a number of *in vitro* test systems. It has also been shown to significantly reduce plasma viral RNA in patients receiving either monotherapy or combination therapy with reverse transcriptase inhibitors. Elevations in CD4 cell counts have also been observed in patients receiving nelfinavir mesylate.

4.3 PRODUCTION LOCATIONS

The new drug substance, nelfinavir mesylate, is the mono-methanesulfonic acid salt of (3S,4aS,8aS)-N-*tert*-butyl-2-[(2R,3R)-3-(3,2-cresotamido)-2-hydroxy-4-(phenylthio)butyl]decahydro-3-isoquinolinecarboxamide (AG1346). AG1346 will be manufactured by two synthetic routes: The Chloroalcohol Route and the AOA Route. AG1346 manufactured from both routes is then converted to its methanesulfonic acid salt and spray dried to provide the drug substance, nelfinavir mesylate.

4.3.1 Synthesis of AG1346 by Chloroalcohol Route and Packaging of AG1346

AG1346 is synthesized by the Chloroalcohol Route and packaged at the following location:

Ganes Chemical, Inc., 33 Industrial Park Road, Pennsville, NJ 08070

AG1346 is manufactured and packed at this location and shipped to Niro, Inc. described in Section 4.3.3. A schematic for the synthesis of AG1346 by Chloroalcohol Route and details of the information on synthesis are provided in confidential Appendix C.

4.3.2 Synthesis of AG1346 by AOA Route and Packaging of AG1346

AG1346 is synthesized by the AOA Route and packaged at the following locations:

1. Fuji Chemical Industry Co., Ltd., Gohkakizawa Factory, 1, Gohkakizawa Kamiichi-machi Nakaniikawa-gun, Toyama Prefecture, Japan 930-03
2. Yonezawa Hamari Chemicals, Ltd., 2-4300-18, Hachimanpara, Yonezawa, Yamagata, Japan

AG1346 will be manufactured and packaged at these locations and shipped to Niro, Inc. as described in Section 4.3.3. A schematic for the synthesis of AG1346 by AOA route and the details of information on synthesis are provided in confidential Appendix C.

4.3.3 Conversion of AG1346 to Nelfinavir Mesylate and Packaging

Conversion of the AG1346 produced at the above locations to the mono-methanesulfonic acid salt (AG1343) bulk drug substance, nelfinavir mesylate, is conducted at the following location:

Niro, Inc., 9165 Rumsey Road, Columbia, MD 21045-1991

The bulk drug substance nelfinavir mesylate is packaged at this location and transported to MOVA Pharmaceuticals Corporation.

4.3.4 Manufacture of Nelfinavir Mesylate Tablets and Packaging

The drug product, nelfinavir mesylate (VIRACEPT™) Tablets, derived from the above bulk drug substance will be formulated and packaged at the following location:

MOVA Pharmaceuticals Corporation, Gialla Blanca Industrial Park, State Road #1, A Street, Kilometer 34.3, Caguas, Puerto Rico 00725

A schematic for the manufacture of nelfinavir mesylate tablets is provided in confidential Appendix C and the manufacturing process described in confidential Appendix C. The drug product will be packaged at this location.

The drug substance and drug product manufacturing locations are depicted in Figure 4-1.

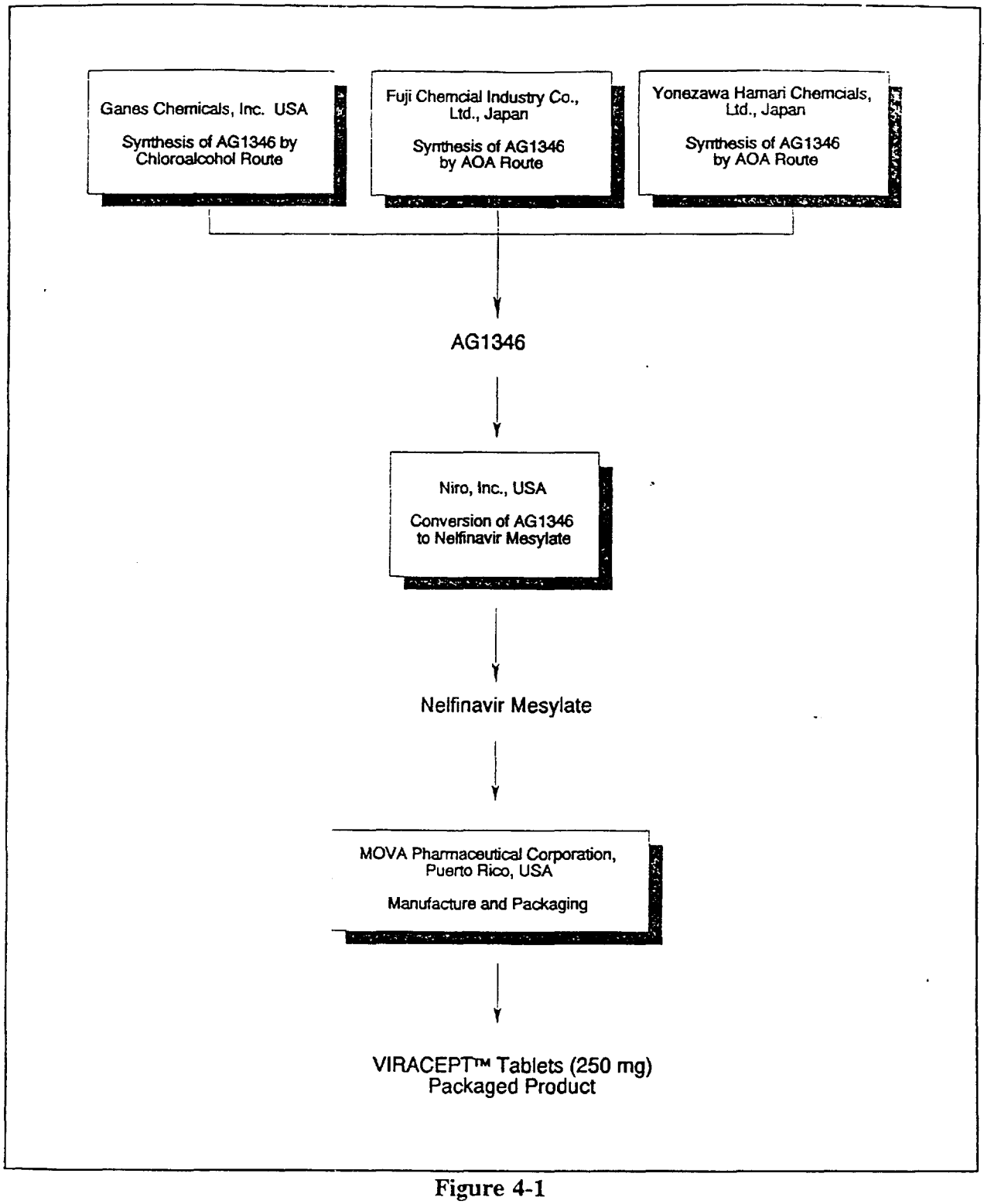


Figure 4-1

Sites for Bulk Drug Synthesis and Drug Product (Tablets) Manufacture and Packaging Operations of VIRACEPT™ Tablets (250 mg)

4.4 Environmental Setting of Production Locations

A brief description of the environments at and adjacent to the manufacturing facilities are provided below.

4.4.1 Gaines Chemicals, Inc., Pennsville, NJ, USA

The synthesis of the bulk drug substance intermediate, AG1346, by Chloroalcohol Route is conducted at the Ganes Chemical Inc. plant located in Pennsville, NJ. This manufacturing facility is situated in a rural area with no nearby industrial activity. Surrounded by woods and wet lands, the Delaware River is approximately 3500 feet to the east and Miles Creek is approximately 1500 feet to the south. The Pennsville community supports this manufacturing facility only. Pennsville and the surrounding area consists of residential, agricultural, and wetlands. The water supply for the factory is from the City of Pennsville. Wastewater that is released from onsite treatment facility is sewered to the Pennsville Treatment Sewerage Facility, a Pennsville Publicly Owned Wastewater Treatment Plant (POWTP), where it is further treated prior to its discharge into the Delaware River (Attachment 15-1A).

4.4.2 Fuji Chemical Industry Co., Ltd., Toyama, Japan

The manufacturing of AG1346 is conducted at Fuji Chemical located in Kamiichi Town, Toyama, Japan. The western part of Fuji Chemical Plant is adjacent to Kamiichi River and the rest of the area is surrounded by the rice fields. The Fuji Chemical Plant has a total area of about 28.3 Acres. The climate of Kamiichi Town is characterized by warm summers 68 to 95°F and cold to moderate winters 23 to 50°F. The average annual rainfall is 110 inches. Most industries in Kamiichi Town obtain potable water from the well

and most residences obtain potable water from the Kamiichi Town municipal water supply. The municipal water is supplied from the 1st Kamiichi Town water purification plant located at about 3 km east of the downtown of Kamiichi Town to which water is pumped from a well 56 feet deep under ground. The Fuji Chemical Plant uses well water which is different from the municipal water. The source of this well water is at 115 feet depth and 980 feet depth under ground within the Fuji Chemical Plant. Wastewater is sewered to an onsite water treatment facility, and then to Kamiichi River (Attachment 15-1B).

4.4.3 Yonezawa Hamari Chemicals, Ltd., Yamagata, Japan

The manufacturing of AG1346 is conducted at Yonezawa Hamari Chemicals located in Yonezawa City, Yamagata, Japan. The Yonezawa Hamari Chemicals Plant is surrounded by industrial park. The Yonezawa Hamari Chemicals Plant has a total area of about 534,000 square feet. The climate of Yonezawa City is characterized by warm summers (68 to 95°F) and cold to moderate winters (23 to 41°F). The average annual rainfall is 447 inches. Most industries and residence in Yonezawa obtain potable water and industrial water from the City of Yonezawa municipal water supply. The source of the municipal water supply is Mizukubo reservoir, which is in the Azuma Mountain Chain. The Yonezawa Hamari Chemicals Plant uses municipal water only. Wastewater is sewered to the onsite water treatment facility and the City of Yonezawa municipal water treatment facility, and then to the Mogami River (Attachment 15-1C).

4.4.4 Niro, Inc., Columbia, MD

Conversion of the bulk drug substance intermediate (AG1346) to nelfinavir mesylate (AG1343) is conducted at Niro Inc.'s Pharmaceutical Technology Center located in

Columbia MD. Located within an industrial park, this modern facility built in 1993, is used to design and test chemical and pharmaceutical processes. There are no significant geographic features, such as mountains, lakes, or rivers in close proximity to the manufacturing facility. The industrial park is served by the Howard County water supply. Wastewater which is sewered from pharmaceutical processes are contained and discharged to an on-site waste treatment plant. This wastewater is treated at the plant prior to being discharged to the local sanitary sewer of Howard County and the POWTP of Howard County for further treatment before it is discharged into the Howard County Reservoir (Attachment 15-1D).

4.4.5 MOVA Pharmaceutical Corporation, Caguas, Puerto

The drug product (VIRACEPT™ Tablets 250 mg) is manufactured at MOVA Pharmaceutical Corporation, Caguas, Puerto Rico.

The manufacturing facility in Caguas, Puerto Rico, is situated in an area which is zoned for industrial purposes (I-1). The facility is surrounded by other manufacturing industries. There are no significant geographic features, such as mountains, in proximity to the manufacturing facilities. The manufacturing site itself drains to the southeast towards the Rio Cagüitas which is approximately 600 meters from the southeast corner of the MOVA property. To date the agricultural and industrial uses (zoning) of land coexist in the region. There is a small residential community located southwest of the facility.

The climate of Puerto Rico is influenced by Puerto Rico's location in the Caribbean. At approximately 18 degrees north latitude, it is situated in the belt of prevailing northeasterly tradewinds. The predominance of tradewinds results in little variability of wind

direction. Puerto Rico's tropical climate also produces only a small range of temperature variation. Typically, the average summer and winter temperatures differ by less than 10 degrees Fahrenheit. The average annual temperature is approximately 78°F.

Precipitation varies widely over the island due to local meteorological effects. On an annual basis, Caguas receives between 50 and 60 inches of rain. Potable water for operations at the MOVA facility is supplied by the Puerto Rico Aqueduct and Sewer Authority (PRASA). Wastewater is treated onsite. Its effluent is piped to PRASA's Regional Wastewater Treatment Plant where it is treated further before being released to the Bairoa River (Attachment 15-1E).

4.5 LOCATIONS OF USE

Nelfinavir mesylate is a protease inhibitor for use in patients with HIV, as stated in Section 4.2. The locations of use by the HIV-infected patients are hospitals, clinics and homes throughout the United States. The injested drug and metabolites will be eliminated through human excreta into domestic sewage and then into a Publicly Owned Wastewater Treatment Plants (POWTP).

4.6 DISPOSAL SITES

Disposal of nelfinavir mesylate occurs during manufacture and human use. Nelfinavir and its metabolites are excreted by the consuming patients predominantly in feces (excreta) with $<1\%$ in urine. These excreta enter the domestic sewage which enters the municipal treatment systems throughout the United States. At U.S. hospitals, pharmacies or clinics, empty or partially empty packages will be disposed of according to the hospital, pharmacy or clinic procedures. Empty or partially empty containers in the homes will be

typically disposed of by a community's solid waste management system which may include recycling, disposal in landfills, or destruction by incineration. Minimal quantities of unused drug may be disposed of in the sewer system. Rejected, expired, or returned drug product are returned to Agouron Pharmaceuticals, Inc., where they are accumulated, classified as appropriate and sent offsite for landfilling as nonhazardous solid waste or disposed of as pharmaceutical waste. Drug substance intermediates and drug substance related wastes will also be similarly disposed, as explained in Section 6.0.

5 IDENTIFICATION OF CHEMICAL SUBSTANCES THAT ARE THE SUBJECT OF THE PROPOSED ACTION

Information on the drug substance, nelfinavir mesylate, is provided below to allow for accurate location of data about the chemical in scientific literature and to allow for identification of closely related compounds. Details of the physical and chemical properties of nelfinavir mesylate are described in confidential Appendix C.

5.1 NOMENCLATURE

5.1.1 Established Name (United States Adopted Name - USAN)

Nelfinavir Mesylate (USAN)

Nelfinavir (INN)

5.1.2 Brand/Proprietary Name

VIRACEPT™

5.1.3 Chemical Name

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

5.1.4 CAS Registry Number

159989-65-8 (Nelfinavir Mesylate); 159989-64-7(AG1346)

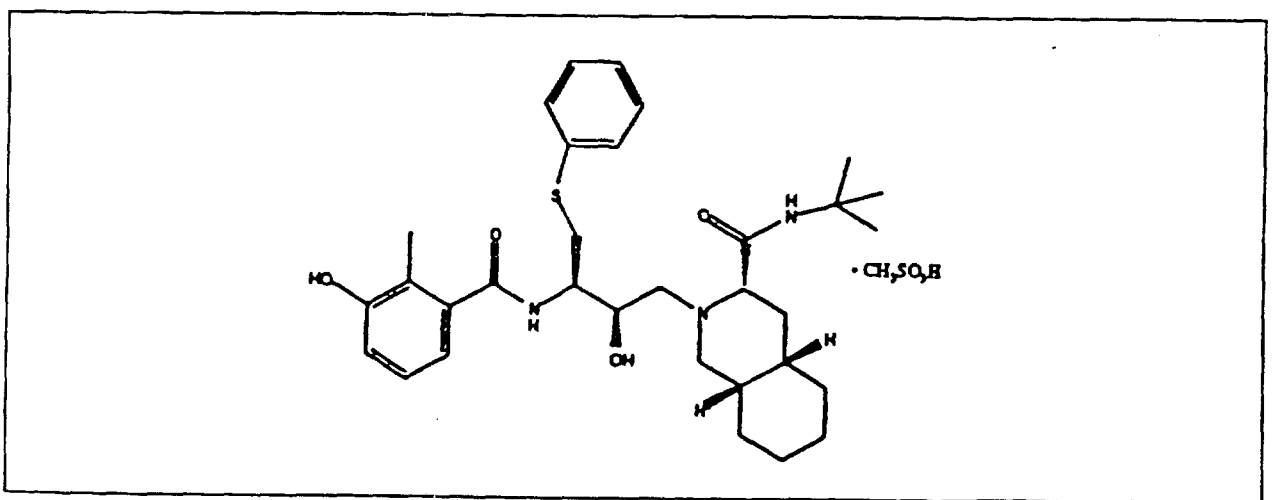
5.1.5 Laboratory Codes and Synonyms

AG1343 (Nelfinavir Mesylate); AG1346 (free base)

5.1.6 Molecular Formula and Weight

Formula = $C_{32}H_{45}N_3O_4S \cdot CH_4O_3S$; Weight = 663.90 (567.79 as the free base)

5.1.7 Chemical Structure



Nelfinavir Mesylate (AG1343)

5.1.8 Physical Description

Nelfinavir mesylate (AG1343) is a white to off-white solid with a characteristic slight pungent odor.

5.1.9 Solubility in Water

≤pH 4: slightly soluble in aqueous solution

≥pH 4: insoluble

5.1.10 Solubility in Solvents

Freely soluble in methanol, acetone, tetrahydrofuran, acetonitrile, dimethyl sulfoxide and ethanol

5.1.11 Log Octanol Water Partition Coefficient (Log k_{ow})

4.07 ± 0.2 at pH 7

5.1.12 Dissociation of the Acid (pK_a)

pKa_1: 6.00 ± 0.10

pKa_2: 11.06 ± 0.10

5.1.13 Melting Point

No melting point was observed. The compound slowly becomes glassy, evolves gas, melts and decomposes over the range of 100°-200°C.

5.1.14 Ultraviolet Absorption Spectrum

Absorption maxima at 204 nm and 255 nm

5.2 ADDITIVES

The excipients (additives) of the drug product, VIRACEPT™ Tablets which are blue colored-compressed tablets, are provided in Table 5-1. The majority of the excipients are naturally occurring and are expected to be metabolized/eliminated in the body of patients

along with the drug substance and released [illegible] degraded. The impact of these excipient related emissions into the environment is discussed under Section 6.

5.3 IMPURITIES

The purity of the nelfinavir mesylate currently used for the manufacture of VIRACEPT™ Tablets is >98.0%. The impurities are no more than 2%. Since the total impurities exceed more than 1%, individual impurities are listed in Table 5-2. As seen from the table, none of the individual impurities exceeded 0.5%, and hence their introduction to the environment or their fate have not been discussed further.

6 INTRODUCTION OF SUBSTANCES INTO THE ENVIRONMENT

The bulk drug substances intermediate AG1346 is manufactured by Ganes Chemcials, Inc., Pennsville, NJ and Fuji Chemical Industry Co., Ltd., Toyama, Japan and Yonezawa Hamara Chemcials, Inc., Yamagata, Japan at the production plant locations indicated in Section 4.3. The intermediate AG1346 is converted to nelfinavir mesylate (AG1343) at Niro, Inc., Columbia, MD. The bulk drug substance, nelfinavir mesylate, is formulated as 250 mg (as the free base) tablets and packaged at MOVA Pharmaceuticals Corporation, Caguas, Puerto Rico. The potential emissions and their controls during manufacturing processes at these locations are described in this section.

6.1 SYNTHESIS OF AG1346 BY CHLOROALCOHOL ROUTE AT GANES CHEMICALS, INC., PENNSVILLE, NJ, USA

Typical waste streams from manufacturing are solid, flammable liquid and aqueous liquid wastes.

Table 5-1

Ingredient
AG1343, anhydrous, solvent-free
Calcium Silicate
Crospovidone, NF
Magnesium Stearate, NF
FD&C Blue #2 Powder (91% dye)
FD&C Blue #2 Aluminum Lake (12-14% dye)
Purified Water, USP

TABLE 5-2

Quantitative Information on Impurities and Degradants

Relative HPLC Retention Time	Identification	Specification Requirements
0.20	AG1382 + AG1401	
0.24	Benzyl Alcohol	
0.28	AG1358	
0.45	AG1361a	
0.49	AG1361b	
0.53	AG1371	
0.74	AG1379	
0.87	AG1404	
0.90	AG1368 + AG1381 + AG1372	
1.28	AG1383	
1.53	AG1356	
1.63	AG1369	
xxx	unknown	
		For any other impurity
	Total Impurities	NMT 2.0%

*NMT - No More Than

5.1.1 Substances Expected to be Emitted

Atmospheric Emissions

The drug substance intermediate AG1346 is made by the Chloroalcohol Route at Ganes Chemicals, Inc. The Chloroalcohol Route is a three step process in which chloroalcohol is converted to AG1346 sequentially through AG1357 → AG1358 → AG1383 as described in confidential Appendix C. The starting materials and reagents of the drug substance synthesis process can be obtained from commercial sources. The chemicals used for a typical batch series are listed in Table 6-1. Estimates of releases of all process substances for a typical batch series are listed in Table 6-2.

Acetone, isoproponal, tetrahydrofuran, methanol, triethylamine, hydrochloric acid and particulates are potential releases to the environment (Table 6-2). To minimize atmospheric pollution, the process equipment is hard piped together and vented to a centralized scrubber. Vacuum systems include condensers and brine-after condensers to improve control efficiency of voaltile organic compounds (VOCs). Flexible fume hoses are situated throughout the plant buildings to minimize fugitive losses during material handling operations.

Ganes Chemicals, Inc. is in the process of obtaining a batch plant air permit for the Manufacturing Building No. 3 where synthesis operations will be carried out (Air Permit Log #01-95-2330) which after a year's review is due for approval by the New Jersey Department of Environmental Protection.

TABLE 6-1

Chemicals Used During the Synthesis of AG1346 at Ganes Chemicals, Inc.

Name
N-CBz-3-Amino-1-Chloro-4-Phenylsulfanybutan-2-ol (Chloroalcohol)
(S)-N-(t-Butyl)Decahydro-3-Isoquinolinecarboxamide (PHIQ)
AG1357
3-Acetoxy-2-Methylbenzoyl chloride
Isopropanol
Methanol
Acetone
Tetrahydrofuran
Triethylamine
Sodium Hydroxide, 30%
Sodium Hydroxide, 50%
Hydrochloric Acid, Reagent grade
Water
Darco S-51
Solka-Floc, BW-40

TABLE 6-2

Release of Process Substances for a Typical Batch Series

Substances	Air (lbs)	Water (lbs)	Liq. Waste (lbs)	Solid Waste (lbs)
Acetone	59.49	2,519	NA	NA
Isopropanol	67.75	3,275	3,516	NA
Tetrahydrofuran	17.37	5	377	NA
Methanol	14.25	15	2,690	NA
Triethylamine	0.95	10	224	NA
Hydrochloric Acid	0.1	277	NA	NA
Particulates	2.03	*	*	NA
Filtering Solids	NA	NA	NA	5

permit from the New Jersey Department of Environmental Protection (NJDEP). The revised air permit (Log #01-95-2330) in preparation for Clean Air Act requirements is currently under review by NJDEP. Records of emissions are maintained and available for inspection. Nonflammable treatable aqueous waste streams from the synthesis process will be sewered. These aqueous waste streams are treated at Ganes's onsite sewage facility before the effluent is released to the Pennsville's POWTP. Flammable liquids are fuel blended at Marisol Inc., Middlesex, NJ. Non-treatable liquid streams are sent to DuPont Environmental Treatment, chamber Works, Deepwater, NJ. Hazardous solid waste are incinerated at Rollins Environmental Inc., Bridgeport, NJ. Nonhazardous solid waste including protective clothing, gloves, dust masks and other will be disposed of in an approved landfill (Attachment 15-1A).

6.1.3 Citations of and Statement of Compliance with Applicable Emission Requirements

Chemicals used in the manufacture of the drug substance are regulated by the Occupational Safety and Health Administration under its responsibility for: (1) permissible exposure limits; (2) safe handling of flammable liquids; (3) safe handling of corrosives; and (4) hazard communication. Handling procedures specified in the Material Safety Data Sheets (MSDS) are followed throughout all operations.

Atmospheric emissions will be in compliance with permitted emission requirements.

Effluents from Ganes Chemicals' treatment plant are discharged in accordance with all applicable state and federal discharge ordinances. Wastewater from manufacturing

Limitation and Standards for Pharmaceutical Manufacturing in 40 CFR Part 439.

Certification of compliance with applicable emission requirements from the facility manager at Pennsville, NJ facility is provided in the FOI, EA document (Attachment 15-2A).

6.1.4 Effect of Approval on Compliance with Current Emission Requirements

No effect is anticipated from approval of the synthesis of AG1346 at Ganes Chemicals on compliance with current emission requirements at the manufacturing facility. All emissions and discharges are within permitted limits established by the governing state and federal authorities. Existing permits are sufficient for the proposed action.

6.2 SYNTHESIS OF AG1346 BY AOA ROUTE AT FUJI CHEMICAL INDUSTRY CO., LTD. JAPAN

At this facility, the drug substance intermediate AG1346 is made by an alternative synthesis process (AOA Route) as indicated in confidential Appendix C. As required by FDA, CDER (1995), EA guidelines for those manufacturing sites located outside the United States, a letter from the plant manager of the manufacturing plant certifying that the facility is in compliance with all local and national regulations has been provided in Attachment 15-2B.

6.3 SYNTHESIS OF AG1346 BY AOA ROUTE AT YONEZAWA HAMARI CHEMICALS, LTD., JAPAN

At this facility, the drug substance intermediate AG1346 is made by an alternative synthesis process (AOA Route) as indicated in confidential Appendix C. As

[illegible] letter from the plant manager of the manufacturing plant certifying that the facility is in compliance with all local and national regulations has been provided in the Attachment 15-2C.

6.4 MANUFACTURE OF THE BULK DRUG SUBSTANCE, NELFINAVIR MESYLATE, AT NIRO, INC., COLUMBIA, MARYLAND, USA

AG1346, manufactured by the Chloroalcohol Route in Pennsville, NJ, USA and by the AOA Route in Japan is transferred to Niro, Inc., USA, where it is converted to its methanesulfonic acid salt and spray dried to provide the drug substance, nelfinavir mesylate. A closed cycle spray dryer will be used to accomplish this process (Attachment 15-1D).

6.4.1 Substances Expected to be Emitted

Atmospheric Emission

Atmospheric emissions are minimized or non-existent due to the use of a closed cycle spray drier. The process requires that an ethanol solution with 26.5% of AG1343 will be pumped into a rotary atomizer. This atomizes the solution into a fine mist inside the drying chamber. Heated nitrogen is mixed with the fine mist which evaporates the ethanol leaving the AG1343 in a powder form.

Aqueous Waste

Once the drug substance manufacture is completed, the closed cycle spray dryer equipment is cleaned with water and detergents. The material balance from the manufacturing process is approximately 100%. Therefore, very little of the drug substance

and discharged to an on-site waste treatment facility.

Solid Waste

Solid wastes from the closed cycle spray dryer will be minimal or none. Non-hazardous solid wastes, such as protective clothing, gloves, etc will be disposed of in a landfill.

6.4.2 Controls Exercised

The closed cycle spray dryer will contain air emissions of nelfinavir mesylate during production of AG1343. The gas and AG1343 powder mixture after leaving the dryer is passed through a cyclone which will separate 90-95% of powder. The remaining powder (5-10% of solids) will be removed as it passes through the baghouses. The material collected in the baghouses will be reprocessed. The gas then passes through HEPA filters to ensure no powder enters the exhaust fan or cooler, thus preventing atmospheric emissions.

Wastewater from equipment cleaning at Niro Inc. is treated to meet Howard County's specifications before being discharged to the local sanitary sewer. If this criteria cannot be met, the wastewater is hauled off-site and disposed of by a certified and audited waste hauler.

When the gas passes through the cooler, the gas is cooled and ethanol vapor is condensed out of the gas. The condenser will recover 98% of the solvents used in the process. The recovered solvents will be removed by a certified and audited waste hauler. The remainder of the solvents will exit in a nitrogen purge stream. This solvent recovery

[illegible] Administration and has been given their approval for installation.

6.4.3 Citation of and Statement of Compliance with Applicable Emission Requirements

Niro, Inc. was granted coverage under the General Discharge Permit effective on September 19, 1992, with an expiration date of September 28, 1997 by the Maryland Department of Environment (MDE). MDE was authorized by USEPA to administer comprehensive state storm water management program. Since there are no releases to the atmosphere and very minimal release in the process water, air and water emissions are in compliance. A statement of General Environmental Compliance by the facility manager of Niro, Inc. is provided in Attachment 15-2D.

6.4.4 Effect of Approval or Compliance with Current Emission Requirements

The manufacture of the bulk drug substance will require Niro to handle 35 to 40 additional drums of ethyl alcohol, 350 kg of methanesulfonic acid, and 2000 kg of additional raw and processed material per month. Niro, Inc.'s facilities have been designed to handle these additional chemicals. The emissions due to this manufacturing process would be minimal since the manufacturing process is a closed cycle process. A schematic showing the closed cycle system is provided in confidential Appendix C illustrating salient features of closed cycle process.

MOVA PHARMACEUTICAL CORPORATION, CAGUAS, PUERTO RICO

The manufacturing and packaging of the drug product, VIRACEPT™ (nelfinavir mesylate) Tablets is conducted at MOVA Pharmaceutical Corporation, Puerto Rico. The manufacturing steps for nelfinavir mesylate tablets are provided in confidential Appendix C. Information on manufacturing facilities is provided in Attachment 15-1E.

6.5.1 Substances Expected to be Emitted

Atmospheric Emissions

Manufacture of nelfinavir mesylate tablets involves no alcohol in granulating or coating steps (Table 5-1). Thus, emission of organic volatiles will be non-existent. The drug substance is relatively stable. A total volatile content of approximately 1% for this compound was observed at 120°C, suggesting that the compound is anhydrous and solvent free (confidential Appendix C). Based on this data, the volatile atmospheric emissions are unlikely during tableting or packaging. Emissions of organics are negligible for the ink used at the label printing stage or packaging. These minor emissions are not subject to any control devices.

Aqueous Waste

The processing areas and equipment are thoroughly hand-scraped prior to washdown. As a result, very little product is sewered. A liquid solution of purified water and FD&C Blue #2 will be discarded at a rate of 17.0 kg per lot. At the manufacturing plant, washdown water is treated onsite in the wastewater treatment plant for primary and

Aqueduct and Sewer Authority (PRASA).

Solid Waste

About 0.5 kg of the product will be lost during the drying step of each batch size of approximately 310 kg. The emissions will be controlled by a filter with an efficiency of 90%. Filters will be disposed of as solid waste. Approximately 5 kg of product waste per batch (batch size of 310 kg) will be generated from the tablet compression process and will be disposed of as a solid waste at the finished good incineration facility stated in Section 6.5.3.

6.5.2 Controls Exercised

Process aqueous wastes and wastes from cleaning of equipment will be discharged to an onsite treatment facility that consists of primary and secondary treatment systems that include solids removal, pH adjustment, biological treatment and filtration. The effluent from onsite wastewater treatment plant is discharged to the PRASA's Regional Wastewater Treatment Plant. Air vent filters, floor and equipment sweepings, and protective clothing worn by operators are disposed of as solid wastes.

6.5.3 Citation of and Statement of Compliance with Applicable Emission Requirements

The wastes generated during the manufacturing activities are packaged according to the Department of Transportation regulations and accumulated in areas designated for those purposes. The hazardous waste is disposed of using Ochoa Environmental Services, EPA ID number PRD090128562. The non-hazardous waste is

generated in a non-hazardous waste incineration facility, Commerical Incineration Corporation, located in Penuelas. The washwaters are pumped to wastewater treatment plant for primary and secondary treatment. The effluent discharge is then sent to PRASA's regional treatment plant for further treatment.

The Environmental Quality Board (EQB) issued the Permit for the Operations of the Emission Sources, PFE-LC-13-0895-1881-I-II-0, in January 30, 1996. This permit will expire October 17, 2000. Federal air regulations do not apply because MOVA is a minor emission source.

MOVA is a large quantity generator of hazardous waste and is inspected yearly by the EQB. The EPA provided the identification number for the generation of hazardous waste as PRD-174-050-377 on April, 1988.

PRASA is the state agency that issued the permit for the industrial discharge of the wastewaters. MOVA's permit was issued in December 24, 1994 and will expire on December 23, 1996. MOVA is in the process of renewal for this permit number GDA-88-602-010. The stormwater discharge permit is PRR-00-A-134 and was issued by EPA in October, 1992. MOVA has a general permit.

A statement of General Environmental Compliance by the facility manager of MOVA Pharmaceutical Inc. is provided in Attachment 15-2E.

6.5.4 Effect of Approval on Compliance with Current Emission Requirements

The drug product tableting and packaging at MOVA, will be within the limits of current permits and emission requirements of those permits.

Occupational safety procedures are similar for all U.S. facilities involved in the manufacture of nelfinavir mesylate and its tableting and packaging. Chemicals used in the manufacture of the drug product are regulated by the Occupational Safety and Health Administration (OSHA). All precautions indicated in the MSDS for starting materials, intermediates and nelfinavir mesylate are followed by workers. All chemical operators are OSHA HAZWOPER trained to control small spills during manufacture. An Emergency Response Team, trained to OSHA, HAZPOWER standards, is available to control larger spills. Personal protective equipment (e.g., gloves, safety shoes, and eye protection) and engineering controls for the equipment are designed to ensure employee safety. Employees are trained in safe and proper handling of equipment and chemicals. All drug substance and drug product containers are labelled adequately for safe handling and packaging and transportation is according to the Department of Transportation (DOT) regulations.

6.7 EXPECTED INTRODUCTION CONCENTRATIONS

Human drugs find their way into the environmental compartments (eg. soil, air, water) through manufacture, use, disposal and accidental spills. The two major sources of environmental exposure of the drug are: 1) the elimination of the drug or its metabolites through excreta (urine and feces) from the patients who are using the drug product, and the consequent release of unchanged drugs or metabolites into the environment; and 2) release of the drug or its precursors through wastewater from the manufacturing plants. In either case, the municipal sewage could be the main recipient of these contaminant sources. The expected introduction concentrations (EIC) estimated in the sections below do not take into

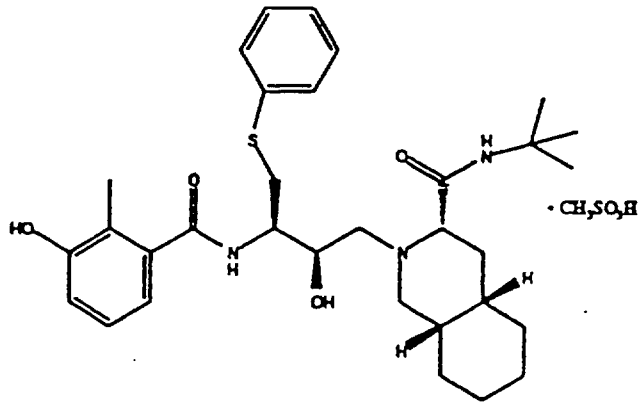
sewage to the wastewater treatment plant, and, therefore, are worst case scenarios. All potential pathways of degradation have been discussed below.

6.7.1 Expected Introduction Concentration From Use

All of the administered nelfinavir mesylate is likely to be excreted in the feces, with less than 1% observed in the urine. Approximately 35% of the administered dose is excreted unchanged as nelfinavir mesylate in the feces under steady-state conditions. The remainder is excreted in the feces as metabolites of nelfinavir. The primary metabolite of Nelfinavir in human and rat plasma has been identified as M1 (molecular weight, 598.4), the 3-O-methyl, 4-hydroxy aryl derivative of nelfinavir. This is also the largest metabolite identified in rat feces. The structure of the parent and the principal metabolite M1 is shown in Figure 6-1. Other metabolites are: AG1361a (molecular weight, 594) and AG1361b (molecular weight, 594). The antiviral activity and cytoxicity of metabolites is significantly less than the parent drug, nelfinavir mesylate. Acute toxicity tests in rodents, dogs and monkeys and microorganisms indicate that nelfinavir mesylate has relatively low toxicity (Section 8).

For a worst case estimate for the Expected Introduction Concentration (EIC) of nelfinavir mesylate in the POWTP, it is assumed that all the drug forecasted for production in the United States (confidential Appendix C) in the year 2001, which is the fifth year of production, is approximately ** ****** **** (****** **) and that all of this will be ingested and eliminated into the POWTP by the U.S. population. The worst case estimates assume that there will be no metabolism in the human body. They will also assume that there will be no degradation in the domestic sewage receiving human excreta containing the drug product.

*Confidential



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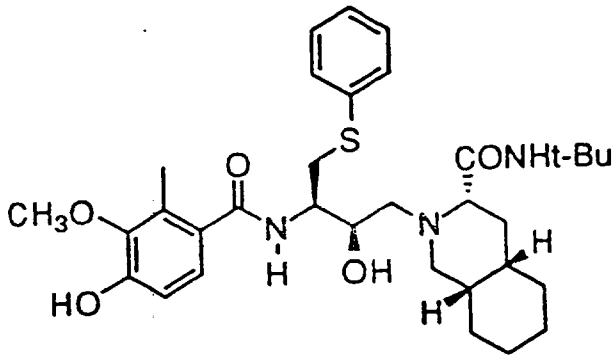


M1

Figure 6-1

Nelfinavir Mesylate and Its Major Metabolite

The 1990 Census gives the population of the United States as 250,378,000. Typical minimum and maximum flow rates for wastewater treatment systems are set by Federal and State agencies to range from 280 to 1,500 L/person/day (Metcalf & Eddy, Inc., 1979).

The EIC from use at the POWTP can be estimated in three different ways. The concentration of the drug is estimated from the dilution of the total drug produced in a year in the total wastewater produced in the United States.

Fifth year production estimate of nelfinavir mesylate = ** ****** **** = ****** ** or = ******* x 10^9 μg.

Total waste water produced in the United States per year:

Liters of waste water per person = 280 L/day
Population of the United States = 250 million
Days in a year = 365 days
= 280 x 250 million x 365 = Liters of total waste water per year

Therefore the EIC for nelfinavir mesylate at the POWTP will be:

$$\frac{****** \; x \; 10^9}{280 \; x \; 250 \; x \; 10^6 \; x \; 365} = **** \; **** \; (***) = **** \; ***$$

An equivalent method for calculating the concentration of drug that would be expected at the POWTP is given in Interim Guidance to the Pharmaceutical Industry for Environmental Assessment Compliance Requirements for the FDA (PMA, 1991) which estimates the EIC in ppm as follows:

*Confidential

... = (A) (B) (C) (D) (E) (F)

A = pounds/year production

B = year/365 days

C = day person/280 L (74 gallons)

D = 1/250 million persons

E = gallons/8.34 pounds

F = one million

Nelfinavir mesylate at POWTP in ppm = ****** lbs (****** **) (A) x 1/365 (B) x 1/74 (C) x 1/(250 x 10^6) (D) x 1/8.34 (E) x 10^6 (F) = ****** *** (= *** ***).

A method for calculating the expected introduction concentration (EIC) of the drug at the POWTP is given in "Guidance for Industry for the Submission of an Environmental Assessment in Human Drug Applications and Supplements" published by the Center for Drug Evaluation Research (CDER), FDA, in November 1995 (FDA 1995). The estimate of the EIC in ppm based on this method is as follows:

EIC-Aquatic (ppm) = (A) (B) (C) (D)

A = kg/year production

B = 1/Liters per day entering POWTP

C = years/365 days

D = 10^6 mg/kg (conversion factor)

EIC of nelfinavir mesylate at POWTP in ppm = ****** (A) x 1/1.115 x 10^{11} (B) x 1/365 (C) x 10^6 (D) = ***** *** = * ***.

The estimation of EIC from the above three methods of calculation ranged from * to *** ***. Almost all of the nelfinavir mesylate residue was excreted in feces in the metabolism studies, of which 35% was excreted as unchanged drug. Based on the metabolism and the

assumed to be environmentally relevant, then the EEC of the more toxic, environmentally relevant component would be **** to **** ***, which is below FDA, CDER guideline limits of 1 ppb for tier 0 classification.

6.8 Expected Introduction Concentration from Disposal

During the synthesis of AG1346 by Chloroalcohol Route, approximately ** ** of process substances/batch are released in wastewater and *-* ******* are released as solid wastes. Most of the process substances (Table 6-2) are readily biodegradable during treatment at Ganes Chemicals, Inc. and at Pennsville's POWTP (Section 6.1.1). AG1346 is synthesized by the AOA Route at the two Japanese facilities and no account of this manufacture is provided as per the guidelines of FDA, CDER (1995) for the foreign manufacturing sites. During the conversion of AG1346 to the drug product nelfinavir mesylate (AG1343) at Niro, Inc., AG1346 or AG1343 are not released into the environment, due to the closed cycle spray dryer process where recovery and reprocessing are practiced, and, therefore, no environmental exposure is anticipated.

Drug product manufacture at MOVA Pharmaceutical Corporation is expected to release *** ** of the drug product during the drying step of each lot and * ** of product waste per lot during the tablet compression process. These wastes are disposed of at an incineration facility for finished goods facility stated under Section 6.5.3. Very little wastes are released into the process sewers from equipment washes because of thorough scraping of equipment used for tableting. Disposal of residues of unused drug product (empty or partially empty packages) after human use will be at homes, hospitals or clinics and this

significant extent. Rejected, expired or returned drug product which are expected to be insignificant are sent to Agouron Pharmaceuticals, Inc. where they are disposed of as pharmaceutical waste limiting any environmental releases. Thus, the exposure of the drug to the environment is limited through the disposal process. The excipients used in the drug product are naturally occurring and many of them are easily biodegradable. Hence no environmental impact is anticipated. EIC estimations due to disposal are not made for drug substance, drug product or its excipients, for the reasons stated above.

7 FATE OF EMITTED SUBSTANCES IN THE ENVIRONMENT

The environmental fate and transport of nelfinavir mesylate is analyzed based on the physical and chemical properties and known mammalian metabolism of the compound. Processes affecting transport between air, water, and soil and processes affecting structural degradation of the compound are also discussed. The physical and chemical properties such as vapor pressure, melting temperature, water solubility, octanol/water partition coefficient, dissociation constant as well as environmentally relevant processes such as biodegradation, hydrolysis, photolysis, oxidation, volatilization, sorption, bioaccumulation, and bioconcentration will be relevant for the prediction of environmental behavior of nelfinavir mesylate. The methodology involved in this evaluation and its application to specific chemicals is discussed in Water-Related Environmental Fate of 129 Priority Pollutants (USEPA, 1979).

7.1 AIR

The melting temperature of nelfinavir mesylate is 100-200°C. Thermogravimetric analysis (TGA) indicated a total volatile content of approximately 1% at 120°C

substance or the manufacture of the drug product no volatilization of nelfinavir mesylate is anticipated. Any particulate matter containing the drug substance collected during the synthesis or manufacture of the drug product in the air filter systems is disposed of as solid wastes. Therefore no air emissions are anticipated. If any drug containing dust should escape the filtration system or in the case of accidental releases, nelfinavir mesylate would dissociate in the atmospheric moisture. The dissociated products will precipitate with rain and would reach surface water where they are likely to biodegrade aerobically by microorganisms present in surface water or anaerobically at surface water and sediment interface by the anaerobic microorganisms present in these matrices. If they reach soil, they would become adsorbed to soil, where they would undergo biodegradation by the microorganisms present in the soil (Hazardous Substances Databank, 1992).

If the solvents used in the synthesis process reach the atmosphere, they are likely to react with the oxidizing radical species of the atmosphere. The environmental fate of these solvents is described in the Hazardous Substances Databank (1992). The atmospheric half-lives (in days) for various solvents are provided in this data base. The solvents used in the synthesis process in the event of their release into the environment are likely to be degraded in the atmosphere or biodegraded when they reach soil and water through precipitation. Therefore, atmospheric emissions are unlikely to have any environmental impact.

Nelfinavir mesylate and its metabolites are released to POWTP through: (1) elimination by consuming patients throughout the United States, and (2) washdown water from the bulk drug manufacture (Ganes Chemicals, Inc. and Niro, Inc.) and formulation and tableting facilities (MOVA Pharmaceuticals, Inc.). Because of the lack of highly biodegradable functional groups in the molecule, nelfinavir mesylate is not likely to biodegrade in water extensively, in the on-site treatment facilities or when it reaches POWTP. However, nelfinavir mesylate may degrade extensively through indirect photodegradation in the POWTP as stated in Section 7.2.3, Photolysis. Because of high octanol/water partition coefficient and consequent adsorption of nelfinavir mesylate to sewage sludge, the drug will partition into sludge and consequently in sludge solids. Anaerobic sludge biodegradation of nelfinavir mesylate and its metabolites in the anaerobic digesters of the POWTP are expected to decrease their concentrations substantially. The sewage effluent or municipal sludge released to surface water or soil, respectively will contain reduced quantities of nelfinavir mesylate and metabolites compared to their entry into POWTP.

The worst-case EIC of nelfinavir mesylate at a typical POWTP in the year 2001, the year of maximum production due to patient usage is expected to be * to *** ***. Dilution factors for effluent can vary because of variations in plant capacity and in rates of surface water flow (depending on geographic location) from about 10^{-7} to essentially no dilution in settling ponds or intermittently dry drainage channels (Metcalf & Eddy, Inc., 1979; Linsley et al., 1975). Based on the literature, the dilution factor of POWTP effluents in surface water for many rivers of the United States is 10^{-3}, and, the worst case EIC of

dilution will be *** ***** *** ******** (*** ***), which is undetectable by analytical techniques. Downstream from the effluent outfall the concentration would be further diluted.

Nelfinavir mesylate and its metabolites could be depleted in the domestic sewage during transport to the POWTP, in the sewage treatment facility, or in the surface water that dilutes the effluent, by environmental processes that include aerobic and anaerobic biodegradation, hydrolysis, photolysis, oxidation, volatilization, adsorption and bioaccumulation and bioconcentration. These processes are discussed individually based on the structural information of nelfinavir mesylate (Section 5) and also its metabolism.

7.2.1 Biodegradation

The primary product of metabolism of nelfinavir mesylate in rats and humans (confidential Appendix C) is identified as the 3-O-methoxy, 4-hydroxy aryl derivative of nelfinavir mesylate. Several other minor metabolites are also formed. Only 35% is released as unchanged drug. The extensive metabolism (>60%) in the human body suggests, that the excreted nelfinavir mesylate and its metabolites may further degrade in the domestic sewage during the transport to the POWTP and at the POWTP into further smaller molecular entities through microbial enzyme-mediated biotransformation. However complete mineralization (degradation to CO_2) is unlikely based on the molecular structure of nelfinavir mesylate and its M1 metabolite which do not show functional groups that are susceptible to mineralization to CO_2. Anaerobic biodegradation may also result in biotransformation and possibly some methane and CO_2 production.

anaerobic conditions may prevail in the domestic sewage due to anoxic conditions, thus facilitating biodegradation by anaerobic microorganisms which may also convert nelfinavir mesylate to methane, CO_2 and biotransformed products of the parent. At the POWTP, in the activated sludge (derived from the domestic sewage) aeration tanks, extensive aeration favors multiplication of microorganisms and consequently enhances the microbial mediated biotransformation of nelfinavir mesylate and its metabolites to other lower molecular weight degradate components. After this process, when the wastewater effluents are separated from the sludge, residues of nelfinavir mesylate that have not been mineralized to CO_2 will be bound to the sludge solids based on the octanol/water partition coefficient of 4.07. Compounds with log K_{ow} of >2 are expected to bind very strongly to organic matter (FDA, TAD 1987). The sludge solids will be subjected to anaerobic biodegradation in the anaerobic sludge digester at the POWTP where anaerobic degradation may lead to CO_2 and methane production and several biotransformed products. Due to these biodegradative processes in the POWTP, nelfinavir mesylate and its metabolites will be depleted significantly from the time they enter the POWTP to the time where the wastewater effluents and processed sludge will leave the POWTP to enter the surface water and soil/landfill, respectively. The mechanism of biodegradation of nelfinavir mesylate and its metabolites would include microbial oxidation by the introduction of an additional hydroxy group into the aromatic ring. Amide linkages are also susceptible to biodegradation (Lyman, et al., 1990).

Nelfinavir mesylate contains two amide linkages that are potentially susceptible to hydrolysis at acidic or basic pH. Hydrolysis of 3-hydroxy-2-methylbenzamide moiety would give the corresponding acid and a second moiety with a terminal amino group. The second amide linkage would give 1,1-dimethylamine and a fragment with terminal carboxylic acid group. Oxidation at the S-linkage would initially yield a sulfoxide followed by a sulfone. In an aqueous environment such as the POWTP followed by hydrolysis, these groups would also be susceptible to biodegradation. These two processes could significantly deplete nelfinavir mesylate and the M1 metabolite from the POWTP.

7.2.3 Photolysis

The ultraviolet/visible (UV/VIS) absorption spectrum of nelfinavir mesylate shows absorption maxima at 204 nm and 253 nm. This absorption is typical of aromatic compounds. The major absorbance is due to the 3-hydroxy-2-methylbenzoic acid residue. No absorption is seen within the wavelength range of natural sunlight, which is normally within the 290-800 nm range, and because of this direct photodegradation by natural sunlight is unlikely to happen in the activated sludge aeration tanks of POWTP, or in the surface water when the wastewater effluent from the POWTP reaches surface water. However, the 3-hydroxy-2-methyl benzoic acid residue which may be responsible for absorption maximum at 255 nm, may photodegrade under basic conditions, if the absorbance is shifted to longer wavelengths in base. In addition, indirect photolysis by sensitizers of bacterial origin present in activated sludge aeration tanks or of humic acid origin in natural surface waters could lead to extensive photodegradation of nelfinavir mesylate and its metabolites. In indirect

other than the target molecule, is transferred to the target molecule but the donor species (the sensitizer) undergoes no net reaction but has a catalytic effect on the reactions that would ensue in the target molecule (Lyman et al., 1990). Indirect photodegradation using a sensitizer is known to occur in a number of pharmaceutical chemicals (Velagaleti, 1996). Nelfinavir mesylate and its metabolites are likely to be depleted by indirect photolysis in the POWTP. Further degradation of components from POWTP is likely in surface water due to indirect photolysis. Photodegradation would, therefore, result in significant depletion of any residues of nelfinavir mesylate and its metabolites in the POWTP and surface water.

7.2.4 Oxidation

Hydroxyl radicals (and alkylperoxy radicals) are generated in surface water from the photolysis of naturally occurring substances that absorb terrestrial sunlight (Lyman, 1990). Photochemically produced hydroxyl radicals in water have been observed to oxidize many organic chemicals. Such oxidation may result in depletion of nelfinavir mesylate and its metabolites.

7.2.5 Volatilization and Dissociation

Nelfinavir mesylate is not likely to transport from water (or soil) to the atmosphere. However it has potential for ionization in aqueous environments. The dissociation constants (pK_a = 6.0; pK_a = 11.06) indicate such a potential. Ionized forms of organic acids are generally adsorbed by sediments to a much lesser degree than in the neutral form.

Based on the partition coefficient (K_{ow}) value of 4.07 (Section 5.1), nelfinavir mesylate is likely to adsorb very tightly to sludge, soil or sediment matrices. The log soil adsorption coefficient (K_{oc}), and the distribution coefficient (K_d) of nelfinavir mesylate can be calculated from the following equation (Lyman et al., 1990):

$$\log K_{oc} = 0.524 \log K_{ow} + 0.855$$
$$(r^2 = 0.84;\ n = 30)$$

where:

K_{ow} = the octanol-water partition coefficient.

r^2 = the coefficient of determination (proportionate reduction in error).

n = the number of chemicals from which the regression was developed.

Based on the log octanol-water partition coefficient of 4.07 for nelfinavir mesylate (Section 5.1) the log K_{oc} is estimated to be 2.99. The distribution coefficient, K_d, is estimated by assuming a 4 percent organic content in the sediment. The relationship between K_{oc} and K_d is given by the following equation (Lyman, et al., 1990):

$$K_d = K_{oc}\ (OC)$$

where:

OC = the fractional amount of organic carbon in sediment

The distribution coefficient, K_d, estimated by this method is 11.96, indicating that nelfinavir mesylate could become adsorbed to sewage sludge, the sediment or soil.

7.2.7 Bioaccumulation/Bioconcentration

Bioconcentration factor (BCF) can be calculated from the following equation (Lyman et al., 1990):

$$\log BCF = 0.76 \log K_{ow} - 0.23$$
$$(r^2 = 0.823;\ n = 84)$$

where:

K_{ow} = the octanol-water partition coefficient.

r^2 = the coefficient of determination (proportionate reduction in error).

n = the number of chemicals from which the regression was developed.

Based on the log octanol-water partition coefficient (4.07) (Section 5.1), the log BCF calculated by this method is 2.86 and the BCF itself is 724. This value indicates that there is a tendency for nelfinavir mesylate to accumulate in aquatic life at parts per billion (ppb) level assuming a 25 to 30 ppb surface water concentration (Lyman et al., 1990). However, nelfinavir mesylate and its metabolites are extensively depleted in the POWTP. The surface water concentration without depletion are estimated at ** ***. With depletion processes these residues may be less than a **** *** ********. So, actual field accumulation in aquatic fish will be non existent and will have no environmental impact.

7.2.8 Probable Fate of Nelfinavir Mesylate and Its Metabolites in Water

The environmental processes that affect nelfinavir mesylate are summarized in Table 7-1. As stated above under various depletion processes, nelfinavir mesylate and its metabolites will be released in ***** *** ****** quantities in surface water, without degradation in the POWTP taken into consideration. With expected degradation and consequent depletion, the residues will be ** *** and are not likely to have any environmental impact.

Table 7-1
Summary of Environmental Fate, Transport and Accumulation of Nelfinavir Mesylate

Environmental Process	Summary Statement
1. Biodegradation	Nelfinavir mesylate is extensively metabolized in humans and rats. Microbial mediated degradation in domestic sewage, POWTP and surface water of nelfinavir mesylate and its metabolites is very likely. Although complete mineralization to CO_2 may not occur, biotransformation to smaller molecular entities is possible.
2. Hydrolysis	Depletion of nelfinavir mesylate due to hydrolysis especially of the amide linkages is likely.
3. Photolysis	Direct photolysis under basic conditions and indirect photolysis under both basic and acidic conditions is likely to deplete nelfinavir mesylate and its metabolites in POWTP and surface water.
4. Oxidation	Hydroxyl radicals (alkyl peroxyl radicals) from naturally occurring chemicals generated by light may help degradation.
5. Volatilization	Volatilization is not expected.
6. Sorption	Because of the high log K_{ow} of 4.07, nelfinavir mesylate is likely to adsorb to sludge strongly.
7. Bioaccumulation/Bioconcentration	Bioaccumulation/bioconcentration is likely. However, surface water concentrations are likely to be ** ***. Hence, accumulation is insignificant, and will have no effect on aquatic species.

Possibilities of depletion are based on criteria discussed in USEPA (1979) and other literature cited (e.g. Lyman et al., 1990) in the reference confidential Appendix B).

Because of high K_{ow} and K_{oc} (Section 7.2.7) of nelfinavir mesylate, the residues of nelfinavir mesylate and its metabolites are likely to be adsorbed to sludge in the POWTP. The sludge from the POWTP may be landfilled or applied to soil. Both in landfills as well as in agricultural soils nelfinavir mesylate and its metabolites are likely to be degraded extensively. Concentrations are expected to be ** *** range because of dilution in soils as well as other wastes in landfills. In soils, aerobic biodegradation by bacteria, fungi, and actinomycetes present in soil will lead to further depletion. In the landfill, however, both aerobic and anaerobic biodegradation are likely, contributing to depletion.

8 ENVIRONMENTAL EFFECTS OF RELEASED SUBSTANCES

The worst case EIC of nelfinavir mesylate and its metabolites in the year 2001 at a typical POWTP is expected to be ** ***. Worst-case concentrations in surface waters that receive the effluent would be ** *** (Section 7.2). No environmental exposure is indicated due to disposal (Section 7.2). These concentrations assume no human metabolism. They also assume no depletion in the POWTP. As stated in the previous sections >60% of nelfinavir mesylate is transformed through human metabolism. Various depletion and dilution processes in the POWTP and surface water will ensure concentrations of ** *** and consequently no environmental impacts due to the drug product use or manufacture are expected.

There was no evidence of mutagenic response or inhibition to nelfinavir mesylate in four strains of *Salmonella typhimurium* and two strains of *Escherichia coli*, when tested up to 1000 μg/plate. These data may imply potential non-toxicity to environmental

microorganisms, which will facilitate biodegradation and consequent depletion of nelfinavir mesylate in the POWTP and in the environment. The LD_{50} for acute oral administration of nelfinavir mesylate in the mouse or rat was >500 mg/kg and in rat the no adverse effect level (NOAEL) was >200 mg/kg/day. These observed toxicity results are several orders of magnitude higher than the estimated EIC in the POWTP, surface water or soil. Hence, no adverse effects to aquatic or terrestrial species are expected. Thus, nelfinavir mesylate will have no environmental impact.

9 USE OF RESOURCES AND ENERGY

Manufacture of the drug substance and the drug product or the packaging of the drug product at the respective facilities will not require large commitment of resources, and would be scheduled to fit with the current operations of the facility.

Based on the discussions in the previous sections ** ***** *** **** ****** of residues may be present in the environment, even if depletion was not accounted. The depletion and dilution would result in concentration levels that would have no effect on threatened or endangered species.

The manufacturing facilities are not near any sites of historical or archaeological significance.

10 MITIGATION MEASURES

No potential adverse environmental impacts have been identified. Therefore, no mitigation measures are planned.

Environmental processes such as biodegradation and photodegradation in the POWTP and surface water are likely to deplete nelfinavir mesylate released both from human use and from manufacturing effluents. No environmental impact is anticipated since the residues are likely to be depleted significantly. Because no adverse environmental impact is expected, alternatives to the proposed action are not being considered. If nelfinavir mesylate is not manufactured, patients with HIV may not have an alternative drug available for treatment.

12 LIST OF PREPARERS

Ranga Velagaleti, Ph.D.
Director, Pharmaceutical Manufacturing Support Group
Analytical-Biochemistry Laboratories, Inc.
7200 E. ABC Lane
Columbia, MO 65202

The undersigned certifies that the information presented is true, accurate, and complete for preparation of the Environmental Assessment Report in accordance with 21 CFR 25.31(a).

Signature Ranga Velagaleti Date November 25, 1996

Title: Director, Pharmaceutical Manufacturing Support Group

13 CERTIFICATION

The undersigned official certifies that the information presented herein and provided to Ranga Velagaleti by Agouron Pharmaceuticals, Inc. (applicant) is true, accurate, and complete to the best of our knowledge.

The undersigned official certifies that this FOI, EA document and Appendices A and B contain non-confidential information and acknowledges that the non-confidential information will be made available to the public in accordance with 40 CFR part 1506.6. Appendix C contains confidential information and was prepared for FDA review and not for public disclosure.

Signature Michael A. Adam Date 11/25/96

Michael A. Adam, Ph.D.

Title Director, Regulatory Affairs

1. Hazardous Substances Data Bank. 1992. MICROMEDEX Inc.

2. Linsley, Jr., R.K., Kohler, M.A., and Paulhus J.L.H. 1975. Hydrology for Engineers - Second Edition. McGraw-Hill Book Company.

3. Lyman, Warren J. Reehl, W.F., and Rosenblatt, D. 1990. Handbook of Chemical Property Estimation Methods. American Chemical Society, Washington, DC.

4. Metcalf & Eddy, Inc. 1979. Wastewater Engineering: Treatment, Disposal, Reuse. Revised by G. Tchobanoglous. New York: McGraw-Hill Book Company.

5. Pharmaceutical Manufacturers Association (PMA). 1991. Interim Guidance to the Pharmaceutical Industry for Environmental Assessment Compliance Requirements for the FDA. PMA Washington, D.C. *Reference not included.

6. U.S. Environmental Protection Agency (USEPA). 1979. Water-Related Environmental Fate of 129 Priority Pollutants. Prepared by M.A. Callahan, M.W. Slimak, N.W. Gabel, I.P. May, C.F. Fowler et al., for the Office of Water Planning and Standards, U.S. Environmental Protection Agency, Washington, D.C., EPA-440/4-79-029ab.

7. U.S. Environmental Protection Agency (USEPA). 1987. Expert Systems Questionnaire. Survey Concerning Biodegradation. Prepared by B. Gregg, N.W. Gabel, and S.E. Campbell (Versar, Inc.) for Office of Toxic Substances, Exposure Evaluation Division, U.S. Environmental Protection Agency, Washington, D.C., EPA Contract No. 68-02-4254.

8. U.S. Food and Drug Administration (USFDA). 1987. Environmental Assessment Technical Assistance Handbook. Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, Washington, D.C.

9. U.S. Food and Drug Administration. 1995. Guidance for the Industry for the Submission of an Environmental Assessment in Human Drug Applications and Supplements. Center for Drug Evaluation Research, (CDER), FDA, Washington, D.C. *Reference not included.

10. Velagaleti, R. 1996. Behavior of Pharmaceutical Drugs (Human and Animal Health) in the Environment. Drug Information Association Journal (In Press).

15 ATTACHMENTS

15-1 Information on the Manufacturing Sites
- 15-1A Ganes Chemicals, Inc.
- 15-1B Fuji Chemical Industry Co., Ltd.
- 15-1C Yonezawa Hamari Chemicals, Ltd.
- 15-1D Niro, Inc.
- 15-1E MOVA Pharmaceutical Corporation

15-2 Certificate of Environmental Compliance from the Facility Managers

15-2A Ganes Chemicals, Inc.

15-2B Fuji Chemical Industry Co., Ltd.

15-2C Yonezawa Hamari Chemicals, Ltd.

15-2D Niro, Inc.

15-2E MOVA Pharmaceutical Corporation

ATTACHMENT 15-1

Information on the Manufacturing Sites

15-1A Ganes Chemicals, Inc.
15-1B Fuji Chemical Industry Co., Ltd.
15-1C Yonezawa Hamari Chemicals, Ltd.
15-1D Niro, Inc.
15-1E MOVA Pharmaceutical Corporation

15-1A Ganes Chemicals, Inc.

September 24, 1996

Overnight mail
Michael A. Adam, Ph.D.
Director, Regulatory Affairs
Agouron Pharmaceuticals, Inc.
10350 North Torrey Pines Road
La Jolla, CA 92037-1020

Re: Environmental Assessment for Viracept™

Dear Mr. Adam,

Please find enclosed the finalized environmental assessment for Viracept™.

Please do not hesitate to call if I can be of further assistance.

Sincerely,

Roman M. Pazdro
Manager, Environmental Affairs

cc: Dave Mohr
Rudy Recla

For Viracept™

The following information is submitted to assess the environmental impact due to the manufacture of Viracept™ at Ganes Chemicals, Pennsville, N.J. facility.

1. The Ganes Chemicals Inc. plant is located in Pennsville, NJ. The facility is situated in a rural area surrounded by woods and wetlands. The Delaware River is approximately 3500 feet to the east and Miles Creek is approximately 1500 feet to the south. There are no areas of archaeological importance. The Ganes facility is the only industry in the Pennsville community. The remainder of the town consists of residential, agricultural and wetlands. The water supply is typical city water. The wastewater discharged from the treatment plant flows to the Pennsville Treatment Sewerage Facility where it is further treated prior to discharge to the Delaware River.

2. The following figures are estimates of releases of all process substances for a typical batch series:

SUBSTANCES	AIR (lbs)	WATER (lbs)	LIQ. WASTE (lbs)	SOLID WASTE (lbs)
Acetone	59.49	2,519	NA	NA
Isopropanol	67.75	3,275	3,516	NA
Tetrahydrofuran	17.37	5	377	NA
Methanol	14.24	15	2,690	NA
Triethylamine	0.95	10	224	NA
Hydrochloric Acid	0.1	277	NA	NA
Particulates	2.03	*	*	NA
Filtering solids	NA	NA	NA	5

* Uncertain at this time, between 10-15% lost to water and liquid waste

3. To minimize atmospheric pollution, the process equipment is hard piped together and vented to a centralized scrubber. Vacuum systems include condensers and brine after-condensers to improve VOC (Volatile organic compound) control efficiency. Flexible fume hoses are situated throughout the plant buildings to minimize fugitive losses during material handling operations.

All chemicals operators are OSHA HAZWOPER trained to control small spills during manufacture. An Emergency Response Team, trained to OSHA HAZWOPER standards, is available to control larger spills. Spill control equipment is located strategically throughout the plant. The facility is in compliance with NJ Department of Environmental Protection's DPCC Regulations (Discharge Prevention and Countermeasures Control Act).

Typical waste streams from manufacturing are solid, flammable liquid and aqueous liquid streams. Solid wastes are incinerated at Rollins Environmental Inc. Bridgeport, NJ, EPA ID No. NJD053288239. Flammable liquids are fuel blended at Marisol Inc., Middlesex, NJ, EPA ID No. NJD002454544. Aqueous liquid streams, which could not be treated in house, are sent to Dupont Environmental Treatment, Chambers Works, Deepwater, NJ, EPA ID No. NJD002385730. Process wastewater is treated on-site prior to discharge to Pennsville's publicly owned treatment works for further treatment. On site-treatment includes neutralization, equalization, biological treatment, clarification, sand filtration and if necessary, granulated activated carbon adsorption and breakpoint chlorination, before discharge to the Pennsville Sewerage facility.

4. Ganes is in compliance with all applicable state regulations. All releases of process substances from the manufacture of this product are within permit limits.

Ganes is currently in the process of converting the old style air permits with a more representative batch plant permit in preparation for Clean Air Act requirements. The air permit for Manufacturing Building No. 3 (air permit Log#01-95-2330) will be approved shortly, after a year's review by the New Jersey Department of Environmental Protection.

Ganes operates as large quantity generator of hazardous waste. The EPA ID No. is NJD064344575.

Ganes operates a tertiary treatment plant for process waste waters. The NJPDES permit No. is NJ0103721.

5. The types and quantity of substances released through the manufacture of this product will not adversely impact compliance with facility permits.

6. For a typical batch series, the following materials are used:

N-CBz-3-Amino-1-Chloro-4-Phenylsulfanylbutan-2-ol	661 lbs
(S)-N-(t-Butyl)Decahydro-3-Isoquinolinecarboxamide	435 lbs
AG1357	882 lbs
3-Acetoxy-2-Methylbenzoyl Chloride	347 lbs
Isopropanol	9,359 lbs
Methanol	2,710 lbs
Acetone	12,078 lbs
Tetrahydrofuran	400 lbs
Triethylamine	235 lbs
Sodium Hydroxide, 30%	230 lbs
Sodium Hydroxide, 50%	995 lbs
Hydrochloric Acid, Reagent grade	278 lbs
Water	26,468 lbs
Darco S-51	20 lbs
Solka-Floc, BW-40	45 lbs

The existing facility is a batch facility with generic, multi-use equipment. There will be no significant increase in energy, water or space due to the manufacture of this product.

7. During a typical production batch series for this product, approximately 2,500 lbs of Isopropanol and 9,500 lbs of Acetone are recycled back into the process.

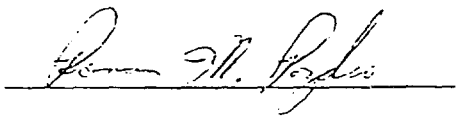
Ganes makes every attempt possible to recycle and recover materials. Due to the nature of many process and FDA requirements, recycling is not always feasible. As a final effort, the materials are sent to a fuel blending facility for energy recovery.

8. The facility is not located on or near areas of historical or archeological significance.

9. The undersigned official declares that the information presented is true, accurate and complete to the best of his knowledge:

Date: September 23, 1996

Signature of Responsible Official:



Roman M. Pazdro
Manager, Environmental Affairs

15-1B Fuji Chemical Industry Co., Ltd.

2-1. Manufacturing Site

a. Address

Gohkakizawa Factory, Fuji Chemical Industry Co.,Ltd.

1 Gohkakizawa Kamiichi-machi Nakaniikawa-gun.

Toyama Pref., Japan 930-03 TEL.: 0764-72-2323

b. Area (Gohkakizawa Factory) ; 28.3 acres. (114,724 m^2)

c. Direction

Gohkakizawa Factory, Fuji Chemical Industry Co., Ltd. is located at 15 km east of the City of Toyama and at 350 km north west of Tokyo. It takes one hour by air from Tokyo to Toyama city. The head office is located near Gohkakizawa Factory.

2-2. Manufacturing Facilities

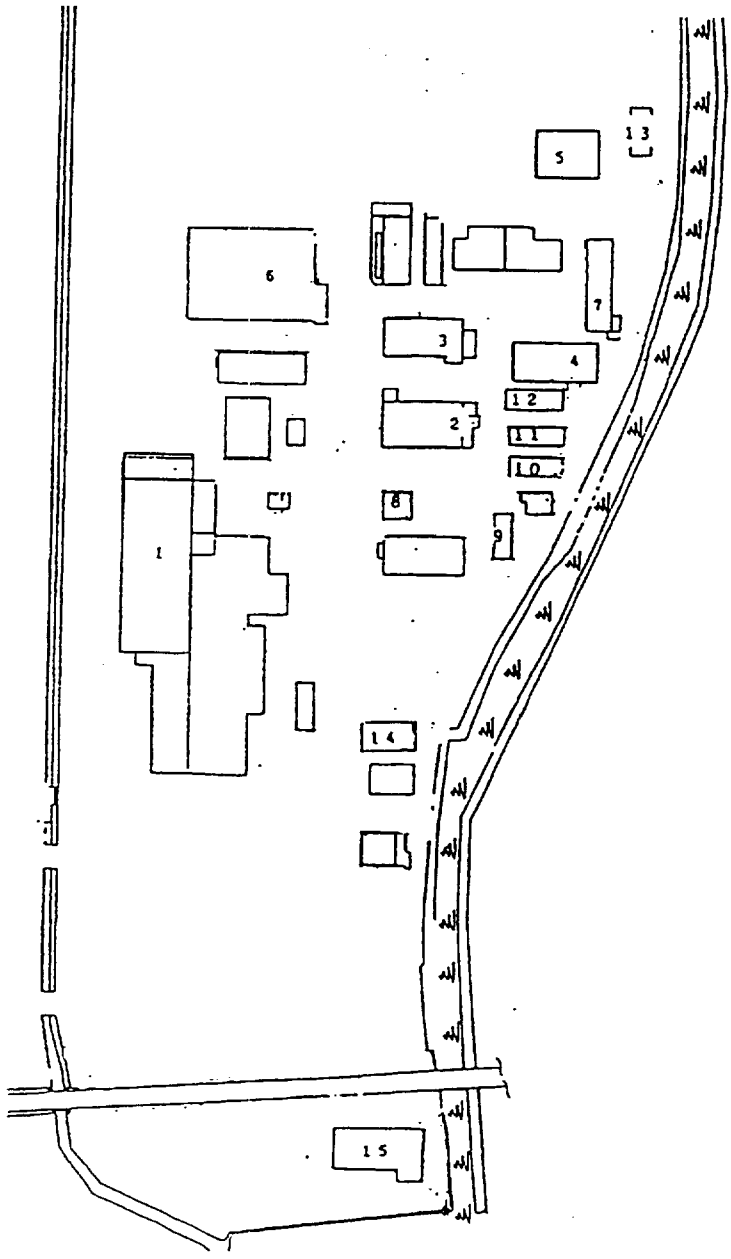
Area and plot plan of the organic synthesis buildings of manufacturing AG1346 (generic name: nelfinavir) are shown in Appendix 1) and 2).

Manufacture and purification of AG1346 (generic name: nelfinavir) are carried out at the buildings called Organic Synthesis (1st) and Organic Synthesis (2nd) , and the drying of in-process materials is carried out at Drying (organics) and the final refining process at Refining (organics,1st).

The tests for release of AG1346 is carried out at Quality control laboratories in the 2nd floor of the building called Pharmaceutical Preparation and QC.

The tests for raw materials used in manufacturing of AG1346 are carried out at

1.Warehouse
2.Organic Synthesis (1st)
3.Organic Synthesis (2nd)
4.Drying (organics)
5.Refining (organics, 1st)
6.Pharmaceutical Preparation and QC
7.Trial Production (organics)
8.Production Control
9.Warehouse for dangerous materials (3rd)
10.Warehouse for dangerous materials (1st)
11.Warehouse for dangerous materials (2nd)
12.Warehouse for poisonous materials (organics)
13.Warehouse for dangerous materials (4th)
14.Waste Water Neutralizing (organics)
15.Waste Water Treatment (organics)



[Figure - 1]

Date : November 7,1996

Agouron Pharmaceuticals, Inc.
10350 North Torrey Pines Road
La Jolla, CA 92037

Dear Sirs:

The manufacturing of AG1346 is conducted at Fuji Chemical located in Kamiichi Town, Toyama, Japan. The western part of Fuji Chemical Plant is adjacent to Kamiichi River and the rest of area is surrounded by the rice field. The Fuji Chemical Plant has a total area of about 28.3 acres. The climate of Kamiichi Town is characterized by warm summers 68 to 95 °F and cold to moderate winters 23 to 50 °F. The average annual rainfall is 110 inches. Most industries in Kamiichi Town obtain potable water from the well and most residence obtain potable water from the Kamiichi Town municipal water supply. The municipal water is supplied from the 1st Kamiichi Town water purification plant located at about 3 km east of the downtown of Kamiichi Town and pumped up at 56 feet depth under the ground. The Fuji Chemical Plant uses well water whitch is different from the municipal water. The source of this well are at 115 feet depth and 980 feet depth under the ground within the Fuji Chemical Plant. Waste water is sewered to an onsite water treatment facility, and then to Kamiichi River.

Sincerely.

Tsutomu Nakagawa

Tsutomu Nakagawa
Vice Director
Production Department

15-1C Yonezawa Hamari Chemicals, Ltd.

ATTN DR. MICHAEL ADAM

I AM SUBMITTING THE FOLLOWING FROM HAMARI'S DMF :

1. OUTLINE OF THE MANUFACTURING SITE (PAGE 3)
2. LAYOUT OF THE PLANT SITE (PAGE 8)
3. DIMENSIONS AND AREA OF BUILDINGS (PAGE 9/10)
4. FACILITIES AND EQUIPMENT CAPABILITY (PAGE 13)
5. MANUFACTURING FACILITIES (PAGE 16)
6. MAIN EQUIPMENT STATEMENT (PAGE 22)
7. QUALITY CONTROL FACILITIES STATEMENT (PAGE 29)
8. MAINTENANCE STATEMENT (PAGE 33)
9. WASTE DISPOSAL STATEMENT (PAGE 34)

IN ADDITION, I HAVE THE FOLLOWING AVAILABLE IF YOU NEED SAME:

A. MAP OF CITY OF OSAKA, WITH SITE NOTED (PAGE 5)
b. MAP WITH CLOSEUP OF SITE NOTED (PAGE 6)
c. MAP OF THE NEIGHBORHOOD OF HAMARI CLOSEUP (PAGE 7)
D. MAIN RAW MATERIALS LIST, DENOTING NORMAL STORAGE FACILITIES, INFLAMMABLES STORAGE A,D POISON STORAGE (PAGE 12)
E. LAYOUT OF PACKAGING AND STORAGE AREA (PAGE 14)
F. LAYOUT OF FIRST MANUFACTURING BUILDINGS (PAGE 15,17/21))
G. LIST OF MAIN EQUIPMENT (PAGE 23/28)
H. LAYOUT OF QC FACILITIES (PAGE 30)
I. LIST OF MAIN EQUIPMENT OF QC (PAGE 3132)

ALL OF THE ABOVE ARE TAKEN FROM A PARTIALLY CORRECTED DRAFT OF THE FIRM'S DMF, AND MAY INCLUDE TYPOS.

THANK YOU FOR THIS OPPORTUNITY TO BE OF SERVICE.

CALL ME IF YOU NEED ME. LOUIE F. TURNER

1. Name and address of the Manufacturing Site.

The plant of Hamari Chemicals, Ltd. is located in 4-29, kunijima 1-chome, Higashiyodogawa-ku, Osaka, 533 Japan and the manufacturing operation of bulk pharmaceutical chemicals is performed (in) this plant site.

Ref: Fig.1, Fig.2, Fig.3, (adn) Fig.4

The site of plant, which covers 2,210,17 sq. meters (0.546 acre) is shown in Fig.5

2. Operational layout and dimensions.

Buildings about 2,000 sq. meters in total floor space for storage, manufacturing and quality control are individually seperated as shown in Fig.5.

The dimensions and area space of the individual building and operational spaces are given in Table 1.

3. Product list.

A list of major products is appended and classified to items export to USA., others for domestic market and intermediates for drug substances manufacturing and specified storage conditions in Table 2.

None of penicillin, cepha antibiotic drugs, nor hormones is handled nor manufactured in this plant.

There is no facility for manufacturing of finished dosage form products in this plant.

4. Main raw materials list.

A list of main raw materials list is appended and classified to the regulatory requirements of Japan in Table 3.

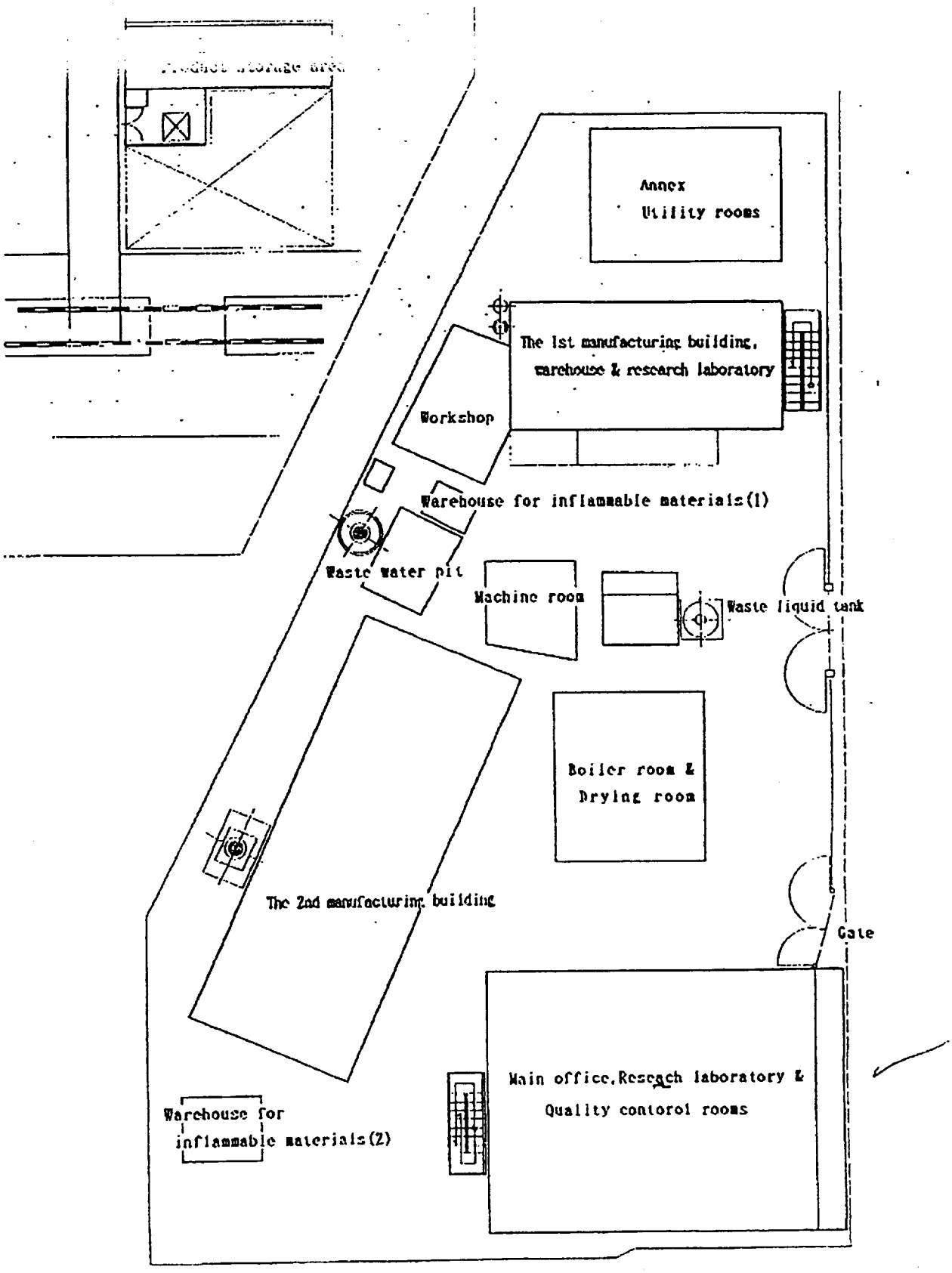


Fig.5. Layout of the plant site.

Building	Dimensions (m)	Area (m^2)
1st manufacturing building ;		352.2
1st floor ;	16.7 x 9.5	158.7
Drying room	4.8 x 3.1	14.9
Packaging & labeling room	2.1 x 2.7	5.7
Pulverizing room	2.1 x 3.1	6.5
Subdividing room	2.0 x 2.5	5.1
Dressing room	2.0 x 2.5	5.1
Storage of raw materials	3.4 x 6.1, 3.4 x 4.0	33.9
Storage of poison and violent poison	1.6 x 2.1	3.4
Sampling room	1.6 x 2.1	3.4
2nd floor ;	16.7 x 5.0	83.5
Drying room	4.8 x 3.1	14.9
Purifying room	3.2 x 3.5 , 2.7 x 1.5	16.2
Pass room	1.5 x 1.0	1.5
3rd floor ;		21.6
Drying room	2.4 x 2.6	6.2
Packaging & labeling room	2.2 x 2.0	4.4
Pulverizing room	2.3 x 2.0	4.6
Mixing room	2.9 x 2.2	6.4
4th floor		
Research laboratory	4.0 x 6.7 , 8.0 x 6.7	80.4

Building	Dimensions (m)	Area (m^2)
2nd manufacturing building ;	24.1 x (10.6+7.1)	426.1
1st floor	24.1 x 10.6	255.5
(Final purification rooms	3.6 x 4.8	17.3)
2nd floor	24.1 x 7.1	171.1
(Final purification rooms	3.6 x 3.2	11.7)
Packaging and product storage area ;		117.7
Sub-dividing and packaging room	4.2 x 3.3	13.9
Packaging and labeling room	6.9 x 3.3	22.8
Packaging material storage	5.0 x 3.3	16.5
Storage of finished products	11.9 x 4.0	47.6
Pre-fabricated refrigerator	3.5 x 2.6	9.1
Office room	2.9 x 2.7	7.8
Boiler and drying building	9.8 x 8.7	85.3
Boiler room	9.4 x 3.3	31.0
Drying room (2)	9.8 x 5.4	52.9
Drying room (1)	2.6 x 2.8	7.3
Main office building	20.0 x 15.0 x 3F + 18.0 x 15.0	1,170.0

III-1. Warehouse and atorage.

* Detailed floor plan of the finished products storage, packaging and laboling facilities is shown in Fig.6.

The construction of these facilities are made of wooden structure with steel sheet roofing and outside walls. The interior finishing of rooms are made of concrete floor painted with epoxy resin and covered with plastic sheet(easily cleanable carpet), plaster board wall of smooth surface and plastor board ceiling at about 2.5 m height.

Air-conditioning and dedusting facilities are individually provided according to the requirments of operation.

A pre-fabricated refrigerator(out-door type) of 20 cubic meter, 3.5m(w) x 2.6(d) x 2.15(h) is installed in the same area as shown in Fig.6 for the products required in cool place storage.

* General raw material storage is located in 1st floor of the 1st manufacturing building as shown in Fig.7 and separated the ordinary materials from the poisons and violent poisons. These facilities are made of concrete floor and slate board wall.

A sampling room for the sample collection of each shipment of each lot of raw material is also provided in this area. This facility is made of painted concrete floor of smooth surface and painted plaster board wall and ceiling of smooth surface and equipped with a ventilator.

* The warehouse for inflammable materials (1) is made of concrete floor, concrete block wall and slate board roofing at 2.1 m height and used for small volume containers of such raw materials.

The warehouse for inflammable materials (2) is made of concrete floor, reinforced concrete block wall of 150 mm thickness and the same roofing at 3.0 m height and used for large volume containers of such materials.

Those warehouses are shown in Fig.5.

* 1st manufacturing building is a four-stories steel frame structure building as shown in Fig.7,8 and 9, and the interior finishing differs with the requirements of operation. Manufacturing rooms in this building are of concrete floor painted with epoxy resin of smooth surface and painted plaster board wall and ceiling. These rooms are air-conditioned for the cotrol of designated conditions and supplied through particulate air filter eqipped with pressure differential monitoring system.

  Purifying room and adjacent pressure filter are equipped with stainless steel platform.

* 2nd manufacturing building is a steel frame structure building with concrete floor and concrete block wall up to 1.2 m height and mortar wall up to slate board roofing at 6.57 m height as shown in Fig.10 and 11. Steel structure platform is constructed at 2.62 m height and 2nd floor is made of iron checker board. METAL GRATES

  Final purification rooms of 1st and 2nd floor are made of concrete floor painted with opoxy rosin of smooth surface and painted plaster board wall and ceiling. These rooms are air-conditioned for the control of designated temperature.

  Spot compulsory ventilation is applied to the equipment or operation which is potential to generate dust or vapor.

* Boiler and drying building is a one story steel structure building with concrete floor and concrete block wall up to slate board roofing as shown in Fig.12.

  Boiler room is separated by concrete block wall from the drying room.

  Drying room is installed with four single end and builet in wall dryers and the interior finishing of drying room is made of painted concrete floor and stainless steel lamina wall and ceiling.

* There are written procedures for controlling access to these manufacturing facilities by visitors, outside or workers from other section of the plant and the brief explanation on the above is given in later Section of "Job description and responsibilities."

* Detailed list of main equipment for the manufacturing is appended and classified to the location correspond to the above manufacturing facilities.

* These equipment are readily used or adapted for a variety of products which are listed in Table 2. and a series of equipment is selected in accordance with the amount of production and requirement of operation.

* These equipment are cleanable according to written procedures and program established for sorts of equipment and with different procedures depending on what product or intermediate was produced.

  An equipment cleaning and use log is provided for the determination of prior use and identification of acceptable cleanliness.

* There is no manufacturing equipment located outdoors but the equipment for utility supply, air-conditioning and waste water treatment are situated out-of-doors.

* Facilities for the quality control is located in the third floor and partially in the second and fourth floors of main office building and a layout plan of the quality control laboratoris are shown in Fig.13.

* The building of main office is a four-stories reinforced concrete structure and the administrative office and resoarch laboratories are also situated in this building.

* The interior finishing of quality control laboratories are made of concrete floor covered with plastic tiles, painted plaster board wall and plaster board ceiling at about 2.5 m height.

* Main equipment of the quality control are given in Table 5 and the calibration of instruments, apparatus and recording devices are carried out at suitable interval in accordance with an established program.

* Rooms for the quality control are provided air conditioning facilitios to maintain required conditions and ventilatin to eliminate vapor and gas generated in the operation.

* Laboratory animals (is) not used in testing components, in-process materials, or products, except for research purpose.

- The buildings are constructed so that they can be maintained in good repair without difficulty.

  Periodic maintenance and cleaning are done in the whole plant three times a year.

- The calibration of instruments, apparatus, and guages are performed according to "Guideline for the maintenance of measuring instruments" and an established written program.

- The rooms and equipment are cleaned after every operation and/or when necessary, per written standard operation procedures and all cleaning operations are recorded.

- Operators check and adjust the equipment before and after operation according to the "Guidlines for the maintenance of equipment and the sanitary conditions of the manufacturing area" .

  Adjustment of equipment and facilities are recorded in the "Inspection file for equipment and facilities" .

- A person responsible for maintenance checks the "Inspection file for equipment and facilities" to confirm proper maintenance, and reports the monthly results to the quality control unit.

(a) Waste water disposal plant

Acid-alkali neutralization method: capacity, 6 m³/hour
Discharging according to the Environmental Pollution Prevention Act

pH : 7.0/day avorage
Biological Oxygon demand (BOD): Not more than 560 ppm
Suspended substance (SS) : Not more than 6 ppm
Verification method: The verification method for draining standard is defined by tho Minister of Environmented controls according to the Act of the Prime Minister's Office, regulating the draining standard.

(b) Treatment of waste matter

Solid waste materials are removed by Partech, Ltd. to a landfill. Wasto solvents and solvent distilled residue are stored in drums, picked up by Partech, Ltd. and incinerated.

Partech provides written assurance as to final disposition of all waste materials

FIRM SIGNED CONTRACT/CONFIRM + DOCUMENT,

FAX : (0238) 28-3605

PHONE : (0238) 28-3601

YONEZAWA HAMARI CHEMICALS, LTD.

2-4300-18, HACHIMANPARA, YONEZAWA, YAMAGATA, JAPAN.

SUBSIDIARY OF

HAMARI CHEMICALS, LTD.

November 8.1996

Agouron Pharmaceuticals.Inc.
10350 North Torrey Pines Road
La Jolla.CA 92037

Dear Sirs:

The manufacturing of AG1346 is conducted at Yonezawa Hamari Chemicals located in Yonezawa City.Yamagata.Japan. The Yonezawa Hamari Chemicals Plant is surrounded by industrial park. The Yonezawa Hamari Chemicals Plant has a total area of about 534.000 square feet. The climate of Yonezawa City is characterized by warm summers (68 to 95 °F) and cold to moderate winters (23 to 41 °F). The average annual rainfall is 447 inches. Most industries and residence in Yonezawa obtain potable water and industrial water from the City of Yonezawa municipal water supply. The source of the municipal water supply is Mizukubo reservoir. which is in the Azuma Mountain Chain. The Yonezawa Hamari Chemicals Plant uses municipal water only. Waste water is sewered to the onsite water treatment facility and the City of Yonezawa municipal water treatment facility. and then to the Mogami River.

sincerely.

M. Sawada

Michikazu Sawada
Plant Manager
Yonezawa Hamari Chemicals.Ltd.

15-1D Niro, Inc.

Md at 9165 Rumsey Road. This facility is located in an industrial park. This facility was built according to Howard and Maryland building codes in 1993. The facility includes pilot plant testing facilities which are utilized for the design and testing of chemical and pharmaceutical processes for a variety of clients. After testing all materials (solids and particulates) are collected and returned to the client.

Once the testing is completed the equipment is cleaned with water and detergents. The wastewater is contained and discharged to a on-site waste treatment facility. At this facility the wastewater is treated to meet Howard County specifications before being discharged to the local sanitary sewer. If this criteria can not be met the wastewater is hauled off-site and disposed of by a certified and audited waste hauler.

2. Releases of Agouron's dust and solvents:
For processing Agouron's AG1343 a closed cycle spray dryer will be used. In this process an ethanol solution with 26.5% of AG1343 will be pumped into a rotary atomizer. This atomizes the solution into a fine mist inside the drying chamber. Heated nitrogen is mixed with the fine mist which evaporates the ethanol leaving the AG1343 in powder form. The gas and powder mixture leaves the bottom of the dryer and enters a cyclone. The cyclone will separate out 95% of the powder. The remaining amount of powder is removed as it passes through the baghouse. The gas also passes through a Hepa filter to ensure no powder enters the exhaust fan or cooler. When the gas passes through the cooler the gas is cooled and the ethanol vapor is condensed out of the gas. Nitrogen exits the cooler and is then recirculated through the system. Attached is a sketch with proposed rates and material balances.

3 & 4. Emission control devices:
To control emissions and to recover the product there are three major devices. The cyclone will be used to recover 90 to 95% of the material produced. This will be on spec material. The baghouse will be used to collect material which is too fine to be used. This material will be reprocessed. The baghouse material will account

solvents will be removed by a certified and audited wastehauler. The remainder of the solvents will exit in a Nitrogen purge stream. This system has been filed with the State of Maryland Department of the Environment, Air and Radiation Management Administration and has been given their approval for installation.

5. There will be no impact on the current compliance of the facility at Niro Inc. The facility has been handling similar type materials and wastes in the past and will continue to do so in the future.

6. The manufacture of Agouron's AG1343 will require Niro to handle 35 to 40 additional drums of Ethyl Alcohol per month. The facility has been designed to handle this additional amount of Ethyl Alcohol. Niro will also be handling 2000 kg of additional raw and processed material per month and 350 kg of Methanesulfonic Acid per month. Niro has an existing limited access warehouse that will easily this increased amount of material. The additional utility requirements of this process will be minimal since this is a closed cycle process.

outfalls in areas associated with industrial activity. Once issued, the individual permit would include numerical discharge limitations for these outfalls and would require periodic sampling to confirm compliance. These regulations also outline the potential for the institution of a general permit but do not identify criteria for a general permit program application. For facilities in Maryland general permit applications are under the direction of the Maryland Department of the Environment (MDE) and Niro must adhere to the provisions of this permit in lieu of an individual permit (See Section 1.03).

1.03 State Regulations Overview

The Maryland Department of the Environment (MDE) was authorized by the USEPA to administer a comprehensive state storm water management program. This program allows industries with specific SIC codes and discharges that are composed entirely of storm water to apply for coverage under a statewide general permit in lieu of an individual permit. The benefit of the general permit is that, currently, no storm water sampling is required and no numerical limitations will be included in the general permit.

According to these regulations, facilities seeking coverage under the statewide general permit must submit a Notice of Intent (NOI) and prepare and implement an SPPP. The NOI for the Niro Inc. facility was submitted to the MDE in September 1992. Niro was granted coverage under the General Discharge Permit effective on September 29, 1992 with an expiration date of September 28, 1997.

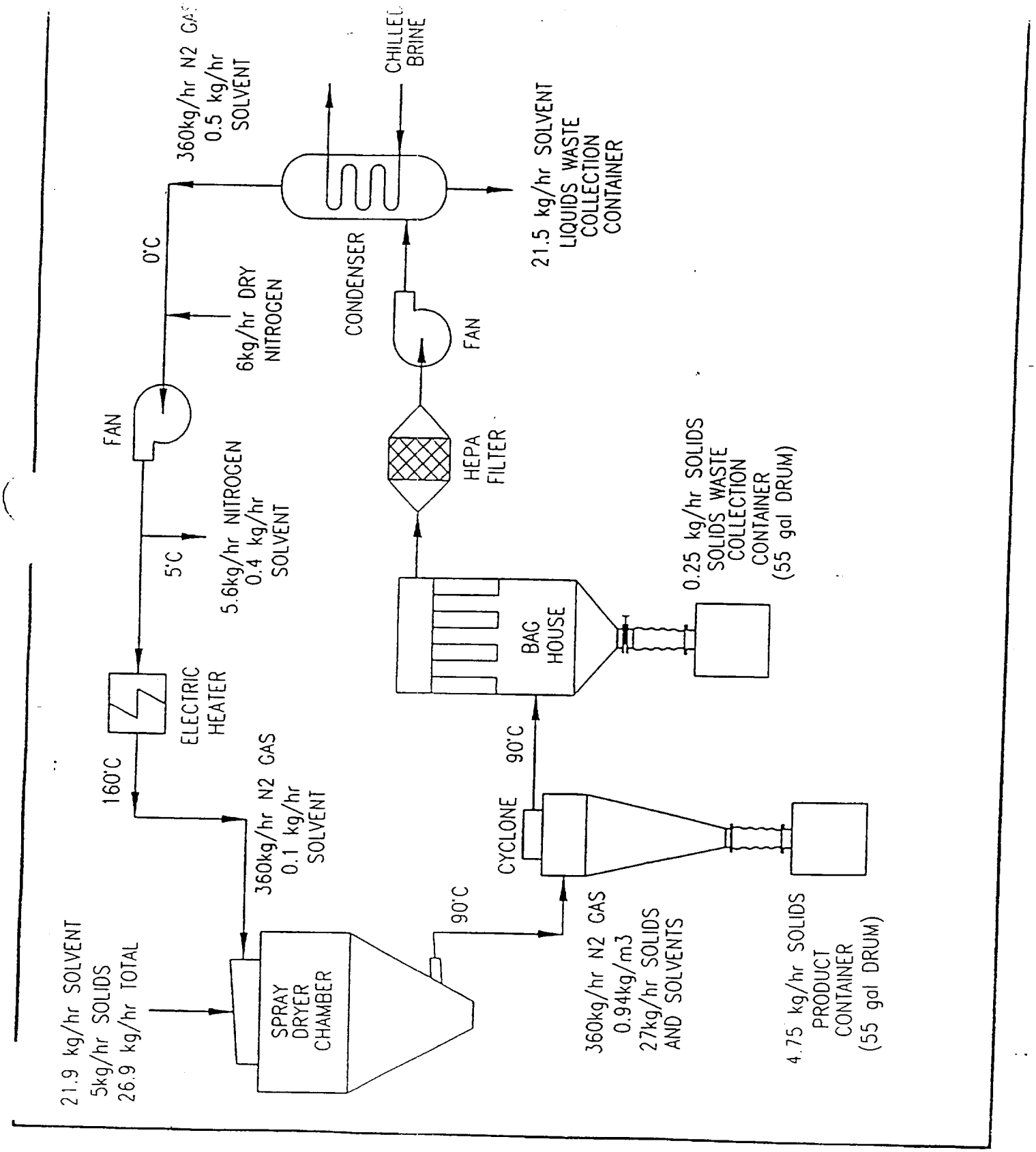
In accordance with the General Discharge Permit No. 92-GP-0001 (General NPDES Permit No. MDR000001), the deadline for completion of the SPPP is twelve months following submittal of the NOI. The SPPP need not be submitted to MDE, but the SPPP should be available for review at the facility. Implementation of the SPPP must occur within eighteen months of the NOI submittal. Upon implementation, annual inspections must be included in the plan documenting that the SPPP has been implemented and is effective.

areas of the State of Maryland with new and existing discharges composed entirely of storm water that are composed in whole or in part of storm water discharges associated with industrial activity.

In July of 1993, O'Brien & Gere Engineers was retained by Niro Inc. to prepare a storm water pollution prevention plan (SPPP) for their Columbia, Maryland facility to maintain compliance with the provisions of the general permit. In accordance with the protocol for production of this plan, a site inspection was conducted by Mr. Brian FitzPatrick of O'Brien & Gere Engineers on August 23, 1993. At this time, storm water discharge points were observed to confirm that no dry weather flow was observable in the storm drainage system. On two additional occasions Niro personnel reportedly revisited the storm water discharge points to confirm that there were no non-storm water contributors to flow (See Section 2.05). This was done to satisfy the requirement that discharges are composed wholly of storm water. Because the storm water discharges which occur at the Niro site are composed entirely of storm water, the site is eligible for coverage under the State of Maryland General Permit.

It was concluded following the site visit that there was limited storm water contact with potentially hazardous or pollutant substances at the Columbia facility since no operations, manufacturing, or storage is conducted outside. Based upon the type of materials managed at the Columbia facility and the type of operations that occur at the site, the most likely sources of contact would be during an accidental spill during unloading or transfers of materials. A summary of potential pollutant sources is provided in Section 2.

SHOW LOCATION OF DUST CLEANING EQUIPMENT IN THE SYSTEM. DRAW OR SKETCH FLOW DIAGRAM SHOWING EMISSION PATH



15-1E MOVA Pharmaceutical Corporation

September 13, 1996

RE: CERTIFICATION OF ENVIRONMENTAL COMPLIANCE

Gentlemen:

This letter certifies that **MOVA** Pharmaceutical Corporation, located at Caguas, Puerto Rico, manufactures solid dosages, oral liquids, and parenteral drugs for sale to pharmaceutical companies including Agouron.

The facility operates in compliance with all environmental permits and to date there are no outstanding non-compliance issues with the environmental regulatory authorities.

It is not anticipated that the on-going manufacturing operations will result in a permit violation as the facility plans to meet the Agouron production demand.

Cordially,

ENVIRONMENTAL ASSESSMENT FOR VIRACEPT (Nelfinavir Mesylate) CMC

1. **Description of the environmental setting:**

MOVA Pharmaceutical Corporation is a pharmaceutical manufacturing facility located in the State Road #1, Km. 34.3, Caguas, Puerto Rico. Its actual location is zoned for industrial purposes I-1 as established by the Permits and Regulations Administration and the Puerto Rico Planning Board.

MOVA is surrounded mostly by other manufacturing industries: Quality Electroplating and Carmela at the North, Zafiro Street at the East, and undeveloped lots at the South and West. There is a small community next to the southwest parking lot corner.

The facility drains naturally to the southeast towards Rio Cagüitas, which is 600 meters from the southeast corner of MOVA.

The potable water is served by the Puerto Rico Aqueduct and Sewer Authority (PRASA). The Department of Natural and Environmental Resources approved a franchise for the well water extraction when PRASA can not supply the water.

The wastewater is treated in our wastewater treatment plant prior to be discharged to the PRASA's Regional Wastewater Treatment Plant. Actually, we discharge an average of 75,000 gallons per day.

2. **Releases of drug and process substances:**

A liquid solution of purified water and FD & C Blue #2 will be discarded at a rate of 17.0 kg per lot that will be handled in our wastewater treatment plant.

About 0.5 kg of product will be lost during the drying step of each lot. These emissions will be controlled by a filter with an efficiency of 90%.

We calculate that 5 kg of product waste per lot will be generated from the tablet compression process. This waste will be disposed in a disposal facility with the permits required by federal and state regulations.

3. **Procedures to control the impact of releases:**

The manufacturing areas are provided with filter and dust collector systems. MOVA has a Contingency and Spill Control and Countermeasure Plan as required by the environmental regulations that contains procedures to prevent and control spill situations.

The wastes generated during the manufacturing activities are packed according to the Department of Transportation regulations and accumulated in areas designated for those purposes. The hazardous waste is disposed by using Ochoa Environmental Services, EPA number PRD090128562. The non hazardous waste is disposed in an industrial non hazardous waste landfill in Peñuelas, PROTECO. The finished goods are disposed in a non hazardous waste incineration facility, Commercial Incineration Corporation, located in Peñuelas. The washwaters are pumped to our wastewater treatment plant for primary and secondary treatment. Our wastewater treatment includes: solids removal, pH adjustment, biological treatment and filtration. The discharge is sent to PRASA's regional treatment plant for further treatment.

4. **Applicable State and U.S. regulations and permits for the manufacturing procedures:**

The Environmental Quality Board (EQB) is the state agency that regulates the environmental impact of our manufacturing procedures. Also, the Environmental Protection Agency (EPA) regulates our activities.

The EQB issued the Permit for the Operation of the Emission Sources, PFE-LC-13-0895-1881-I-II-0, in January 30, 1996. This permit will expire in October 17, 2000. **MOVA** is not affected by air federal regulations because we are a minor source.

The EPA provided the identification number for the generation of hazardous waste, PRD-174-050-377 on April, 1988. **MOVA** is a large quantity generator and is inspected yearly by the EQB.

PRASA is the state agency that issued the permit for the industrial discharge of the wastewaters. **MOVA**'s permit was issued in December 24, 1994 and will expire on December 23, 1996. **MOVA** is in the process of renewal for this permit number GDA-88-602-010.

The stormwater discharge permit is PRR-00-A-134 and was issued by the EPA in October, 1992. **MOVA** has a general permit.

The Department of Natural and Environmental Resources issued in July 18, 1996, a franchise for the use of well water. This franchise will expire on July 18, 1999.

5. **Impact of the new product manufacturing:**

The addition of this new product will not impact significantly the compliance with existing environmental regulations. **MOVA** will experience and increment of 4,000 gallons per day in the wastewaters discharges and an increase of 1.21 tons per year in air emissions.

6. **List of Raw Materials:**

a. Nelfinavir Mesylate
b. Calcium Silicate
c. FD & C Blue #2
d. Purified Water

7. **Recycling of the materials:**

MOVA is recycling materials such as cardboard, paper, glass and metal.

8. **Historical and Archaeological Preservation Sites**

A consultation was made to the Institute of Puertorrican Culture on June, 1995, to determine that there are no national historic sites within a 15 km radius of the manufacturing facility.

ATTACHMENT 15-2

Certificate of Environmental Compliance from the Facility Managers

- **15-2A Ganes Chemicals, Inc.**
- **15-2B Fuji Chemical Industry Co., Ltd.**
- **15-2C Yonezawa Hamari Chemicals, Ltd.**
- **15-2D Niro, Inc.**
- **15-2E MOVA Pharmaceutical Corporation**

15-2A Ganes Chemicals, Inc.

Date: November 7, 1996

Company: Ganes Chemicals Inc.

Facility: Pennsville, NJ

Dear Sirs:

I hereby certify that the manufacturing facility noted above is:

1. in compliance with all state and federal environmental laws;
2. in compliance with, or on an enforceable schedule to be in compliance with all emission requirements set forth in all permits; and
3. approval and the subsequent increase in production at this facility is not expected to affect compliance with current emission requirements of compliance with environmental laws.

Furthermore, this manufacturing facility and the surroundings are not on sites of any historic or archeological significance per the Department of Natural Resources or other state agencies.

Signature: C A Kramer

Name (printed): C. A. KRAMER

Title: V.P. TECHNICAL OPERATIONS

15-2B Fuji Chemical Industry Co., Ltd.

Semptember 20, 1996

Agouron Pharmceuticals, Inc.
10350 North Torrey Pines Road
La Jolla, C A 92037

Dear Sirs:

We hereby certify that the manufacturing facility of Fuji Chemical Industry . Co.,Ltd. 1 Gohkakizawa Kamiichi, Toyama, Japan is:

1) in compliance with the environmental laws and regulations of Japan;

2) in compliance with all emission requirements set forth in all permits of prefectural and country;and

We further certify that approval and the subsequent increase in production at the facility is not expected to affect compliance with current emission requirements or compliance with environmental laws.

Sincerely.

masaya yamada

Masaya Yamada
Director
Production Department

15-2C Yonezawa Hamari Chemicals, Ltd.

FAX : (0238) 28-3805

PHONE : (0238) 28-3801

米沢浜成

YONEZAWA HAMARI CHEMICALS, LTD.

2-4300-18, HACHIMANPARA, YONEZAWA, YAMAGATA, JAPAN.

SUBSIDIARY OF

HAMARI CHEMICALS, LTD.

September 17, 1996

Agouron Pharmaceuticals, Inc.
10350 North Torrey Pines Road
La Jolla, CA 92037
U. S. A.

Dear Sirs:

We hereby certify that the manufacturing facility of Yonezawa Hamari Chemicals, Ltd. 2-4300-18, Hachimanpara, Yonezawa, Yamagata, Japan is:

1) in compliance with the environmental laws and regulations of Japan;

2) in compliance with, or is on an enforceable schedule to be in compliance with, all emission requirements set forth in all permits of local authority; and

We further certify that approval and the subsequent increase in production at the facility is not expected to affected compliance with current emission requirements or compliance with environmental laws.

Sincerely,
Yonezawa Hamari Chemicals, Ltd.

高梨時郎

Tokiro Takami
President

15-2D Niro, Inc.

Date: 07 NOVEMBER 96

Company: NIRO INC.

Facility: Pharmaceutical Technology Center - Columbia, MD.

Dear Sirs:

I hereby certify that the manufacturing facility noted above is:

1. in compliance with all state and federal environmental laws;
2. in compliance with, or on an enforceable schedule to be in compliance with all emission requirements set forth in all permits; and
3. approval and the subsequent increase in production at this facility is not expected to affect compliance with current emission requirements of compliance with environmental laws.

Furthermore, this manufacturing facility and the surroundings are not on sites of any historic or archeological significance per the Department of Natural Resources or other state agencies.

Signature: Donald F Adams 07 NOV 96

Name (printed): DONALD F. ADAMS

Title: PRODUCTION MANAGER

15-2E MOVA Pharmaceutical Corporation