CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-503

STATISTICAL REVIEW(S)

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research
Office of Pharmacoepidemiology and Statistical Science
Office of Biostatistics

STATISTICAL REVIEW AND EVALUATION

NEW DRUG APPLICATION

CLINICAL STUDIES

NDA/Serial Number:	21-503 / N000
Drug Name:	VIRACEPT® (nelfinavir mesylate) 625 mg tablets
Indication(s):	Treatment of HIV infection
Applicant:	Agouron Pharmaceuticals, Inc.
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Keywords:	HIV infection, antiretroviral naïve (48-week data), PK-safety analysis, time to discontinuation, AUC, Pooling.

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1. EXECUTIVE SUMMARY

1.1 Conclusions and Recommendations

Sensitivity analyses of the PK-safety data in subjects who received the VIRACEPT 250-mg suggest that frequency and episode of eye disorder, ACNE, and diarrhea showed a tendency to increase with increased exposure to NFV. In addition, higher exposure of NFV may also increase the likelihood of NFV-related discontinuations. Other treatment emergent adverse events were not associated with increased exposure of NFV.

Based on analysis of the VIRACEPT 250-mg PK-safety data, the above results could be inferred to the VIRACEPT 625-mg formulation, using appropriate statistical modeling on data from the bioequivalence studies between the 250-mg and the 625-mg formulations.

1.2 Brief Overview of Clinical Studies

VIRACEPT® 250-mg (nelfinavir mesylate, NFV) was approved in 1997 for the treatment of HIV infection. A new VIRACEPT 625-mg formulation with a higher strength was developed to reduce pill burden and increase drug compliance. Clinical data require statistical review were data from pharmacokinetic-safety (PK-safety) studies. The PK-safety analysis was performed on subjects from two pooled populations PP-1 and PP-2, for whom both safety and plasma concentration of nelfinavir data were available.

In PP-1, 317 subjects who received nelfinavir were from two pivotal clinical studies AG1343-511 and AG1343-542, and two clinical pharmacokinetic studies, AG1343-503 and AG1343-510. The study population was predominantly male (91%), with a median age 36 years. The majority of subjects were Caucasian (85%). The median baseline HIV RNA was 4.85 $\log_{10}$, the median CD4+ cell count was 297 cells/mm^3.

In PP-2, 22 subjects from M3331-0073A and 173 from M3331-0073B received nelfinavir and delavirdine. The study population was predominantly male (81%), with a median age was 36 years. The majority of subjects were Caucasian (64%). The median baseline HIV RNA was 4.82 $\log_{10}$, and the median CD4+ cell count was 298 cells/mm^3.

1.3 Statistical Issues and Findings

The statistical review included (1) the applicant's final report in Integrated Safety Summary provided in Section 8 Clinical Data and in Summary of section 3; (2) adverse event tables in the NDA submission requested by the medical reviewer; and (3) data sets primarily on PP-1.

The applicant indicated that, of the adverse events typically associated with nelfinavir (NFV), only diarrhea showed a tendency to increase with increased exposure, yet the rate of diarrhea in these studies varied widely. Nelfinavir-associated diarrhea is generally a manageable reaction and rarely interrupts therapy; patients infrequently discontinued due to diarrhea. No unexpected adverse events appeared outside the safety profile associated with the use of VIRACEPT.

In response to the medical reviewer's request, the statistical reviewer verified the applicant's findings in safety analysis on treatment-emergent adverse events. In addition, the statistical reviewer conducted sensitivity analysis using 33 and 67 percentiles of plasma NFV AUC concentration (<37, 37-51, >51 mg.h/L) to regrouping the study subjects, so that a tendency of treatment emergent adverse event with increasing NFV exposure could be re-examined and detected.

In the past, only frequency of subjects with more than 2% adverse events were reported. The quantitative nature of these adverse events had never been investigated. There arose a need to compare safety outcome quantitatively among AUC subgroups, via episode of treatment-emergent adverse events, defined as person-time treatment-emergent adverse events. The safety data of pooled populations in this submission permitted us to quantitatively conduct such an analysis.

Up to now, sponsor only reported adverse event related discontinuations and the reasons for discontinuation had to be re-adjusted. A patient may drop out for "lack of efficacy" or "other reasons" and there were adverse events just before dropping out (or just after),. There was a need to scrutinize these discontinuations more closely. This reviewer felt that all discontinuations, not just those due to adverse events may furnish information regarding the safety of NFV. Therefore, analyzing time to discontinuation by reason and AUC group ought to be done when the data are available.

Based on the available PK-safety data on PP-1 from four nelfinavir studies 511, 542, 503 and 510, revealed the following findings:

1. Analyses shows 43 (12%) of the study subjects in PP1 had a total of 88 cardiovascular events. There was no sufficient evidence to show frequency or episode of cardiovascular events was associated with NFV exposure and NFV studies in PP1, $p>0.05$ by the Chi-square test and the Wilcoxon test, respectively.

2. Sensitivity analyses on all causalities and all grades treatment emergent adverse events by alternative category of AUC (<37, 37-51, >51 mg.h/L) show that (1) increased frequency of eye disorder and frequency of ACNE may also be associated with increasing exposure of NFV, $p<0.05$, by the Chi-square test; and (2) increased episode of eye disorder, ACNE, and diarrhea may be associated with increasing exposure of NFV, $p<0.05$, by the Wilcoxon test. No associations were found between NFV exposure and frequency or episode of Asthenia, Lymphadenopathy, Nausea, Flatulence, rash and other adverse event.

3. Analyses using Kaplan-Meier approach and non-parametric competing risk models evaluating time and probability of discontinuation that were NFV-associated or not-NFV-associated, by NFV exposure category (<37,37-51,>51 mg.h/L) show the following results:

   a) Time to all causality discontinuation may be associated with increased exposure of NFV, $p<0.05$, by the Log-rank test;

   b) Time to NFV associated discontinuation may also be associated with increased NFV exposure; i.e., more subjects in medium and higher AUC groups discontinued than those in the lower AUC group; and

   c) The relationship between NFV exposure and time to non-NFV associated discontinuation was not clear using the competing risk model.

4. Sensitivity analyses of NFV-associated diarrhea in PP1 using 33 and 67 percentiles of AUC for AUC categories, show the following:

   a) Increasing frequency of NFV-related diarrhea may be associated with increasing exposure to NFV, $p=0.0799$ for Grade 1+ diarrhea, $p=0.0337$ for Grade 2+ diarrhea, by the Cochran-Mantel-Haenszel test. Results from alternative analysis using logistic regression models support the associations.

   b) A subject in PP1 had an average of 1.5 episodes of Grade 1+ diarrhea that were possibly associated with NFV. Stratifying by AUC subgroup and study, the mean episode of diarrhea ranged from 1 to 3.2 episodes per person. Subjects in AUC > 51 group had 50% –70% more episodes than subjects in the lower and medium AUC groups. Increasing episode of NFV-related diarrhea is associated with increasing exposure to NFV, $p=0.0106$, by the Wilcoxon tests. Results from alternative analysis using linear regression model support that the episode of diarrhea increases as NFV exposure increases.

   c) Variations in frequency of diarrhea were observed from study-to-study. Frequency of Grade 1+ or Grade 2+ diarrhea had no significant difference among studies. Subjects in two PK studies 510 and 503 had more episode of diarrhea than those in the two pivotal studies 511 and 542, $p=0.0045$, by the Wilcoxon test. Mean episode of diarrhea per subject presented a trend, the higher the NFV exposure, the more the episode of diarrhea, except in Study 542.

5. Additional analysis of ever/never NFV-associated diarrhea in PP1 using logistic regression models with covariates AUC, Age, gender, Caucasian or not, baseline CD4+ cell count, and baseline HIV RNA show the following:

a) Increased NFV exposure (AUC) was associated with increasing probability of NFV-associated Grade 1+, or Grade 2+ diarrhea. Age, gender, baseline CD4+ cell count, and baseline HIV RNA may not be associated with NFV-associated Grade 1+, or Grade 2+ diarrhea.

b) White (Caucasian or not) is associated with higher probability of Grade 1+ diarrhea, not associated with Grade 2+ diarrhea. Race may be a confounding factor for pk-Grade 1+ diarrhea data, i.e., 80.6% of Caucasian had Grade 1+ NFV-associated diarrhea, compared it with 63.3% in non-Caucasian subjects, p=0.007, by the Chi-Square test. However, the mean AUC was 49.3 mg.h/L in Caucasian subjects, much lower than 55.4 in non-Caucasian subjects, p=0.0002 by the Wilcoxon test.

c) These results in 5.a) and 5.b) were supported by the results of statistical modeling controlling for NFV studies. However, the analyses were retrospective in nature, and the original NFV study design did not address the race issue. Interpretation of these results should be cautious.

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2. INTRODUCTION

2.1 Overview

VIRACEPT® (nelfinavir mesylate, NFV) is an inhibitor of the human immunodeficiency virus (HIV) protease, and is indicated for the treatment of HIV-1infection. VIRACEPT® was approved by the FDA on March 14, 1997, on a 250 mg strength under NDA 20-779, and on a 50 mg/g strength oral powder under NDA 20-778. The current recommended dose for VIRACEPT tablets in adults is 1250 mg (i.e., five 250 mg tablets) twice daily or 750 mg (i.e., three 250 mg tablets) three-times daily in combination with nucleoside analogues.

In this submission NDA21503, applicant Agouron Pharmaceuticals Inc. is to receive a traditional approval for a VIRACEPT® 625 mg strength tablet in order to decrease the pill burden from ten tablets to four tablets daily for HIV-infected patients using the most commonly administered twice daily regimen of VIRACEPT. Two single-dose bioequivalence studies AG1343-712 and AG1343-713 have been conducted comparing the commercially available VIRACEPT 250 mg tablets to the proposed 625 mg NFV tablets in both fasted and fed conditions, respectively. The pharmacokinetical properties were evaluated via AUC and C_{max}. An increase in AUC and an increase in C_{max} were found with the 625 mg tablets, indicating that higher exposures may be achieved with the 625 mg tablet.

In the NDA submission, the applicant reported a retrospective assessment of safety data from two populations in which patients received nelfinavir and patients received nelfinavir and delavirdine. No assessment of efficacy was needed.

The pharmacokinetic-safety (PK-safety) analysis was performed on two pooled populations, PP-1 and PP2, for subjects for whom both safety and plasma concentration of nelfinavir data were available. The PP-1 included 317 subjects who received nelfinavir from four clinical studies AG1343-511, AG1343-542, AG1343-503 and AG1343-510. The PP-2 included 195 subjects who received nelfinavir and delavirdine from studies M3331-0073A and M3331-0073B as RESCRIPTOR-VIRACEPT studies. The applicant stated that, "of the adverse events typically associated with nelfinavir, only diarrhea showed a tendency to increase with increased exposure, yet the rate of diarrhea in these studies varied widely; and nelfinavir-associated diarrhea is generally a manageable reaction and rarely interrupts therapy; patients infrequently discontinued due to diarrhea. No unexpected adverse events appeared outside the safety profile associated with the use of VIRACEPT". The applicant concluded that the 625 mg formulation does not present any increased risk to patients of unexpected or significant medical events and presents a favorable benefit to its risk profile.

Different from other NDA submissions, there had no controlled or comparative studies evaluating the virologic response to the NFV 625-mg formulation-contained regimen. Therefore, the statistical review focuses on safety issues related to PK-safety data from previous NFV studies for PP-1: consist of two pivotal clinical studies, AG1343-511 and AG1343-542, and two clinical PK studies, AG1343-503 and AG1343-510.

2.2 Data Sources

2.2.1 Pharmacokinetical-Safety Data for Pooled Populations 1 & 2

The applicant reported PK-safety analysis on two-pooled populations:

Pooled Population 1 (PP-1)

Including four NFV studies AG1343-503, AG1343-510, AG1343-511, and AG1343-542. The Studies 511 and 542 were pivotal clinical studies, and the Studies 503 and 510 were phase II clinical pharmacokinetic studies. PP-1 had 317 subjects for whom both pharmacokinetic and safety data were available.

Pooled Population 2 (PP-2)

Including 153 subjects from two NFV/delavirdine (DLV) studies 73A and 73B. Study 0073A had 23 subjects. Study 0073B had 173 subjects, 130 received NFV/DLV, and 43 received NFV without DLV. Delavirdine is an inhibitor of NFV clearance. Therefore, subjects who received NFV/DLV were exposed to considerably higher levels of nelfinavir.

2.2.2 Categories in Plasma Concentration of Nelfinavir (AUC)

2.2.2.1 AUC Categories by the Applicant

One of the pharmacokinetic parameters measuring nelfinavir concentration is Area Under the Curve 'AUC_{24}' mg.h/L (AUC). According to the applicant, the lower and upper quartile values of AUC_{24} of 41 and 61 mg.h/L respectively were considered a representative exposure range of nelfinavir in HIV patients at steady-state. In addition, AUC_{24} value above 61 mg.h/L may be considered as high exposure and critical for the assessment of safety in order to address and increase in adverse events with increasing exposure. Throughout the submission, the pharmacokinetic-safety data were summarized according to subjects' AUC_{24} levels (<41, 41-61 and >61 mg.h/L).

2.2.2.2 AUC Categories by The Reviewer

In PP-1, the lower and upper quartile values were 34 and 55 mg.h/L respectively. Using the applicant's grouping rules, 39% of the study subjects in PP1 had AUC <41 mg.h/L, and 16% of the study subjects had AUC >61 mg.h/L. Therefore, it is unclear where the applicant obtained the cutpoints of AUC_{24} for grouping.

In order to obtain an even number of patients per AUC subgroup, this reviewer conducted a sensitivity analysis on PK-safety data using AUC cutpoints of 33 percentile (37 mg.h/L) and 67 percentile (51 mg.h/L). These cutpoints are close to the geometric means of two PK studies identified by the PK reviewer.

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3. STATISTICAL EVALUATION

3.1 Evaluation of Efficacy

VIRACEPT® (nelfinavir mesylate) 250 mg tablets were approved in 1997 under NDA20-779, where the efficacy of VIRACEPT® was evaluated by studies AG1343-511, AG1343-542, ACTG-364, and Avanti 3. No new clinical studies of the nelfinavir 625-mg formulation were conducted for the assessment of efficacy.

3.2 Evaluation of Safety

This reviewer verified the applicant's findings in PK-safety analysis on treatment-emergent adverse events for PP-1. Note that the treatment-emergent adverse events have been reported in a NDA submission and labeling if it occurred in more than 2% of the study population. The statistical review work including the following aspects:

- A sensitivity analysis on nelfinavir PK-safety data was carried out to determine whether there is an increased trend with increasing NFV exposure. Subjects in PP-1 were re-grouped into an alternative category using the 33 and 67 percentiles of NFV exposure level (AUC).

- Additional analysis on episode of a treatment-emergent adverse event amongst AUC subgroups was considered using basic statistical approaches. In the past, only a percentage of subjects with a frequent treatment-emergent adverse event (>2%) was reported and the quantitative nature of these adverse events had been neglected. In the safety databases of PP1 in this submission, a quantitative analysis on adverse events is permissible.

- Linear and logistic regression models were utilized to estimate the magnitude of association between diarrhea and NFV exposure. Covariates, such as age, gender, race (Caucasian or non-Caucasian), baseline CD4+ cell count, baseline plasma HIV viral load, and NFV study, were discussed.

- Time to discontinuation analysis was conducted using non-parametric competing risk models and Kaplan-Meier approach. Reasons for discontinuation were classified into nelfinavir-related or not. So far sponsor only reported adverse event related discontinuation although the safety data consist of many categories for subject's discontinuation.

3.2.1 Study Design

The original study design of the PP-1 and PP-2 are listed below.

Study AG1343-511

Title: 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

A total of 297 antiretroviral naive HIV-1 infected patients were enrolled and randomized to one of three arms: zidovudine (ZDV; 200 mg TID) and lamivudine (3TC; 150 mg BID) plus 2 doses of VIRACEPT (750 mg n=99, and 500 mg TID, n=97) to zidovudine (200 mg TID) and lamivudine (150 mg BID) alone (n=101). The median age was 35 years, 89% male and 78% Caucasian. Mean baseline CD4+ cell count was 288 cells/mm^3 and mean baseline plasma HIV RNA was 5.21 log_{10} copies/mL. Duration of therapy was 24 weeks with an extension to 12 months.

The pharmacokinetic of nelfinavir was studied in a subset of patients from each dose regimen. Plasma samples for full PK profiles were collected at selected sites before and at multiple times after the morning dose at week 4 (up to 12 hours after dosing for the BID regimen and 8 hours after dosing for the TID regimen).

Study AG1343-542

Title: "A Phase III Study Comparing BID and TID Dosing of VIRACEPT™ in Combination with Stavudine (d4T) +Lamivudine (3TC) in HIV Positive Patients".

A total of 542 subjects were randomized to VIRACEPT 1250 mg BID (n=336) and VIRACEPT 750 mg TID (n=206) in combination of stavudine (d4T; 30-40 mg BID) and lamivudine (3TC; 150 mg BID). Patients had a median age of 36 years (range 18 to 83). 84% was male, and 91% was Caucasian. Patients had received less than 6 months of therapy with nucleoside transcriptase inhibitors and were naïve to protease inhibitors. Mean baseline CD4+ cell count was 296 cells/mm^3 and mean baseline plasma HIV RNA was 5.0 log_{10} copies/mL. This study was conducted in 29 sites across 11 European countries. This study lasted 3 years with a minimum of 48 weeks of therapy.

Study AG1343-503

Title: "A Phase II, Open-Label, Dose Ranging-Finding Study in HIV Positive Subjects".

This study was conducted at three sites within the United States. A total of 65 HIV positive subjects were enrolled and completed the 28 day core study. Among them 54 subjects continued to received nelfinavir in the protocol extension period, 25 receiving

nelfinavir on a BID regime (500 mg, 600 mg, and 750 mg) and 29 on a TID regime (500 mg, 750 mg, and 1000 mg). The duration of study was 18 months following the 28-day core study.

Study AG1343-510

Title: "A Phase I/II Pilot Study of VIRACEPT (AG1343) in Combination with Stavudine (d4T) Versus Stavudine (d4T) Alone in HIV-positive Patients".

Study 510 was conducted in three sites within the United States. An 8-week pilot study was designed to evaluate the safety and efficacy of 3 dosages of VIRACEPT (500, 750, and 1000 mg TID) plus stavudine versus stavudine alone. A total of 32 HIV positive subjects were enrolled this study after completion of the 8-week pilot study (n=39). Patients were followed for 12-months or until VIRACEPT became commercially available.

Study M3331-0073A

Title: "An open-label randomized study of delavirdine mesylate (DLV, RESCRIPTOP®) plus nelfinavir (NFV), didanosine (ddI), and stavudine (d4T) in triple and quadruple treatment regimens in HIV-1 infected individuals".

Study 73A was conducted in 6 sites of the United States. 22 HIV-1 infected patients were enrolled and randomized to DLV 400 mg TID, and DLV 600 mg TID groups plus nelfinavir (NFV 750 mg TID), didanosine (ddI, 12 mg or 200 mg BID) and stavudine (d4T, 30 mg or 40 mg BID) regimens. Patients were to be treated for 24 weeks with the option of continuing study for an additional 24 weeks at the discretion of the investigator. The mean age was 37.5 years, approximately 82% were male, and 91% were Caucasian. Mean baseline HIV RNA was 4.9 in $\log_{10}$ and mean baseline CD4+ cell count was 372 cells/mm^3. PK evaluation was performed on all patients at Week 4.

Study M3331-0073B

Title: "An open-label randomized study of delavirdine mesylate (DLV, RESCRIPTOP®) plus nelfinavir (NFV), didanosine (ddI), and stavudine (d4T) in triple and quadruple treatment regimens in HIV-1 infected individuals".

Study 73B was conducted in 39 sites of the United States. A total of 137 HIV-1 infected patients were enrolled: 35 patients in the DLV+NFV+ddI group, 34 patients in the DLV+NFV+d4T group, 34 patients in the NFV+d4T+ ddI group, and 34 patients in the DLV+NFV+d4T+ddI group (NFV: 400 mg or 600 mg TID). Duration of study was 24 weeks with three optional extensions of 24-week period. No PK evaluation was performed.

3.2.2 Overall Safety Profile

The applicant performed PK-safety analysis on two pooled studies, VIRACEPT/delavirdine studies 73A and 73B, and in VIRACEPT studies 503, 510, 511, 542. In the case of PP1, the safety analysis presents treatment-emergent adverse events split by the AUC categories (<41, 41-61, >61 mg.h/L). In the case of PP2, the safety analysis presents treatment-emergent adverse events between subjects receiving VIRACEPT/delavirdine and subjects receiving nelfinavir without delavirdine. The following safety outcomes were evaluated for each pooled population:

- All-causality treatment-emergent adverse events of at least Grade 1 in severity, where there is a trend for increased reporting with increasing exposure the nelfinavir.
- The relationship to exposure of the drug-related adverse events most commonly associated with nelfinavir (diarrhea, nausea, flatulence, and rash).
- Discontinuation due to adverse events.
- Laboratory abnormalities.

3.2.3 Demographics and Baseline Characteristics

Tables 1 & 2 list demographics and baseline characteristics for pooled population 1 and 2. Overall, the study population was predominantly male (91%), with a median age 36 years. Caucasian (85%) was the lead group, followed by Black patients (8%), and other origin (7%). At baseline, the median HIV RNA was 4.85 $\log_{10}$ and the median CD4+ cell counts was 297 cells/mm^3.

The four NFV studies in PP1 had similar age, racial and gender distributions. The baseline CD4+ cell count was statistically significant among four studies, $p<0.01$, by the Wilcoxon test. Likewise, the baseline HIV-1 RNA level was also statistically significant among four studies, $p<0.0001$, by the Wilcoxon test. Study 542 had lowest mean or median CD4+ cell count, and greatest mean or median baseline HIV-1 RNA in $\log_{10}$ c/mL.

Table 1. Demographics

	STUDY		Gender		Race		Age	
		n	Male	%	White	%	Mean (sd)	Median (range)
Pooled Population 1								
PK	**503**	**63**	**61**	**97**	**59**	**94**	**38.8 (7.5)**	**38 (24-58)**
PK	**510**	**23**	**21**	**91**	**19**	**83**	**37.0 (6.2)**	**36 (25-48)**
Pivotal	**511**	**174**	**155**	**89**	**136**	**78**	**36.8 (9.0)**	**34 (21-63)**
Pivotal	**542**	**57**	**53**	**93**	**54**	**95**	**37.6 (9.1)**	**36 (23-66)**
	Total	**317**	**290**	**91**	**268**	**85**	**37.3 (8.6)**	**36 (21-66)**
P-value*			**NS**		**NS**		**NS**	
Pooled Population 2								
	73A	**22**	**18**	**82**	**20**	**91**	**37.5 (10.2)**	**36 (25-64)**
	73B	**137**	**140**	**81**	**104**	**61**	**37.2 (9.8)**	**36 (18-75)**
	Total	**159**	**158**	**81**	**124**	**64**	**37.2 (9.9)**	**36 (18-75)**
P-value*			**NS**		**0.049**		**NS**	

***. P-value by the Chi-square test for gender and race comparisons; P-value by the Wilcoxon test for age comparisons.**

The two NFV/DLV studies in PP2 had similar age, gender, baseline CD4+ cell count, and baseline HIV-1 RNA. Study 73A appears to have more Caucasian than Study 73B. The baseline CD8+ cell count was higher in subjects in 73A, $p<0.05$, by the Wilcoxon test.

Table 2. Baseline HIV RNA, CD4 and CD8 Cell Count

	STUDY	n	mean	Std	Median	min	max	P-value**
Pooled Population 1								
HIV RNA	503	63	4.84	0.33	4.81	4.22	5.63	
Log_{10}	510	23	4.68	0.29	4.76	4.18	5.22	
	511	174	4.87	0.47	4.83	3.35	6.14	
	542	57	5.10	0.39	5.11	4.06	5.83	
	total	317	4.89	0.43	4.85	3.35	6.14	<0.0001
CD4+	503	63	334.87	132.61	325.00	133.00	1008.33	
	510	23	341.85	100.68	325.33	150.33	567.00	
	511	174	294.38	215.20	273.50	10.00	1066.00	
	542	57	258.94	161.35	223.50	13.00	820.00	
	Total	317	299.50	186.59	296.67	10.00	1066.00	<0.01
CD8+	503	63	1101.88	489.47	957.33	352.33	2583.00	
	510	23	963.28	426.98	821.50	278.33	1971.00	
	511	174	1029.57	632.54	887.25	181.00	5255.00	
	542	57	1028.07	540.37	956.00	155.00	3355.00	
	Total	317	1038.86	575.99	924.67	181.00	5255.00	NS
Pooled Population 2								
HIV RNA	73A	22	4.84	0.41	4.84	4.27	5.43	NS
Log_{10}	73B	163	4.90	0.43	4.82	4.21	6.12	
		185	4.89	0.43	4.82	4.21	6.12	
CD4+	73A	21	372.05	198.51	383.50	79.50	757.50	NS
	73B	162	310.52	195.56	291.25	37.00	1421.00	
		183	317.58	196.34	297.5	37.00	1421.00	
CD8+	73A	21	1100.12	466.39	1015.00	540.00	2602.00	0.046
	73B	162	953.96	522.29	820.00	327.50	3788.00	
		183	970.73	517.11	839.00	327.50	3788.00	

*. P-value by the Chi-square tests; ** P-values by the Wilcoxon tests.

3.2.4 Nelfinavir Plasma Concentration AUC_{24}

The histograms of AUC_{24} mg.h/L (AUC) by study in the PP1 are shown in Figure 1. Table 3 shows basic statistics of AUC by study. Study 542 had lowest mean and median AUC, and Study 510 had greatest mean and median AUC. AUC was not evenly distributed among different studies in PP-1, $p<0.0001$, by the Wilcoxon test.

The applicant used the lower and upper quartile values for AUC_{24} of 41 and 61 mg.h/L to quantify lower, medium, and higher levels of AUC. The lower and upper quartile values appeared to be *34 and 55* mg.h/L in PP-1.

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Table 4 shows contingency table of AUC by study using the applicant's AUC categories. Note that 45% of subjects had AUC <41, 39% had AUC between 41 and 61, and 16% had higher AUC (>61). The study population Study 542 had more subjects (63%) with low AUC (<41), than those in Study 511 (45%), Study 503 (37%) and Study 510 (17%). The percentages of lower, medium and higher AUC were not evenly distributed across studies, p=0.0003, by the Chi-square test.

Table 3. AUC_{24} (mg.h/L) by VIRACEPT Studies in Pooled Population 1*

STUDY	N	Mean	Std	Median	Minimum	Maximum
503	63	52.49	25.11	45.80	12.90	132.30
510	23	57.29	17.42	52.50	34.50	97.50
511	174	46.81	18.28	43.63	21.81	132.81
542	57	39.14	10.59	37.07	15.62	64.18
Total**	317	47.32	19.30	43.89	12.90	132.81

*** P<0.0001 by the Wilcoxon test. **. Lower and upper quartiles were 34 and 55 mg.h/L.**

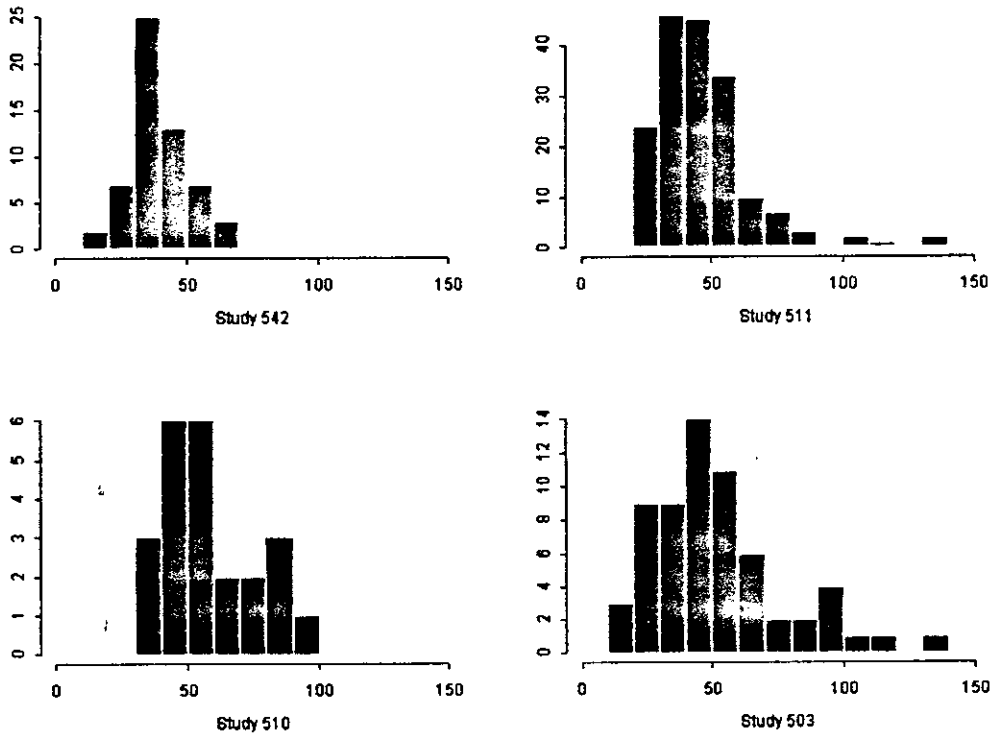


Figure 1: Frequecies in AUC_{24} by Study for Pooled Population 1

Table 4. AUC Categories by Nelfinavir Studies

STUDY	<41	41-61	>61
503	23 (36.5)	23 (36.5)	17 (27.0)
510	4 (17.4)	11 (47.8)	8 (34.8)
511	79 (45.4)	70 (40.2)	25 (14.4)
542	36 (63.2)	19 (33.3)	2 (3.5)
Total*	142 (44.8)	123 (38.8)	52 (16.4)

*. P=0.0003, Chi-Square, p=0.0683, by the Mantel-Haenszel Chi-Square.

3.3 Safety Findings by the Applicant

Section 3.3.1 lists safety findings by individual studies in PP-1. Sections 3.3.2-3.3.5 summarize the adverse events in at least 2% of patients, laboratory abnormalities were reported in PP-1 in above 1% of the subjects. The exposure-related trends were reported.

3.3.1 Safety Findings by Individual Study in PP-1

In Study 511, VIRACEPT was shown to be well tolerated over the entire 12 months of treatment. The most frequently reported adverse event of moderate or greater intensity was diarrhea. The incidence of new treatment-emergent cases of diarrhea appeared to decrease in the extension period in patients receiving the triple combination with VIRACEPT 750 mg (2/81, 2%) when compared to the core study (20/99, 20%). The incidence of moderate or greater diarrhea over the full 12 months of the study, was 21% and 20%, respectively, in patients originally on the triple combination with VIRACEPT 750 mg and VIRACEPT 500 mg.

In Study 542, the adverse event profile for patients in VIRACEPT BID and TID regimens are similar, and comparable to other studies of VIRACEPT in triple-combination therapy with d4T, 3TC, AZT, and ddI. The 750 mg TID, 1250 BID with d4T and 3TC was well tolerated over the entire 48 weeks of treatment and through week 120. 7% of the patients discontinued due to drug-related adverse events. The most frequently reported adverse events was diarrhea, where 23% of patients in BID regimen and 21% in TID regimen had diarrhea during the study period. No differences in clinical disease progression or discontinuations due to treatment failure were found between the BID and TID groups.

In Study 503, Nelfinavir was safe and well tolerated by patients in the core study and

protocol extension period. Four subjects had new grade 2+ diarrhea during the extension period. Five subjects experienced a grade 3 or 4 treatment emergent adverse event, including diarrhea, elevated liver function and asthma, three were treatment related- one with diarrhea and two with elevated liver function tests.

In Study 510, there were no patients died or discontinuation due to treatment-emergent adverse events. Two subjects had serious adverse events that were not considered as VIRACEPT related by the investigators. 9 patients had marked change in one or more laboratory values in the extension period.

In Study 73A over the 48 weeks of treatment, all of the 22 patients experienced at least one treatment-emergent adverse event. Most of these events (>93%) were rated as ACTG Grade 1 or 2 in severity. Diarrhea (59%), nausea (45%), upper respiratory infection (36%), sinusitis (32%), and rash (32%) were the most commonly reported treatment-emergent adverse events. Drug-related adverse events were observed in 15 patients (8 DLV 400 mg TID and 7 DLV 600 mg TID): diarrhea (27%), nausea (27%), and rash (23%) were the most commonly reported adverse events attributed to DLV. Most of these events (>95%) were rated as ACTG Grade 1 or 2 in severity. Serious adverse events were observed in one DLV 600 mg TID-treated patient (an ACTG Grade 3 pancreatitis). Withdrawal due to adverse events was found in 4 patients (2 DLV 400 mg TID and 2 DLV 600 mg TID) withdrew from the study due to adverse events. None of these events were considered by the investigator to be related to DLV. Laboratory test results: ACTG Grade 3/4 abnormal laboratory measurements were reported for 5 patients (2 DLV 400 mg TID, 3 DLV 600 mg TID). The patients experienced one ACTG Grade 4 laboratory abnormality and 9 ACTG Grade 3 laboratory abnormalities. There was no statistical difference in the frequency of any events between treatment groups. There were no clinically significant differences between the treatment groups.

In Study 73B, there was no significant difference between the four treatment groups in the overall frequency of all medical events. The number of DLV-related medical events was similar for each of the three patient groups receiving one of the DLV-containing regimens. More patients receiving the four-drug regimen discontinued because of medical events, although the difference between the groups did not achieve statistical significance. However, there was a statistically significant difference in the frequency of discontinuation because of ACTG Grade 3 or 4 medical events, with more such event occurring in patients receiving four drug treatment. One death and 23 serious adverse events were reported, none of them were attributed to study drug. There was no increase in the frequency of NFV-associated diarrhea.

3.3.2 Treatment-emergent Diarrhea, Nausea, Flatulence and Rash in PP-1

Table 5 shows the treatment-emergent Diarrhea, Nausea, Flatulence and Rash in PP-1 by the applicant. Treatment-emergent diarrhea was reported in 247/317 (77.9%) subjects in the PP-1. In patients with at least Grade 2 treatment-related diarrhea, incidence increased with plasma concentration of nelfinavir. Nausea (137/317; 43.2%), flatulence (65/317;

20.5%), and rash (70/317; 22.1%) were reported less frequently, and without a relationship of exposure to nelfinavir.

Table 5. Treatment-emergent Diarrhea, Nausea, Flatulence, and Rash Split by AUC

Adverse Event COSTART Term	AUC mg.h/L <41 N=142 n (%)	41-61 N=123 n (%)	>61 N=52 n (%)	Total N=317 n (%)
Diarrhea				
All grades/all causalities	106 (74.6)	98 (79.7)	43 (82.7)	247 (77.9)
Grade 2+ (treatment related)	21 (14.8)	29 (23.6)	16 (30.8)	66 (20.8)
Nausea				
All grades/all causalities	63 (44.4)	55 (44.7)	19 (36.5)	137 (43.2)
Grade 2+ (treatment related)	4 (2.8)	6 (4.9)	2 (3.8)	12 (3.8)
Flatulence				
All grades/all causalities	23 (16.2)	32 (26.0)	10 (19.2)	65 (20.5)
Grade 2+ (treatment related)	3 (2.1)	4 (3.3)	0	7 (2.2)
Rash				
All grades/all causalities	29 (20.4)	32 (26.0)	9 (17.3)	70 (22.1)
Grade 2+ (treatment related)	1 (0.7)	2 (1.6)	1 (1.9)	4 (1.3)

Source: Appendix 3 Tables 3 and 4.

3.3.3 Treatment-emergent Cardiac Adverse Events

Treatment-emergent Cardiac Adverse Events were found in 0.3% to 3.2% of subjects in PP-1. The most frequent cardiac event was Tachycardia (3.2%) and there was no relationship to exposure.

3.3.4 Discontinuation Due to Adverse Events

There was no relationship between discontinuations due to adverse events and exposure to NEV in PP 1. There were 10/142 (7.0%), 5/123 (4.1%), and 3/52 (5.8%) subjects who discontinued with at least 1 adverse event in the <41, 41 to 61 and > 61 mg.h/L groups respectively.

3.3.5 Laboratory Abnormalities

The highest percentage of marked changes were seen in serum lipase (3/13=23.1%), creatine kinase (5.6%), neutrophils (4.1%), lymphocytes (3.5%), AST [SGOT] (2.5%), and triglycerides (3.3%). The high rate of serum lipase abnormalities may be due to small number in the denominator. Therefore, it may not be a safety signal. In addition, marked change in hemoglobin (2/52=3.8) in the higher exposure group (AUC >61 mg.h/L), compared with 1/142 (0.7) and 1/123 (0.8) in the lower and medium AUC groups were observed. Likewise, neutrophils occurred in 4.2%, 3/3% and 5.8% of lower, medium and higher AUC groups, respectively. The clinical significance of these observations are not clear.

3.4 Statistical Reviewer's Findings

In order to verify the applicant's findings in PK-safety analysis on PP-1, this reviewer performed a sensitivity analysis on safety in PP-1, using 33 and 67 percentiles of AUC measurement to regrouping study subjects.

In the past, only subjects with more than 2% adverse events were reported and the quantitative nature of these adverse events had never been investigated. There is a need to compare safety outcome quantitatively among AUC subgroups, i.e., via episode of treatment emergent adverse event, defined as person-time treatment emergent adverse event. The safety data of pooled populations in this submission permitted this reviewer to quantitatively conduct such analysis.

So far sponsor only reported adverse event related discontinuations. Reasons for discontinuation need to be re-adjusted. A patient may drop out for "lack of efficacy" or "other reasons" and there might be adverse events just before dropping out (or just after), these discontinuations should be counted as drug related discontinuation. In other words, all discontinuations, not just those due to adverse events may contribute information regarding the safety of the drug. Therefore, analyzing time to discontinuation by reason and AUC group ought to be done whenever the data are available.

3.4.1 All Causalities and All Grades Cardiovascular Events

The association between NFV exposure (AUC) and cardiovascular events was examined. Using an alternative approach, this reviewer obtained episode of cardiovascular event per person and used this quantity for comparisons among different AUC subgroups. According to the medical reviewer, all causalities and all grades cardiovascular events include Tachycardia, Syncope, Arrhythmia, Bradycardia, Sinus Bradycardia, Abnormal ECG,

Atrial Fibrillation, Extrasustoles, Supraventricular Extrasystoles, Heart Failure, and Right Heart Failure.

Table 6 shows distribution of episode of cardiovascular events by AUC subgroups and study. Overall, 43 (12%) of study subjects had a total of 88 cardiovascular events: 9/63 (14%) in Study 503, 1/23 (4%) in Study 510, 30/174 (17%) in Study 511, and 3/57 (5%) in Study 542. Episode of cardiovascular events was not associated with AUC levels or study, $p>0.05$, by the Wilcoxon tests.

Table 6. AUC by Number of Cardiovascular Events[5]

Number	AUC	N	Minimum	Maximum	Mean
503	< 41	23	0	2	0.13
	41 ~ 61	23	0	2	0.26
	> 61	17	0	2	0.18
510	< 41	4	0	0	0
	41 ~ 61	11	0	2	0.18
	> 61	8	0	0	0
511	< 41	79	0	3	0.24
	41 ~ 61	70	0	10	0.47
	> 61	25	0	6	0.48
542	< 41	36	0	8	0.22
	41 ~ 61	19	0	1	0.11
	> 61	2	0	0	0

[5] $p>0.05$, by the Wilcoxon tests.

3.4.2 All Causalities and All Grades Treatment Emergent Adverse Event

Table 7 shows all causalities and all grades treatment emergent adverse events by AUC <37, 37-51, >51 in sixteen specific adverse events. The first row in Table 7 lists the number and percentage of subjects with a particular treatment emergent adverse event, and the second row shows mean episode of a particular adverse event.

It was observed that (1) an increased episode of diarrhea, eye disorder and ACNE was associated with increasing exposure of NFV respectively, $p<0.05$, by the Wilcoxon test; and (2) an increased % of subjects with eye disorder or ACNE was also associated with increasing exposure of NFV respectively, $p<0.05$, by the Chi-Square test.

Table 7. Treatment-emergent Adverse Events Split by AUC 33 and 67 percentiles

AUC (mg.h/L) n		<37 104	37-51 107	>51 106	Total 317	p-value*
Asthenia	#(%)	28 (27)	36 (34)	33 (31)	97 (31)	NS
	Mean	0.4	0.4	0.5	0.4	NS
Lymphadenopathy	#(%)	12 (12)	11 (11)	14 (13)	37 (12)	NS
	Mean	0.2	0.1	0.2	0.2	NS
Pharyngitis	#(%)	11 (11)	10 (9)	15 (14)	36 (11)	NS
	Mean	0.2	0.1	0.4	0.2	NS
Rental Discharge	#(%)	6 (6)	8 (7)	14 (13)	28 (9)	0.1370
	Mean	0.1	0.1	0.2	0.1	0.1339
Dermal Fung	#(%)	3 (3)	7 (7)	9 (8)	19 (6)	NS
	Mean	<0.1	0.1	0.1	0.1	NS
Eye Disorder	#(%)	4 (4)	3 (3)	11 (10)	18 (6)	0.0356
	Mean	0.1	<0.1	0.2	0.1	0.0368
Infection Viral	#(%)	3 (3)	4(4)	9 (8)	16 (5)	0.1342
	Mean	0.1	<0.1	0.2	0.1	0.1281
ACNE	#(%)	2 (2)	3 (3)	10 (9)	15 (5)	0.0192
	Mean	<0.1	<0.1	0.2	0.1	0.0183
Ecchymosis	#(%)	2 (2)	5 (5)	7 (7)	14 (4)	NS
	mean	<0.1	<0.1	0.1	0.1	NS
Flu Syndrome	#(%)	4 (4)	3 (3)	6 (6)	13 (4)	NS
	Mean	<0.1	<0.1	0.1	0.1	NS
Hypertension	#(%)	4 (4)	3 (3)	5 (5)	12 (4)	NS
	Mean	0.1	<0.1	0.1	0.1	NS
Weight Decreased	#(%)	2 (2)	5 (5)	5 (5)	12 (4)	NS
	Mean	<0.1	0.1	0.1	0.1	NS
Diarrhea	#(%)	78 (75)	79 (74)	90 (85)	247 (78)	0.1022
	Mean	1.8	1.9	2.6	2.1	0.0301
Nausea	#(%)	44 (42)	48 (45)	45 (42)	137 (43)	NS
	Mean	0.9	0.9	0.9	0.9	NS
Flatulence	#(%)	17 (16)	23 (22)	25 (24)	65 (21)	NS
	Mean	0.3	0.4	0.3	0.3	NS
Rash	#(%)	21	225)	21(20)	70 (22)	NS
	mean	0.3	0.3	0.4	0.3	NS

*. Chi-square for first row and Kruskal-Wallis test for second row.

3.4.3 Discontinuation

3.4.3.1 Reclassification of Discontinuation: NFV-related or not

Reasons for discontinuation need to be re-adjusted so that all discontinuations that may be contributed by the study drug, not just those due to adverse events, should be examined more closely.

Table 8 shows the frequencies of discontinuation by fourteen reasons in 137 subjects. It appears that toxicity related to study drug, progression of disease, termination by sponsor or patient, protocol noncompliance, and treatment failure per protocol, etc., may be NFV-related; toxicity related to other drug, inter-current illness, protocol violation, and current use of another drug, may not be NFV-related.

Following this, the fourteen reasons were regrouped into two categories: NFV-related discontinuation and not-NFV-related discontinuation. Table 9 shows the frequencies of no-discontinuation (code=0), NFV-related discontinuation (code=1) and non-NFV-related-discontinuation (code=2) by AUC groups. There were 58 subjects who discontinued from studies due to NFV treatment.

- Note in this data set, 10 subjects in Study 503 were coded as still on study at Week 48. However, their last dates of lab test were within a month (=28 days) from the first date of NFV treatment.

On average, 58/317 (18%) subjects discontinued due to reasons related to NFV. Using the applicant's AUC grouping method, there were 24/142 (17%), 19/123 (15%), and 15/52 (29%) subjects who discontinued due to reasons related to NFV in the <41, 41 to 61 and > 61 mg.h/L groups respectively. The proportion of subjects without discontinuation was 12%-14% greater in the lowest or medium NFV exposure groups, than that in the highest NV exposure group. Additionally, the percentage of subjects who discontinued due to reasons that were not-NFV-related was also the greatest in the highest NFV exposure group. It is likely that AUC value was associated with subject's discontinuation status, p=0.05, by the Chi-square test.

A sensitivity analysis using AUC cutpoints of 37 and 51 was somewhat supportive for the above finding but the significance level reduced to 0.07. There were 18/104 (17%), 16/107 (15%), and 24/106 (23%) subjects who discontinued from study due to reasons related to study drug in the <37, 37 to 51 and > 51 mg.h/L groups respectively. Also, an increased proportion in non-NFV-related discontinuation was found as NFV exposure increased.

Table 8. Reasons for Discontinuation

Reasons	#	%
Related To Other Drug*, Other	2	1.46
Related To Other Drug*, Toxicity	2	1.46
Current Use Of Another Drug	14	10.22
Inter-current Illness	3	2.19
Other	24	17.52
Protocol Noncompliance	8	5.84
Protocol Violation	26	18.98
Related To Study Drug, Other	1	0.73
Related To Study Drug, Toxicity	10	7.30
Toxicity	1	0.73
Treatment Failure Per Protocol	6	4.38
Progression Of Disease	7	5.11
Patient Request	16	11.68
Termination By Sponsor	17	12.41
Total	137	100.00

Table 9. Discontinuation Status by NFV Study in Pooled Population 1

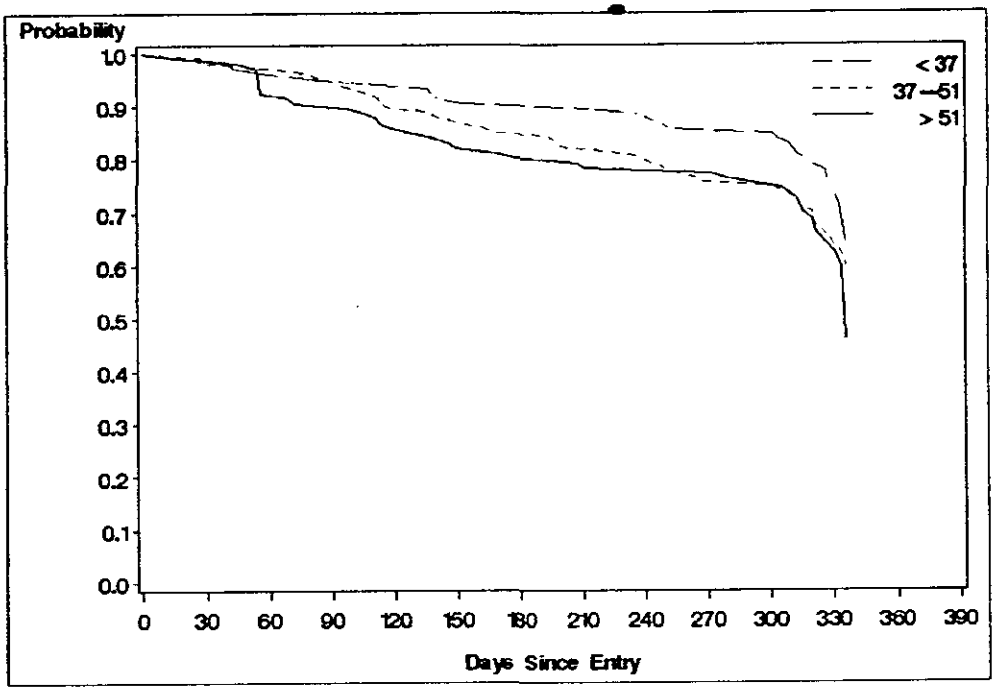
AUC	Not Disc (0)	Disc (1)	Disc (2)	p-value
< 41	89 (63)	24 (17)	29 (20)	0.0513
41 ~ 61	70 (57)	19 (15)	34 (28)	
> 61	21 (40)	15 (29)	16 (31)	
< 37	67 (64)	18 (17)	19 (18)	0.0714
37 ~ 51	64 (60)	16 (15)	27 (25)	
> 51	49 (46)	24 (23)	33 (31)	
Total	180 (57)	58 (18)	79 (25)	

3.4.3.2 Time to Discontinuation: Kaplan-Meier Analysis

In this section, the association between NFV exposure and time to discontinuation, regardless the reason of discontinuation whether it was NFV-related or not, was considered. A time of 365 days was assigned to a subject who was on study at Week 48 regardless of the last date of his (her) lab test. Figure 2 shows the Kaplan-Meier curves by NFV exposure.

- Subjects in AUC <37 group had the greatest probability of staying on study, followed by subjects in AUC 37-51 and >51 groups. In other word, on average, the subjects in AUC >51 group tended to drop out of the study sooner than subjects in lower or moderate NFV exposure groups. It appears that NFV exposure is associated with probability and time to discontinuation, p=0.032, by the Log-rank test.

Figure 2: Kaplan-Meier Estimates of the Discontinuation-Free Probability by AUC



3.4.3.3 Time to NFV-related and Non-NFV-related Discontinuation Analysis

One drawback for the analysis of time to discontinuation, regardless NFV-related or not, is a strong assumption that the distributions due to different reasons are homogeneous. In this submission concerning NFV treatment, discontinuation due to NFV-related causes might be of primarily concern. Discontinuation as a result of NFV treatment, and as a result of not-NFV-related, are competing risks. In statistical modeling on time to NFV-related discontinuation, one could not consider those subjects who discontinued due to a reason that was not-NFV-related, as censoring at the time of discontinuation.

This reviewer modeled the discontinuation data for PP1 using the non-parametric competing risk models by Lawless[1] (1992) in order to determine the association between NFV exposure and time to two types of discontinuations.

Figure 3 shows the probability of NFV-related discontinuation by AUC groups. Up to 8 months since first dose date of study drugs, the probability of NFV-related discontinuation-free curves for AUC subgroups are well separated, more subjects in AUC<37 group appear to stay on study than subjects in AUC 37-51 and >51 groups. Additionally, it would take longer time for subjects in lower or medium NFV exposure groups to drop out of study because of NFV treatment, than those in the higher NFV exposure group.

Figure 4 shows the probability of not NFV-related discontinuation by AUC groups. Three curves cross over around 6 months since first dose of study drugs, indicating a qualitative interaction. No clear association between AUC and non-NFV related discontinuation was observed.

- This reviewer repeated the above analyses on a modified data set – for subjects with no discontinuation status, time to last date of laboratory test (+14 days) were assigned. Although this data set had slightly shorter time to discontinuation, and shorter time to censor for those subjects without discontinuation, slightly differences were observed.

Figure 3: Time to NFV-related Discontinuation

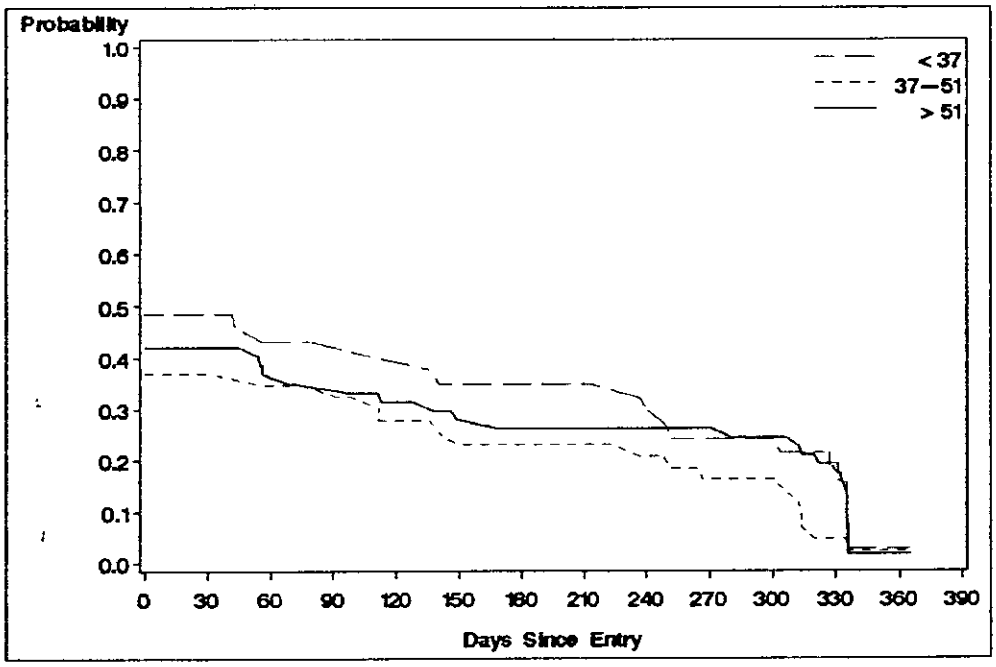


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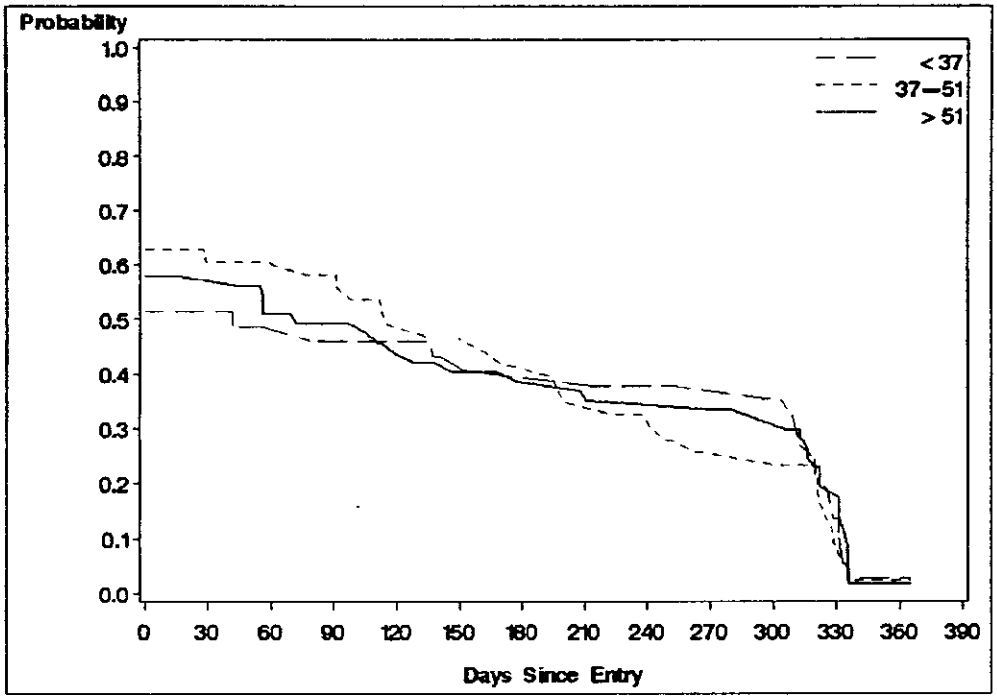
Figure 4: Time to Discontinuation Not NFV-related



3.4.3.4 Discussion: Limitations

Using the above method of classification of discontinuation categories, it appears that the discontinuation due to study drug NFV is associated with AUC.

We are aware of the limitation of this analysis. Determining a reason whether it was drug-related or not may be subjective. It is difficult to determine a real cause of discontinuation without further investigation. For example, if a subject requested to be withdrawn from the study or if there was a protocol violation, etc. The nature of a different study design - including combination HIV-1 therapy with NFV also obscures the causes for study discontinuation.

3.4.4 Diarrhea Associated or Possibly Associated with NFV

The most frequently reported adverse event of moderate or greater intensity was diarrhea. Two data sets were generated for Grade 1-4 diarrhea and Grade 2-4 diarrhea that were associated or possibly associated with NFV. Overall, there were 662 Grade 1 or above diarrhea. Excluding 175 records that were not associated with VIRACEPT/NFV treatment or duplicates, the remaining 487 episodes include 127 episodes that were associated and 360 episodes that were possibly associated with VIRACEPT/NFV treatment. In addition, there were 92 episodes of diarrhea with intensity of Grade 2 and above.

Table 10 shows distribution of Grade 1+ diarrhea (data set 1) by AUC subgroups and study. Findings are as follows:

- On average, subject in PP-1 1 had 1.5 episodes of NFV-associated diarrhea;
- Stratifying by AUC subgroup and study, the mean episode of diarrhea ranged from 1 to 3.2 episodes per person.
- On average, subject in AUC > 51 group had about 50%-70% more episode than those in the lower and medium NFV exposure groups.
- Frequency of diarrhea is associated with NFV exposure, p=0.0106, by the Wilcoxon tests.
- Variations in frequency of diarrhea were observed from study to study. Studies 510 and 503 had more subjects with frequent diarrhea than the two pivotal studies, p=0.0045, by the Wilcoxon test.
- Mean episode per subject presented a trend, the higher the NFV exposure, the more the episodes of diarrhea, except for Study 542.

Using the Cochran-Mantel-Haenszel test, we have the following findings:

- For subject with ever/never Grade 1+ diarrhea stratified by study, no association was found between diarrhea and different levels of AUC, p=0.4721.
- For subject with ever/never Grade 1+ diarrhea stratified by AUC (<37, 37-51, >51), there is an association between diarrhea and different study, p=0.0799.
- For subject with ever/never Grade 2+ diarrhea stratified by study, no association was found between diarrhea and different levels of AUC, p=0.2873.
- For subject with ever/never Grade 2+ diarrhea stratified by AUC (<37, 37-51, >51), there is an association between diarrhea and different study, p=0.0337.

Table 10. AUC by Episode of Grade 1+ Diarrhea[5]

Study	AUC	#	%	Mean	Median	Min.	Max.
503	<37	12	71	1.41	1	0	5
	37-51	12	63	1.63	1	0	6
	>51	21	78	2.07	2	0	8
510	<37	2	67	1.33	2	0	2
	37-51	6	86	2.71	2	0	7
	>51	12	92	3.23	3	0	8
511	<37	37	66	1.21	1	0	5
	37-51	40	66	1.34	1	0	5
	>51	43	75	1.75	1	0	12
542	<37	19	68	1.21	1	0	5
	37-51	11	55	0.95	1	0	2
	>51	8	89	1.22	1	0	2
Total	<37	70	67	1.25	1	0	5
	37-51	69	64	1.41	1	0	7
	>51	84	79	1.97	1.5	0	12

[5] **Comparisons of Episode of Diarrhea using Wilcoxon tests – p=0.0106, for within study comparisons; p=0.0045 for between study comparisons.**

3.4.4.1 Comparisons of Rate of Diarrhea Possibly Associated with NFV Adjusting for Sizes of Clinical Study

The observed overall rates of subject with Grade 1+ diarrhea are 70%, 69% and 84% for AUC <37, 37-51 and >51 subgroups respectively. The rates adjusting for the size of clinical studies by ever/never Grade 1+ or Grade 2+ diarrhea are listed in Table 11. The findings are as follows.

- The percentage of diarrhea adjusting for the sizes of NFV studies is lower than the observed one.

- The difference in adjusted percentage of Grade 1+ diarrhea between two AUC subgroups ranges from 3.6% to 13.9%, the lower the AUC, the lower the adjusted percentage of Grade 1+ diarrhea. Similar results were seen for Grade 2+ diarrhea.
- For the comparisons of adjusted rate among different AUC subgroups, no difference was statistically significantly different except for one situation. For Grade 1+ diarrhea, the adjusted rate for subject with AUC >51 is 79.4% that is statistically significantly greater than 65.5% for subjects with AUC < 37 at alpha=0.05 level.

Table 11. Percentage of Diarrhea Adjusting for NFV Studies

AUC	<37 P_1(%)	37-51 P_2(%)	>51 P_3(%)	P_I-P_k (%)	95% CI P_I-P_k(%)
Grade 1+ Diarrhea					
	65.5		**79.4**	**-13.9**	**-26.0,-1.7**
		67.4	**79.6**	**-12.2**	**-24.7,0.3**
	63.7	**67.3**		**-3.6**	**-16.4,9.3**
Grade 2+ Diarrhea					
	15.9		**25.7**	**-9.85**	**-21.9,2.2**
		20.6	**26.3**	**-5.73**	**-16.9,5.4**
	17.4	**17.7**		**-0.25**	**-10.8,10.3**

3.4.4.2 Modeling Ever/Never Diarrhea Possibly Associated with NFV

In PP-1, diarrhea that were associated or possibly associated with NFV treatment include 223 (70%) subjects had ever experienced Grade 1+.diarrhea, and 66 (21%) subjects had ever experienced Grade 2 or above diarrhea. This reviewer fitted logistic models for this binary response variable from the two diarrhea data sets in PP-1.

Table 12 shows the results for two logistic models with AUC only in the model. The slope estimates are significantly different from zero by the Wald Chi-square test. The concordance rate, which measures the percentage of agreement between the observed event (diarrhea in this case) and predicted event, was 56.7% in Grade 1+ diarrhea data set and 59.4% in Grade 2+ diarrhea data set.

Figures 5 and 6 plot the probability and 95% confidence interval of Grade 1+ and Grade 2+ diarrhea vs. AUC measurement, respectively. The 95% CI for the probability of experienced diarrhea around AUC 37-51 mg.h/L was the narrowest.

Figure 5: Probability of VIRACEPT Associated Diarrhea Grade 1+ vs. AUC

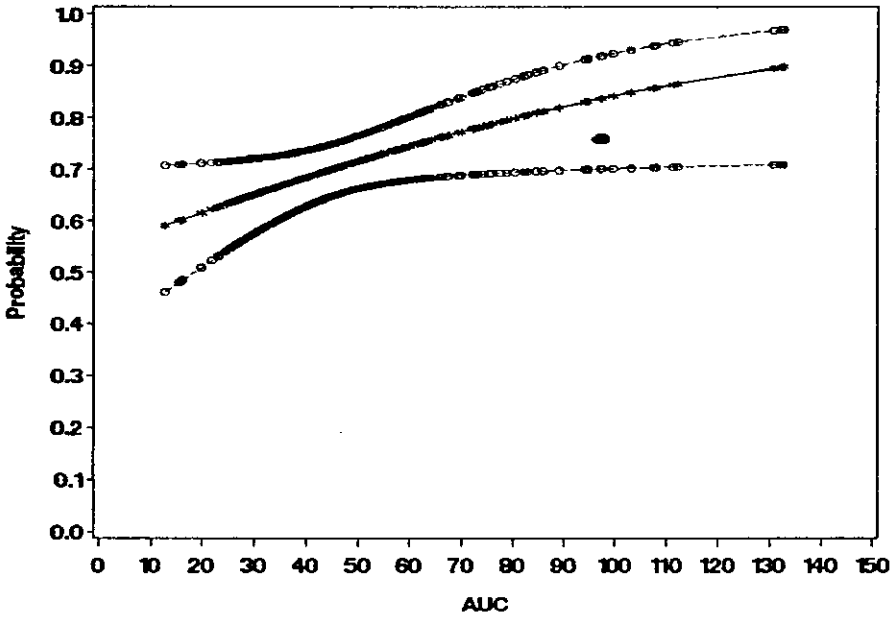


Figure 6: Probability of VIRACEPT Associated Diarrhea Grade 2+ vs. AUC

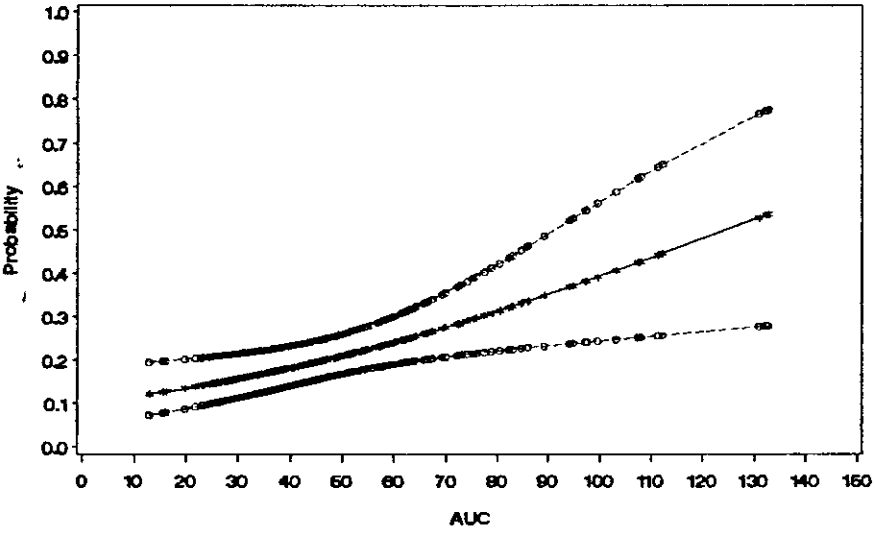


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Table 12. Results from Logistic Regression Models* on Ever/Never Diarrhea

Model	Parameter	β	SE	χ2	Pr	% concordance
		Diarrhea Grade 1 and above (70%)				
1	Intercept	0.1688	0.3509	0.2309	0.6309	56.7
	AUC	0.0151	0.0073	4.2582	0.0391	
		Diarrhea Grade 2 and above (21%)				
2	Intercept	-2.2703	0.3656	38.5583	<.0001	60.3
	AUC	0.0186	0.0066	7.8707	0.0050	

3.4.4.3 Comparison of Episode of Diarrhea Possibly Associated with NFV Using ANOVA

As an alternative, this reviewer examined the association between episode of diarrhea and NFV exposure via the analysis of variance (ANOVA) and non-parametric statistics. In Grade 1+ diarrhea data set, grouping episode of diarrhea by 0, 1-2, 3-4 and 5+ four groups, the mean AUC values are 43.8, 46.9, 54.5 and 51.7, respectively, p=0.016 by the F-test, the mean scores are 143, 157,186,and 193, respectively, p=0.024 by the Kruskal-Wallis test. Figure 7 plots episode of Grade 1+ diarrhea vs. AUC values.

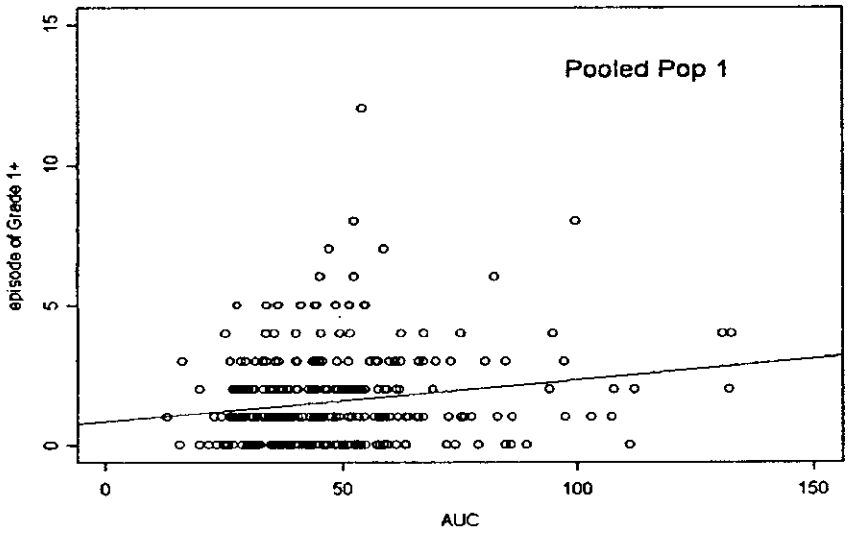
Likewise, in Grade 2+ diarrhea data set, the results from ANOVA showed that the mean AUC values were 45.8, 53.2, 45.3 and 99.6, respectively, for episode of diarrhea 0, 1-2, 3-4 and 5+, p=0.002 by the F-test. The result from non-parametric comparisons showed that the mean scores were 152, 184, 171 and 309, respectively, p=0.038 by the Kruskal-Wallis test.

3.4.4.4 Modeling Episode of Diarrhea Possibly Associated with NFV Using Linear Regression

Linear regression models were fitted to explore whether episode of diarrhea is also associated with NFV exposure. Covariates included age, gender, Caucasian or not, baseline CD4+ cell count, and baseline HIV-1 RNA. Note with all covarites in the model, only the parameter estimate of AUC was statistically significant, p=0.0044, by the Wald test in Grade 1+ diarrhea data set.

For Grade 2+ diarrhea data set, similar results were observed: only the parameter estimate of AUC and Caucasion or not were statistically significant, p<0.05.

Figure 7: Episode of Grade 1+ Diarrhea vs. AUC



4. FINDINGS IN SPECIAL/SUBGROUP POPULATIONS

4.1 Gender, Race and Age

Previously, no safety issues by gender, race and age were identified in NFV studies. Nevertheless, this reviewer fitted logistic models for two PK-diarrhea data sets. Covariates included age, gender, White (Caucasian or not), AUC, baseline CD4+ cell count and baseline HIV RNA. The parameter estimates of gender and age were not statistically significantly different from zero, $p>0.05$, by the Wald test. For Caucasian (85% in PP-1) and non-Caucasian category, however, the parameter estimate was statistically significantly different from zero by the Wald test in Grade 1+ diarrhea data set ($p=0.0116$), not in Grade 2+ diarrhea data set ($p=0.4622$). Table 13 shows the results from fitting logistic regression on ever/never NFV-related Grade 1+ diarrhea data.

- Race may be a confounding factor for PK-Grade 1+ diarrhea data, i.e., 80.6% of Caucasian subjects had Grade 1+ NFV-associated diarrhea, compared it with 63.3% in non-Caucasian subjects, p=0.007, by the Chi-Square test. However, the mean AUC was 49.3 mg.h/L in Caucasian subjects, much lower than 55.4 mg.h/L in non-Caucasian subjects, p=0.0002 by the Wilcoxon test.

Table 13. Results from Logistic Regression Model on Ever/Never Grade 1+ Diarrhea

The LOGISTIC Procedure

Analysis of Maximum Likelihood Estimates

Parameter	DF	Standard Estimate	Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	0.1735	2.1481	0.0065	0.9356
CD4	1	0.000796	0.000838	0.9016	0.3423
HIVRNA	1	0.0514	0.3561	0.0209	0.8852
AUC	1	0.0177	0.0086	4.2868	0.0384
AGE	1	-0.0234	0.0162	2.0849	0.1488
GENDER	1	-0.0482	0.4996	0.0093	0.9232
White	1	0.8939	0.3543	6.3662	0.0116

Odds Ratio Estimates

Effect	Point Estimate	95% Wald Confidence Limits	
CD4	1.001	0.999	1.002
HIVRNA	1.053	0.524	2.116
AUC	1.018	1.001	1.035
AGE	0.977	0.946	1.008
GENDER	0.953	0.358	2.537
white	2.445	1.221	4.895

4.2 Other Special/Subgroup Populations

4.2.1 Modeling PK-Diarrhea in NFV Study of PP-1

4.2.1.1 Two PK Studies

For ever/never diarrhea data sets, this reviewer fitted two logistic regression models on two PK studies 503 and 510 combined. Three covariates baseline CD4+, HIV RNA and AUC were included. Only the parameter estimates for AUC were statistically significantly different from zero, $p<0.05$, by the Wald test.

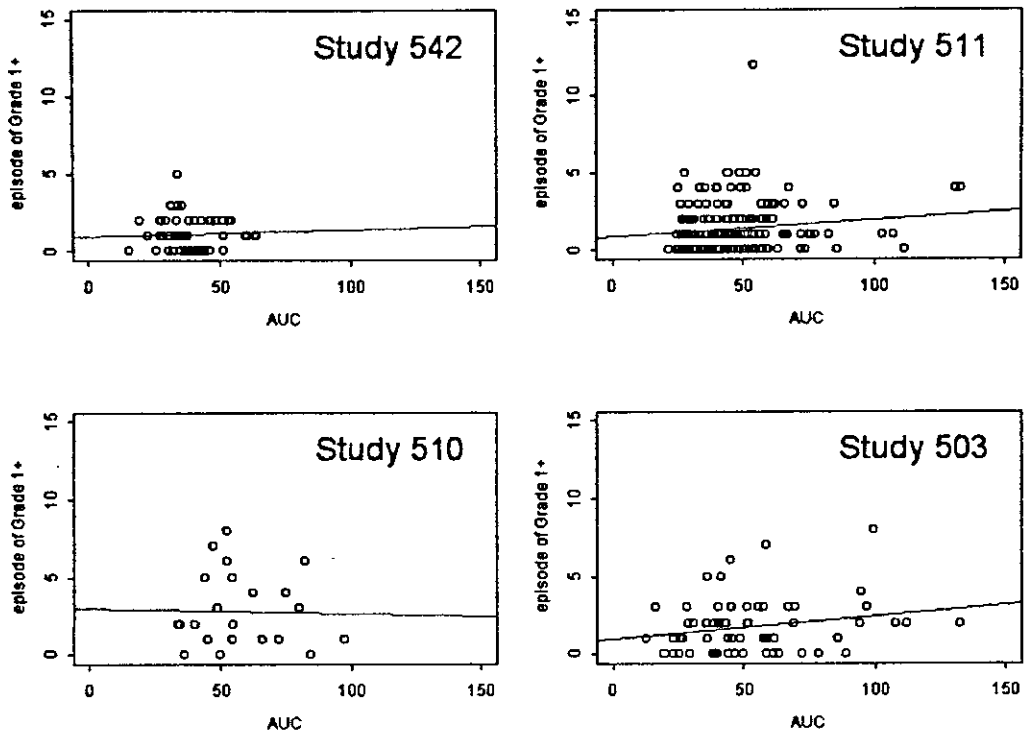
4.2.1.2 Two Phase III Clinical Studies

For ever/never diarrhea data sets, two logistic regression models on two phase III pivotal studies 511 and 542 were obtained. Three covariates baseline CD4+, HIV RNA and AUC were included. The parameter estimates for AUC were not statistically significantly different from zero at alpha=0.05 level, $p=0.06$ and 0.07, respectively, by the Wald test.

4.2.1.3 Individual Study

For episode of Grade 1+ diarrhea data sets, Figure 8 shows linear regression models fitted to individual study in PP1 with only AUC in the models. The parameter estimates for AUC were not statistically significantly different from zero for studies 542 and 510, but statistically significantly different from zero for studies 511 and 503, by the Wald test.

Figure 8: Episode of Grade 1+ Diarrhea vs. AUC by Study



4.2.1.4 Modeling Ever/Never Diarrhea Controlling for Baseline Covariates

This reviewer fitted backward stepwise logistic models for the response of subject who had diarrhea during the clinical trials to verify whether other baseline characteristics were associated with diarrhea. Covariates included age, gender, Caucasian or not, baseline CD4+, baseline HIV RNA, study dichotomous (0-1) indicators- S542, S511, and S503. In these modeling, two pooled PK-diarrhea data sets (Grade1+, Grade 2+ diarrhea) were used. Here is a list of findings:

- For Grade 1+ diarrhea data set, the final logistic model included AUC, Caucasian or not, S542, S511, and S503. The parameter estimate of AUC was not significant, p=0.08. Only the parameter estimate of Caucasian was statistically significant at 0.05 level.

- For Grade 2+ diarrhea data set, the final logistic model included AUC, gender, and study indicators S542, S511, and S503. The parameter estimates of AUC, S542, S511, and S503 were significant at 0.05 level.

4.2.2 Poolability

Pooling safety results from Studies 542, 511, 510 and 503 may lead to a more precise estimate of an adverse event of interest and to increase the power to obtain a significant finding. However, one major limitation for such retrospective analysis of safety is the availability of relevant studies. In the following, we discuss the strength and weakness of such a pooling in this NDA.

The strength of PP-1 is as follows.

- All four NFV studies were sponsored by Agouron Pharmaceuticals, Inc. Thus, usual biases found in other meta analysis including definition and diagnosis biases, coding biases, and reporting biases are minimal for this pooling.

- The start and stop dates were: 7/8/95-8/6/96 for Study 503, 11/16/95-6/3/97 for Study 510, 2/8/96-6/27/97 for Study 511, and 3/11/97-2/29/2000 for Study 542, indicating some overlapping in study periods.

- The demographic characteristics such as age, race, and gender were not statistically significantly different across studies. Except for Study 542, which was conducted in the European countries, all other studies were conducted in the United States.

The weakness or discrepancies were also seen.

- First, the study design was different. 503 and 510 were PK studies, while studies 511 and 542 were phase III clinical studies. The duration of study in Study 503 was the shortest, followed by those in studies 511, 510 and 542. Study 511 was a double-blind study, and the rest were open-label trials. Subjects in Study 503 had only VIRACEPT treatment, while subjects in other studies received VIRACEPT plus double or triple combination treatment. Study 542 was conducted in European countries, while 511, 503 and 510 were conducted in the United States. The baseline CD4+ cell count, and baseline HIV-1 RNA measurement were statistically significantly different among studies.

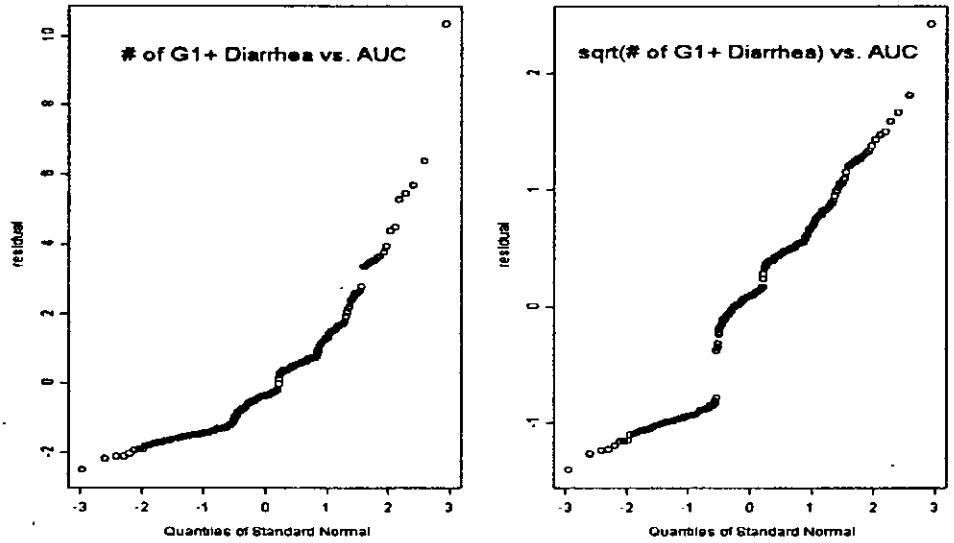
- The PP-1 did not have safety data from active controls, i.e., non-NFV treatment arms.

- Note in PK-safety analysis, the study populations included subjects treated with NFV or NFV/DLV for whom both pharmacokinetic and safety data were available. Therefore, not all the study subjects in Studies 542, 511, 503 and 510 were included in PP1. For example, only 57 (10.5%) subjects in two study arms of Study 542 were included in the PK-safety analysis.

4.2.3 Evaluation of Model Fitting

Figure 9 shows Q-Q plots for PK-episode of Grade 1+ diarrhea data set. The one on the left was for episode of Grade 1+ diarrhea, and the one on the right was for square root transformation of the episode of Grade 1+ diarrhea. It appears that the PK-diarrhea data violate normality in modeling fitting.

Figure 9: Q-Q Residual Plots: Episode of Grade 1+ Diarrhea vs. AUC



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5. SUMMARY AND CONCLUSIONS

5.1 Statistical Issues and Collective Evidence

The statistical review included (1) the applicant's final report in Integrated Safety Summary provided in Section 8 Clinical Data and in Summary of section 3; (2) adverse event tables in the NDA submission requested by the medical reviewer; and (3) data sets primarily on PP-1.

In response to the medical reviewer's request, the statistical reviewer verified the applicant's findings in safety analysis on treatment-emergent adverse events. In addition, the statistical reviewer conducted sensitivity analysis using 33 and 67 percentiles of plasma NFV AUC concentration category (<37, 37-51, >51 mg.h/L) to obtain even number of subjects per subgroup, so that a tendency of treatment emergent adverse event with increasing NFV exposure could be re-examined and detected.

In the past, only frequency of subjects with more than 2% adverse events were reported. The quantitative nature of these adverse events had not been investigated. There arose a need to compare safety outcome quantitatively among AUC subgroups, via episode of treatment-emergent adverse events, defined as person-time treatment-emergent adverse events. The safety data of pooled populations in this submission permitted us to quantitatively conduct such an analysis.

Up to now, sponsor only reported adverse event related discontinuations and the reasons for discontinuation had to be re-adjusted. A patient may drop out for "lack of efficacy" or "other reasons" and there were adverse events just before dropping out (or just after),. There was a need to scrutinize these discontinuations more closely. This reviewer felt that all discontinuations, not just those due to adverse events may furnish information regarding the safety of NFV. Therefore, analyzing time to discontinuation by reason and AUC group ought to be done when the data are available.

Based on the available PK-safety data on PP-1 from four nelfinavir studies 511, 542, 503 and 510, revealed the following findings:

- Analyses shows 43 (12%) of the study subjects in PP1 had a total of 88 cardiovascular events. There was no sufficient evidence to show frequency or episode of cardiovascular events was associated with NFV exposure and NFV study in PP1, $p>0.05$ by the Chi-square test and the Wilcoxon test, respectively.

- Sensitivity analyses on all causalities and all grades treatment emergent adverse events by alternative category of AUC (<37, 37-51, >51 mg.h/L) show that (1) increased frequency of eye disorder and frequency of ACNE may also be associated with increasing exposure of NFV, $p<0.05$, by the Chi-square test; and (2) increased episode

of eye disorder, ACNE, and diarrhea may be associated with increasing exposure of NFV, $p<0.05$, by the Wilcoxon test. No associations were found between NFV exposure and frequency or episode of Asthenia, Lymphadenopathy, Nausea, Flatulence, rash and other adverse event.

- Analyses using Kaplan-Meier approach and non-parametric competing risk models evaluating time and probability of discontinuation that were NFV-associated or not-NFV-associated, by NFV exposure category (<37,37-51,>51 mg.h/L) show the following results:

  - Time to all causality discontinuation may be associated with increased exposure of NFV, $p<0.05$, by the Log-rank test;

  - Time to NFV associated discontinuation may also be associated with increased NFV exposure; i.e., more subjects in medium and higher AUC groups discontinued than those in the lower AUC group; and

  - The relationship between NFV exposure and time to non-NFV associated discontinuation was not clear using the competing risk model.

- Sensitivity analyses of NFV-associated diarrhea in PP1 using 33 and 67 percentiles of AUC for AUC categories, show the following:

  - Increasing frequency of NFV-related diarrhea may be associated with increasing exposure to NFV, $p=0.0799$ for Grade 1+ diarrhea, $p=0.0337$ for Grade 2+ diarrhea, by the Cochran-Mantel-Haenszel test. Results from alternative analysis using logistic regression models support the associations.

  - A subject in PP1 had an average of 1.5 episodes of Grade 1+ diarrhea that were possibly associated with NFV. Stratifying by AUC subgroup and study, the mean episode of diarrhea ranged from 1 to 3.2 episodes per person. Subjects in AUC > 51 group had 50% –70% more episodes than subjects in the lower and medium AUC groups. Increasing episode of NFV-related diarrhea is associated with increasing exposure to NFV, $p=0.0106$, by the Wilcoxon tests. Results from alternative analysis using linear regression model support that the episode of diarrhea increases as NFV exposure increases.

  - Variations in frequency of diarrhea were observed from study-to-study. Frequency of Grade 1+ or Grade 2+ diarrhea had no significant difference among studies. Subjects in two PK studies 510 and 503 had more episode of diarrhea than those in the two pivotal studies 511 and 542, $p=0.0045$, by the Wilcoxon test. Mean episode of diarrhea per subject presented a trend, the higher the NFV exposure, the more the episode of diarrhea, except in Study 542.

- Alternative analyses of ever/never NFV-associated diarrhea in PP1 using logistic regression models, show the following:

  - Increased NFV exposure was associated with increasing probability of NFV-associated Grade 1+, or Grade 2+ diarrhea.

  - Age, gender, baseline CD4+ cell count, and baseline HIV RNA were not associated with NFV-associated Grade 1+, or Grade 2+ diarrhea.

  - White (Caucasian or not) was associated with higher probability of Grade 1+ diarrhea. Race may be a confounding factor for PK-Grade 1+ diarrhea data, i.e., 80.6% if Caucasian had Grade 1+ NFV-associated diarrhea, compared it with 63.3% in non-Caucasian subjects, p=0.007, by the Chi-Square test. However, the mean AUC was 49.3 mg.h/L in Caucasian subjects, much lower than 55.4 in non-Caucasian subjects, p=0.0002 by the Wilcoxon test.

5.2 Conclusions and Recommendations

Sensitivity analyses of the PK-safety data in subjects who received the VIRACEPT 250-mg suggest that eye disorder, ACNE, and diarrhea showed a tendency to increase with increased exposure of NFV. In addition, NFV-related discontinuations may also be associated with increased exposure of NFV. In general, the 250-mg formulation of VIRACEPT presented a favorable benefit to its risk profile.

Based on analysis of the VIRACEPT 250-mg PK-safety data, the above results could be inferred to the VIRACEPT 625-mg formulation, using appropriate statistical modeling on data from the bioequivalence studies between the 250-mg and the 625-mg formulations.

6. APPENDICES

References:

1. J.F.Lawless, Statistical Models and Methods for Lifetime Data. John Wiley & Sons, 1992.

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