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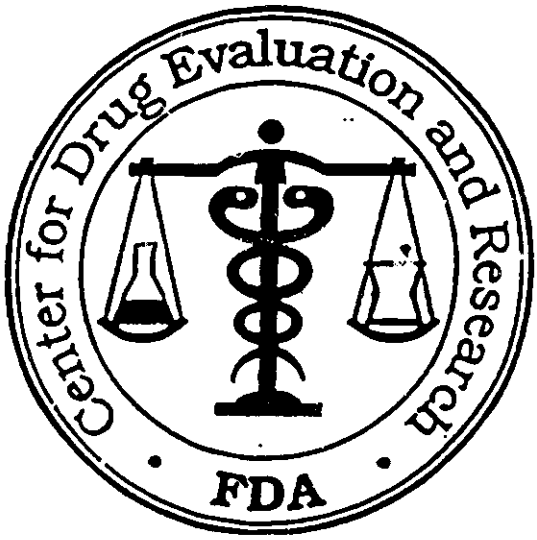
NDA 20-636
Viramune® Tablets
(nevirapine tablets)



DIVISION OF ANTIVIRAL
DRUG PRODUCTS
CSO: Anthony Zeccola

301-827-2335

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville MD 20857

NDA 20-636

JUN 21 1996

Boehringer Ingelheim Pharmaceuticals, Inc.
Attention: Pamela S. Strode
900 Ridgebury Road
P.O. Box 368
Ridgefield, Connecticut

Dear Ms. Strode,

Please refer to your February 23, 1996 new drug application (NDA) submitted under section 505(b) of the Federal Food, Drug, and Cosmetic Act for Viramune® (nevirapine) Tablets, 200 mg.

We acknowledge receipt of your amendments dated:

March 7, 1996	March 22, 1996	March 26, 1996	March 28, 1996 (2)
March 29, 1996	April 9, 1996 (2)	April 12, 1996	April 19, 1996
April 24, 1996	April 25, 1996	April 29, 1996	April 30, 1996
May 1, 1996	May 3, 1996	May 10, 1996	May 15, 1996
May 21, 1996	May 24, 1996	May 28, 1996	May 31, 1996
June 12, 1996	June 18, 1996(2)	June 19, 1996(3)	June 20, 1996(2)
June 21, 1996			

Viramune® (nevirapine) is indicated in combination with nucleoside analogues for the treatment of HIV-1 infected adults who have experienced clinical and/or immunologic deterioration.

We have completed the review of this application including the submitted draft labeling and have concluded that adequate information has been presented to demonstrate that the drug product is safe and effective for use as recommended in the draft labeling submitted June 21, 1996. Accordingly, the application is approved effective on the date of this letter.

We acknowledge your commitment to comply with the conditions of Accelerated Approval as stated in your June 20, 1996 letter. Additionally, we acknowledge your commitment to conduct the phase 4 studies also listed in the above letter.

The final printed labeling (FPL) must be identical to the June 21, 1996 draft labeling. Marketing the product with FPL that is not identical to this draft labeling may render the product misbranded and an unapproved new drug.

Please submit twenty 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 NDA 20-636. Approval of this labeling by FDA is not required before it is used.

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

Validation of the regulatory methods has not been completed. At 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 deficiencies that may occur.

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

Under section 736(a)(1)(B)(ii) of the Prescription Drug User Fee Act of 1992, this letter triggers the remaining 50% of the fee assessed for this application. You will receive an invoice for the amount due within the next month. Payment will be due within 30 days of the date of the invoice.

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, Anthony M. Zeccola, Regulatory Management Officer, at (301) 827-2335

Sincerely yours,

David W. Feigal, Jr., M.D., M.P.H.
Director
Division of Antiviral Drug Products
Center for Drug Evaluation and Research

Boehringer Ingelheim

Boehringer Ingelheim
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a subsidiary of
Boehringer Ingelheim Corporation
900 Ridgebury Rd.
P.O. Box 368
Ridgefield, Connecticut 06877-0368

Celebrating 25 YEARS in the U.S.

June 20, 1996

Center for Drug Evaluation and Research
Food and Drug Administration
HFD-530, Document Control Room
9201 Corporate Boulevard
Rockville, MD 20850

Attention: David W. Feigal, Jr., M.D., Director
Division of Antiviral Drug Products

Re: VIRAMUNE® (nevirapine) Tablets, 200 mg
NDA 20-636/Amendment No. 029

Dear Dr. Feigal:

Our NDA 20-636 for VIRAMUNE® (nevirapine) Tablets, 200 mg, submitted on February 23, 1996, has been reviewed per the regulations outlined in 21 CFR 314 Subpart H, "Accelerated Approval of New Drugs for Serious or Life-Threatening Illnesses".

In accordance with these regulations, we hereby commit to the following as conditions of accelerated approval:

1. Study ACTG 193a* is scheduled to complete clinically in June, 1996.

2 Pages
Purged

DRAFT U.S. PACKAGE INSERT
VIRAMUNE® (nevirapine) Tablets

FINAL DRAFT
June 21, 1996

VIRAMUNE®
(nevirapine)
Tablets

WARNING:
VIRAMUNE® (NEVIRAPINE) IS INDICATED FOR USE IN COMBINATION WITH NUCLEOSIDE ANALOGUES FOR THE TREATMENT OF HIV-1 INFECTED ADULTS WHO HAVE EXPERIENCED CLINICAL AND/OR IMMUNOLOGIC DETERIORATION. THIS INDICATION IS BASED ON ANALYSIS OF CHANGES IN SURROGATE ENDPOINTS IN STUDIES OF UP TO 48 WEEKS DURATION. AT PRESENT, THERE ARE NO RESULTS FROM CONTROLLED CLINICAL TRIALS EVALUATING THE EFFECT OF VIRAMUNE® WITH NUCLEOSIDE ANALOGUES ON THE CLINICAL PROGRESSION OF HIV-1 INFECTION, SUCH AS THE INCIDENCE OF OPPORTUNISTIC INFECTIONS OR SURVIVAL.

THE DURATION OF BENEFIT FROM ANTIRETROVIRAL THERAPY MAY BE LIMITED. ALTERATION OF ANTIRETROVIRAL THERAPIES SHOULD BE CONSIDERED IF DISEASE PROGRESSION OCCURS WHILE PATIENTS ARE RECEIVING VIRAMUNE®.

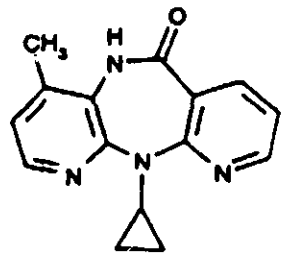
RESISTANT VIRUS EMERGES RAPIDLY AND UNIFORMLY WHEN VIRAMUNE® IS ADMINISTERED AS MONOTHERAPY. THEREFORE, VIRAMUNE® SHOULD ALWAYS BE ADMINISTERED IN COMBINATION WITH AT LEAST ONE ADDITIONAL ANTIRETROVIRAL AGENT.

VIRAMUNE® HAS BEEN ASSOCIATED WITH SEVERE RASH, WHICH IN SOME CASES HAVE BEEN LIFE-THREATENING. WHEN SEVERE RASH OCCURS, VIRAMUNE® MUST BE DISCONTINUED.

DESCRIPTION: VIRAMUNE® is the brand name for nevirapine (NVP), a non-nucleoside reverse transcriptase inhibitor with activity against Human Immunodeficiency Virus Type 1 (HIV-1). Nevirapine is structurally a member of the dipyridodiazepinone chemical class of compounds.

VIRAMUNE® is available as tablets for oral administration. Each tablet contains 200 mg of nevirapine and the inactive ingredients microcrystalline cellulose, lactose monohydrate, povidone, sodium starch glycolate, colloidal silicon dioxide and magnesium stearate.

The chemical name of nevirapine is 11-cyclopropyl-5,11-dihydro-4-methyl-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one. Nevirapine is a white to off-white crystalline powder with the molecular weight of 266.3 and the molecular formula $C_{15}H_{14}N_4O$. Nevirapine has the following structural formula:



MICROBIOLOGY: ***Mechanism of Action:*** Nevirapine is a non-nucleoside reverse transcriptase inhibitor (NNRTI) of HIV-1. Nevirapine binds directly to reverse transcriptase (RT) and blocks the RNA-dependent and DNA-dependent DNA polymerase activities by causing a disruption of the enzyme's catalytic site. The activity of nevirapine does not compete with template or nucleoside triphosphates. HIV-2 RT and eukaryotic DNA polymerases (such as human DNA polymerases α, β, γ, or δ) are not inhibited by nevirapine.

In Vitro HIV Susceptibility: The relationship between *in vitro* susceptibility of HIV-1 to nevirapine and the inhibition of HIV-1 replication in humans has not been established. The *in vitro* antiviral activity of nevirapine was measured in peripheral blood mononuclear cells, monocyte derived macrophages, and lymphoblastoid cell lines. IC_{50} values (50% inhibitory concentration) ranged from 10-100 nM against laboratory and clinical isolates of HIV-1. In cell culture, nevirapine demonstrated additive to synergistic activity against HIV in drug combination regimens with zidovudine (ZDV), didanosine (ddI), stavudine (d4T), lamivudine (3TC) and saquinavir.

Resistance: HIV isolates with reduced susceptibility (100-250-fold) to nevirapine emerge *in vitro*. Genotypic analysis showed mutations in the HIV RT gene at amino acid positions 181 and/or 106 depending upon the virus strain and cell line

employed. Time to emergence of nevirapine resistance *in vitro* was not altered when selection included nevirapine in combination with several other NNRTIs.

Phenotypic and genotypic changes in HIV-1 isolates from patients treated with either nevirapine (n=24) or nevirapine and ZDV (n=14) were monitored in Phase I/II trials over 1 to ≥12 weeks. After 1 week of nevirapine monotherapy, isolates from 3/3 patients had decreased susceptibility to nevirapine *in vitro*; one or more of the RT mutations at amino acid positions 103, 106, 108, 181, 188 and 190 were detected in some patients as early as 2 weeks after therapy initiation. By week eight of nevirapine monotherapy, 100% of the patients tested (n=24) had HIV isolates with a >100-fold decrease in susceptibility to nevirapine *in vitro* compared to baseline, and had one or more of the nevirapine-associated RT resistance mutations; 19 of 24 patients (80%) had isolates with a position 181 mutation regardless of dose. Nevirapine+ZDV combination therapy did not alter the emergence rate of nevirapine-resistant virus or the magnitude of nevirapine resistance *in vitro*; however, a different RT mutation pattern, predominantly distributed amongst amino acid positions 103, 106, 188, and 190, was observed. In patients (6 of 14) whose baseline isolates possessed a wild type RT gene, nevirapine+ZDV combination therapy did not appear to delay emergence of ZDV-resistant RT mutations. The clinical relevance of phenotypic and genotypic changes associated with nevirapine therapy has not been established.

Cross-resistance: Rapid emergence of HIV strains which are cross-resistant to NNRTIs has been observed *in vitro*. Data on cross-resistance between the NNRTI nevirapine and nucleoside analogue RT inhibitors are very limited. In four patients, ZDV-resistant isolates tested *in vitro* retained susceptibility to nevirapine and in six patients, nevirapine-resistant isolates were susceptible to ZDV and ddI. Cross-resistance between nevirapine and HIV protease inhibitors is unlikely because the enzyme targets involved are different.

ANIMAL PHARMACOLOGY: Animal studies have shown that nevirapine is widely distributed to nearly all tissues and readily crosses the blood-brain barrier.

CLINICAL PHARMACOLOGY: ***Absorption and Bioavailability in Adults:*** Nevirapine is readily absorbed (>90%) after oral administration in healthy volunteers and in adults with HIV-1 infection. Absolute bioavailability in 12 healthy adults following single-dose administration was 93 ± 9% (mean ± SD) for a 50 mg tablet and

91 ± 8% for an oral solution. Peak plasma nevirapine concentrations of 2 ± 0.4 µg/mL (7.5 µM) were attained by 4 hours following a single 200 mg dose. Following multiple doses, nevirapine peak concentrations appear to increase linearly in the dose range of 200 to 400 mg/day. Steady state trough nevirapine concentrations of 4.5 ± 1.9 µg/mL (17 ± 7 µM), (n = 242) were attained at 400 mg/day.

When VIRAMUNE® (200 mg) was administered to 24 healthy adults (12 female, 12 male), with either a high fat breakfast (857 kcal, 50 g fat, 53% of calories from fat) or antacid (Maalox® 30 mL), the extent of nevirapine absorption (AUC) was comparable to that observed under fasting conditions. In a separate study in HIV-1-infected patients (n=6), nevirapine steady-state systemic exposure (AUCτ) was not significantly altered by ddI, which is formulated with an alkaline buffering agent. VIRAMUNE® may be administered with or without food, antacid or ddI.

Distribution: Nevirapine is highly lipophilic and is essentially nonionized at physiologic pH. Following intravenous administration to healthy adults, the apparent volume of distribution (Vdss) of nevirapine was 1.21 ± 0.09 L/kg, suggesting that nevirapine is widely distributed in humans. Nevirapine readily crosses the placenta and is found in breast milk. (see PRECAUTIONS, *Nursing Mothers*) Nevirapine is about 60% bound to plasma proteins in the plasma concentration range of 1-10 µg/mL. Nevirapine concentrations in human cerebrospinal fluid (n=6) were 45% (± 5%) of the concentrations in plasma; this ratio is approximately equal to the fraction not bound to plasma protein.

Metabolism/Elimination: *In vivo* studies in humans and *in vitro* studies with human liver microsomes have shown that nevirapine is extensively biotransformed via cytochrome P450 (oxidative) metabolism to several hydroxylated metabolites. *In vitro* studies with human liver microsomes suggest that oxidative metabolism of nevirapine is mediated primarily by cytochrome P450 isozymes from the CYP3A family, although other isozymes may have a secondary role. In a mass balance/excretion study in eight healthy male volunteers dosed to steady state with nevirapine 200 mg given twice daily followed by a single 50 mg dose of ^{14}C-nevirapine, approximately 91.4 ± 10.5% of the radiolabeled dose was recovered, with urine (81.3 ± 11.1%) representing the primary route of excretion compared to feces (10.1 ± 1.5%). Greater than 80% of the radioactivity in urine was made up of glucuronide conjugates of hydroxylated metabolites. Thus cytochrome P450 metabolism, glucuronide conjugation, and urinary excretion of glucuronidated metabolites represent the primary route of nevirapine biotransformation and elimination in humans. Only a small fraction (<5%) of the radioactivity in urine

(representing < 3% of the total dose) was made up of parent compound; therefore, renal excretion plays a minor role in elimination of the parent compound.

Nevirapine has been shown to be an inducer of hepatic cytochrome P450 metabolic enzymes. The pharmacokinetics of autoinduction are characterized by an approximately 1.5 to 2 fold increase in the apparent oral clearance of nevirapine as treatment continues from a single dose to two-to-four weeks of dosing with 200-400 mg/day. Autoinduction also results in a corresponding decrease in the terminal phase half-life of nevirapine in plasma from approximately 45 hours (single dose) to approximately 25-30 hours following multiple dosing with 200-400 mg/day.

Special Populations: Renal/Hepatic Dysfunction: The pharmacokinetics of nevirapine have not been evaluated in patients with either renal or hepatic dysfunction.

Gender: In one Phase I study in healthy volunteers (15 females, 15 males), the weight-adjusted apparent volume of distribution (Vdss/F) of nevirapine was higher in the female subjects (1.54 L/kg) compared to the males (1.38 L/kg), suggesting that nevirapine was distributed more extensively in the female subjects. However, this difference was offset by a slightly shorter terminal-phase half-life in the females resulting in no significant gender difference in nevirapine oral clearance or plasma concentrations following either single- or multiple-dose administration(s).

Race: An evaluation of nevirapine plasma concentrations (pooled data from several clinical trials) from HIV-1-infected patients (27 Black, 24 Hispanic, 189 Caucasian) revealed no marked difference in nevirapine steady-state trough concentrations (median Cminss = 4.7 µg/mL Black, 3.8 µg/mL Hispanic, 4.3 µg/mL Caucasian) with long-term nevirapine treatment at 400 mg/day. However, the pharmacokinetics of nevirapine have not been evaluated specifically for the effects of ethnicity.

Age: Nevirapine pharmacokinetics in HIV-1-infected adults do not appear to change with age (range 18-68 years); however, nevirapine has not been extensively evaluated in patients beyond the age of 55 years. Nevirapine is metabolized more rapidly in pediatric patients than in adults. (see PRECAUTIONS, *Pediatric Use*)

Drug Interactions: No dosage adjustments are required when VIRAMUNE® is taken in combination with ZDV, ddI, or ddC. When the ZDV data were pooled from two studies (n=33) in which HIV-1-infected patients received VIRAMUNE® 400 mg/day either alone or in combination with 200-300 mg/day ddI or 0.375 to 0.75

mg/day ddC on a background of ZDV therapy, nevirapine produced a non-significant decline of 13% in ZDV AUC and a non-significant increase of 5.8% in ZDV Cmax. In a subset of patients (n=6) who were administered nevirapine 400 mg/day and ddI on a background of ZDV therapy, nevirapine produced a significant decline of 32% in ZDV AUC and a non-significant decline of 27% in ZDV Cmax. Paired data suggest that ZDV had no effect on the pharmacokinetics of nevirapine. In one crossover study, nevirapine had no effect on the steady-state pharmacokinetics of either ddI (n=18) or ddC (n=6).

Available data on the potential interactions between nevirapine and other CYP3A substrates are limited and preliminary; therefore, recommendations for dose adjustments cannot be made. (see PRECAUTIONS, *Drug Interactions*, for recommendations regarding rifampin, rifabutin, protease inhibitors and oral contraceptives)

In vitro: Studies using human liver microsomes indicated that the formation of nevirapine hydroxylated metabolites was not affected by the presence of dapsone, rifabutin, rifampin, and trimethoprim/sulfamethoxazole. Ketoconazole significantly inhibited the formation of nevirapine hydroxylated metabolites.

In vivo: Monitoring of steady-state nevirapine trough plasma concentrations in patients who received long-term VIRAMUNE® treatment in combination with ketoconazole (n=11) revealed no evidence of a significant inhibitory effect on nevirapine metabolism. Steady-state nevirapine trough plasma concentrations were elevated in patients who received cimetidine (+21%, n=11) and macrolides (+12%, n=24), known inhibitors of CYP3A.

Steady-state nevirapine trough concentrations were reduced in patients who received rifabutin (-16%, n=19) and rifampin (-37%, n=3), known inducers of CYP3A. Nevirapine is an inducer of CYP3A, with maximal induction occurring within 2-4 weeks of initiating multiple-dose therapy. Other compounds that are substrates of CYP3A may have decreased plasma concentrations when co-administered with VIRAMUNE®. Therefore, careful monitoring of the therapeutic effectiveness of CYP3A-metabolized drugs is recommended when taken in combination with VIRAMUNE®. (see PRECAUTIONS)

INDICATIONS AND USAGE: VIRAMUNE® (nevirapine) in combination with nucleoside analogues is indicated for the treatment of HIV-1 infected adults who have

experienced clinical and/or immunologic deterioration. This indication is based on analysis of changes in surrogate endpoints in studies of up to 48 weeks duration. At present, there are no results from controlled clinical trials evaluating the effect of VIRAMUNE® with nucleoside analogues on the clinical progression of HIV-1 infection, such as the incidence of opportunistic infections or survival.

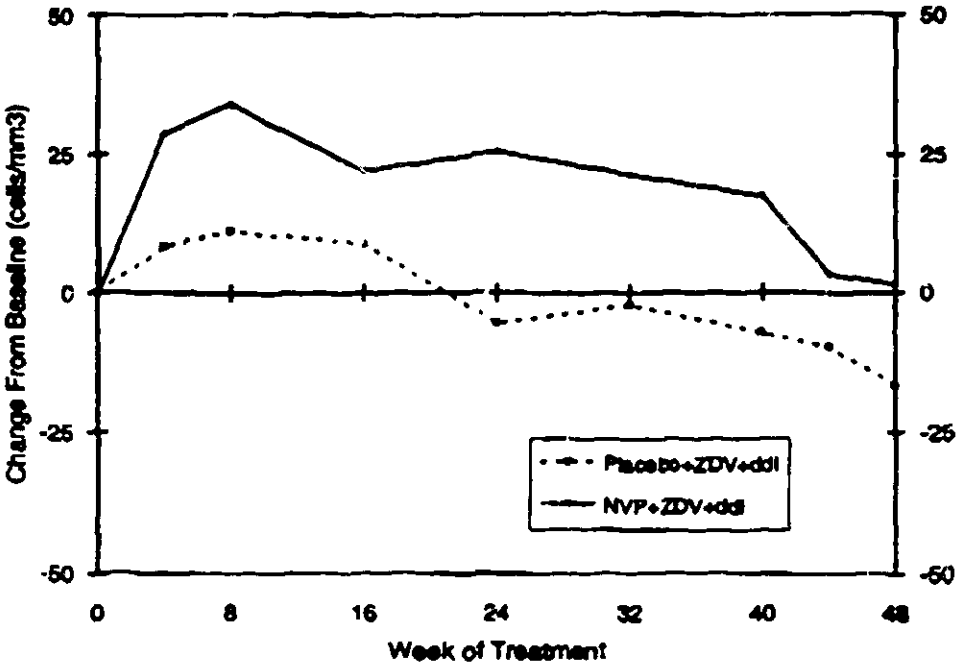
The duration of benefit from antiretroviral therapy may be limited. Alteration of antiretroviral therapy should be considered if disease progression occurs while patients are receiving VIRAMUNE®.

Resistant virus emerges rapidly and uniformly when VIRAMUNE® is administered as monotherapy. Therefore, VIRAMUNE® should always be administered in combination with at least one additional antiretroviral agent.

Description of Clinical Studies:

Patients with a prior history of nucleoside therapy:
ACTG 241 compared treatment with VIRAMUNE®+ZDV+ddI versus ZDV+ddI in 398 HIV-1-infected patients (median age 38 years, 74% Caucasian, 80% male) with CD4+ cell counts ≤350 cells/mm^3 (mean 153 cells/mm^3) and a mean baseline plasma HIV-1 RNA concentration of 4.59 $\log_{10}$ copies/mL (38,905 copies/mL), who had received at least 6 months of nucleoside therapy prior to enrollment (median 115 weeks). Treatment doses were VIRAMUNE®, 200 mg daily for two weeks, followed by 200 mg twice daily, or placebo; ZDV, 200 mg three times daily; ddI, 200 mg twice daily. Mean changes in CD4+ cell counts are shown in Figure 1. For 198 patients in the virology sub-study, mean HIV-1 RNA concentration changes from baseline are shown in Figure 2.

258 **Figure 1: Mean Change from Baseline for CD4+ Cell Count (absolute**
259 **number of CD4+ cells/mm3), Trial ACTG 241**



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Number of patients
with CD4 cell counts at each timepoint

	Baseline	Week 16	Week 32	40-48 Weeks
NVP+ZDV+ddI	196	177	157	161
Placebo+ZDV+ddI	196	176	160	167

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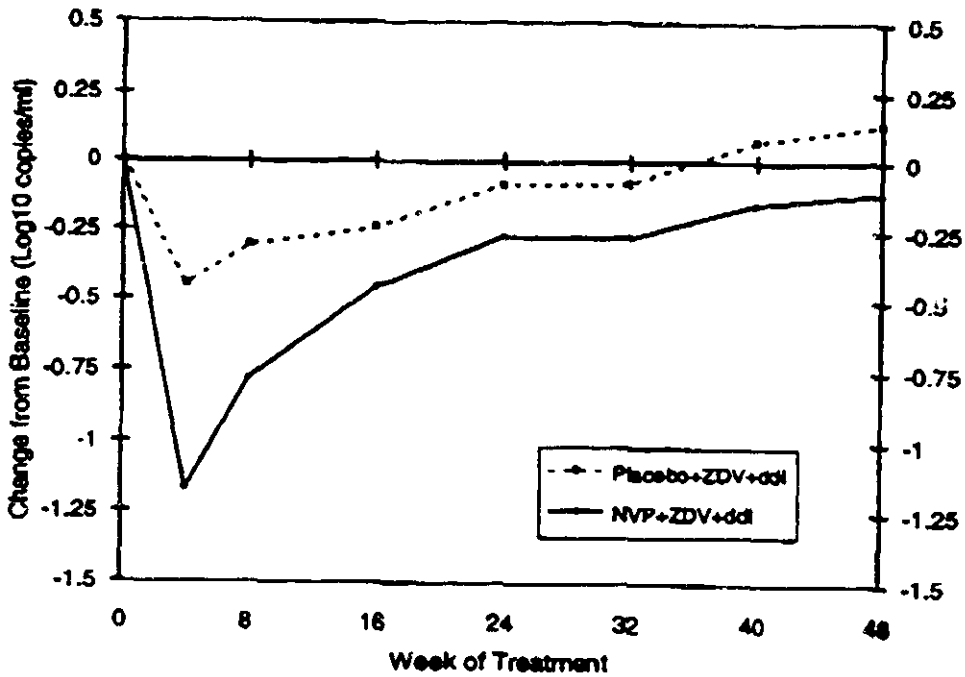
263 Figure 2: Mean Change from Baseline in HIV-1 RNA* Concentrations
264 (Log_{10} copies/mL), Virology Sub-study of Trial ACTG 241



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Number of patients
with HIV-1 RNA data at each timepoint

	Baseline	Week 16	Week 32	40-48 Weeks
NVP+ZDV+ddI	95	84	75	74
Placebo+ZDV+ddI	93	82	75	75

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267
268 * the clinical significance of changes in serum viral RNA measurements during treatment with VIRAMUNE® has not been established
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270 Trial BI 1037 compared treatment with VIRAMUNE®+ZDV versus ZDV in 60
271 HIV-1-infected patients (median age 33 years, 70% Caucasian, 93% male) with
272 CD4+ cell counts between 200 and 500 cells/mm^3 (mean 373 cells/mm^3) and a mean
273 baseline plasma HIV-1 RNA concentration of 4.24 log_{10} copies/mL (17,378
274 copies/mL), who had received between 3 and 24 months of prior ZDV therapy
275 (median 35 weeks). Treatment doses were VIRAMUNE® 200 mg daily for 2 weeks,
276 followed by 200 mg twice daily, or placebo; ZDV, 500-600 mg/day. Mean changes in
277 CD4+ cell counts are shown in Figure 3. Mean HIV-1 RNA concentration changes
278 from baseline are shown in Figure 4.
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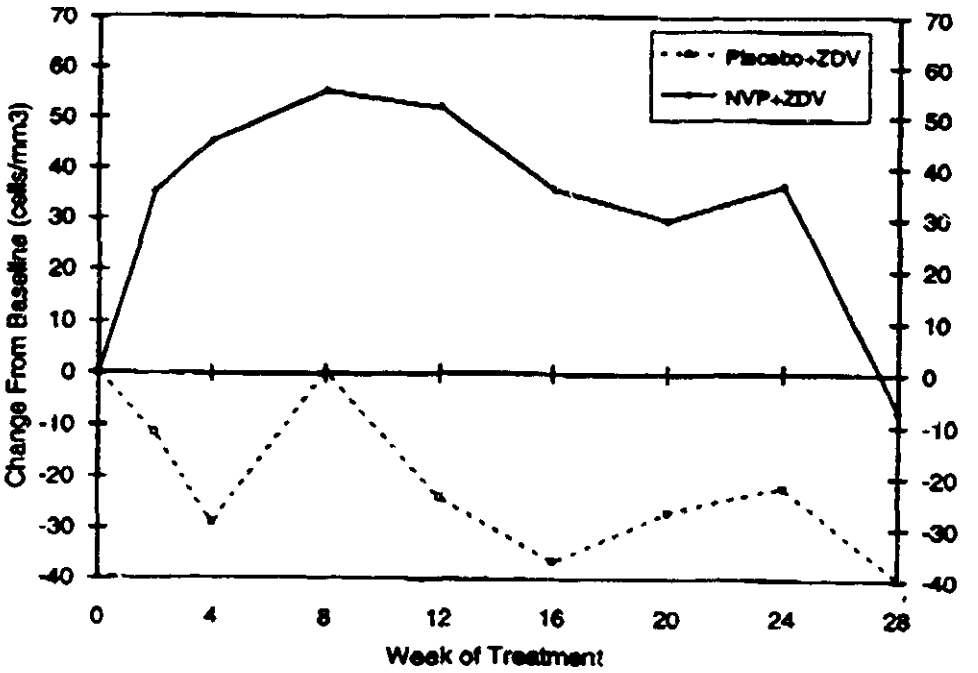
280 Figure 3: Mean Change from Baseline for CD4+ Cell Count (absolute
281 number of CD4+ cells/mm³), Trial BI 1037



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Number of patients
with CD4 cell counts at each timepoint

	Baseline	Week 8	Week 16	20-28 Weeks
NVP+ZDV	30	28	26	26
Placebo+ZDV	30	30	28	29

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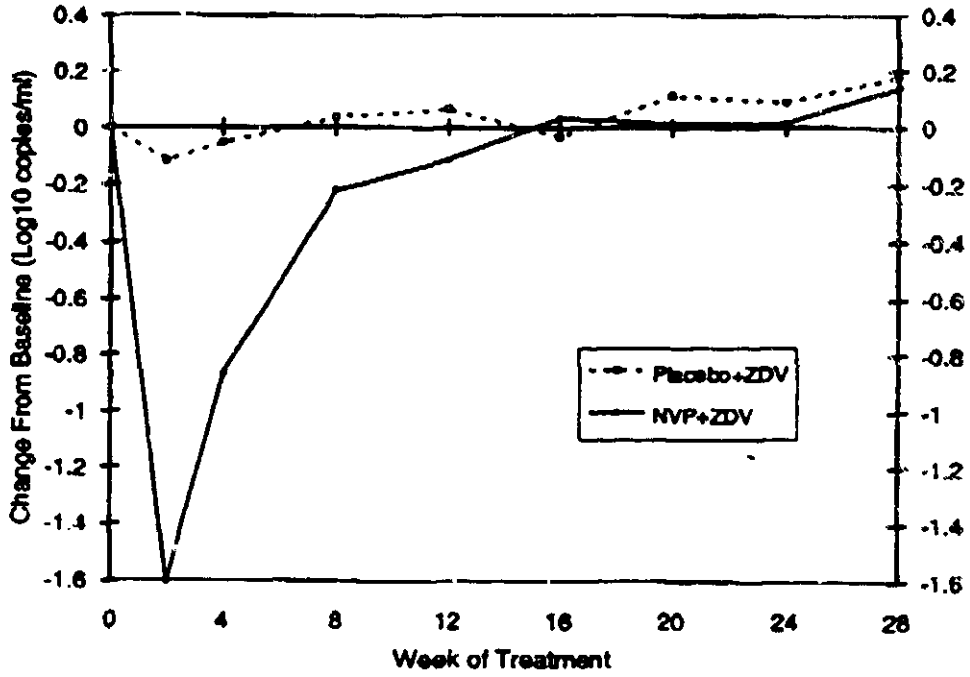
Figure 4: Mean Change from Baseline in HIV-1 RNA Concentrations (Log_{10} copies/mL), Trial BI 1037



	Number of patients with HIV-1 RNA data at each timepoint			
	Baseline	Week 8	Week 16	20-28 Weeks
NVP+ZDV	30	27	26	26
Placebo+ZDV	30	29	28	29

Patients without a history of prior antiretroviral therapy:

BI Trial 1046 compared treatment with VIRAMUNE®+ZDV+ddI versus VIRAMUNE® +ZDV versus ZDV+ddI in 151 HIV-1-infected patients (median age 36 years, 94% Caucasian, 93% male) with CD4+ cell counts of 200-600 cells/mm^3 (mean 376 cells/mm^3) and a mean baseline plasma HIV-1 RNA concentration of 4.41 log_{10} copies/mL (25,704 copies/mL). Treatment doses were VIRAMUNE®, 200 mg daily for two weeks, followed by 200 mg twice daily, or placebo; ZDV, 200 mg three times daily; ddI, 125 or 200 mg twice daily. Changes in CD4+ cell counts at 24 weeks: mean levels of CD4+ cell counts in those randomized to VIRAMUNE®+ZDV+ddI and ZDV+ddI remained significantly above baseline; however there was no significant difference between these arms. Changes in HIV-1 viral RNA at 24 weeks: there was no significant difference as measured by mean changes in plasma viral RNA between those randomized to VIRAMUNE®+ZDV+ddI and ZDV+ddI. However, the proportion of patients whose HIV-1 RNA decreased below the limit of detection (400 copies/mL) was significantly greater for the VIRAMUNE®+ZDV+ddI group (27/36 or

75%), when compared to the ZDV+ddI group (18/39 or 46%) or the VIRAMUNE® +ZDV group (0/28 or 0%); the clinical significance of this finding is unknown.

CONTRAINDICATIONS: VIRAMUNE® is contraindicated in patients with clinically significant hypersensitivity to any of the components contained in the tablet.

WARNINGS: Severe and life-threatening skin reactions have occurred in patients treated with VIRAMUNE®, including Stevens-Johnson syndrome (SJS). VIRAMUNE® must be discontinued in patients developing a severe rash or a rash accompanied by constitutional symptoms such as fever, blistering, oral lesions, conjunctivitis, swelling, muscle or joint aches, or general malaise. (see PRECAUTIONS, Information for Patients; ADVERSE REACTIONS)

VIRAMUNE® therapy must be initiated with a 14-day lead-in period of 200 mg/day, which has been shown to reduce the frequency of rash. Dose escalation should not occur if rash is observed during this lead-in period until the rash has resolved. (see DOSAGE AND ADMINISTRATION)

PRECAUTIONS: ***General:*** When administering VIRAMUNE® as part of an antiretroviral treatment regimen, the complete product information for each therapeutic component should be consulted before initiation of treatment.

While nevirapine is extensively metabolized by the liver and nevirapine metabolites are extensively eliminated by the kidney, the pharmacokinetics of nevirapine have not been evaluated in patients with either hepatic or renal dysfunction. Therefore, VIRAMUNE® should be used with caution in these patient populations.

Abnormal liver function tests have been reported with VIRAMUNE®, some in the first few weeks of therapy, including cases of hepatitis. VIRAMUNE® administration should be interrupted in patients experiencing moderate or severe liver function test abnormalities until liver function tests return to baseline values. VIRAMUNE® treatment should be permanently discontinued if liver function abnormalities recur on readministration.

Drug Interactions: Although clinical studies have not been conducted, induction of CYP3A by nevirapine may result in lower plasma concentrations of other concomitantly administered drugs that are extensively metabolized by CYP3A. (see

CLINICAL PHARMACOLOGY) Thus, if a patient has been stabilized on a dosage regimen for a drug metabolized by CYP3A, and begins treatment with VIRAMUNE®, dose adjustments may be necessary.

Rifampin/Rifabutin: There are insufficient data to assess whether dose adjustments are necessary when nevirapine and rifampin or rifabutin are coadministered. Therefore, these drugs should only be used in combination if clearly indicated and with careful monitoring.

Protease Inhibitors: Nevirapine may decrease plasma concentrations of protease inhibitors. Therefore, until clinical data are available that evaluate the need for dose adjustments, these drugs should not be administered concomitantly with VIRAMUNE®.

Oral Contraceptives: There are no clinical data on the effects of nevirapine on the pharmacokinetics of oral contraceptives. Nevirapine may decrease plasma concentrations of oral contraceptives (also other hormonal contraceptives); therefore, these drugs should not be administered concomitantly with VIRAMUNE®.

Information for Patients: Patients should be informed that VIRAMUNE® is not a cure for HIV-1 infection, and that they may continue to experience illnesses associated with advanced HIV-1 infection, including opportunistic infections. Treatment with VIRAMUNE® has not been shown to reduce the incidence or frequency of such illnesses, and patients should be advised to remain under the care of a physician when using VIRAMUNE®.

Patients should be informed that the long-term effects of VIRAMUNE® are unknown at this time. They should also be informed that VIRAMUNE® therapy has not been shown to reduce the risk of transmission of HIV-1 to others through sexual contact or blood contamination.

Patients should be instructed that the major toxicity of VIRAMUNE® is rash and should be advised to promptly notify their physician of any rash. The majority of rashes associated with VIRAMUNE® occur within the first 6 weeks of initiation of therapy. Therefore, patients should be monitored carefully for the appearance of rash during this period. Patients should be instructed that dose escalation is not to occur if any rash occurs during the two-week lead-in dosing period, until the rash resolves. Any patient experiencing severe rash or a rash accompanied by constitutional symptoms such as fever, blistering, oral lesions, conjunctivitis,

swelling, muscle or joint aches, or general malaise should discontinue medication and consult a physician.

Patients should be informed to take VIRAMUNE® every day as prescribed. Patients should not alter the dose without consulting their doctor. If a dose is missed, patients should take the next dose as soon as possible. However, if a dose is skipped, the patient should not double the next dose.

VIRAMUNE® may interact with some drugs; therefore, patients should be advised to report to their doctor the use of any other medications.

Patients should be instructed that oral contraceptives and other hormonal methods of birth control should not be used as a method of contraception in women taking VIRAMUNE®.

Carcinogenesis, Mutagenesis, Impairment of Fertility: Long-term carcinogenicity studies of nevirapine in animals are currently in progress. In genetic toxicology assays, nevirapine showed no evidence of mutagenic or clastogenic activity in a battery of *in vitro* and in vivo assays including microbial assays for gene mutation (Ames: Salmonella strains and *E. coli*), mammalian cell gene mutation assays (CHO/HGPRT), cytogenetic assays using a Chinese hamster ovary cell line and a mouse bone marrow micronucleus assay following oral administration. In reproductive toxicology studies, evidence of impaired fertility was seen in female rats at doses providing systemic exposure, based on AUC, approximately equivalent to that provided with the recommended clinical dose of VIRAMUNE®.

Pregnancy: Pregnancy Category C: No observable teratogenicity was detected in reproductive studies performed in pregnant rats and rabbits. In rats, a significant decrease in fetal body weight occurred at doses providing systemic exposure approximately 50% higher, based on AUC, than that seen at the recommended human clinical dose. The maternal and developmental no-observable-effect level dosages in rats and rabbits produced systemic exposures approximately equivalent to or approximately 50% higher, respectively, than those seen at the recommended daily human dose, based on AUC. There are no adequate and well-controlled studies in pregnant women. VIRAMUNE® should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Nursing Mothers: Preliminary results from an ongoing pharmacokinetic study (ACTG 250) of 10 HIV-1-infected pregnant women who were administered a single

oral dose of 100 or 200 mg VIRAMUNE® at a median of 5.8 hours before delivery, indicate that nevirapine readily crosses the placenta and is found in breast milk. Consistent with the recommendation by the U.S. Public Health Service Centers for Disease Control and Prevention that HIV-infected mothers not breast-feed their infants to avoid risking postnatal transmission of HIV, mothers should discontinue nursing if they are receiving VIRAMUNE®.

Pediatric Use: Safety and effectiveness of VIRAMUNE® in pediatric patients have not been established.

VIRAMUNE® has been studied in two open-label, uncontrolled trials (BI 882, BI 892) in 37 HIV-1-infected pediatric patients with a median age of 0.9 years (range: 0.1 to 15 years) who were treated for a median duration of 20.7 months. Seven patients developed rashes while receiving VIRAMUNE®. In an ongoing, controlled trial of VIRAMUNE® combination therapy in HIV-1-infected pediatric patients (ACTG 245), one of approximately 268 patients treated with VIRAMUNE® experienced Stevens-Johnson syndrome.

Because there are no data on multi-dose pharmacokinetics in children, no recommendation on dosing can be made. Based on single-dose pharmacokinetics in 9 HIV-1-infected pediatric patients (age 9 mos. to 14 years) who were administered nevirapine in a suspension formulation, it appears that oral clearance is approximately 2-fold greater in children when compared to adults.

ADVERSE REACTIONS: The most frequently reported adverse events related to VIRAMUNE® therapy were rash, fever, nausea, headache, and abnormal liver function tests.

The major clinical toxicity of VIRAMUNE® is rash, with VIRAMUNE®-attributable rash occurring in 17% of patients in combination regimens in Phase II/III controlled studies. Thirty-seven percent of patients treated with VIRAMUNE® experienced rash compared with 20% of patients treated in control groups of either ZDV+ddI or ZDV alone (Table 1). Severe or life-threatening rash occurred in 7.6% of VIRAMUNE®-treated patients compared with 1.2% of patients treated in the control groups.

Rashes are usually mild to moderate, maculopapular erythematous cutaneous eruptions, with or without pruritus, located on the trunk, face and extremities. The majority of severe rashes occurred within the first 28 days of treatment; 25% of the

patients with severe rashes required hospitalization; and one patient required surgical intervention. All patients recovered. Overall, 7% of patients discontinued VIRAMUNE® due to rash.

Table 1: Percentage of patients with rashes in controlled trials[a]

	ACTG 241[b]		BI 1037		BI 1011		COMBINED DATA	
	NVP+ZDV +ddI	ZDV+ddI	NVP+ZDV	ZDV	NVP+ZDV	ZDV	NVP	CONTROL
n	197	201	30	30	25	24	252	255
Rash events of all Grades and all causality	39.6%	23.9%	26.7%	6.7%	32.0%	4.2%	37.3%	20.0%
Grade 3 or 4 rash events; all causality	8.1%	1.5%	3.3%	0%	8.0%	0%	7.6%	1.2%

a At recommended dose of one 200 mg tablet daily for the first 14 days followed by one 200 mg tablet twice daily

b Trial ACTG 241 was designed to report Grade 3/4 (severe or life-threatening) events; except for several pre-specified events including rash for which all grades are reported

Table 2 lists treatment-related clinical adverse events that occurred in patients receiving VIRAMUNE® in ACTG 241 and in Trials BI 1037 and BI 1011.

Table 2: Comparative Incidence of Selected Drug-Related Events in Controlled Trials

	ACTG 241		Trial BI 1037 and BI 1011	
	Grade 3/4 events		All severities	
	NVP+ZDV +ddI	ZDV+ddI	NVP+ZDV	ZDV alone
Number of patients	197	201	55	30
Overall incidence of related adverse events	31%	23%	42%	33%
Rash	8	2	20	3
Fever	3	3	11	3
Nausea	5	4	9	3
Headache	3	3	11	0
Diarrhea	2	2	0	0
Abdominal pain	1	2	2	0
Ulcerative stomatitis	0	0	4	0
Peripheral Neuropathy	0	2	0	0
Paraesthesia	1	0	2	0
Myalgia	1	0	2	7
Hepatitis	1	0	4	0

Laboratory Abnormalities: Table 3 summarizes marked laboratory abnormalities occurring in three controlled studies.

Table 3: Percentage of patients with marked laboratory abnormalities

	Data combined for controlled trials ACTG 241, BI 1037 & BI 1011	
	VIRAMUNE® n=252	Control n=255
<u>Hematology</u>		
Decreased Hg (<8.0 g/dL)	1.2%	2.0%
Decreased platelets (<50,000/mm^3)	0.8	0.8
Decreased neutrophils (<750/mm^3)	11.1	10.2
<u>Blood chemistry</u>		
Increased ALT (>250 U/L))	3.4	3.5
Increased AST (>250 U/L)	2.0	2.4
Increased GGT (>450 U/L)	2.4	1.2
Increased total bilirubin (>2.5 mg/dL)	0.4	1.2

Asymptomatic elevations in GGT levels are more frequent in VIRAMUNE® recipients than in controls. Because hepatitis has occasionally been reported in VIRAMUNE®-treated patients, monitoring of liver function tests should be considered.

OVERDOSAGE: There is no known antidote for VIRAMUNE® overdosage. No acute toxicities or sequelae were reported for one patient who ingested 800 mg of VIRAMUNE® for one day.

DOSAGE AND ADMINISTRATION: The recommended dose for VIRAMUNE® is one 200 mg tablet daily for the first 14 days **(this lead-in period should be used because it has been found to lessen the frequency of rash)**, followed by one 200 mg tablet twice daily, in combination with nucleoside analogue antiretroviral agents. For concomitantly administered nucleoside therapy, the manufacturer's recommended dosage and monitoring should be followed.

Monitoring Of Patients: Clinical chemistry tests, which include liver function tests, should be performed prior to initiating VIRAMUNE® therapy and at appropriate intervals during therapy.

Dosage Adjustment: **VIRAMUNE® should be discontinued if patients experience severe rash or a rash accompanied by constitutional findings (see WARNINGS). Patients experiencing rash during the 14-day lead-in period of 200 mg/day should not have their VIRAMUNE® dose increased until the rash has resolved. (see PRECAUTIONS, *Information for Patients*)**

VIRAMUNE® administration should be interrupted in patients experiencing moderate or severe liver function test abnormalities (excluding GGT), until the liver function test elevations have returned to baseline. VIRAMUNE® may then be restarted at half the previous dose level. VIRAMUNE® should be permanently discontinued if moderate or severe liver function test abnormalities recur. (see PRECAUTIONS)

Patients who interrupt VIRAMUNE® dosing for more than 7 days should restart the recommended dosing, using one 200 mg tablet daily for the first 14 days (lead-in) followed by one 200 mg tablet twice daily.

No data are available to recommend a dosage of VIRAMUNE® in patients with hepatic dysfunction, renal insufficiency, or undergoing dialysis.

HOW SUPPLIED: VIRAMUNE® (nevirapine) Tablets, 200 mg, are white, oval, biconvex tablets, 9.3 mm x 19.1 mm. One side is embossed with "54 193", with a single bisect separating the "54" and "193". The opposite side has a single bisect.

VIRAMUNE® Tablets are supplied in bottles of 100 (NDC 0054-4647-25) and individually blister-sealed unit-dose cartons of 100 tablets as 10 x 10 cards (NDC 0054-8647-25).

Store at 15°C - 30°C (59°F - 86°F). The bottles should be kept tightly closed.

Manufactured by: Boehringer Ingelheim Pharmaceuticals, Inc.
Ridgefield, CT 06877

Distributed by: Roxane Laboratories, Inc.
Columbus, OH 43216

CAUTION FEDERAL LAW PROHIBITS DISPENSING WITHOUT PRESCRIPTION

Version 12A June 21, 1996

13.0 PATENT INFORMATION

1)	Name of Drug Product	VIRAMUNE®
2)	Active Ingredient(s)	nevirapine (the chemical name for which is 11-cyclopropyl-5,11-dihydro-4-methyl-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one)
3)	Strength(s)	200 mg
4)	Dosage Form	tablet
	Route of Administration	oral
5)	Name of Applicant	Boehringer Ingelheim Pharmaceuticals, Inc.
6)	NDA Number	20-636
7)	Applicable Patent Numbers and Expiration Date of Each	U.S. Patent No. 5,366,972 November 22, 2011
8)	Type of Patent	drug, drug product and method of use
9)	Name of Patent Owner	Boehringer Ingelheim Pharmaceuticals, Inc. and Dr. Karl Thomae GmbH, as joint owners

10) Declaration

The undersigned declares that Patent No. 5,366,972 covers the formulation, composition, and/or method of use of Viramune. This product is the subject of this application for which approval is being sought.

By: Alan Stempel

Alan Stempel

Capacity: Attorney for Patent Owner and Applicant

Date: October 20, 1995

EXCLUSIVITY SUMMARY FOR NDA # 20,636 SUPPL # _____

Trade Name Viramune® Generic Name nevirapine

Applicant Name Boehringer Ingleheim Pharmaceuticals, Inc. HFD # 530

Approval Date If Known June 21, 1996

PART I IS AN EXCLUSIVITY DETERMINATION NEEDED?

1. An exclusivity determination will be made for all original applications, but only for certain supplements. Complete PARTS II and III of this Exclusivity Summary only if you answer "yes" to one or more of the following question about the submission.

a) Is it an original NDA?

YES /X/ NO /___/

b) Is it an effectiveness supplement?

YES /___/ NO /X/

If yes, what type? (SE1, SE2, etc.)

c) Did it require the review of clinical data other than to support a safety claim or change in labeling related to safety? (If it required review only of bioavailability or bioequivalence data, answer "no").

YES /X/ NO /___/

If your answer is "no" because you believe the study is a bioavailability study and, therefore, not eligible for exclusivity, EXPLAIN why it is a bioavailability study, including your reasons for disagreeing with any arguments made by the applicant that the study was not simply a bioavailability study.

_____

_____

If it is a supplement requiring the review of clinical data but it is not an effectiveness supplement, describe the change or claim that is supported by the clinical data:

_____

_____

d) Did the applicant request exclusivity?

X/ NO /___/

If the answer [illegible] ", how many years of exclusivity did the applicant request?

Sponsor did not specify, requested standard period as described in regulations.

YOU HAVE ANSWERED "NO" TO ALL OF THE ABOVE QUESTIONS, GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8.

2. Has a product with the same active ingredient(s), dosage form, strenght, route of administration, and dosing schedule, previously been approved by FDA for the same use?

YES /___/ NO /___/

If yes, NDA # _____ Drug Name _____

IF THE ANSWER TO QUESTION 2 IS "YES", GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8.

3. Is this drug product or indication a DESI upgrade?

yes /___/ NO /X/

IF THE ANSWER TO QUESTION 3 IS "YES", GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8 (even if a study was required for the upgrade).

PART II FIVE-YEAR EXCLUSIVITY FOR NEW CHEMICAL ENTITIES

(Answer either #1 or #2 as appropriate)

1. Single active ingredient product.

Has FDA previously approved under section 505 of the Act any drug product containing the same active moiety as the drug under consideration? Answer "yes" if the active moiety (including other esterified forms, salts, complexes, chelates or clathrates) has been previously approved, but this particular form of the active moiety, e.g., this particular ester or salt (including salts with hydrogen or coordination bonding) or other non-covalent derivative (such as a complex, chelate, or clathrate) has not been approved. Answer "no" if the compound requires metabolic conversion (other than deesterification of an esterified form of the drug) to produce an already approved active moiety.

YES /___/ NO/X/

If "yes", identify the approved drug product(s) containing the active moiety, and, if known, the NDA #(s).

NDA # _____ _____

NDA # _____ _____

NDA # _____ _____

2. Combination product.

If the product contains more than one active moiety (as defined in Part II, #1), has FDA previously approved an application under section 505 containing any one of the active moieties in the drug product? If, for example, the combination contains one-never-before-approved active moiety and one previously approved active moiety, answer "yes". (An active moiety that is marketed under an OTC monograph, but that was never approved under an NDA, is considered not previously approved).

YES /___/ NO/ X /

If "yes", identify the approved drug product(s) containing the active moiety, and, if known, the NDA #(s).

NDA # _____ _____

NDA # _____ _____

NDA # _____ _____

IF THE ANSWER TO QUESTION 1 OR 2 UNDER PART II IS "NO", GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8. IF "YES", GO TO PART III.

PART III THREE-YEAR EXCLUSIVITY FOR NDA'S AND SUPPLEMENTS

To qualify for three years of exclusivity, an application or supplement must contain "reports of new clinical investigations (other than bioavailability studies) essential to the approval of the application and conducted or sponsored by the applicant". This section should be completed only if the answer to PART II, Question 1 or 2 was "yes".

1. Does the application contain reports of clinical investigations? (The Agency interprets "clinical investigations" to mean investigations conducted on humans other than bioavailability studies). If the application contains clinical investigations only by virtue of a right of reference to clinical investigations in another application, answer "yes", then skip to question 3(a). If the answer to 3(a) is "yes" for any investigation referred to in another application, do not complete remainder of summary for that investigation.

YES /___/ NO /___/

IF "NO", GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8.

2. A clinical investigation is "essential to the approval" if the Agency could not have approved the application or supplement without relying in that investigation. Thus, the investigation is not essential to the approval if 1) no clinical investigation is necessary to support the supplement or application in light of previously approved applications (i.e., information other than clinical trials, such as bioavailability data, would be sufficient to provide a basis for approval as an ANDA or 505(b)(2) application because of what is already known about a previously approved product), or 2) there are published reports of studies (other than those conducted or sponsored by the applicant) or other publicly available data that independently would have been sufficient to support approval of the application, without reference to the clinical investigation submitted in the application.

(a) In light of previously approved applications, is a clinical investigation (either conducted by the applicant or available from some other source, including the published literature) necessary to support approval of the application or supplement?

YES /___/ NO /___/

If "no", state the basis for your conclusion that a clinical trial is not necessary for approval AND GO DIRECTLY TO SIGNATURE BLOCK ON PAGE 8:

_____

_____

(b) Did the applicant submit a list of published studies relevant to the safety and effectiveness of this drug product and a statement that the publicly available data would not independently support approval of the application?

YES /___/ NO /___/

(1) If the answer to 2(b) is "yes", do you personally know of any reason to disagree with the applicant's conclusion?

YES /___/ NO /___/

If yes, explain: _____

_____

(2) If the answer to 2(b) is "no", areyou aware of published studies not conducted or sponsored by the applicant or other publicly available date that could independently demonstrate the safety and effectiveness of this drug product?

YES /___/ NO /___/

If yes, explain: _____

_____

(c) If the answers to (b)(1) and (b)(2) were both "no", identify the clinical investigations submitted in the application that are essential to the approval:

_____

_____

Studies comparing two products with the same ingredient(s) are considered to be bioavailability studies for the purpose of this section.

3. In addition to being essential, investigations must be "new" to support exclusivity. The agency interprets "new clinical investigation" to mean an investigation that 1) has not been relied on by the agency to demonstrate the effectiveness of a previously approved drug for any indication and 2) does not duplicate the results of another investigation that was relied on by the agency to demonstrate the effectiveness of a previously appproved drug product, i.e., does not redemonstrate something the agency considers to have been demonstrated in an already approved application.

a) For each investigation identified as "essential to the approval", has the investigation been relied on by the agency to demonstrate the effectiveness of a prevviously approved drug product? (If the investigation was relied on only to support the safety of a previously approved drug, answer "no").

Investigation #1 YES /___/ NO /___/

Investigation #2 YES /___/ NO /___/

If you have answered "yes" for one or more investigations, identify each such investigation and the NDA in which each was relied upon:

_____ _____

_____ _____

b) For each investigation identified as "essential to the approval", does the investigation duplicate the results of another investigation that was relied on by the agency to support the effectiveness of a previously approved drug product?

Investigation #1 YES /___/ NO /___/

Investigation #2 YES /___/ NO /___/

If you have answered "yes" for one or more investigations, identify the NDA in which a similar investigation was relied on:

_____ _____

_____ _____

c) If the answers to 3(a) and 3(b) are "no", identify each "new" investigation in the application or supplement that is essential to the approval (i.e., the investigations listed in #2(c), less any that are not "new"):

_____ _____

_____ _____

4. To be eligible for exclusivity, a new investigation that is essential to approval must also have been conducted or sponsored by the applicant. An investigation was "conducted or sponsored by" the applicant if, before or during the conduct of the investigation, 1) the applicant was the sponsor of the IND named in the form FDA 1571 filed with Agency, or 2) the applicant (or its predecessor in interest) provided substantial support for the study. Ordinarily, substantial support will mean providing 50 percent or more of the cost of the sudy.

a) For each investigation identified in response to question 3(c): if the investigation was carried out under an IND, was the applicant identified on FDA 1571 as the sponsor?

Investigation #1

IND # _____ YES /___/ NO /___/ Explain: _____

_____

Investigation #2

IND # _____ YES /___/ NO /___/ Explain: _____

_____

b) For each investigation not carried out under an IND or for which the applicant was not identified as the sponsor, did the applicant certify that it or the applicant's predecessor in interest provided substantial support for the study?

Investigation #1

YES /___/ Explain _____ NO /___/ Explain _____

_____ _____

_____ _____

Investigation #2

YES /___/ Explain _____ NO /___/ Explain _____

_____ _____

_____ _____

(c) Not withstanding an answer of "yes" to (a) or (b), are there other reasons to believe that the applicant should not be credited with having "conducted or sponsored" the study? (Purchased studies may not be used as the basis for exclusivity. However, if all rights to the drug are purchased (not just studies on the drug), the applicant may be considered to have sponsored or conducted the studies sponsored or conducted by its predecessor in interrest)

YES /___/ NO /___/

If yes, explain: _____

_____

Anthony M. Zeccola 6/21/96
Anthony M. Zeccola Date
Consumer Safety Officer

_____ _____
David W. Feigal, Jr., M.D., MPH Date
Division Director

Revised 5-90
cc: Orig NDA
Div File
HFD-85

EXCLUSIVITY INFORMATION

1) The applicant, Boehringer Ingelheim Pharmaceuticals, Inc., believes that after approval of the New Drug Application, VIRAMUNE® Tablets will be entitled to a period of marketing exclusivity under the provisions of 21 CFR 314.108, and is, therefore, claiming exclusivity.

2) Reference is made to 21 CFR 314.108(b)(2) to support the applicant's claim to exclusivity for VIRAMUNE® Tablets.

3) The applicant claims exclusivity under 21 CFR 314.108(b)(2), and accordingly must submit information to show that, to the best of the applicant's knowledge or belief, a drug has not previously been approved under section 505(b) of the Federal Food, Drug and Cosmetic Act containing any active moiety in the drug for which the applicant is seeking approval. This information is as follows: The sole active moiety in the drug for which the applicant is seeking approval, VIRAMUNE® Tablets, is nevirapine (the chemical name for which is 11-cyclopropyl-5,11-dihydro-4-methyl-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one). To the best of the applicant's knowledge and belief, no drug containing nevirapine as an active moiety has previously been approved under section 505(b) of the Federal Food, Drug and Cosmetic Act.

BOEHRINGER INGELHEIM PHARMACEUTICALS, INC.

By: Martin M. Kaplan

Martin M. Kaplan, M.D., J.D.

Title: Director, Drug Regulatory Affairs

Date: February 5, 1996

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

NDA # 20,636 Trade (generic) names Viramune® (nevirapine)

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).

X 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.

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

X (1) Studies are ongoing.
___ (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:_____

Anthony M. Zeccola
Consumer Safety Officer

6/17/96
Date

cc: Orig NDA
HFD-530/Div File
NDA Action Package

CERTIFICATION REQUIREMENT

SECTION 306(k)(1) OF THE ACT
21 U.S.C.335a(k)(1)

The undersigned certifies, that, to the best knowledge and belief of the undersigned, Boehringer Ingelheim Pharmaceuticals, Inc. did not and will not use in any capacity the services of any person debarred under subsection (a) or (b) [Section 306(a) or (b)], in connection with VIRAMUNE® Tablets.

Signature

Name of the Applicant: Martin Kaplan, M.D.
Director, Drug Regulatory Affairs
Boehringer Ingelheim Pharmaceuticals, Inc.

Mailing Address: Boehringer Ingelheim Pharmaceuticals, Inc.
900 Ridgebury Road
P.O. Box 368
Ridgefield, CT 06877-0368

NDA 20-636

DRAFT

Date NDA submitted: 23 Feb 96
Date NDA received: 23 Feb 96
Date assigned: 26 Feb 96
Review completed: 1 9Jun draft

Medical Officer's Review
(Original NDA for NME)

Applicant: Boehringer Ingelheim Pharmaceuticals, Inc.
900 Ridgebury Road
P.O. Box 368
Ridgefield, CT 06877-0368

Drug: Chemical: 5,11-dihydro-11-cyclopropyl-4-methyl-6H-dipyrido-[3,2-b:2',3'-e][1,4]diazepin-6-one
Generic: Nevirapine
Trade: VIRAMUNE®

Route: oral

Dosage form: 200 mg (tablet)

Proposed indication: "Indicated in combination with nucleoside analogues for the treatment of HIV-1 infected adults who have experienced clinical and/or immunologic deterioration"

Related INDs:
(saquinavir, Hoffman-LaRoche)

Related documents: Major amendments
BM: 7Mar96: Information for Inspection decisions
BM: 12Apr96: Study 1046
Four-month safety update

Minutes of meetings dated:
4 Apr 95: (closed session of Advisory Committee)
7 Aug 95: (pre-NDA)
18 Mar 95:(post-submission)
Date: (labeling)
7 Jun 95: (Advisory Committee)

Medical reviews dated:
Date (ACTG 241/1100.1031)
Date (1100.1037)
Date (1100.1011)
Date (1100.744)
Date (1100.834)

TABLE OF CONTENTS

1. Summary Viramune® (nevirapine) is the first non-nucleoside inhibitor of HIV-1 reverse transcriptase to be submitted to the FDA for regulatory consideration in the treatment of HIV infection (new molecular entity, NME). The clinical portion of the submission consists of six studies in HIV-infected subjects which provide information on the effect of this drug on laboratory values, including particularly the numbers of circulating CD4 lymphocytes, and on measures of viremia, particularly HIV-1 RNA levels in serum, which are generally considered to be surrogate endpoints that are reasonably likely to predict clinical benefit in this patient population. The applicant has requested approval of nevirapine for the indication "In combination with nucleoside analogues is indicated for the treatment of patients with HIV-1 infection who have experienced clinical and/or immunologic deterioration."

Rachel's comment: This section needs to be expanded. It should summarize the whole review. It should state in advance that Safety and Efficacy will be summarized at the end of the review.

2. **Background - Regulatory History**

The original IND for nevirapine (IND) was submitted on (date), and the adult Phase I/II program was initiated. Controlled clinical trials with surrogate marker endpoints in adults were initiated in (mo, yr). A closed session meeting with the Antiviral Drugs Advisory Committee was held on ( ) Apr, 1995 to discuss whether the completed studies were sufficient to justify submission of a marketing application. A pre-NDA meeting was held on ( ) 1995. The NDA was submitted to the FDA on 23 Feb 1996. The NDA post-submission meeting between the Applicant and DAVDP representatives was held on 18 Mar 96, and the Applicant was asked if additional viral surrogate endpoint data was available that might be submitted to the NDA. On 12 April 96, the Applicant submitted to the NDA a 6-month analysis of activity data from an international non-IND trial, Study 1046.

Nevirapine is not approved for marketing in any country.

Materials Reviewed: NDA 20-636 consists of 239 volumes. Major amendments include summary information on a new trial 1100.1046 (dated 12 April 96) and a Safety Update (dated 29 Apr 96).

Chemistry/Manufacturing Controls: Please refer to Dr. Steven Miller's review.

Animal Pharmacology/Toxicology: Please refer to Dr. Peter Verma's review.

Microbiology: Please refer to Dr. Walla Dempsey's review

Statistics: Please refer to Dr. Michael Elashoff's review.

3. Summary of NDA clinical section

The clinical section of this application includes the study reports of three randomized controlled clinical trials in HIV-infected adults, and two uncontrolled studies. Also included is safety information from 18 clinical trials; these studies are either completed but partially analyzed, or are ongoing. The NDA also summarizes information from 10 clinical pharmacology studies, and safety information is summarized for one of these.

Of the three randomized, controlled trials, two (Study 1100.1031/ACTG 241 and 1100.1037) were double blinded, and their reports are submitted as the primary evidence of efficacy for this NDA, while Study 1100.1011, an open-label study, is submitted as providing supportive evidence[1]. Table 1 summarizes major features of these studies.

Table 1. Comparison of controlled clinical studies of nevirapine in anti-retroviral experienced, HIV+ subjects

Trial	1031 (ACTG 241)	1037	1011
Location	US (16 centers)	US (7 centers)	Aust/Ital/Neth (11 centers)
Total no.	398	60	49
Design	Randomized, double-blind	Randomized, double-blind	Controlled, open label
Population	≥13 y CD4 ≤350 ≥6 m prior nucleosides	≥13 y, asymptomatic CD4 200-500 3-24 m prior ZDV	≥18 y CD4<500 ZDV >6 m
Stratification	CD4≤50 (n=100) CD4 51-200 (n=150) CD4 201-350 (n=150)	None	None
Treatment groups	NVP 400 mg+ddI 400 mg +ZDV 600 mg *vs* ddI 400 mg +ZDV 600 mg	NVP 400 mg+ZDV 500-600 mg *vs* ZDV 500-600 mg	NVP 400 mg QD+ 500-600 mg ZDV
Primary endpoint	CD4 at 16, 24, 48 wks; HIV RNA (PCR); PBMC microculture	CD4 at 3, 6 mo; HIV RNA (PCR); PBMC microculture	p24 antigen response
Follow-up	48 wks	6 mo	28 wks (ext to 92)

In addition, safety and efficacy information from two non-comparative, Phase 1/2 dose-escalation trials (Study 1100.744/ ACTG 164, 1100.834/ACTG 168) are included:

Table . Comparison of non-controlled phase 1/2 clinical studies of nevirapine in HIV+ subjects

Trial	744 (ACTG 164)	834 (ACTG 168)
Location	US (6 centers)	US (5 centers)
Total no.	63	83
Design	Uncontrolled, dose escalation	Uncontrolled, dose escalation
Population	HIV-infected adults, CD 4 < 400	HIV-infected adults, CD4 <400
Treatment groups	12.5 mg 11 subjects 50 mg 10 200 mg 9 400 mg 22 600 mg 11	12.5mg 10 subjects 50mg 10 200mg 12 400mg 25* 600mg 26* *most received 200 mg NVP lead-in for 2 or 4 wks
Objective	Dose-finding	Dose-finding
Primary endpoint	PK, clinical toxicity	PK, clinical toxicity
Follow-up	24 wks	24 wks

[1]The trial designation prefix *1100.* denotes a nevirapine study, and will be omitted hereafter.

During the course of the review of this NDA, and at the request of the FDA, summary information from a recently completed trial that evaluated the effect of NVP therapy in combination with nucleosides in an anti-retroviral-naive HIV + population, Study 1046, was submitted. Features of this study are summarized in Table .

Table . Study 1046: Controlled clinical trial of nevirapine in anti-retroviral naive, HIV + subjects	
Locations	Canada, Australia, Netherlands, Italy
Total no.	152
Design	Randomized, double-blind
Population	HIV +, antiretroviral naive, CD4 200-600
Stratification	By country
Treatment groups	NVP + ddI + ZDV vs NVP + ZDV vs ddI + ZDV (1:1:1)
Primary endpoint	CD4 and HIV RNA at 16 and 28 weeks
Follow-up	28 weeks

4. Safety and efficacy in adults with nucleoside anti-retroviral therapy experience

4.1 Clinical Trial 1031 (ACTG 241)

A. Design The study is a placebo-controlled, randomized, double-blind study conducted at 16 US sites that evaluates the efficacy (CD4 and HIV viral load surrogate markers and preliminary data on clinical efficacy), safety, quality-of-life effects and pharmacokinetics of nevirapine, when used in combination with ddI + ZDV in HIV-positive subjects. Eligible subjects had more than 6 months of prior nucleoside therapy (monotherapy or in combination) and CD4 cell count ≤ 350/mm³. The primary surrogate endpoints were the changes in absolute CD4 count and in HIV-1 measures (virus titer in quantitative PBMC micro-cultures and decline in p24 antigenemia).

This study was initiated on 12 May 1993 and the study report includes data collected through 30 Jul 94. Surrogate endpoint analyses were conducted when the last patient randomized completed the 48 week evaluation.

B. Results: Study population and subject disposition

1. *Study Population:* A total of 400 patients were enrolled and 398 received study drug.

a. *Baseline characteristics*: These are summarized in Table :

Table . Baseline demographics, by study arm

	NVP + ddI + ZDV	PLA + ddI + ZDV
N	197	201
Age, mean (SD) (range)	39.2 (7.8) (16-61)	38.4 (8.6) (21-65)
Sex-male, N (%) female	163 (82.7) 34 (17.3)	156 (77.6) 45 (22.4)
Race-white, N (%) -black -Hispanic	147 (74.6) 19 (9.6) 28 (14.2)	148 (73.6) 21 (10.4) 30 (14.9)
CD4, mean (SD) median (range)	155.8 (109.5) 137.8 (2-443)	149.7 (109.3) 138 (1-416)
Prior nucleoside usage	N (%)	N (%)
<51 weeks	36 (18.3)	34 (16.9)
51-100	47 (23.9)	54 (26.9)
101-150	36 (18.3)	38 (18.9)
151-200	42 (21.3)	41 (21.4)
201-350	17 (8.6)	18 (9)
>350	19 (9.6)	16 (8)

Comment: *A subset of subjects (approximately 9%) had CD4 cell counts > 350. This raises the question as to how the baseline CD4 was established. According to the protocol, 3 determinations of CD4 cells, at least 72 hrs apart, were to be made before initiation of study treatment. Study eligibility was to be determined based solely on the first of these; the baseline CD4 value was established by averaging the second and third. This method is considered appropriate for such trials. The fact that a minority of subjects had CD4 counts > 350 seems unlikely to materially influence the outcome or interpretation of the study.*

b. *Stratification.* Subjects were stratified by CD4 count: CD4 = 201-350, 150 subjects; CD4 = 51-200, 150 subjects; CD4 ≤ 50, 100 subjects.

> Comment: *The numbers of subjects per study arm are similar by center and center subunit. (Study Table 2.3).*

2. *Disposition of subjects*

a. *Time on study medication and follow-up* is summarized in the following table:

Table . Time on study drug and time to last follow-up

Study arm	NVP + ddI + ZDV	PLA + ddI + ZDV
Exposure to NVP or PLA, mo. *Median (range)*	10.85 (0.26-13.41)	10.85 (0-12.66)
Exposure to ddI, mo. *Median (range)*	10.75 (0.26-13.41)	10.78 (0-12.62)
Exposure to ZDV, mo. *Median (range)*	10.82 (0.26-13.41)	10.82 (0-12.62)
Exposure to any study drug, mo. *Median (range)*	11.01 (0.26-13.41)	11.01 (0-12.98)

> Comment: *The study arms appear to be well balanced with respect to aggregate time of exposure to study drugs.*

A summary of subjects treated, taking nevirapine or placebo, participating in trial, and completing study is summarized in Table . (See Study Table 3.1)

Table . Subjects on NVP + ddI + ZDV vs PLA + ddI + ZDV, and participating in trial, by study arm and study week

No.Treated	At Wk:	4	8	12	16	20	24	28	32	36	40	44	48	Completed Study
NVP + ddI + ZDV	On NVP:	194	186	175	169	161	154	146	144	140	138	133	94	
n = 197	In Trial:	197	194	194	193	190	188	182	181	177	176	173	157	172 (87.3 %)
PLA + ddI + ZDV	On PLA:	194	184	180	173	164	160	160	156	149	147	136	96	
n = 201	In Trial:	199	195	192	188	184	181	179	178	176	175	170	156	169 (84.1%)

> Comment: *The Applicant was questioned on the relatively large decrease in subjects in the trial between weeks 44 and 48 (n = 30). It appears that the last scheduled study visit (Wk 48 visit) actually occurred during weeks 46 or 47 of the study, due to clinic and subject scheduling constraints for the large majority of the 30 subjects in question. Thus, these subjects completed the study in a normal fashion, and were not lost to follow-up. The two study arms remain balanced throughout the trial.*

b. *Study terminations* are summarized in the following Table:

Table . Study terminations, number, %, and cause , by treatment group

Treatment group	Terminations- No. (%)	Deaths	Refuses contact	Unable to contact
NVP + ddI + ZDV	25/197 (12.7%)	8	9	8
PLA + ddI + ZDV	32/201 (15.9%)	8	17	7

> Comment: *The treatment groups are similar, except that more placebo recipients refused contact than did NVP recipients. A review of the line listings failed to disclose a reason*

for this difference; NVP-associated toxicity (rash) was not disproportionately greater in NVP recipients who refused contact or could not be contacted.

c. *Study drug discontinuations* are summarized in the following table:

Table . Study drug discontinuations

Treatment group	No treated	No. Participating in trial (% of treated)			No. Taking all trial meds (% of treated)			No. taking ddI &/or ZDV (% of treated)			No. taking NVP/PLA (% of treated)		
Week		24	44	48	24	44	48	24	44	48	24	44	48
NVP +ddI + ZDV	197	188 (95.4)	173 (87.8)	157 (79.7)	152 (77.2)	126 (64.0)	90 (45.7)	154 (78.2)	133 (67.5)	95 (48.2)	154 (78.2)	133 (67.5)	94 (47.7)
PLA +ddI + ZDV	201	181 (90.0)	170 (84.6)	156 (77.6)	155 (77.1)	125 (62.2)	87 (43.3)	160 (79.6)	135 (67.2)	96 (47.8)	160 (79.6)	136 (67.7)	96 (47.8)

Comment: *Drug exposure to both NVP/PLA and to nucleoside remains balanced in both arms of the trial through Week 48. There is a pronounced fall-off of subjects participating in the trial and in those taking NVP/Pla and background nucleosides between weeks 44 and 48. However, this appears to relate to the timing of the last scheduled visit (see Time on Study Medication and Follow-up, above).*

Reasons for discontinuation of all study drugs are summarized in Table :

Table . Discontinuation of all study medication, by treatment group and by cause

	NVP + ddI + ZDV	PLA + ddI + ZDV
Received study medication	197	201
Permanently completed all study medication	129 (65.5%)	134 (66.7%)
Discontinuations due to :		
Death while on study drug(s)	4 (2%)	2 (1%)
Treatment toxicity, protocol-defined	8 (4.1)	5 (2.5)
Treatment toxicity, non-protocol-defined	14 (7.1)	16 (8)
Clinical endpoints, protocol-defined	0 (0)	0 (0)
Treatment with experimental drugs having antiviral activity	4 (2)	0(0)
Lost to follow-up	7(3.6)	7(3.5)
Subject's request	25(12.7)	33(16.4)
Investigator's request	0(0)	1(0)
Miscellaneous, protocol-defined	3(1.5)	1(0.5)
Other	1(0.5)	1(0.5)
Unknown	2(1)	1(0.5)

Comment: *A relatively high proportion of subjects completed this 48-week study. Fewer were lost to follow-up among nevirapine recipients than controls; this is not expected to materially influence the outcome or interpretation of the study.*

C. Results: Efficacy

1. *Applicant's analysis*

Primary endpoints defined by this ACTG study protocol are: (i) change in absolute CD4 count (all subjects); (ii) change in quantitative PBMC HIV-1 microculture titer (subset of subjects); (iii) decline in p24 antigenemia; (iv) the time to the occurrence of and AIDS-defining event or death.

Comment: *For accelerated approval, there has been more experience projecting clinical benefit of an experimental antiretroviral from the effect on absolute CD4 count than from effects on other surrogate endpoints, including measures of viral load. Further, CD4 data were collected for all subjects, whereas data on viral markers was collected on a subset of subjects. Therefore CD4 measures are given greater emphasis than viral load measures in the analysis of this study.*

a. *Change in CD4 count and %* are summarized in Tables :

Table . Change in immunologic markers from baseline to wks 40-48, by study treatment

Marker	NVP + ddI + ZDV	PLA + ddI + ZDV	Difference between groups (95% CI)	p-value
Change in absolute CD4 count (cells/mm³)				
LS Mean	5.81	-15.60	21.41 (8.0[illegible], 34.8)	.002
SE	5.04	4.88	6.83	
Median	-4.80	-17.14		
N	161	167		
Absolute CD4 count (AUCMB)*				
LS Mean	19.53	-0.33	19.86 (10.88,28.84)	<0.001
Standard Error	3.28	3.27	4.58	
Median	12.28	-3.08		
N	196	196		
% CD4 cells (AUCMB)*				
LS Mean	1.32	-0.21	1.53 (1.04, 2.02)	<0.001
Standard Error	0.18	0.18	0.25	
Median	0.99	-0.14		
N	196	196		

*AUCs include all subjects with post-treatment results

Comment: *The between-treatment difference in mean CD4 counts at 48 weeks is 21 cells, in favor of the nevirapine-containing study treatment; the p-value is significant and the 95% confidence intervals do not include zero. Analyses of other parameters produce similar results, and support this conclusion.*

The effect of therapy on changes in immunologic response by baseline CD4 stratum was also examined. The results at Week 40-48 are shown by CD4 stratum and treatment group in Table .

Table . Immunologic responses by CD4 stratum and treatment group

	CD4 350-201			CD4 51-200			CD4 ≤50		
Variable	NVP + ddI + ZDV	PLA + ddI + ZDV	Diff (95% CI)	NVP + ddI + ZDV	PLA + ddI + ZDV	Diff (95% CI)	NVP + ddI + ZDV	PLA + ddI + ZDV	Diff (95% CI)
Change, Abs.. CD4									
LS Mean	19.14	3.08	16.06	0.03	-39.59	39.62	-11.19	-5.31	-5.89
Standard Error	8.15	7.79	11.27	7.51	7.74	10.78	11.52	10.33	15.47
Median	2.38	11.97	(-6.03, 38.15)	2.59	-41.99	(18.49, 60.75)	-6.67	-5.05	(-36.21,24.43)
N	59	64		72	66		30	37	
% Change, Abs.CD4									
LS Mean	8.25	0.05	8.2	-3.06	-35.00	31.94	-22.36	-19.21	-3.15
Standard Error	5.86	5.60	8.1	5.40	5.56	7.75	8.28	7.42	11.12
Median	0.74	3.87	(-7.68, 24.08)	1.95	-40.88	(16.75,47.13)	-30.61	-35.63	(-24.95, 18.65)
N	59	64		72	66		30	37	

Change, %CD4									
LS Mean	1.19	-0.41	1.6	0.23	-2.75	2.98	-0.89	-.40	-0.49
Standard Error	0.48	0.45	0.66	0.44	0.45	0.63	0.67	0.60	0.9
Median	1.52	-0.41	(0.31, 2.89)	-0.04	-2.43	(1.75, 4.21)	-0.41	0.00	(-2.25, 1.270)
N	59	64		72	66		30	37	

Comment: *The treatment effect on CD4 measures by entry CD4 count was most clearly evident in subjects having CD4 51-200 at entry. The reasons for this are unclear.*

Other analyses conducted on the effect of study treatment on immunological markers, by baseline CD4 stratum and treatment group included absolute CD4 (AUCMB and NAUC), %CD4 cells (AUCMB).

b. *Viral endpoint responses* are summarized in Table .

Table . Change in surrogate virologic endpoints from baseline to weeks 40-48, by study regimen

Marker	NVP + ddI + ZDV	PLA + ddI + ZDV	Difference between groups (95% CI)	p-value
Change in log_{10}PBMC (IU/10^6 cells)				
LS Mean	-0.28	-0.02	-0.26 (-0.520, 0.002)	=0.052
Standard Error	0.10	0.09	0.13	
Median	-0.08	-0.07		
N	76	79		
Change in log_{10} HIV-RNA (copies/ml)				
LS Mean	-0.14	0.11	0.26 (-0.48, -0.04)	=.024
Standard Error	0.08	0.08	0.11	
Median	-0.07	0.17		
N	74	75		
log_{10} PBMC (AUCMB)				
LS Mean	-0.36	-0.15	-0.21 (-0.39, -0.03)	=0.026
Standard Error	0.07	0.07		
Median	-0.34	-0.06		
N	93	95		
log_{10} HIV-RNA (AUCMB)				
LS Mean	-0.43	-0.17	-0.26 (-0.44, -0.08)	=.003
Standard Error	0.06	0.06	0.09	
Median	-0.27	-0.07		
N	95	93		

* AUCs include all subjects with post-treatment results

Comment: *In these treatment groups, comparisons of mean measures of circulating HIV, including HIV-RNA (quantitative PCR) and viral infectivity (PBMC assay) support the interpretation, based on CD4 cell data, that NVP when used in combination with ddI + ZDV is superior to placebo.*

However, there are limitations to the virological data. The virology endpoints were performed on all the subjects at half the centers, chosen in advance. Since each center enrolled approximately 25 subjects, only about half of all study subjects were tested. Approximately equal numbers of subjects were tested in each of the treatment groups. Approximately three-fourths of subjects had evaluable virological data at 40-48 weeks. A further limitation of the virological data is that samples for HIV RNA determination using the PCR assay were tested as two batches, and that aliquots of the baseline sample that were tested in each batch were sufficiently different that the Applicant adjusted all values of one batch on the basis of this difference in baseline samples. For these reasons, the

virological data in this study are considered to be supportive.

c. *Subgroup analyses*

Effect of therapy by prior nucleoside therapy. A secondary analysis of immunologic and virologic markers by prior nucleoside therapy and by treatment group was conducted. Analyses of immunologic markers are summarized in Table .

Table . Immunologic response by prior nucleoside therapy and by treatment group

Variable	Prior ZDV only			Any prior ddI and/or ddC		
	NVP +ddI +ZDV	PLA +ddI +ZDV	Diff (95% CI)	NVP +ddI +ZDV	PLA +ddI +ZDV	Diff (95% CI)
Change, Abs., CD4						
LS Mean	30.67	3.31	27.36	-5.15	-27.15	22.00
Standard Error	11.05	11.25	14.31	5.12	4.90	6.96
Median	14.74	7.47	(-0.69, 55.41)	-7.51	-26.32	(8.36, 35.64)
N	54	52		107	115	
% Change, Abs.CD4						
LS Mean	8.57	-6.51	15.08	-7.23	-26.29	19.06
Standard Error	6.38	6.50	8.27	4.49	4.30	6.10
Median	13.01	3.87	(-1.13, 31.29)	-11.16	-28.50	(710, 31.02)
N	54	52		107	115	
Change, %CD4						
LS Mean	1.42	-0.39	1.82	-0.21	-2.05	1.84
Standard Error	0.65	0.67	0.85	0.31	0.29	0.41
Median	1.66	0.39	(0.15, 3.49)	-0.41	-1.25	(1.04, 2.64)
N	54	52		107	115	

The effect of study treatment on immunological responses, by prior nucleoside therapy (ZDV only), CD4 stratum, and study treatment are summarized in Table .

Table . Immunologic response, by CD4 stratum and study treatment, in subjects with prior nucleoside therapy of ZDV only

	CD4 201-350			CD4 51-200			CD4≤50		
	NVP +ddI +ZDV	PLA +ddI +ZDV	Diff (95% CI)	NVP +ddI +ZDV	PLA +ddI +ZDV	Diff (95% CI)	NVP +ddI +ZDV	PLA +ddI +ZDV	Diff (95% CI)
Change, Abs., CD4									
LS Mean	51.4	32.28	19.12	29.35	-32.44	61.79	-1.27	-14.87	13.61
Standard Error	15.16	15.17	21.44	16.93	20.93	26.92	28.26	31.71	42.48
Median	45.52	35.34	(-22.90, 61.14)	15.16	-43.62	(9.03, 114.55)	-11.51	-4.14	(-69.65, 96.87)
N	25	29		21	16		8	7	
%Change, Abs.CD4									
LS Mean	19.10	14.05	5.06	17.27	-29.15	46.41	-27.12	-13.60	-13.53
Standard Error	8.84	8.85	12.51	9.88	12.21	15.71	16.49	18.50	24.79
Median	17.60	14.73	(-19.46, 29.58)	13.93	-43.69	(15.62, 77.20)	-37.56	-33.05	(-62.12, 35.06)
N	25	29		21	16		8	7	

Change, %CD4									
LS Mean	2.64	1.23	1.41	2.1	-2.05	4.15	-2.37	0.22	-2.58
Standard Error	0.88	0.88	1.25	0.99	1.22	1.57	1.65	1.85	2.47
Median	2.61	1.17	(-1.04, 3.86)	2.74	-2.90	(1.07, 7.23)	-1.22	-0.41	(-7.42, 2.26)
N	25	29		21	16		8	7	

2. *FDA analysis*

The FDA analyses generally concurred with those of the applicant, and will not be reiterated.

D. Results: Safety

1. Applicant's Analysis

a. *Deaths* A total of 16 deaths are reported that were related to events with onsets while on study drug, or within 7 days of last study drug. One of these (ID# 181351) was ascribed by the investigator as possibly related to study treatment.

Table . Events leading to death having onsets while on study drug or within 7 days of last study drug, by treatment group							
NVP/ddI/ZDV				ddI/ZDV			
ID # (Age/Sex/Race)	Treatment Time to Event (da)	Time to Death (da)	Event	ID # (Age/Sex/Race)	Treatment Time to Event (da)	Time to Death (da)	Event

Comment: *Acute hepatitis (ID# 620212): Hepatitis B virus and hepatitis C virus infections were diagnosed in this subject, and hepatitis is presumed to be virus- induced, and not specifically related to study treatment. Myocardial infarction (ID#320634) and esophageal malignancy of unspecified type (ID#320197) are uncommon causes of death in patients with AIDS, but are presumed to be unrelated to study drug treatment. The cause of death in subject # 181351 is listed as a lymphoma, is unlikely to be nevirapine-related, but is consistent with lymphoma that is AIDS-associated. Other deaths appear to be of typical causation for patients with AIDS.*

A further 9 deaths occurred due to events having onsets more than 7 days after last study drug (on-study, off-drug):

Table . Events leading to death having onsets off study drug (>7 days after last study drug), by treatment group							
NVP/ddI/ZDV				ddI/ZDV			
ID # (Age/Sex/Race)	Treatment Time Pre-Event (da)	Time to Death (da)	Event	ID # (Age/Sex/Race)	Treatment Time Pre-Event (da)	Time to Death (da)	Event

Comment: These appear to be typical AIDS-related deaths.

d. *More severe adverse events*

More common adverse events ≥ Grade 3[2], regardless of causality are summarized in Table . Severe adverse events are defined as Grade 3, and life-threatening events are defined as Grade 4.

Table . Adverse events, Grade 3 or 4, occurring in ≥2% of subjects in either treatment group[3]		
	NVP/ddI/ZDV	ddI/ZDV
Rash[1], *N (%)*	16 (8.1)	3 (1.5%)
Granulocytopenia	12 (6.1)	10 (5)
Liver function test abnormalities	10 (5.1)	6 (3.0)
Nausea	9 (4.6)	7 (3.5)
Fever	6 (3.0)	5 (2.5)
Headache	5 (2.5)	5 (2.5)
Diarrhea	4 (2.0)	3 (1.5)
Vomiting	3 (1.5)	4 (2.0)
Abdominal pain	2 (1.0)	4 (2.0)
PCP	1 (0.5)	4 (2.0)

[1]Includes rash, rash maculo-papular, Stevens-Johnson syndrome, urticaria, rash erythematous, erythema multiforme and allergic reaction with rash

e. *Specific adverse events*

Rash

All rash and allergy events, regardless of grade or causality are summarized (from the Clinical Trial Report, Appendix 15.9.2.5) in Table :

Table . All rash and allergy			
	NVP/ddI/ZDV	PLA/ ddI/ZDV	Total
Allergic reaction	7 (3.6)	1 (0.5)	8 (2.0)
Allergic reaction with rash	3 (1.5)	1 (0.5)	4 (1.0)
Rash	64 (32.5)	48 (23.9)	112 (28.1)
Rash maculo-papular	12 (6.1)	4 (2.0)	16 (4.0)
Folliculitis	8 (4.1)	3 (1.5)	11 (2.8)
Pruritis	7 (3.6)	2 (1.0)	9 (2.3)

[2]Severe adverse events are defined as Grade 3, and life-threatening events are defined as Grade 4.

Skin disorder	4 (2.0)	7 (3.5)	11 (2.8)
Urticaria	4 (2.0)	2 (1.0)	6 (1.5)
Rash erythematous	3 (1.5)	0	3 (0.8)
Stevens Johnson syndrome	2 (1.0)	0	0 (0.5)
Dermatitis	1 (0.5)	0	1 (0.3)
Pruritis, genital	1 (0.5)	0	1 (0.3)

Grade 3 or 4 rash events of all types, having onsets while on study drug or within 7 days of its discontinuation are summarized in the following table:

Table . Subjects having rash, Grade 3 or 4, with onset while on study drug, by treatment group			
	NVP/ddI/ZDV	PLA/ ddI/ZDV	Total
Rash maculo-papular, *N (%)*	6 (3.0)	1 (0.5)	7 (1.8)
Rash	2 (1.0)	0	2 (0.2)
Stevens-Johnson Syndrome	2 (1.0)	0	2 (0.5)
Urticaria	2 (1.0)	1 (0.5)	3 (0.8)
Rash erythematous	1 (0.5)	0	1 (0.3)
Erythema multiforme	0	1 (0.5)	1 (0.3)

Comment: *It is clear in this controlled trial that treatment with NVP in combination with ddI + ZDV is associated with disproportionately more rash and rash-related allergic events than is control treatment with ddI + ZDV. This includes events classified as allergic reaction, allergic reaction with rash, rash, maculopapular rash, erythematous rash, Stevens-Johnson syndrome, pruritis, urticaria, and folliculitis. Further, severe and life-threatening rash (Grades 3 and 4) were more frequent in nevirapine-treated subjects than in controls.*

Liver function test abnormalities and Liver and Biliary System disorders

Subjects having Grade 3 or 4 liver function test elevations are summarized in Table . This includes 2 subjects (# 81846, #181405) noted to have Grade 4 Increased LFTs, and for whom it is assumed that SGPT, SGOT, GTT, Alk Phos, and TBili all reached Gr 3. Events are restricted to those occurring between Day 0 and 7 days following study drug discontinuation, or the Week 48 visit.

Table . Subjects having Grade 3 or 4 LFT elevations, by treatment group		
	NVP + ddI + ZDV	PLA + ddI + ZDV
GTT, *N*	9	5
Alk Phos	1	4
Total Bilirubin	2	4
SGPT	13	8
SGOT	10	9
GTT, only	8	3
Other LFT (with or without GTT,)	17	17

Subjects having Grade 3 or 4 liver function test elevations are summarized in the following table . This information was provided in response to reviewers' request that information similar to that provided in the NDA for Study 1037 be provided in a consistent manner for all studies.

Table . No of subjects with reported LFT elevations by type, grade, treatment group while on-treatment[1]									
NVP + ddI + ZDV (N =)					ddI + ZDV (N =)				
Grade		2	3	4	Grade		2	3	4
SGOT-on treatment	8	4	4	0	SGOT-on treatment	8	2	4	2
SGPT-on treatment	10	4	4	2	SGPT-on treatment	7	1	4	2
GTT-on treatment	8	3	4	1	GTT-on treatment	7	4	2	1
Total Bili-on treatment	1	0	0	1	Total Bili-on treatment	3	0	0	3

[1]Note: for Trial 1031 (ACTG 241), lab values were not routinely collected. Investigators were to report only Gr 3/4 abnormalities. Values reported by DAIDS were recalculated by BIPI.

Liver and biliary system disorders irrespective of causality or grade (from Clinical Trial Report Appendix 15.9.2.5), are summarized in Table :

Table . Subjects having liver and biliary system disorders, by treatment group			
	NVP + ddI + ZDV	ddI + ZDV	Total
Liver function tests abnormal numbers	10 (5.1)	7 (3.5)	17 (4.3)
Gamma-GT increased	3 (1.5)	0	3 (0.8)
Hepatitis	2 (1.0)	0	2 (0.5)
Cholecystitis	1 (0.5)	1 (0.5)	2 (0.5)
Cholelithiasis	1 (0.5)	2 (1.0)	3 (0.8)
Hepatomegaly	1 (0.5)	1 (0.5)	2 (0.5)
Jaundice	1 (0.5)	0	1 (0.3)

Comment: The available LFT data from Study 1031 is limited. With the exception of GGT, no excess of LFT elevations is noted in the NVP-containing study treatment group. The two cases of hepatitis were HBV-associated. There is no evident excess of liver disease in NVP recipients. No evidence of NVP-induced hepatitis was found in this study.

Musculoskeletal system disorders

Musculoskeletal system disorders irrespective of causality or grade (from Clinical Trial Report Appendix 15.9.2.5), are summarized in Table :

Table . Subjects having musculoskeletal system disorders			
	NVP + ddI + ZDV	ddI + ZDV	Total
Myalgia	7 (3.80	5 (2.5)	12 (3.0)
Arthralgia	4 (2.0)	3 (1.5)	7 (1.8)
Muscle weakness	3 (1.5)	0	3 (0.8)
Arthropathy	1 (0.5)	0	1 (0.3)
Arthrosis	1 (0.5)	0	1 (0.3)
Muscle malformation	1 (0.5)	0	1 (0.3)
Myositis	1 (0.5)	0	1 (0.3)
Skeletal pain	1 (0.5)	0	1 (0.3)
Arthritis aggravated	0	1 (0.5)	1 (0.3)

Comment: *More nevirapine recipients had an assortment of musculoskeletal system disorders than did controls, but examination of the specific disorders listed did not suggest*

a consistent underlying pattern of disease.

2. FDA analysis

Review of Case Report forms: A random selection of Case Report Forms were examined. In most instances these appeared to be appropriately filled out, were reasonably complete, with few corrections.

Deaths: The case report forms for all deaths were reviewed. There is no evidence of rash-associated deaths. Acute hepatitis (grade 2) developed in subject No. 620212 while on NVP + ddI + ZDV (treatment Day 223), and subsequently, subject was found to be HBV + and HCV +. Last study treatment was received on Day 281; death occurred on Day 477. Death was not ascribed to NVP by the investigators. In several instances, the case report forms did not include the form listing the cause of death. In these instances, the subject line listings were consulted, and it appeared that these subjects had ceased active participation in the study, but that information related to the cause of death had been pursued and in general the information supplied appeared to be reasonably complete.

Comment: *There was no evidence that NVP is the cause of death of any of these subjects.*

Grade 3 and 4 Rashes: Case report forms (n = 20) of Grade 3 and 4 rashes were reviewed. Of these, 17 were in recipients whose study treatment included NVP, while 3 were in placebo recipients. Of these 17 subjects who received NVP and developed Grade 3 or 4 rash, 13 appeared to be related to study treatment, as follows: 11/13 had onsets occurring before the Week 8 study visit (most before the Week 4 visit) and 2/13, while onsets occurred later in the study, recurred on NVP rechallenge. Three additional NVP recipients had late (post Week 8) rashes, in which there was neither a close temporal relationship with onset of study treatment, nor of rechallenge. One subject was described as having Gr 1 rash and dry mouth on several study visits, yet Stevens-Johnson syndrome was mentioned at one point in the case report form; it is unclear to the reviewer how this description justifies use of Stevens-Johnson syndrome and classification as a Grade 3/4 rash. In recipients of the NVP placebo, the 3 subjects with Grade 3/4 rashes likewise had rash onset prior to Week 8.

Comment: *The excess rash that is NVP-related is mostly occurring early (first few weeks) after initiation of study treatment. Rashes having onset after Week 8 may also relate to NVP treatment, as rash recurrence in several NVP-rechallenged subjects indicates.*

E. Conclusions

With 398 subjects, Study 1031 (ACTG 241) is the largest trial in this application. The study was well-balanced with respect to baseline demographic characteristics of the subjects and their prior nucleoside usage. Approximately 85% of subjects completed the 48-week trial, which is unusually high for a trial of this duration in this population. The study arms remained balanced throughout the 48-week period.

In this relatively advanced (median CD4, 138), rather extensively nucleoside-experienced (median prior nucleosides, 115 weeks) population, treatment with NVP in combination with ddI + ZDV, resulted in greater mean CD4 increases by approximately 30 cells at 20-28 weeks and by 20 cells at 40-48 weeks, when compared to ddI + ZDV. For NVP + ddI + ZDV, the CD4 cell increase was sustained above baseline for the 48-week trial duration.

For assay and for other virological measures, subjects at 8 of the 16 trial centers were assayed. Other limitations of the HIV RNA data included that samples were tested in two batches, and values in one batch were adjusted. The magnitude of the difference between mean changes in the trial arms was relatively small (approximately 0.3 $\log_{10}$). The limitations of this data

are discussed in Dr. Elashoff's review. If these limitations are ignored, the differences between treatment groups for mean HIV RNA changes are statistically significant, and in the NVP + ddI + ZDV treatment group, HIV-RNA remained below baseline for the 48 week duration of the trial. The other virological data, included HIV infectivity in an *in vitro* peripheral blood mononuclear cell assay, showed greater reduction in the NVP + ddI + ZDV group as well. Thus, despite their limitations, these data do support the CD4 data in showing that treatment with NVP + ddI + ZDV is superior to ddI + ZDV with respect to surrogate endpoint improvements in the population tested in this trial.

In examination of the safety information, none of the deaths were due to rash and none appeared to be otherwise related to study treatment. Rash was the primary nevirapine-associated toxicity that was evident, although liver function test abnormalities were more frequently observed in nevirapine recipients than in controls. Isolated GGT elevations were seen in particular, although a modest disproportion of transaminase elevations may have occurred in nevirapine recipients. More nevirapine recipients had an assortment of musculoskeletal system disorders than did controls, but examination of the specific disorders listed did not suggest a consistent underlying pattern of disease.

4.2 Clinical Trial 1037

A. Design This placebo-controlled, randomized, double-blind study was conducted at 6 US centers, to evaluate the efficacy (CD4 and HIV viral load surrogate markers), safety, and quality-of-life effects of nevirapine, when used in combination with ZDV in asymptomatic HIV-positive subjects. Eligible subjects had 200-500 CD4 cells and 3-24 months prior ZDV experience. Exclusion criteria included the diagnosis of AIDS, clinically important disease other than HIV-1 infection, and previous treatment with antiretrovirals other than ZDV.

The primary surrogate endpoints were the changes in CD4 count and in HIV-1 measures (HIV RNA by PCR and virus titer in PBMC microculture). Treatment effects were evaluated at 12-16 weeks and at 20-28 weeks. Changes in CD4 count and in HIV titer and RNA were summarized using area under the activity variable-treatment duration curves, adjusted for the pre-treatment value of the activity variable.
The study was initiated in Sept, 1993 and the study report includes data collected through July, 1995. Surrogate endpoint analyses were conducted when the last patient randomized completed the Week 28 evaluation.

B. Results: Study population and subject disposition

1. *Study population*: *Baseline characteristics* are summarized in Table .

Table . Baseline demographics, by study arm

	NVP + ZDV	ZDV
Age, mean (SD)	33.6 (6.4)	34.7 (8.4)
median (range)	32.5 (24-51)	33.5 (21-52)
Sex-male, N (%)	26 (87)	30 (100)
female	4 (13)	0 (0)
Race-white, N (%)	21 (70)	21 (70)
-black	7 (23)	6 (20)
-Hispanic	2 (7)	3 (10)
Yrs since seropositive, mean	1.9	2.2
median (range)	1.4 (0.2-4.7)	1.3 (0.5-8.8)
range, interquartile	2.4	1.4
HIV-1 risk factor, N (%)		
homosexual sex, male	19 (63)	24 (80)
IV drug user	4 (13)	4 (13)
sex partner is HIV-infected	7 (23)	2 (7)
transfusion	0	0
other	4 (13)	4 (13)
unidentified	1 (3)	1 (3)
CD4, mean	390	356
median (interquartile range)	398 (120)	342 (156)
min-max	211-616	158-543
CD4, %, mean	23	20
median (interquartile range)	24 (9)	20 (6)
min-max	9-35	12-38
HIV RNA (log 10), mean	4.3	4.1
median (interquartile range)	4.4 (0.5)	4.2 (1.2)
min-max	3.3-5.7	2.3-5.8
PBMC (log10), mean	1.0	1.1
median (interquartile range)	0.9 (1.0)	0.9 (0.9)
min	-0.4 - 3.1	-0.5 - 3.1

Prior nucleoside usage, *median, wks*	*28.5 wks*	*34.0 wks*
0-26 wks, N (%)	11 (37)	11 (37)
>26-52	9 (30)	14 (47)
>52-78	5 (17)	1 (3)
>78-104	3 (10)	4 (13)
>104	2 (7)	0

Comment: *The study arms appear well-balanced, except that a higher proportion of subjects in the NVP+ZDV arm (12/30 vs 5/30) had more than 1 year prior nucleoside therapy. This is unlikely to have had an impact on the outcome of the study.*

2. *Disposition of subjects*

Sixty subjects were randomized, 30 to each arm, and all received study medication. 54 (90%) completed the study.

a. *Study terminations, study drug discontinuations, time on study drug and follow-up*: — —

54 of 60 subjects (90%) completed the study. Reasons for termination are summarized in the following Table. Two subjects treated with NVP and ZDV had adverse events that were considered to be drug-related. Subject 2028 experienced Stevens-Johnson syndrome, discontinued nevirapine on Day 16, and was discontinued from the study on Day 57. Subject 2058 experienced hepatitis, discontinued nevirapine on day 27, and was discontinued from the study on Day 57.

Table . Study terminations by treatment group and cause, number (%)

Treatment group, *No*	NVP+ZDV, *30*	PLA+ZDV, *30*	Total, *60*
Protocol completion	26 (87)	28 (93)	54 (90)
Adverse event	2* (6.7)	0	2 (3.3)
Protocol violation	0	1 (3.3)	1 (1.7)
Lost to follow-up	2 (6.7)	1 (3.3)	3 (5)

Table . Time on study drug and time to last follow-up

Study arm	NVP+ZDV	PLA+ZDV	Total
Exposure to study drug, weeks. *Mean (range)*	*24.8 (2.3-29.3)*	*27.1 (15-30.1)*	*25.9 (2.3-30.1)*
No. with exposure (total)	(30)	(30)	(60)
>2-4 wks	3	0	3
>8-12	1	0	1
>12-16	0	1	1
>16-20	0	1	1
>20-24	1	0	1
>24-28	13	19	32
>28	12	9	21

Comment: *In the NVP+ZDV arm, there were 2 subjects with nevirapine-related toxicities (Stevens-Johnson syndrome, hepatitis) developing in the first 4 weeks of treatment, and for whom study treatment was discontinued. This accounts for the wider range of study drug exposures in this treatment group. However, the mean exposure to study treatment remains relatively similar in both study arms.*

C. **Results: Efficacy**

1. *Applicant's analysis*

Primary endpoints defined by the study protocol are: (i) change in CD4 count, and (ii) changes in HIV RNA by PCR and virus titer in PBMC microculture. Changes in CD4 count and in HIV titer and RNA were summarized using area under the activity variable-treatment duration curves, adjusted for the pre-treatment value of the activity variable.

a. *Change in CD4 count and %* are summarized in the following Table; all values except p-value rounded to nearest tenth:

Table . Change in immunologic markers from baseline to Wks 12-16 and Wks 20-28, by study treatment

Variable	Wks 12-16			Wks 20-28		
	NVP+ZDV	ZDV	p-value	NVP+ZDV	ZDV	p-value
CD4 change from baseline						
mean	44.3	-32.5	<.001	24.2	-28.5	0.009
median (IQ range)	52.9 (82.1)	-31.1 (78.2)		14.3 (89.6)	-30.7 (86.3)	
SD	74.7	61.2		76.0	65.1	
N	26	29		26	29	
%CD4 change from baseline						
mean	2.8	0.21	0.002	2.1	-0.5	0.003
median (IQ range)	2.5 (3.5)	0 (3.5)		2.2 (4.2)	-0.7 (3.5)	
SD	2.8	2.8		3.2	3.1	
N	26	29		26	29	

The median CD4 $AUCMB_{0\text{-}16wks}$ was 44 for NVP+ZDV and -11 for ZDV; the $AUCMB_{0\text{-}28wks}$ was 22 for NVP+ZDV and -25 for ZDV.

The median CD4 $NAUC_{0\text{-}16wks}$ was 1.11 for NVP+ZDV and 0.97 for ZDV; the $NAUC_{0\text{-}28wks}$ was 1.06 for NVP+ZDV and 0.93 for ZDV. For AUCMB and NAUC between-group comparisons for both time intervals, significant differences favoring NVP+ZDV ($p<0.001$) were reported by the applicant.

In a responder analysis, 22 NVP+ZDV-treated subjects were responders, *vs* 10 ZDV recipients.

> Comment: *The applicant's analysis is consistent in showing a nevirapine-related treatment benefit as regards CD4 measures. The maximum magnitude of this benefit over ZDV+placebo appears to be approximately 50 CD4 cells. The duration of this benefit appears to be for at least 28 weeks.*

b. *Viral markers* ($\log_{10}$ HIV RNA by PCR, $\log_{10}$ HIV titer by quantitative microculture) are summarized in the following Table; all values (except p-value) rounded to nearest hundredth.

Table . Change in virologic markers from baseline to Wks 12-16 and Wks 20-28, by study treatment

Variable	Wks 12-16			Wks 20-28		
	NVP+ZDV	ZDV	p-value	NVP+ZDV	ZDV	p-value
HIV RNA (log 10) change from baseline						
mean	-0.04	0.01	0.525	0.05	0.13	0.590
median (IQ range)	0.02 (0.53)	0.01 (0.35)		0.16 (0.39)	0.12 (0.31)	
SD	0.43	0.38		0.37	0.35	
N	26	29		26	29	

PBMC (log 10) change from baseline						
mean	-0.24	-0.13	0.913	-0.25	0.19	0.235
median (IQ range)	0.03 (1.22)	-0.06 (0.87)		-0.10 (0.95)	0.15 (1.09)	
SD	0.83	0.68		0.79	0.69	
N	26	29		26	29	

The applicant reports that while the median change from baseline in HIV RNA by PCR was not statistically significant at Wks 12-16 or 20-28, there were statistically significant between-group differences at 2, 4 and 8 weeks.

For HIV-1 RNA (log_{10}) in the [illegible] he median $AUCMB_{0-16wks}$ was -0.381 for NVP+ZDV recipients and -0.005 for ZDV recipients ($p<.001$); the median $AUCMB_{0-28wks}$ was -0.161 for NVP+ZDV and 0.040 ($p=.001$). In a responder analysis, 23 NVP+ZDV recipients were responders, *vs* 13 ZDV recipients.

For HIV-1 virus titers in microcultures, there was no statistically significant difference between treatment groups for AUCMB for either 0-16 or 0-28 week comparisons, or for responder analysis ~~between~~-group comparison for the 0-28 week interval.

ICD P24 antigen comparisons were restricted to those subjects who were p24 antigen-positive at baseline (N=20), although one subject had no subsequent data available. An examination of HIV-1-induced syncytial cell formation in an *in vitro* assay was also conducted.

> Comment: *The nevirapine-related effects on HIV-RNA (quantitative [illegible] and on HIV in a quantitative infectivity assay (PBMC test) at 16 and 28 weeks do not reach statistical significance by the applicant's analysis. The median HIV-RNA AUCMB for 0-16 and 0-28 weeks do reach statistical significance. In general, other analyses show no consistent nevirapine-related viral surrogate endpoint effect, or are best considered as exploratory analyses.*

D. Results: Safety

1. Applicant's Analysis

a. *Deaths.* There were no deaths reported during this study (Study 1037, Part I).

b. *Serious Adverse events* (fatal, immediately life threatening, permanently or severely disabling, or requires or prolongs hospitalization; includes congenital anomaly, cancer and overdose), regardless of causality, are summarized in Table .

Table . Serious adverse events, regardless of causality

	NVP+ZDV	ZDV	Total
Total treated	30	30	60
Total subjects with ≥1 serious event	6	2	8
Gastrointestinal	1 (3.3)	0	1 (1.7)
Dysphagia	1 (3.3)	0	1 (1.7)
Stomatitis ulcerative	1 (3.3)	0	1 (1.7)
Liver and biliary system	1 (3.3)	0	1 (1.7)
Hepatitis	1 (3.3)	0	1 (1.7)

Neoplasms	0	1 (3.3)	1 (1.7)
Brain neoplasm benign	0	1 (3.3)	1 (1.7)
Psychiatric Disorders	2 (6.7)	0	2 (3.3)
Depression aggravated	1 (3.3)	0	1 (1.7)
Depression psychotic	1 (3.3)	0	1 (1.7)
Resistance mechanism	1 (3.3)	0	1 (1.7)
Cellulitis	1 (3.3)	0	1 (1.7)
Respirator system	1 (3.3)	0	1 (1.7)
Sinusitis	1 (3.3)	0	1 (1.7)
Skin and appendages	2 (6.7)	0	2 (3.3)
Skin disorder	1 (3.3)	0	1 (1.7)
Stevens Johnson syndrome	1 (3.3)	0	1 (1.7)

f. *Severe Adverse Events, Specific*

Rash

All rash and allergy events, regardless of grade and causality are summarized (from the Clinical Trial Report, Table 13.11.1) in Table .

Table . Rash and selected other skin disorders, and allergy, regardless of grade or causality, by treatment group

	NVP + ZDV	ZDV	Total
Allergic reaction	1 (3.3)	0	1 (1.7)
Allergic reaction with rash	0	1 (3.3)	1 (1.7)
Allergy	1 (3.3)	1 (3.3)	2 (3.3)
Allergy aggravated	0	1 (3.3)	1 (1.7)
Rash	1 (3.3)	0	1 (1.7)
Rash maculo-papular	3 (10)	3 (10)	6 (10)
Pruritis	1 (3.3)	1 (3.3)	2 (3.3)
Urticaria	1 (3.3)	0	1 (1.7)
Rash erythematous	3 (10.0)	0	3 (5.0)
Stevens Johnson syndrome	1 (3.3)	0	1 (1.7)

Subjects having Grade 3 or 4 rash events of all types, having onsets while on study drug or within 7 days of its discontinuation are summarized by subject number in the following table:

Table Subjects having rash, severe (I presume Gr 3 or 4) with onset while on study drug, by treatment group

NVP + ZDV		ZDV	
Subject No	Event	Subject No	Event
2028	Stevens Johnson	None	
2042	Rash Skin disorder (cutaneous sarcoid) stomatitis ulcerative		

Note: The information summarized here is abstracted from CTR table 13.12.1, and is noted to be "severe adverse events", not specifically stated to be ≥Gr3

Liver function test abnormalities and Liver and Biliary System disorders

LFT elevations by type, pretreatment vs maximum on-treatment grade, and by treatment group are summarized in the following table (From CTR Appendix 15.9.2.5.3 Table 1):

Table . No of subjects with LFT elevations by type, grade, treatment group and on-treatment status

NVP + ZDV (N = 30)							ZDV (N = 30)						
Grade		<1	1	2	3	4	Grade		<1	1	2	3	4
SGOT	N						SGOT	N					
pretreatment	30	28	1	1	0	0	pretreatment	30	25	5	0	0	0
on treatment	30	22	5	2	0	1	on treatment	30	19	9	2	0	0
SGPT							SGPT						
pretreatment	30	25	3	2	0	0	pretreatment	30	21	5	4	0	0
on treatment	30	17	7	4	1	1	on treatment	30	21	3	3	3	0
GTT,							GTT,						
pretreatment	30	28	2	0	0	0	pretreatment	30	28	1	1	0	0
on treatment	30	16	6	5	2	1	on treatment	30	26	2	2	[illegible]	0
Total Bili							Total Bili						
pretreatment	30	28	2	0	0	0	pretreatment	30	30	0	0	0	0
on treatment	27	27	0	0	0	0	on treatment	30	26	4	0	0	0

Comment: With the possible exception of Grade 1 and 2 SGPT elevations, the NVP-related LFT elevations appear largely to be restricted to GTT,.

2. FDA analysis

a. *Deaths:* There were no deaths in this study.

b. *Toxicity:* Grade 3 and 4 rash: One subject treated with NVP + ZDV developed Stevens-Johnson syndrome, 2 days after NVP was increased to 400 mg/day.

E. Conclusions

With 60 subjects, Study 1037 is the smallest of the controlled double-blind clinical trials in this application that were designed to test for surrogate endpoint effects with nevirapine therapy, and is therefore viewed as a supportive study. The study was well-balanced with respect to baseline demographic characteristics of subjects between treatment groups, and relatively well balanced with respect to baseline ZDV usage. 90% of subjects completed this 28-week study, and the study arms remained balanced throughout the study period.

In this less-advanced (median CD4, 367 cells), ZDV-experienced population (31 weeks median prior ZDV), treatment with NVP in combination with ZDV, resulted in greater mean CD4 cell increases (approximately 50 cells at 28 weeks) than did treatment with ZDV alone. NVP + ZDV recipients returned to CD4 baseline between Weeks 24 and 28. In the comparison of virological effects of NVP + ZDV to ZDV alone, NVP recipients experienced an approximately 1.6 $\log_{10}$ mean decrease in viral RNA which was maximal at Week 2; HIV RNA returned to baseline between weeks 12 and 16, however. Nevirapine recipients experienced an approximately 0.4 $\log_{10}$ greater decrease in HIV RNA over the 28 weeks of the study, compared to controls.

In examination of the safety information, 2 nevirapine-treated subjects developed severe rash, including one who had Stevens-Johnson syndrome. With respect to LFT elevations, there were disproportionately more GGT elevations in nevirapine recipients than controls.

4.3 Clinical Trial 1011

This is an open-label trial which enrolled just 49 patients, only 69% of whom completed the trial. For these reasons this study is considered to be supportive and will be briefly summarized. It was placebo-controlled, randomized, and was conducted at 10 centers in the Netherlands, Italy and Australia. The purpose was to evaluate the efficacy (primary endpoint, change in HIV-1 ICD p24 antigen), safety, and tolerance of nevirapine, when used in combination with ZDV in HIV-infected adults. Eligible subjects had CD4 <500, p24 antigen ≥ 25 pg/ml, and had used ZDV ≥ 500 mg/day for at least 6 months. Exclusion criteria included clinically important disease other than HIV-1 infection, and selected HIV-associated active opportunistic infections, visceral Kaposi's sarcoma requiring treatment, and others. The primary surrogate endpoint was change in ICD p24 antigen at 12-16 and 20-28 weeks.

The 49 subjects who were enrolled were a median age of 32 years, predominantly white males, had been seropositive for about 5 years and had median CD4 counts = 202 and median prior nucleoside usage of 70 weeks. All received study drug. The median exposure to study drug was 5.5 months in both treatment groups. Thirty-four subjects (69%) completed the study. Those discontinuing the study included 9 (36%) of NVP + ZDV recipients and 6 (25%) of ZDV recipients. Twenty-eight percent of NVP recipients and 4% of controls discontinued the study because of adverse events.

Change in CD4 count and percent are summarized in Table :

Table . Change in immunologic markers from baseline to wks12-16 and 20-28, by study treatment

	Weeks 12-16			Weeks 20-28		
Marker	NVP + ZDV	PLA + ZDV	p value[1]	NVP + ZDV	PLA + ZDV	p value[1]
Change in abs. CD4 count (cells/mm³)						
Mean	8.15	-28.61	0.96	-32.27	-47.31	0.52
Median	-20.5	-11.0		-8.0	-10.25	
N	20	19		20	20	
%change in abs.. CD4 count (% of baseline)						
Mean	0.69	-0.65	0.18	0.26	-1.81	0.064
Median	0.28	-1.00		0.00	-1.215	
N	20	19		20	20	
Absolute CD4 count (AUCMB)						
Mean	20.54	-15.72	0.18	1.48	-26.56	0.34
Median	13.38	-8.28		3.69	-11.98	
N	20	20		20	20	

[1]Blocked Wilcoxon Rank Sum

Changes in viral surrogate endpoints are summarized in Table :

Table . Change in virologic markers from baseline to Wks 12-16 and Wks 20-28, by study treatment

Variable	Wks 12-16			Wks 20-28		
	NVP + ZDV	ZDV	p-value	NVP + ZDV	ZDV	p-value
log_{10} HIV RNA change from baseline						
mean	0.05	0.23	0.09	0.13	0.24	0.31
median	0.03	0.20		0.03	0.32	
N	19	19		16	10	
log_{10}HIV RNA (AUCMB)						
mean	-0.36	0.10	0.0000	-0.12	0.19	0.0002
median	-0.33	0.08		-0.15	0.14	
N	19	19		19	17	

ICD p24 antigen %change from baseline						
mean	-34.92	0.91	0.003	-30.31	0.05	0.014
median	-35.59	0.00		-30.52	-8.56	
N	19	19		20	20	

Comment: *The efficacy data provide only very limited evidence of a nevirapine effect. This may be because these subjects had had previous ZDV experience for a minimum of 6 months, so the effect was largely due to nevirapine, and was transient. This could be explained by rapid onset of nevirapine resistance. No data on NVP resistance was provided on subjects in this study report, however.*

Safety

There was one death during this trial. The subject was a 49 year old male who had progressive PML and swallowing disturbance, and died after receiving NVP+ZDV for 3 days.

The important adverse events regardless of causality and grade, by system and treatment group are summarized in Table :

Table . Selected adverse events, regardless of cause and grade, by system and treatment group

	NVP+ZDV	ZDV	Total
Subjects treated	25	24	49
Liver and biliary	3	0	3
Skin and appendages	17	9	26

Of adverse events involving skin, there were 9 subjects with rash among nevirapine recipients (36%), vs 2 in controls (8%). Five subjects developed Gr 3 or 4 rash. Of these, 4 were being treated with NVP, and rash began within 4 weeks of initiation of NVP. The other subject had received only ZDV.

LFT elevations by type, pretreatment vs maximum on-treatment grade, and by treatment group are summarized in Table :

Table . No of subjects with LFT elevations by type, grade, treatment group and on-treatment status

NVP+ZDV (N=25)							ZDV (N=24)						
Grade		<1	1	2	3	4	Grade		<1	1	2	3	4
SGOT	N						SGOT	N					
pretreatment	25	21	4	0	0	0	pretreatment	24	17	6	1	0	0
on treatment	25	15	8	1	1	0	on treatment	24	8	9	5	2	0
SGPT							SGPT						
pretreatment	25	21	2	2	0	0	pretreatment	24	12	9	2	1	0
on treatment	25	10	12	2	0	1	on treatment	24	10	7	4	3	0
GTT,							GTT,						
pretreatment	25	21	1	3	0	0	pretreatment	24	19	3	1	1	0
on treatment	25	5	10	6	2	2	on treatment	24	14	5	3	1	1
Total Bili							Total Bili						
pretreatment	25	25	0	0	0	0	pretreatment	24	24	0	0	0	0
on treatment	25	25	0	0	0	0	on treatment	24	20	4	0	0	0

Comment: *The single death appears to be unrelated to nevirapine treatment. Rash, including Grade 3 and 4 rash is related. LFT elevations do not appear to occur selectively in the NVP-containing treatment group.*

4.4 Clinical trials 744 (ACTG 164) and 834 (ACTG 168)

Trials 744 and 834 are early open-label dose escalation trials that did not include placebo controls. Data from these trials were used to determine the dose of nevirapine used in the surrogate endpoint trials 1031, 1037 and 1046, which would become the dose proposed for marketing.

In Study 744, nevirapine as monotherapy was tested in 52 ZDV-experienced adults, using 12.5, 50, 200 and 400 mg QD doses for 20 weeks. HIV-1 p24 responses were measured at 1-week intervals for the first 6 weeks, and at 2-week intervals for the remainer of the trial. At the 12.4, 50 and 200 mg QD dose levels, p24 responses were transient, returning to approximately baseline levels in 2 to 5 weeks. At the 400 mg QD dose level, p24 antigen levels decreased to about -75% of baseline by week 3 and the effect was sustained throughout the duration of the trial. indicating that doses below 400mg QD were unlikely to be useful.

In Study 834 compared 12.5, 50, 200, 400 and 600 mg NVP in combination with ZDV in 83 ZDV-experienced adults for 24 weeks, using p24 antigen as the endpoint. P24 antigen effects were relatively sustained for trial duration in those subjects who received the 400 and 600 mg NVP doses but had dissipated prior to 24 weeks in subjects receiving lower doses.

By this point, it had become apparent that rash was an important nevirapine-associated toxicity, and that rash, which was seen in approximately 10-15% of subjects receiving nevirapine doses of 200 mg QD or less, increased in frequency to about 60% in recipients of the 400 mg QD dose. A subset of patients received a 200mg nevirapine lead-in dose for 2 weeks, followed by 400mg or 600mg QD doses; about 20% and 30% of these, respectively, developed rash.

Conclusion: These studies provided evidence that a 200 mg QD lead-in for 2 weeks, followed by 400 gm QD would be the optimal dose for further studies, considering both the surrogate endpoint evidence and the data relating to nevirapine-associated toxicity. They provide evidence that nevirapine-associated rash is dose-related.

5. Safety and Efficacy in Anti-retroviral Naive Adults

5.1 Clinical Trial 1046

In view of the limited viral marker data available in Studies 1031, 1037 and 1011, the Applicant was asked at its NDA post-submission meeting with DAVDP representatives (18 Mar 96) if additional viral marker data was available that might be submitted to the NDA. In response, on 12 April 96 the Applicant submitted to the NDA a 6-month analysis of activity data from an international non-IND trial, Study 1046. The trial was not clinically complete until 15 May 96. Information from this analysis is summarized below.

A. Design

The study is a randomized, placebo controlled, double blind, multi-national trial comparing the immunologic and virologic effects of NVP+ddI+ ZDV *vs* ddI+ZDV *vs* NVP+ZDV in the treatment of antiretroviral naive HIV-1 infected subjects with CD 4200-600 and no AIDS-defining diseases. The trial was conducted in Canada, the Netherlands, Italy and Australia.

B. Results: Study population and subject disposition (Applicant's analysis)

1. *Study population: Baseline characteristics*

Table. Baseline demographic characteristics, by treatment group

	NVP+ddI+ZDV	ddI+ZDV	NVP+ZDV	p-value
Number	51	53	47	
Age, mean (SD)	38 (10.7)	36.4 (8.1)	37.8 (9.1)	
range	22-62	21-54	25-65	
Sex-male, N (%)	47 (92)	50 (94)	43 (91)	
female	4 (8)	3 (6)	4 (9)	
Race- white, N (%)	49 (96)	51 (96)	42 (90)	
-black	0	0	0	
-hispanic	0	0	1 (2)	
-asian	0	1 (2)	0	
-other	2 (4)	1 (2)	4 (8)	
HIV disease type				
-asymptomatic	50 (98)	51 (96)	45 (96)	
-ARC	1 (2)	2 (4)	2 (4)	
-AIDS	0	0	0	
Years seropositive				
-median, IQ range	1.7 (3.3)	1.5 (2.9)	1.1 (3.8)	
-min-max	0.1-10.0	0.2-9.7	0-10.2	
CD4 count				
-median, range	340 (200-683)	380 (145-755)	395 (185-610)	p=0.09
-interquartile range	140	151	188.5	
%CD4 (SD)	21.7 (7.3) (n=51)	21.6 (7.4) (n=52)	20.9 (5.8) (n=47)	p=0.80
log_{10} HIV RNA (SD)	4.24 (0.55) (n=51)	4.47 (0.63) (n=50)	4.52 (0.69)(n=47)	p=0.03
ICD p24 Ag (SD)	226 (151) (n=10)	152 (111) (n=13)	193 (176) (n=18)	p=0.93
β_2 microglobulin (SD)	2.49 (1.08) (n=48)	2.57 (1.02) (n=46)	2.81 (0.82) (n=43)	p=0.19

Baseline immunologic and virologic characteristics by country are summarized in Table:

Table . Baseline immunologic and virologic status by country

Mean (SD)	Australia	Canada	Italy	Netherlands	p-value

CD4 count, N	375 (118) N = 36	394 (120) N = 69	406 (108) N = 16	317 (113) N = 29	p = 0.02
% CD4	21.3 (6.4) N = 36	23.1 (7.0) N = 69	22.7 (4.3) N = 16	17.2 (6.5) N = 29)	p = .001
log_{10} HIV RNA	4.64 (0.64) N = 34	4.21 (0.60) N = 69)	4.38 (0.70) N = 16	4.62 (0.53) N = 29	p = 0.001
ICD p24 Ag	233 (171) N = 11	90 (42) N = 11	104 (74) N = 6	272 (161) N = 13	p = 0.02
β, microglobulin	2.30 (0.48)N = 35	2.25 (0.98) N = 59	3.61 (1.05) N = 16	3.26 (0.75) N = 27	p < 0.001

2. *Disposition of subjects* is summarized in Table :

Table . Disposition of subjects by treatment group

	NVP + ddI + ZDV	ddI + ZDV	NVP + ZDV
Number	51	53	47
Completed first 6 mo	41	38	28
Failed to complete due to:			
Adverse event	7	6	10
worsening of disease under study	0	1	0
worsening of previous disease	1	0	1
other adverse event	6	5	9
Lack of efficacy	0	0	0
Protocol violation	1	3	2
Lost to follow-up	1	4	2
Withdrawal	1	2	4
Other	0	0	1

a. *Time on study medication and follow-up*

b. *Study discontinuations*

Table . Study discontinuations by treatment group

	NVP/ddI/ZDV	ddI/ZDV	NVP/ZDV	Total
Total treated	51	53	47	151
Discontinued study, N (%)	10 (20)	15 (28)	19 (40)	44 (29)
Completed study, N (%)	41 (80)	38 (72)	28 (60)	107 (71)

c. *Study drug discontinuations* are summarized in Table :

d. *Protocol violations:* Fifty-one percent of subjects had at least one protocol violation. Nearly all were violations involving missed laboratory tests.

C. Results: Efficacy

1. *Applicant's analysis*

The applicant has provided analyses of CD4 cell counts and HIV-RNA levels at 16 and 28 weeks, using both DAVG and mean change from baseline at 16 or 20-28 week approaches. Treatment comparisons of each of the three treatment groups at these times were

a. *Change in CD4 cell count* of selected analyses and comparisons are summarized in Table :

Table . CD4 change from baseline by treatment group

	Change, DAVG, Weeks 0-28	Change at Week 20-28
NVP + ZDV	47 cells	22

ddI + ZDV	67	78
NVP + ddI + ZDV	87	113
p-balue by treatment comparison NVP + ddI + ZDV vs ddI + ZDV NVP + ddI + ZDV vs NVP + ZDV	 0.23 0.18	 0.02 0.18
N for analysis	150	116

b. *HIV RNA measures* of selected analyses and comparisons are summarized in Table :

Table. HIV RNA $\log_{10}$ copies /ml change from baseline, by treatment group		
	Change, DAVG, Weeks 0-28	Change at Week 20-28
NVP + ZDV	-0.85	-0.55
ddI + ZDV	-1.41	-1.43
NVP + ddI + ZDV	-1.63	-1.72
p-balue by treatment comparison NVP + ddI + ZDV vs ddI + ZDV NVP + ddI + ZDV vs NVP + ZDV	 0.12 0.0001	 0.10 0.0001
N for analysis	149	112

2. *FDA analysis*
a. *Conduct of the Study*
b. *Analyses Performed*

D. **Results: Safety**

1. Applicant's Analysis: The information provided on adverse events is limited to information provided in Amendments 10 and 14; the latter includes line listings of adverse events by center and subject.

a. *Deaths* There was one death during the first 6 months of the trial, a probable suicide. The case report form is not provided.

b. *Serious Adverse Events* In the line listings, one serious, non-rash was found:

Subject, #3415, developed hepatitis with Grade 4 liver enzyme elevations during NVP + ZDV treatment, resolving during nevirapine interruption, and recurring during nevirapine rechallenge.

c. *Rash* is summarized by treatment group, frequency, severity and timing in Table :

Table. Trial 1046: Summary of rash by treatment group			
Treatment group	NVP + ddI + ZDV (n = 51)	ddI + ZDV (n = 53)	NVP + ZDV (n = 47)
Patients with rash	14 (28%)	7 (13%)	16 (34%)
Severity Mild Moderate Severe	 11 1 2	 7 0 0	 10 4 2

Timing			
Days 1-14	9	2	8
Days 15-28	2	2	4
Days 29-56	1	2	1
After Day 56	2	1	3

d. *Liver function test abnormalities and Liver and Biliary System disorders*

LFT elevations by type, pretreatment vs maximum on-treatment grade, and by treatment group are summarized in the following table (From):

Table . No of subjects with LFT elevations by type, grade, treatment group and on-treatment status

NVP + ddI + ZDV (N = 51)							NVP + ddI (N = 47)							ddI + ZDV (N = 53)						
Grade		< 1	1	2	3	4	Grade		< 1	1	2	3	4	Grade		< 1	1	2	3	4
SGOT	N						SGOT							SGOT						
	51	43	7	1	0	0		47	39	6	1	0	1		53	44	8	1	0	0
pretreatment	51	35	11	3	1	1	pretreatment	47	25	12	4	2	4	pretreatment	52	42	8	1	0	1
on treatment							on treatment							on treatment						
SGPT							SGPT							SGPT						
	51	37	12	1	1	0		47	32	12	2	0	1		53	38	14	1	0	0
pretreatment	51	32	12	5	0	2	pretreatment	47	26	10	3	2	5	pretreatment	52	28	20	3	1	0
on treatment							on treatment							on treatment						
GTT,							GTT,							GTT,						
	51	43	8	0	0	0		47	40	4	3	0	0		52	47	5	0	0	0
pretreatment	51	27	16	5	1	2	pretreatment	47	19	14	7	4	3	pretreatment	51	44	6	0	1	0
on treatment							on treatment							on treatment						
Total Bili							Total Bili							Total Bili						
	51	50	1	0	0	0		47	43	3	1	0	0		53	51	1	1	0	0
pretreatment	51	44	6	0	0	1	pretreatment	47	38	6	1	1	1	pretreatment	52	43	7	1	1	0
on treatment							on treatment							on treatment						

E. Conclusions

This study enrolled 151 antiretroviral-naive subjects into three treatment groups, with approximately 50 subjects per group. The study was well-balanced with respect to baseline demographic characteristics of the subjects. The median baseline CD4 count and mean HIV RNA levels were lower in the NVP + ddI + ZDV group than in either of the other treatment groups. Overall only about 70% of subjects completed the study, with disproportionately more study discontinuations in the NVP + ZDV treatment group.

All treatment groups experienced a significant increase in CD4 cells from baseline.

When NVP + ddI + ZDV was compared to ddI + ZDV, differences in mean changes for CD4 and HIV RNA were not significant, raising the question of whether nevirapine adds anything to the ddI + ZDV combination. But the magnitude of the CD4 and HIV RNA effects were similar to those seen in nucleoside-experienced individuals in Study 1031. Study 1046 is only a little more than one-third the size of Study 1031, however, and thus sample size could explain the failure to find a difference between these two treatment groups.

When NVP + ddI + ZDV was compared to NVP + ZDV, significant differences in mean changes for CD4 and HIV RNA were found, which provides evidence that nevirapine combined with two nucleoside RT inhibitors is superior to nevirapine in combination with a single nucleoside.

The safety data is extremely limited. No nevirapine-associated deaths were seen. Rash frequency, onset, and duration were similar to that observed in other studies. The line listings note that one nevirapine recipient developed hepatitis and Grade 4 LFT elevations that subsided on drug interruption and that recurred on nevirapine rechallenge.

6. Studies in pediatric patients

The applicant has provided pharmacokinetic data in 9 pediatric patients, and safety data in 37 subjects.

> Comment: *This data is insufficient to support expansion of the indication to include pediatric patients.*

7. Studies in other special patient populations

Although nevirapine induces p450 enzymes in the liver and is extensively metabolized, no data has been provided in subjects having hepatic or renal failure.

> Comment: *Absence of data in these patient populations was not deemed to be of sufficient concern to withhold approval at this time. These studies will be requested as part of the Applicant's Phase IV commitments.*

8. Summary of Efficacy and Safety

Efficacy

Safety

DEATHS

A review of deaths which included examination of all the case report forms indicated that no deaths appeared to be due to nevirapine treatment. In trials 774, 834, 1011, 1037 and 1031, no severe rashes were noted to be present in subjects who died. The single subject who developed hepatitis and died developed evidence of acute hepatitis after 223 days on treatment (Trial 1031, NVP+ddI+ZDV treatment group) was subsequently found to be HBV+ and HCV+; hepatitis is not considered to relate to nevirapine treatment in this subject.

RASH

1. *Relationship of NVP dose to rash incidence.* Rash was first identified as a complication of NVP therapy in dose ranging trials (744 and 834). The relationship of NVP dose to rash incidence is summarized in Table .

Table . Relationship of NVP dose to rash incidence, Trials 744 and 834

Dose	Rash incidence
12.5 mg QD	14%
50 mg QD	15%
200 mg QD	10%
400 mg QD	59%

On the basis of cumulative evidence from Trials 744 and 834 and subsequently Trial 854, the applicant states that the incidence of rash was higher (32%) in 600 mg QD recipients than in 400 mg QD recipients (19%).

2. *Effect of 200 mg NVP lead-in on rash incidence.* In trial 854, extended prior exposure to NVP at lower doses followed by NVP, 400 mg QD resulted in fewer rashes (7% were NVP-related) and no temporal clustering. In preclinical studies, autoinduction of NVP was nearly complete after 2 weeks of treatment. For this reason, a 200 mg QD lead-in for 2 weeks was instituted, followed by NVP treatment at 400 mg QD. The modification of 400 mg QD NVP dosing to 200 mg BID was based on pharmacokinetic considerations, not rash

3. *Incidence of rash by grade in comparative trials.* The incidence of rash by severity in Trials 1011, 1031 and 1037 is summarized in the following Table .

Table . Incidence of rash in comparative trials by treatment group

	NVP, 200 mg lead-in, then 400 mg (N=252)	Control (N=255)
Subjects with rash, N (%)	94 (37)	51 (20)
Grade 1	50 (20)	34 (13)
Grade 2	25 (10)	14 (5)
Grade 3	14 (5.6)	2 (0.8)
Grade 4	5 (2.0)	1 (0.4)

4. *Temporal relationship of rash onset to initiation of NVP therapy.* The onset of rash by severity in Trials 1011, 1031 and 1037 is summarized in the following Table .

Table . Onset of rash following NVP therapy in comparative trials				
NVP recipients = 252, Controls = 255	Days 0-14	Days 15-28	Days 29-42	After Day 42
Subjects having rash, irrespective of grade *No. (%)*				
NVP 200 mg lead-in, then 400 mg (N = 94)	27 (10.7)	20 (7.9)	10 (4.0)	37 (14.7)
Control (N = 51)	17 (6.7)	8 (3.1)	5 (2.0)	21 (8.2)
Subjects having Grade 3 & 4 rash				
NVP 200 mg lead-in, then 400 mg (N = 19)	6	8	0	5
Control (N = 3)	2	0	0	1

5. *Rash outcome.* As noted above, there were no deaths resulting from rash

Severe rash. In the Integrated Safety Summary, (Table 20, Vol. 1.149, p. 663/126, ff), the applicant summarizes information on subjects experiencing Grade 3 and 4 rashes in all NVP clinical trials through 1 Aug 95. This includes subjects in all unblinded and blinded trials, and includes subjects first exposed to NVP when rolled over to an NVP-containing regimen following unblinding of a controlled trial. For trials remaining blinded as of 1 Aug 95, all rashes are considered to be NVP-related.

In this table, 84 subjects are listed with Grade 3 or 4 rashes. Of these, 78 are in adults and 6 are in children. Of these, 42 cases of rash are subjects whose study treatment remains blinded. These are summarized in Table

Table . Grade 3 and 4 rashes, all trials, through 1 August 95						
Subjects unblinded to treatment assignment				Subjects blinded to treatment assignment		
NA	Gr 2	Gr 3	Gr 4	NA	Gr 3	Gr 4
3/42	3/42	30/42	6/42	8/42	26/42	8/42

Hospitalizations. As a measure of the seriousness of these rash events, 23 of 84 subjects with Grade 3 and 4 rashes were hospitalized. Hospitalizations are summarized in Table .

Table . Grade 3 and 4 rashes with hospitalization, all trials, through 1 August 95						
Subjects unblinded to treatment assignment				Subjects blinded to treatment assignment		
NA	Gr 2	Gr 3	Gr 4	NA	Gr 3	Gr 4
0/3	0/3	7/30	2/6	0/8	8/26	4/8

In the 3 controlled trials submitted (1011, 1037, 1031) Grade 3 and 4 rashes, regardless of causality, occurring while on study drug or within 7 days of its discontinuation, are listed by trial number and treatment group in the following Table.

Table.								
Trial No / Treatmnt[1]	Case No	NVP dose	Rash Gr	Onset, Da	Rash Dur, Da	NVP/PLA Disc.	Rechall result	Description

[redacted]

[1]Study 1031: NVP/ddI/ZDV vs PLA/ddI/ZDV
Study 1037: NVP/ZDV vs PLA/ZDV
Study 1011: NVP/ZDV vs PLA/ZDV

9. Reviewer's assessment/conclusions

A. Risk-benefit assessment

Risk. The principle toxicity-associated risk of nevirapine therapy is rash. In controlled clinical trials, severe and life threatening (Grades 3 and 4) rashes, including Stevens-Johnson syndrome, occurred more frequently in nevirapine recipients than in controls (7.6% vs 1.2%). To date, none of the rashes observed have led to death although 27% of patients with Grade 3 or 4 rashes were hospitialized. Subjects developing Gr 3 and 4 rashes recovered following discontinuation of study medication. Since rash is a toxicity that would be expected to be recognized by the patient, if patients are instructed to seek medical attention for any rash and to discontinue nevirapine therapy if severe rash occurs, this would appear to provide a margin of safety in the use of this medication. Because the large majority of rashes observed were of mild or moderate degree and resolved during continued nevirapine treatment, these do not appear to require interruption or discontinuation of nevirapine.

The other nevirapine-associated adverse event of note is liver function test abnormalities. Isolated GGT elevation was disproportionately more frequent in nevirapine recipients than in controls, occurring in 6.3% vs 1.6%. GGT elevation may relate to the auto-induction of liver enzymes by nevirapine. Transaminase elevations (particularly SGPT) also were observed more frequently in nevirapine recipients than in controls (9% vs 5%). However, the evidence to date from controlled trials does not indicate an increased number of hepatitis cases in nevirapine treatment groups when compared to controls.

A further risk of nevirapine relates to the development of resistance. It is clear that NVP as monotherapy and NVP in combination with ZDV leads to rapid emergence of resistant HIV-1 isolates (1-12 weeks). Resistance develops more rapidly than has generally been seen for other antiretrovirals. Preliminary data in two antiretroviral-naive patients treated with NVP in combination with ddI + ZDV raise the possibility that NVPin combination with antiretrovirals may delay the emergence of NVP-resistant strains, but more data will be required to evaluate this possibility.

Benefit. The chief therapeutic effect demonstrated thus far to be associated with nevirapine therapy is a surrogate endpoint effect, with increased CD4 cell counts and reduced viral measures, including plasma HIV RNA levels, in nevirapine recipients.

In nucleoside-experienced patients, nevirapine in combination with ddI and ZDV, when compared to ddI + ZDV alone, showed a mean CD4 advantage of approximately 30 cells at 28 weeks and of 20 cells at 48 weeks, with differences that were statistically significant. The virological data, including plasma HIV RNA levels determined by the while having limitations, likewise showed a benefit in favor of nevirapine. These surrogate endpoint effects were shown to be sustained for the entire trial duration of 48 weeks, considerably longer than has typically been demonstrated for other antiretrovirals for treatment of HIV.

Another study in ZDV-experienced subjects also showed mean CD4 cell and HIV RNA surrogate endpoint differences in favor of nevirapine when NVP + ZDV was compared to ZDV alone; these differences were statistically significant. These NVP-associated surrogate endpoint effects, however, were sustained for a shorter duration than was observed observed in the three-drug combination of NVP + ddI + ZDV.

In nucleoside-naive subjects, all treatment groups (NVP + ddI + ZDV vs ddI + ZDV vs NVP + ZDV) experienced a significant increase in CD4 cells from baseline; however, the comparison of mean CD4 and HIV-RNA effects between NVP in combination with ddI/ZDV to ddI/ZDV did not reach statistical significance But the magnitude of the CD4 and HIV RNA effects were similar to those seen for this treatment comparison in nucleoside-experienced subjects, which was statistically significant. Since the study in nucleoside-naive subjects had one-fourth the number of subjects per treatment group compared

to the trial in nucleoside-experienced individuals (approximately 50 vs 200), this could explain the failure to detect statistical significance in this comparison in nucleoside-naive patients.

In conclusion, treatment with nevirapine in combination with ddI and ZDV, when compared to ddI and ZDV, appears to confer a surrogate endpoint advantage (by CD4 and virological measures) in nucleoside-experienced, and probably also nucleoside-naive individuals. By analogy with information from clinical endpoint studies of other antiretrovirals in HIV-infected subjects, one would expect that the nevirapine-associated surrogate endpoint effect to translate into a clinical benefit, when appropriately designed and successfully conducted clinical endpoint trials are completed and analysed. The risk associated with rash, on the other hand, while not insignificant, appears to be acceptable, as does LFT elevations. Resistance to NVP is of concern, but is also a concern for other antiretrovirals. For this reason, one may conclude that the risk-benefit comparison for nevirapine favors its approval for marketing.

B. Therapeutic use.

The rapid emergence of NVP-resistant HIV isolates, particularly when NVP is used in monotherapy, but also when used in combination with ZDV in ZDV-experienced individuals, argues that NVP should not be used as monotherapy , and that when NVP is used in combination with ZDV in ZDV-experienced patients, the duration of a demonstrable treatment effect may also be quite limited. Based on the limited information available in the trials conducted to date, it would appear that NVP may be most effective when used in combination with 2 nucleosides, particularly when used in nucleoside-naive individuals or, in nucleoside-experienced patients, when NVP treatment is instituted in combination with at least one new nucleoside.

10. Recommended regulatory action

This application, NDA20-636, for nevirapine, 200 mg BID, in combination with nucleoside analogues for the treatment of adults with HIV-1 infection who have experienced clinical and/or immunologic disease progression, is recommended for approval.

John R. Martin
Medical Officer

concurrences:

HFD-530/DivDir/ DFeigal
HFD-530/TL/RBehrman

cc: NDA
HFD-530
HFD-530/DivDir/ DFeigal
HFD-530/TL/RBehrman
HFD-530/CSO/AZeccola
HFD-530/Chem/SMiller
HFD-530/Micro/WDempsey
HFD-530/Biopharm/CSahajwalla
HFD-530/PharmTox/PVerma
HFD-530/Stats/MElashoff
HFD-530/MO/JMartin

Appendix 1. Labelling Discussion

A series of labelling discussions were conducted both preceeding and following the Advisory Committee meeting on June 7, 1996. The major parts of the initial draft label that were modified based on these discussions included the indication, inclusion of a black box warning, selection of the clinical trial information that appropriately represented the key surrogate endpoint effects observed in the major clinical trials, an appropriate representation of the safety information, and an appropriate representation of the clinically relevant HIV resistance information.

The indication as originally proposed was to restrict nevirapine use to treatment of patients with advanced HIV-1 infection whose current antiretroviral therapy is no longer deemed adequate. Based on efficacy data in nucleoside-naive population subjects submitted during the review, the indication was expanded to a broader patient population, including those who have experienced clinical and/or immunological deterioration.

A black box warning was added to the draft label to emphasize that: (i) the indication is based solely on surrogate endpoint data, (ii) the duration of benefit may be limited and that alternate therapy should be considered if disease progression occurs (iii) because viral resistance emerges rapidly with nevirapine therapy, combination therapy should be used, and (iv) that nevirapine causes rash which can be life threatening, and that nevirapine must be discontinued if rash is severe.

Graphical representation of surrogate endpoint efficacy data was restricted to the Studies 1031 and 1037, in which efficacy endpoints showed significant mean differences between nevirapine-containing treatment and control. Because of the failure for mean surrogate endpoint data in Study 1046 to show a statistically significant difference between nevirapine in combination with ddI + ZDV vs ddI + ZDV , the description of this information was limited to text.

Representation of the safety data was modified to emphasize data from controlled trials, particularly rash.

Resistance information was modified to emphasize the available clinical data, including the settings in which nevirapine-resistant virus has been observed to emerge, including its rapid emergence in patients treated with nevirapine monotherapy.

Appendix 2. Phase IV

In the Phase IV (post-marketing) stage of nevirapine development, the applicant is requested to provide commitments as follows:

Clinical endpoint trials:

Other studies:

DRAFT

Statistical Review and Evaluation

NDA# : 20-636

APPLICANT: Boehringer Ingelheim Pharmaceuticals, Inc.

NAME OF DRUG: Viramune® (nevirapine)

INDICATION: Treatment of patients with advanced HIV-1 infection whose current antiretroviral therapy is no longer deemed adequate.

DOCUMENTS REVIEWED: Volumes 2.1, 2.99–2.154; Amendments 9, 10, 13, 15, 18. 20, 21.

MEDICAL INPUT: John Martin, M.D.

1 Summary

Nevirapine belongs to a new class of antiretroviral agents called non-nucleoside reverse transcriptase inhibitors. Three studies were submitted under accelerated approval to support the claim that the addition of nevirapine to one or more nucleoside drugs provides an improvement in surrogate markers for HIV disease. Two of the studies were conducted in a nucleoside experienced population, and one in a nucleoside naive population. One study had results out to 48 weeks with a high degree of followup, longer than has been seen in recent applications. Together, the studies provide support for the applicant's claim. The addition of nevirapine to the combination of ZDV and ddI increased subjects' CD4 count by approximately 20-30 cells and decreased subjects' RNA levels by approximately .2-.3 log copies. These effects appeared to be durable out to the end of the studies. The data suggest that nevirapine is more effective in the context of a three drug regimen, where at least one of the other drugs is one to which the patient is naive. Further, as will be discussed, the HIV-RNA results should be viewed with caution.

2 Study Designs

The NDA submission contained results from two randomized, blinded clinical trials, BI1031 (ACTG 241) and BI1037. Additionally, the applicant submitted preliminary results from study BI1046. These studies were designed to assess the impact of adding nevirapine to one or more nucleosides through an analysis of surrogate marker changes. This section summarizes the relevant aspects of the study designs for each of these three trials.

2.1 Study 1037

Study 1037 was a randomized, double-blind, placebo-controlled trial that compared ZDV/NVP to ZDV monotherapy. Nevirapine was given as 200 mg once daily for the first two weeks, increasing to 200 mg bid after that lead-in period. Subjects must have had between 3 and 24 months prior ZDV therapy, and between 200 and 500 CD4 cells at screening. Subjects were randomized by center, using permuted blocks of size 4. Sixty subjects were to be randomized. Subjects were to be followed for 28 weeks after the start of therapy, with visits scheduled for 2, 4, 8, 12, 16, 20, 24, and 28 weeks after the start of therapy.

2.2 Study 1031 (ACTG 241)

Study 1031 was a randomized, double-blind, placebo-controlled trial that compared ZDV/DDI/NVP to ZDV/DDI. The dose schedule was the same as for study 1037. Subjects must have had greater than 6 months of prior nucleoside (ZDV, ddI, or ddC) experience, and less than 350 CD4 cells at screening. Subjects were stratified by their screening CD4 cell count (0-50, 51-200, 201-350), with the goal of 100 subjects in the lower CD4 strata and 150 subjects in each of the other two strata. Sixteen ACTG centers participated, and randomization was carried out within each ACTG center. Eight of the sixteen centers were designated to participate in the virology substudy, in which virologic marker data including HIV-RNA would be collected in addition to the other surrogate markers. A total of 400 subjects were to be randomized, of which 200 would be in the virology substudy. Subjects were to be followed for 48 weeks after the start of therapy, with visits scheduled for 2, 4, 8, 16, 24, 32, 40, 44, and 48 weeks after the start of therapy.

2.3 Study 1046

Study 1046 was a randomized, double-blind, placebo-controlled trial that compared ZDV/DDI/NVP to ZDV/DDI to ZDV/NVP. The dose escalation schedule for nevirapine was the same as that in the other two trials. The study was conducted in Australia, Canada, Italy, and the Netherlands. Patients were to be initially nucleoside naive, with between 200 and 600 CD4 cells at screening. One hundred twenty patients were to be randomized equally among the three treatment arms. Randomization was done within country using permuted blocks of size 6. Subjects were to be followed for 52 weeks after the start of therapy, with visits scheduled for weeks 1, 2, 4, 8, and every 4 weeks following.

2.4 Planned Analyses

For each study, the primary efficacy variables were change from baseline CD4 cell count and HIV-RNA level in an eight week window of time at the end of the studies. Since the applicant's terminology for the various pre-treatment measurements used in the calculation of baseline was used quite inconsistently, a redefinition of terms is necessary. For the purposes of this review, the screening value is the value used to determine eligibility for the studies. The entry value is the value at the start of therapy, and a pre-entry value is any other value. Table 1 illustrates which values were to be collected for CD4 cell count and HIV-RNA level. Note that RNA values were

Table 1: Pre-Treatment Schedule

Study	Marker	Screening	Pre-Entry	Entry
All	CD4	X	X	X
1046	RNA		X	X
1031	RNA Batch 1		X	X
	RNA Batch 2			X
1037	RNA Batch 1		X	X
	RNA Batch 2		X	

not used for eligibility in any trial, although they may have been collected at the same time as screening CD4 counts. Also, in trial 1031 and 1037, RNA was sent to the lab in two batches.

For both markers, the log transform was used, and the *baseline* value was defined as the mean of the entry value and the latest pre-entry value. For RNA, only batch 1 values were used, and if either the entry or pre-entry value was missing, the other was used by itself. For CD4, if one of the values was missing, the screening value was substituted for that value and then the mean was calculated. The *final* value of a marker was defined as the mean of all values obtained during the eight week interval of interest. Analysis was to be based on linear models incorporating treatment and center, using log-transformed marker values. Of specific interest in 1046 were the two comparisons of the nevirapine containing arms to the ZDV/DDI arm. Clinical endpoints were to be analyzed using the logrank test. There were no interim efficacy analyses.

3 BIPI Analysis

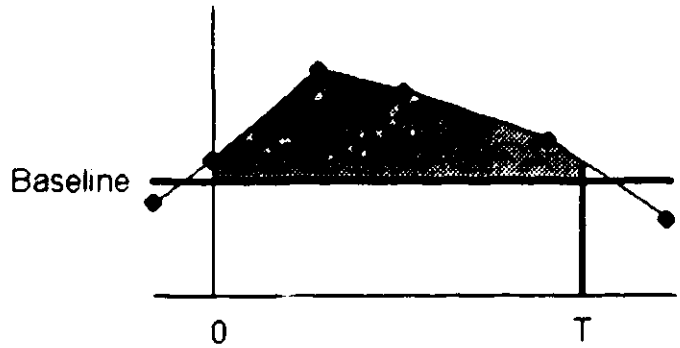
This section summarizes the analyses submitted by BIPI. I will focus on the CD4 and HIV-RNA results from the three trials discussed in the previous section.

3.1 Study 1031

3.1.1 Definition of Endpoints

There were several differences between the protocol-specified analysis (the ACTG analysis) and the applicant's analysis. First, the applicant used untransformed CD4 counts instead of log transformed counts. Also, the applicant reclassified 20 patients' CD4 stratum based on their baseline CD4, instead of using the assigned CD4 stratum based on the screening value. Baseline CD4 was calculated as the geometric mean of the entry value and the pre-entry value. Final CD4 was the geometric mean of all observations within the 8 week window of time at the end of the study (weeks 40, 44, and 48). If no observations were in the window, the subject was treated as missing. HIV-RNA was treated as detailed above, with the following exceptions. Week 44 data was only used when either week 40 or 48 was missing (week 44 data was only measured in 30 subjects, of which 6 were used). Also, several subjects were classified as having "uninterpretable" RNA data if the Batch 1 and Batch 2 values were "very different." The applicant did not provide a definition of "very

Figure 1: AUCMB Calculation



different." These subjects were excluded from the applicant's analysis. For each of the analysis modifications, the applicant provided an analysis using the protocol-specified method. Very little difference was observed in either the estimated treatment effects or their p-values. Thus only the applicant's primary analysis is presented in this section.

Figure 1 illustrates the computation of the Area Under The Curve Minus Baseline (AUCMB) for time T. Data points are connected by lines, and the area of the shaded region is calculated. Recall that the baseline was the mean of the pre-entry and entry (time 0) values. This area is then divided by T to yield the AUCMB. When a subject had dropped out prior to time T, the AUCMB was calculated only up to the dropout time. When the entry value was missing, the applicant set it equal to the value at pre-entry.

3.1.2 Main Results

Four hundred subjects were randomized, 197 to triple therapy and 203 to ZDV/DDI. Two subjects randomized to ZDV/DDI did not receive study drug and had no followup data, and were excluded from the analysis. Four other subjects received incorrect study medication, and were analyzed according to the medication they received. The result of these adjustments was that the analysis contained 197 subjects in the triple therapy arm and 201 in the ZDV/DDI arm. Of these, 99 in each arm were enrolled in the virology substudy.

Subjects in study 1031 were primarily male (80%) and white (74%), and averaged 39 years of age. The median duration of prior nucleoside therapy was 115 weeks, and the median CD4 cell count was 138 cells. No significant differences were observed between the treatment groups in any of the baseline variables. No differences were observed between subjects in the virology subgroup and subjects not in the virology subgroup.

Of the 197 subjects in the triple therapy arm and 201 subjects in the ZDV/DDI arm, 172 and 169 completed the trial, respectively. The dropout rate was fairly constant throughout the trial, with between 2 and 8 subjects dropping out each month.

Analysis of variance (ANOVA) models, including treatment, baseline CD4 strata, and center (plus treatment/CD4 strata and treatment/center interactions) were used to assess the treatment effect. The estimated treatment effect was a weighted average of the effects over the CD4 strata and

Table 2: Trial 1031: Results of BIPI Analysis

Endpoint	Metric	N	ZDV/DDI/NVP	ZDV/DDI	p-value
CD4	Mean Change Week 20-28	328	26	-5	.001
	Mean Change Week 40-48	328	6	-16	.002
	AUCMB Week 28	392	23	6	.001
	AUCMB Week 48	392	20	0	.001
RNA	Mean Change Week 20-28	155	-.27	-.08	137
	Mean Change Week 40-48	149	-.14	.11	.024
	AUCMB Week 28	188	-.57	-.27	.001
	AUCMB Week 48	188	-.43	-.17	.003

Table 3: Trial 1031: Week 40-48 CD4 Treatment Effect by Subgroup

CD4 Strata	ZDV Only	Any ddI or ddC	Total
≤ 50	14	2	-6
51-200	62	35	40
201-350	19	20	16
Total	27	22	21

centers, using weights proportional to the sample sizes (SAS: PROC GLM with Type II sums of squares). The results for trial 1031 are shown in Table 2. For both 24 and 48 weeks, and for both the mean change from baseline and the AUCMB, the estimated CD4 treatment effect was about 20 cells, and was statistically significant. With the exception of the mean change from baseline at 24 weeks, triple therapy also resulted in a significantly greater reduction in HIV-RNA levels than did double therapy.

3.1.3 Subgroup Analyses

The estimated treatment effects across the three CD4 strata and the type of prior nucleoside experience are shown in Table 3. This table contains the week 40-48 CD4 estimated treatment effects. The greatest treatment effect was seen in the middle CD4 strata for both types of prior nucleoside experience. The treatment effect was generally seen to be greater in the ZDV only subgroup.

3.1.4 Clinical Results

Although trial 1031 was designed primarily to assess surrogate marker changes, the clinical endpoint data can be analyzed as well. Thirty two subjects in the triple therapy arm and 29 subjects in the ZDV/DDI arm experienced clinical progression and/or death. This difference was not found to be

significant ($p = .71$) using the logrank test. A significant treatment by prior nucleoside use (less than 2 years vs. greater than 2 years) was observed. In the less than 2 year category the triple therapy had fewer clinical endpoints, with the reverse in the greater than 2 years category.

The applicant also defined criteria for being a responder. A CD4 responder is defined as a patient with CD4 greater than baseline, and an RNA responder is defined as a patient with RNA below baseline. At 24 weeks, the triple therapy arm had more CD4 responders (33% vs. 21%) and more RNA responders (44% vs. 37%). By 48 weeks, these percentages had dropped to 15% vs. 8% for CD4 response and 30% vs. 24% for RNA response. The differences in the CD4 response rates were significant.

In addition, the applicant examined the time to the first of two consecutive values below/above baseline for CD4/RNA. The median time for the CD4 cell counts to fall below baseline was 32 weeks in the triple therapy arm and 17 weeks in the ZDV/DDI arm. The median time for the RNA values to go above baseline was 41 weeks in the triple therapy arm and 32 weeks in the ZDV/DDI arm.

3.1.5 Conclusions

The applicant made the following conclusions:

Over a 48 week treatment period, combination therapy with NVP/ZDV/DDI was more effective than therapy with ZDV/DDI in decreasing HIV load and improving immune markers in patients with advanced HIV infection (CD4 cell counts <350 cells/mm^3) and extensive prior nucleoside therapy. Greater proportions of NVP/ZDV/ddI recipients were CD4 cell count and HIV-RNA responders as compared to the ZDV/ddI treatment group.

3.2 Study 1037

3.2.1 Endpoints

The endpoints were defined in the same manner as described for study 1031, with the exception that the baseline value was substituted for the entry value in the AUCMB calculations.

3.2.2 Main Results

Subjects in study 1037 were primarily male (85%) and white (70%), and averaged 34 years old. Subjects in the ZDV/NVP arm had a median duration of prior nucleoside therapy of 28 weeks, compared to 34 weeks for subjects in the ZDV arm. Subjects in the ZDV/NVP arm had a somewhat higher CD4 cell count (median 398 cells vs. 342 cells) and HIV-RNA value (median 4.4 log copies vs. 4.2 log copies). However, there were no significant differences between the two treatment groups with respect to any demographic or baseline variable.

ANOVA models (using Type II sums of squares as in study 1031) were used to analyze the CD4 and RNA data. The ANOVA models included treatment and "virtual site". The virtual sites resulted

Table 4: Trial 1037: Results of BIPI Analysis

Endpoint	Metric	N	ZDV/NVP	ZDV	p-value
CD4	Mean Change Week 12-16	55	53	-31	.001
	Mean Change Week 20-28	55	14	-31	.009
	AUCMB Week 16	60	44	-11	.001
	AUCMB Week 28	60	22	-24	.001
RNA	Mean Change Week 12-16	55	.03	.01	.525
	Mean Change Week 20-28	55	.16	.12	.590
	AUCMB Week 16	60	-.38	-.01	.001
	AUCMB Week 28	60	-.16	.04	.001

from the grouping of the 6 sites into 3 pairs of approximately equal sample size. The applicant provided an analysis using actual site and found little difference between the two.

Table 4 shows the results for trial 1037. Nearly all randomized subjects were followed for the full 28 weeks and had values in both time windows (26/30 in the ZDV/NVP arm and 28/30 in the ZDV arm). Both the AUCMB and the mean change for CD4 were significant, while for RNA only the AUCMB was significant. This was due to the rapid diminishing of the initial RNA difference by 8-12 weeks.

3.2.3 Conclusions

The applicant made the following conclusions:

NVP+ZDV was more effective than continued ZDV therapy in decreasing viral load and improving immune parameters in HIV-1 infected patients with CD4 cells between 200 and 500 cells/mm^3. Although differences between treatment groups for HIV-RNA PCR were lost by weeks 12-16, HIV-RNA PCR decreased initially and AUC measurements for HIV-RNA PCR were significantly better for the NVP+ZDV group. CD4 cells were significantly improved in the NVP+ZDV group by week 2 and remained significantly improved through week 20. Virologically and immunologically, patients benefited from adding NVP to their ZDV for six months.

3.3 Study 1046

A preliminary analysis of trial 1046 was submitted by the applicant after the submission of the NDA. The analysis covered only the first 6 months of data. In addition, the depth and scope of the analysis were less than in the trials covered in the NDA submission.

Table 5: Trial 1046: Baseline Means by Country

Marker	Australia	Canada	Italy	Netherlands	p-value
CD4	375	394	406	317	.02
RNA	4.64	4.21	4.38	4.62	.001

3.3.1 Endpoints

Endpoints were treated the same as in study 1031. Recall that all RNA data in this trial were analyzed in a single batch.

3.3.2 Main Results

A total of 152 subjects were randomized, 51 to triple therapy, 53 to ZDV/DDI, and 47 to ZDV/NVP. Subjects in study 1046 were primarily male (93%) and white (94%), and averaged 37 years of age. The median CD4 cell count was 362 cells. There was a significant difference ($p = .03$) between the treatment groups noted in baseline HIV-RNA. The triple therapy arm had an average RNA value of 4.47 log copies, compared to 4.24 for the ZDV/DDI arm and 4.52 log copies for the ZDV/NVP arm.

Subjects had significantly different baseline marker values between the four countries in which the trial was conducted. As Table 5 illustrates, average baseline CD4 cell counts ranged from 317 cells in the Netherlands to 406 cells in Italy, while average baseline HIV-RNA values ranged from 4.21 log copies in Canada to 4.64 log copies in Australia. The pattern of baseline values across countries was different for CD4 than it was for HIV-RNA, as well as for other immunologic and virologic markers.

The trial 1046 results are shown in Table 6. The p-values are for the pairwise comparisons, and are from ANOVA models with treatment, country and treatment/country interaction terms. Highly significant country effects were observed in the HIV-RNA models. There was some suggestion of a country/treatment interaction in the CD4 models.

At the agency's request, the applicant provided a breakdown of the mean changes by country and treatment arm. Inspection of these results revealed that the CD4 results were indeed variable from country to country. In Australia, the triple therapy arm had a smaller response than either of the two double therapy arms. This contrasts with the Netherlands, where triple therapy was strikingly better than the two double therapy arms. Canada and Italy had patterns similar to the overall results. Baseline CD4 differences between the countries would not seem to account for the observed effect since, for example, Australia and the Netherlands had the lowest baseline CD4 counts. The HIV-RNA results did not show this country/treatment interaction.

As a further, exploratory analysis, the applicant provided analysis of covariance (ANCOVA) models incorporating baseline CD4 and RNA values into the analyses. Baseline CD4 was a significant predictor in the CD4 models, and baseline RNA was a significant predictor in the RNA models. Interestingly, the inclusion of these variables did not change the significance level of either the country/treatment interaction in the CD4 models or the country term in the RNA models.

Table 6: Trial 1046: Results of BIPI Analysis

Endpoint	Metric	Z/D/N	Z/D	Z/N	p_{ZDN-ZD}	p_{ZD-ZN}	p_{ZDN-ZN}
CD4	Mean Change 12-16	117	95	44	.44	.08	.01
	Mean Change 20-28	113	78	22	.18	.05	.001
	AUCMB Week 16	72	62	57	.58	.77	.39
	AUCMB Week 28	87	67	47	.23	.28	.02
RNA	Mean Change 12-16	-1.76	-1.55	-.56	.35	.001	.001
	Mean Change 20-28	-1.72	-1.43	-.55	.14	.001	.001
	AUCMB Week 16	-1.61	-1.44	-.99	.24	.002	.001
	AUCMB Week 28	-1.63	-1.41	-.85	.15	.001	.001

The reduction in variability induced by the extra variables did result in the triple vs. ZDV/DDI comparisons for the RNA results achieving statistical significance. The applicant argued that the ANCOVA models incorporating baseline RNA should be preferable to the ANOVA models since the treatment groups differed with respect to baseline RNA values.

For both endpoints and at all times and metrics studied, triple therapy was better than double therapy, but none of the comparisons between triple and ZDV/DDI were statistically significant. Triple therapy was significantly better than ZDV/NVP for both CD4 and RNA, while ZDV/DDI was significantly better than ZDV/NVP only for the RNA comparisons.

3.3.3 Responder analysis

The applicant compared the percentage of subjects in each treatment groups whose RNA values were below 200 copies. During the 20-28 week period, 67% of subjects on triple therapy and 29% of subjects on ZDV/DDI had an average RNA value below 200 copies. A Mantel-Haentzel test, stratified by country, found that this difference was statistically significant ($p = .001$).

3.3.4 "Compliance" analysis

The applicant examined the effect of an interruption in study medication on eventual outcome and concluded: *For both ZDV+ddI and triple therapy, missed treatment was associated with failure to sustain undetectable levels of virus, as evidenced by the fact that all except one of the non-compliant patients in these groups fail to reach the limit of detection.*

3.3.5 Conclusions

The applicant made the following conclusions:

Both ZDV+DDI and ZDV+DDI+NVP were superior to ZDV+NVP in immunologic and virologic activity. The triple therapy was consistently superior to ZDV+DDI, though differences were not consistently statistically significant. Sustained suppression of viral replication to below the limit of detection was achieved in more than half of patients treated with this triple therapy regimen.

4 Reviewer Comments

While many small details of the applicant's analyses are somewhat non-standard, the applicant provided alternative analyses in the NDA to address these concerns. These alternate analyses include using the screening CD4 count instead of the baseline CD4 count to determine CD4 strata in 1031, using arithmetic means rather than geometric means for CD4 counts, using actual rather than virtual sites in 1037, using HIV-RNA values without a batch adjustment in 1031 and 1037, and not dropping the four subjects in 1031 due to batch differences. These analyses indicate that the applicant's results are essentially the same as those from a more standard approach. Thus, this section will focus on some of the limitations of the data and some caveats to the conclusions based on the data, rather than on a reworking of the results section.

4.1 HIV-RNA Issues

It is important to examine the effects of the lower limit of detection on the analysis of the HIV-RNA data. The phrase "lower limit of detection" is used to mean 3 different things. The first meaning is the lowest non-zero number that the laboratory reports. This seems to be a number based on the methodology used to generate the data (dilution factor, machine sensitivity, etc.). All numbers reported should be integer multiples (including 0) of this number. This value was 20 copies in 1031 and 1037 and 1 copy in 1046. This might be termed the *laboratory lower limit*. Second, there is the lower limit reported with the assay, which is a consensus number based on experimental data. The interpretation of this number seems to be that values reported below this number should not be completely trusted. Each study used the same assay, and that assay's lower limit has been set at 400 copies. This may be termed the *accuracy lower limit*. Lastly, there is the number which the applicant substituted for reported values below that number. This was 20 copies in 1031 and 1037 and 200 copies in 1046. This may be termed the *statistical lower limit*. It is this number that affects the estimates of mean changes and of the treatment effect.

Table 7 shows the estimated treatment effect in terms of mean change from baseline in study 1046. The estimated treatment effect is much greater when 0 copies is used as the *statistical lower limit* than when 200 or 400 copies are used. This results from the fact that more subjects in the triple therapy arm had values below 200 or 400 than in the ZDV/DDI arm. Using higher values for the limit of detection result in a markedly smaller estimate of the treatment effect. This means that the the only added effect of nevirapine on RNA levels is to make low levels lower.

While the results of the applicant's percent below the lower limit (responder) analysis in study 1046 may be interesting, the analysis does not convey much information. First, the same as for the analysis of mean changes, the percentages depend on the lower limit chosen. Additionally, since there is no known clinical relevance of 200 copies or any other cutoff value, the responder analysis simply reflects the observed difference in the distributions of change from baseline between

Table 7: Trial 1046: Week 20-28 RNA Results for Different Statistical Lower Limits of Detection

Lower Limit (copies)	0	200	400	1000
Treatment Effect	-1.06	-.32	-.19	-.05
p-value	.006	.110	.304	.772

the two treatment groups. The significance of the responder analysis results from the reduction in variability from using a binary endpoint instead of a highly variable continuous endpoint. That is, in the absence of a clinically relevant cutoff, the two analyses are fundamentally similar.

The fact that in studies 1031 and 1037, the RNA data was assayed in two batches illustrates how relatively small the RNA differences are between the treatment groups. In study 1031, Batch 1 consisted of the pre-entry sample and entry, weeks, 4, 16, 24, 32, and 48. Batch 2 consisted of entry, weeks 8, 40, and 44. The entry sample was assayed in both batches to determine if differences existed between the batches. It was determined that there was in fact a significant difference between the batches of about .1 log copy ($p = .02$), with Batch 2 having the higher median RNA value. The applicant subtracted .1 log copies from all Batch 2 values. In study 1037, Batch 2 (pre-entry, weeks 2, 4, and 8) was found to have a median RNA value which was *lower* than Batch 1 (pre-entry, entry, weeks 12, 16, 20, 24, and 28) by about .16 log copies. This number was added to all Batch 2 values. The effect of the adjustment of RNA values in both studies, and the exclusion of 4 subjects in 1031, on either the estimated treatment effects or their significance levels was minimal, but it points out that the observed treatment effect of about .20-.25 log copies is relatively quite small.

4.2 Loss to Followup

Long term followup was quite good in each of the three trials discussed. In trial 1037, 54/60 subjects completed the full 28 weeks on study. In trial 1031, 341/398 subjects completed the full 48 weeks on study. Trial 1046 was still ongoing when the 6 month results were submitted. In each of the studies, the dropout rate was similar in the treatment arms. Importantly, the AUCMB analyses, which included all subjects, yielded similar treatment effects to the analysis of mean changes.

4.3 "Compliance" Analysis: 1046

For trial 1046, the applicant made the argument that compliance with study medication was an important predictor of overall benefit. However, it appears that the cause and effect relationship suggested may be reversed, that is, non-compliance may result from poor initial benefit. For example, subjects who stayed on study medication during weeks 16 through 28 had experienced an average drop of 120 CD4 cells during the first 16 weeks on study. In contrast, subjects who went off study medication a some point during weeks 16 to 28 had experienced an average drop of only 40 CD4 cells during the first 16 weeks on study, Additionally, it is worth pointing out that those who went off study medication did so primarily by dropping ddI and not nevirapine. In conclusion, greater compliance may well result in improved response, but in this trial that cause and effect relationship cannot be separated from the opposite cause and effect relationship described above.

Table 8: Trial 1031: Alternative Subgroup Analysis of Week 40-48 Treatment Differences

Marker	ddI Naive	ddI Experienced
CD4	30 cells	7 cells
RNA	-.38 log copies	-.03 log copies

4.4 Population

In drawing overall conclusions, it is important to keep in mind the relative sample sizes of the studies.

While the applicant provided an analysis of the subgroup in study 1031 who had only received ZDV prior to entering the study, an alternate way to assess the impact of prior nucleoside therapy is to split up the study into a ddI naive subgroup and a ddI experienced subgroup. Table 8 shows the estimated 40-48 week effects comparing triple therapy to ZDV/ddI by prior ddI subgroup. From these results it appears that the overall significance level of the treatment difference was driven by the ddI naive subgroup. Based on this finding, and the fact that the magnitude of the difference between triple therapy and ZDV/ddI in study 1046 was similar to the ddI naive subgroup in 1031, it appears that the effect of nevirapine is most pronounced when given with at least one drug to which the patient is naive. The lack of significance in 1046 may be attributed to the much smaller sample size (50/arm versus 200/arm).

Although study 1037 showed an effect for adding nevirapine to just one drug, the apparently limited durability of this effect (8-12 weeks for RNA and 24-28 weeks for CD4) would indicate that a two drug combination may not be the optimal use for this drug. The comparatively poor performance of the NVP/ZDV arm in 1046 supports this idea. Both the magnitude and duration of the CD4 and RNA effects in 1046 were much less in the NVP/ZDV arm than in either of the ddI containing arms.

One can speculate on the reasons behind the observations that nevirapine seems to work better when started with two other drugs, where at least one of these is a new drug for the subject. It is known that resistance sets in very rapidly when nevirapine is given as monotherapy. In addition, all subjects on the combination of nevirapine and ZDV who were tested become resistant to nevirapine. Since the onset of resistance is slower for lower levels of the virus, a drug combination that reduces the virus to very low levels can avoid the rapid onset of nevirapine resistance. Clearly, the more drugs, especially new drugs, the lower the RNA levels will be, and thus the longer nevirapine will be effective.

4.5 Conclusions

The addition of nevirapine to one or more nucleosides has been shown to produce an increase in CD4 cell counts and a small decrease in HIV-RNA levels. The durability of this effect appears longer than has been shown in recent applications. These findings, from three clinical trials, justify the applicant's claim that nevirapine, in combination with nucleoside analogues, provides a significant surrogate marker benefit.

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CLINICAL PHARMACOLOGY/BIOPHARMACEUTICS REVIEW

Nevirapine (Viramune), 200 mg Tablets
NDA 20-636
Applicant: Boehringer Ingelheim Pharmaceuticals, Inc.
Submission Dates: 2/23/95, Review Date: 5/15/96
Reviewer: Chandrahas Sahajwalla, Ph.D. Final: 6/xx/96

SYNOPSIS

INTRODUCTION

Nevirapine (chemically related to dipyridodiazepinone class) is described by the applicant as a highly potent non-nucleoside with activity against Human Immunodeficiency Virus (HIV-1) enzyme reverse transcriptase (RT). Nevirapine binds directly to HIV-1 RT and appears to block RT polymerase by altering the position of critical amino acids within the enzyme's catalytic site, resulting in slowing of the chemical reaction catalyzed by the RT. Nevirapine does not block the activity of mammalian polymerase (including human polymerase alpha, beta, delta and gamma). The in vitro IC_{50}s ranged from 10 nM to 100 nM when tested against several HIV-1 strains and clinical isolates.

Nevirapine is proposed to be used in combination with nucleoside analogues for the treatment of patients with advanced HIV-1 infection whose current antiretroviral therapy is no longer deemed adequate. The proposed dose is one 200 mg tablet q.d. for the first 14 days (lead-in period due to autoinduction), followed by one 200 mg tablet b.i.d., in combination with nucleoside analogue antiretroviral agents.

The applicant has adequately studied the pharmacokinetics of nevirapine at the proposed dose. However, some of the limitations of this NDA submission include; lack of characterization of pharmacokinetics in hepatic dysfunction and renal insufficiency, including the effect of dialysis; absence of in vivo drug interaction studies of nevirapine with the drugs commonly used by AIDS patients.

Nevirapine undergoes autoinduction and on multiple dosing apparent oral clearance increases by about 70% (from 22 mL/Kg/h to 36 mL/kg/h), terminal phase half-life of nevirapine decreases from about 45 hours following single dose to about 25-30 hours following multiple dosing with 200 mg/day.

ABSORPTION:

Nevirapine tablets are well absorbed with an absolute bioavailability (50 mg tablet) of greater than 90%. A high fat diet and antacid significantly affect the rate of absorption without significantly affecting the extent. Thus nevirapine could be taken without regards to food. The dose (12.5 mg to 600 mg per day) proportionality studies in patients indicate that the pharmacokinetics of a 400 mg dose appear to be proportional to 200 mg dose. However, compared to the lower

doses (12.5 and 50 mg) the higher doses have lower bioavailability. Further, AUC and Cmax showed a better proportionality when dose is adjusted for body weight.

Results from a single dose rising study in HIV patients indicated that; the AUC or Cmax plotted against dose deviates from linearity; if dose is adjusted for weight, Cmax and AUC appear to show linearity with higher doses resulting in shorter half-life; patients with a history of intravenous drug use had longer half-lives (compared to patients with no history of i.v. drug use); patients taking concomitant antifungals had the highest AUC for their doses.

DISTRIBUTION:
Nevirapine is highly lipophilic and is about 60% bound to plasma protein binding over the plasma concentration range of 1-10 μg/mL. The volume of distribution is similar following i.v. and oral administrations and is not substantially affected by gender. The mean Vdss following 50 mg i.v. infusion, oral solution and oral tablets was 1.34 ± 0.12, 1.33 ± 0.11 and 1.37 ± 0.14 L/kg, respectively. In a study to assess gender effects, apparent oral Vdss following 200 mg oral dose was 1.54 ± 0.12, 1.38 ± 0.11, 1.46 ± 0.14 for 15 females, 15 males and overall (N = 30), respectively.

METABOLISM:
Nevirapine undergoes autoinduction and on multiple dosing, apparent oral clearance increases by about 70%, AUC decreased by about 40% and half life decreased from about 41 hours to 30 hours. Autoinduction appear to continues beyond 14 days of nevirapine however, most of the autoinduction occurs by Day 14 of dosing.

In a mass balance study under the conditions of autoinduction, about 81% of the radiolabelled dose was recovered in the urine and 10% in the feces. Less than 5% of the administered dose was recovered unchanged in the urine. Nevirapine is extensively biotransformed via cytochrome P450 (oxidative) metabolism to several hydroxylated metabolites. The major urinary metabolites were 2/3 hydroxy nevirapine glucuronide (55%) and hydroxymethyl nevirapine glucuronide (29%). Based on chromatographic signal, nevirapine accounted for about 75% of the species circulating in the plasma. In vitro activity for the cytochrome P450 indicates that CYP4503A is the isoenzyme primarily responsible for metabolism of nevirapine and CYP2D6 is implicated to a very minor extent.

EXCRETION:
Most of the radioactivity (80%) in urine was represented by glucuronide conjugates of hydroxylated metabolites. Less than 5% of the dose is excreted unchanged in the urine. Thus, metabolism, including glucuronide conjugation, and urinary excretion of the metabolites is the primary route of nevirapine elimination in humans.

HEALTHY VERSUS PATIENTS:
Nevirapine appears to have similar pharmacokinetics in adult patients and healthy volunteers.

PEDIATRICS:
Based on single dose data in HIV infected children (9 months to 14 years) receiving 7.5 to 120 mg/mm^2 (N=3 per dose group), the AUC and Cmax increased proportionally with dose. Further, children appear to have a two fold higher clearance, shorter half-life (about 24 hours), lower AUC and Cmax compared to adults. However, since nevirapine undergoes autoinduction, clinical relevance of single dose data in children is unknown. The sponsor has indicated that a multiple dose trial in pediatric patients and ongoing.

DRUG INTERACTIONS:
At clinically relevant doses of nevirapine, mean steady state zidovudine (ZDV) pharmacokinetic parameters were not significantly affected. In another study using triple therapy (ZDV + ddI + NEV), a 32% decline in ZDV was noted in patients. Nevirapine did not appear to affect ddC pharmacokinetics. Nevirapine oral clearance when coadministered with ddi+ZDV was 2.32 ± 1.34 L/hr compared to 4.21 ± 2.22 L/h when nevirapine was administered with ddc + ZDV thus, the mean nevirapine clearance in the ddi triple therapy is about one half of that in ddc + ZDV triple therapy group. This finding suggests that patients may benefit from reduction in nevirapine dose (three patients had CL/F of 0.55, 1.15 and 1.39 producing Cminss of greater than 10 μg/mL). These two groups (ddi and ddc) differed even in ZDV pharmacokinetics. The reason for this difference is unknown.

Preliminary data (N=11) indicate that saquinavir exposure (AUC) is reduced by about 20% (range -60% to +86%; saquinavir change in AUC for 11 patients 2, 29, 73, 86, -16, -18, -24, -38, -39, -54, -60%) when co-administered with nevirapine. This observation suggests that the effect of autoinduction produced by nevirapine was vaiable and will affect the clearance of protease inhibitors, which are substrates of CYP3A.

In vitro Drug Interactions:
In vitro drug interaction studies with human liver microsomes indicate the following: nevirapine pharmacokinetics is less likely to be affected by dapsone, rifabutin and trimethoprim; and is likely to be affected by rifampin, ketoconazole and sulfamethoxazole. Further, in vitro nevirapine incubation with human liver microsomes does not appear to affect the pharmacokinetics of sulfamethoxazole or trimethoprim. Nevirapine is likely to affect the metabolism of rifabutin. The effects of nevirapine on dapsone, rifampin and ketoconazole were not assessed in vitro. Since nevirapine undergoes autoinduction, relevance of its in vitro effects on metabolism of other drugs is unknown.

DOSE-RESPONSE/BLOOD LEVEL RESPONSE:
In an attempt to correlate Dose-Response/Blood Level Response (12.5 to 400 mg dose), the applicant measured immune complex dissociation (ICD) p24 antigen levels (virological activity, up to 24 weeks of treatment) and decline to less than 50% of their baseline as indicator of response to nevirapine therapy. If the response was seen on two consecutive visits, patients were defined as responders. Patients showing an increase in CD4+ counts of 50 or more on two consecutive visits were defined as having immunologic response. The applicant reports that three of the four patients with the shortest duration of p24 suppression below 50% of their baseline had 3 of the 4 lowest steady state trough concentrations. Results indicated that a 400 mg/day dose of nevirapine reduced ICD p24 antigen levels in 68% of the patients (13/19). Immunologic activity (increase in CD4+ counts) was not impressive for any of the doses tested. Although CD4+ and ICD p24 were the surrogate markers used at the time the study was conducted, the recent trend is to monitor the viral load and RNA genotype as well.

SUBPOPULATION ANALYSIS:
Nevirapine steady state trough concentrations were monitored in several studies and the individual patient median Cminss (N=387 patients) was utilized for determinations of relationship of Cminss to demographics, disease state and drug interactions. Regression analysis (performed by applicant at this reviewer's request) of the data from two studies indicated that Cminss and AUC are correlated with an R-Squared of 0.856, suggesting that examining Cminss is a reasonable initial approach in assessing pharmacokinetic differences.

Median Cminss for 200, 400 and 600 mg/day appeared to increase proportionally.

None of the nevirapine treatment regimens in combination with ZDV (n=185) or ZDV + ddI (n=176) had an effect on the nevirapine Cminss compared to nevirapine monotherapy (n=26[illegible] However, in a formal drug interaction study, steady state apparent clearar[illegible] ecreased in patients receiving ddI (compared to historic control). This finding ques[illegible] the validity of using median Cminss to look for the differences in pharmacokinetics.

No trend in increased or decreased median Cminss was noted based on age (18 to 63 years of age), gender, weight, ethnicity or baseline disease factors (AIDS, ARC, Hepatitis B). In a formal study assessing gender effect, body weight adjusted apparent clearance for females was about 24% higher compared to males suggesting higher metabolic capacity for female subjects. Volume of distribution was significantly different, however, differences were less than 20%.

Median Cminss were also used for assessment of drug interactions. Median Cminss for all the patients were compared to the median Cminss for patients who were also concomitantly taking rifampin (n=2), rifabutin (n=18), cimetidine (n=12), ranitidine

(n=18), erythromycin (n=3), clarithromycin (n=14), azithromycin (n=7), ketoconazole (n=11).

It was noted that cimetidine and macrolides, known inhibitors, elevated nevirapine median Cminss whereas, rifampin and rifabutin, known inducers of CYP3A, slightly lowered median Cminss compared to the overall median Cminss. It also appears that cimetidine, macrolides and rifampin would have a significant interaction because most of the median Cminss in these groups were outside the range of 75% (upper or lower limit of the boxplots) of Cminss for the overall patients. However reliability of Cminss as a measure for interactions is questionable. With high variability in Tmax, the relationship of Cminss to the overall exposure (AUC) is unknown.

DISSOLUTION:
Nevirapine has better solubility at lower pH, the dissolution is much faster when using 0.1N HCL as the dissolution media. The applicant has requested that phosphate buffer at pH of 2.0 be used as the dissolution media, as it gives better discrimination ability. The dissolution method and specifications proposed by the applicant are acceptable.

FORMULATION:
The production batch of tablet formulation contains drug substance whereas, the clinical batch contains drug substance.

Tablets are equally bioavailable compared to solution or pediatric suspension that was used to study single dose pharmacokinetics in children. The proposed to-be marketed tablets were bioequivalent to the tablets that were used in the pivotal clinical trials.

Comments/Recommendation:
1. Pharmacokinetics has not been characterized in hepatic impairment or in patients undergoing dialysis. It is suggested that the sponsor characterize the pharmacokinetics of nevirapine in these populations

2. The maximum plasma concentration (Cmax) and AUC adjusted for weight appear to show better proportionality than absolute dose. This suggests that patients lighter

in weight may tend to have more toxicity related to high drug concentrations and patients heavier in weight may show decreased efficacy. At this reviewer's request the applicant performed analysis to assess relationship between body weight and adverse events and efficacy markers. The results showed that rash was more frequent in the low weight (<64.25 Kg) patients (58% in nevirapine group and 28% in control group) and less frequent in higher weight (>80.2 kg) patients (29% in nevirapine group and 17% in control group). However, this analysis was confounded by the fact that patients in low weight group were sicker (lower CD4 counts) patients. This hypothesis needs to be further explored, by looking at low and high weight patients within a same CD4 range. Based on the results, assessment to lower nevirapine dose in low weight patients can be made.

3. Preliminary data suggest that, a history of I.V. drug use appears to increase half-life. For better care and patient management clinical relevance of this finding should be further explored.

4. The in-vitro drug interactions were generally conducted to study effect of other drugs on the pharmacokinetics of nevirapine. Since, nevirapine is an autoinducer and autoinduction is difficult to establish in-vitro, it is recommended that the applicant conduct in-vivo drug interactions for relevant drugs.

Preliminary data (N=11) indicate that saquinavir exposure (AUC) is highly variable and the average AUC is reduced by about 20% when co-administered with nevirapine. The sponsor has also attempted to look at the median Cminss for patients in their data base who were on concomitant medications. Nevirapine treatment regimens in combination with ZDV (n=185) or ZDV + ddi (n=176) had an effect on the nevirapine Cminss compared to nevirapine monotherapy (n=26). Whereas, in study 1009 decreased steady state apparent clearance was noted for patients receiving ddi as compared to that of monotherapy. It is recommended that the effect of nevirapine on the pharmacokinetics of other drugs be studied. Further, effect of nevirapine on cimetidine and macrolides, known inhibitors; and rifampin and rifabutin, known inducers of CYP3A be assesses (quantitatively). The effect of nevirapine on oral contraceptives should also be assessed.

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5. Apparent clearance of nevirapine in patients concomitantly taking ddI is reduced to half compared to patients taking monotherapy or combination with ZDV. This finding indicates that patients taking ddI concomitantly will have much higher concentrations compared to patients taking ddc or patients not taking ddI. This issue has been discussed with the reviewing medical officer to explore the clinical relevance (increased toxicity in ddi treated group) of this finding.

6. The dissolution method and specification proposed by the applicant is acceptable.

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60 minutes.

Review Related Issues:
Following is a list of additional information requested from the applicant:

1. Since better dose proportionality was observed for AUC and Cmax when dose...
(This Section Will Summarize our input in the data analysis - TO BE ADDED)

DRAFT

Chandrahas G. Sahajwalla Ph. D.
Division of Pharmaceutical Evaluation III
Office of Clinical Pharmacology & Biopharmaceutics

Concurrence:
Janice Jenkins, Ph.D.
Team Leader, Antiviral Drug Products Section
Division of Pharmaceutical Evaluation III

cc:
/NDA 20-636
/HFD 530/Div File
/HFD 530/CSO/Zeccola
/HFD 530/MO/MartinJ
/HFD 530/Biopharm/Sahajwalla
/HFD 530/Biopharm/Jenkins
/HFD 880/Fleischer
/
/

DRAFT

General Notes:
A secondary peak generally occurring between 12 to 24 hours after administration of nevirapine was noted in plasma concentration time profiles in all the studies.

Unless specified otherwise, oral formulations used were 50, 100 and 200 mg tablets (for batch number reference please refer to Appendix at the end of the review).

The drop outs mentioned in the reports dropped out for acceptable reasons.

TABLE OF CONTENTS

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MASS BALANCE/METABOLISM:
Title: Open-Label, Multiple-Dose Study to Characterize the Pharmacokinetics and Excretion/Mass Balance of ^{14}C-Labeled Nevirapine and Metabolites in Healthy Volunteers (Study # 1100.816, Volume 71, Page 1)

The objectives of the study were to: (1) characterize the pharmacokinetics and excretion/mass balance of ^{14}C-labeled nevirapine and metabolites; (2) isolate, identify and quantify major metabolites of nevirapine in plasma and urine; (3) determine the partitioning of radioactivity between plasma and red blood cells. The study also was designed to further assess safety/tolerance of the multiple-dose regimen using healthy volunteers.

In an open label study, 11 healthy volunteers (8 completed the study) received 200 mg qd nevirapine for two weeks followed by 200 mg q12 (400 mg/day) for two weeks and an additional 50 mg dose of ^{14}C-nevirapine solution (200 mL) 24 hours after the last 200 mg dose. Blood, plasma, urine and fecal samples collected over 0-240 hours were analyzed for total radioactivity, parent compound and metabolites. Plasma nevirapine trough concentrations were also determined (Day 14, 26 and 27 AM and Day 26 PM) to assess compliance.

Results:
The excretion/mass balance of ^{14}C-labeled nevirapine was determined under conditions of autoinduction and the data for individual subjects are provided in Table 1 and the results summarizes in the following table:

Parameter Mean ± SD (N = 8)	Radioactivity Plasma	Radioactivity Whole Blood
Cmax DPM/mL	3496 ± 501	3425 ± 467
AUC(0-inf) DPM.h/mL	92889 ± 17744	92515 ± 18537
Cl/F ml/kg/hr	36.1 ± 6.5	36.4 ± 7.1
Half-life hours	21.3 ± 3.6	21.8 ± 2.5
MRT hours	30.9 ± 5.5	30.1 ± 5.3
Total % Radioactivity Recovered	91.4 ± 10.5	
% Radioactivity in Urine	81.3 ± 11.1	
% Radioactivity in Feces	10.1 ± 1.53	

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Trough nevirapine concentrations on days 14, 26 and 27 were suggestive of volunteers' compliance (data not shown).

The mean blood to plasma ratio of AUC(0-inf, radioactivity) was 0.99 CL/F, $T_{1/2}$, MRT, and Cmax obtained for plasma and whole blood were also similar. The results from an in-vitro study (not part of this study) to determine partitioning of nevirapine (1-10 μg/mL) into whole blood and red blood cells indicated that nevirapine partitions (rbc/plasma 1.56 ± 0.11 and 1.27 ± 0.11 at 1 and 10 μg/mL, respectively) into red blood cells in an inverse concentration dependent manner.

Plasma nevirapine $T_{1/2}$ and Cmax were 24.4 ± 3.94 h and 3768 ± 1570 ng/mL, respectively. Radioactivity in plasma was detectable up to 96 hours whereas, nevirapine plasma levels were detectable up to 144 hours. Plasma nevirapine half-life was higher compared to plasma radioactivity half-life, possibly because the assay was more sensitive than the assay for radioactivity measurements.

The metabolites isolated/identified from the urine samples are presented in the table below. In the urine, the glucuronide conjugates of nevirapine hydroxylated metabolites accounted for about 85% of the total radioactivity. In most subjects less than 3% of the radioactivity was recovered (in one subject 11.7% recovered) in the urine as parent compound.

Metabolite	Position of OH Group	% Recovered in Urine
2/3-hydroxy-^{14}C-nevirapine glucuronide	2 or 3	54.5 ± 5.1
hydroxymethyl-^{14}C-nevirapine glucuronide	methyl-(4)	29.1 ± 6.5
8-hydroxymethyl-^{14}C-nevirapine glucuronide	8	1.6 ± 0.2
M5 (unidentified)	-	2.9 ± 0.6
3-hydroxy-^{14}C-nevirapine	3	1.5 ± 0.3
hydroxymethyl-^{14}C-nevirapine	methyl-(4)	0.7 ± 0.3
^{14}C-Nevirapine	-	3.3 ± 3.5

To determine plasma concentrations of the metabolites, plasma samples for all the subjects were pooled and concentrations approximated using nevirapine plasma calibration curve. Nevirapine accounted for about 75% of the total chromatographic signal. Metabolites identified in plasma were 2/3-hydroxy nevirapine glucuronide (12%), hydroxymethyl nevirapine glucuronide (5%), hydroxymethyl nevirapine (4%) and 3-hydroxy nevirapine (0.6%) (Figures 1 and 2). Nevirapine and metabolites combined accounted for 95% of the plasma radioactivity.

Nine of the 11 volunteers reported abnormalities in the liver function test, including

one case of clinical hepatitis. There were 28 other adverse events experienced by 9 of the 11 subjects classified by the applicant as non-serious.

The **in vitro activity for the cytochrome P450** was investigated by the applicant and Figure 2A summarizes the results. Based on the percentage of dose as individual metabolites excreted in the urine, about 70% of nevirapine's contribution can be attributed to CYP3A. To a minor extent, CYP2D6 may also be involved in the metabolism of nevirapine.

In conclusion, under the conditions of autoinduction, nevirapine is extensively metabolized with about 91% of the radioactivity recovered in 96 hours. In the urine most of the nevirapine is excreted as hydroxylated glucuronide, whereas, in plasma unchanged nevirapine was the predominant circulating species. In vitro findings indicate that nevirapine is mainly metabolized by CYP3A and to a very minor extent by CYP2D6.

PHARMACOKINETICS:

Title: Open Label Study to Assess the Single- and multiple-Dose Pharmacokinetics of Nevirapine 200 mg in Healthy Volunteers (Study # 1100.1056, Volume 79, Page 1)

The objectives of the study were to: (1) characterize the single- and multiple-dose pharmacokinetics of nevirapine in healthy volunteers; (2) further assess safety and tolerance of the clinical regimen; (3) further characterize the effect of gender on nevirapine pharmacokinetics.

Thirty (15 M, 15 F) healthy volunteers received 200 mg nevirapine on Day 0 of the study; 200 mg qd nevirapine for two weeks (Days 7 - 20) followed by 200 mg bid (q12h, 400 mg/day) for two weeks (Days 21 to 35 AM). Trough plasma concentrations were determined on Days 10, 12, 15, 25 and 30. To characterize the effects of autoinduction on pharmacokinetic disposition, plasma and urine samples were collected over 0 to 168 hours on Days 0 and 35, and between 0 to 24 hours on Day 20.

Results:

The study was terminated due to three cases of toxic hepatitis in female volunteers receiving 200 mg bid dose. It was possible to evaluate pharmacokinetics in 30 volunteers after single dose, 18 volunteers following 200 mg qd for 14 days and 4 volunteers following 200 mg bid for 14 days.

The pharmacokinetic data for individual subjects are provided in Tables 2-4. The mean pharmacokinetic parameters obtained following a single 200 mg dose of nevirapine are summarized in the following table:

	Females N = 15	Males N = 15	All Subjects N = 30	P Value Male vs. Female
Cmax μg/mL	2.1 ± 0.4	1.9 ± 0.3	2.0 ± 0.4	0.300
AUC μg*h/mL	147.2 ± 42.4	130.4 ± 27.4	138.8 ± 36.1	0.340
CL/F (L/h)	1.5 ± 0.4	1.6 ± 0.3	1.5 ± 0.4	0.340
CL/F mL/Kg/h	24.6 ± 7.7	19.9 ± 3.9	22.2 ± 6.5	0.082
Vdss/F (L)	93.9 ± 15.3	111.6 ± 18.4	102.8 ± 18.9	0.011
Vdss/F (L/Kg)	1.54 ± 0.12	1.38 ± 0.11	1.46 ± 0.14	0.001
MRT (h)	70.7 ± 30.5	71.9 ± 15.5	71.3 ± 23.6	0.362
Half-Life (h)	41.2	47.1	44.0 ± 12.9	0.300

Clearance (CL/F), Cmax, MRT, $T_{1/2}$, and AUC were not affected by gender. Body weight adjusted apparent clearance for females was about 24% higher compared to males suggesting higher metabolic capacity for female subjects. Volume of distribution was significantly different, however, differences were less than 20%. Results of this analysis indicate that single dose pharmacokinetics of nevirapine are not significantly affected by gender.

Multiple dose pharmacokinetics in healthy volunteers:
Pharmacokinetic parameters (Day 20) obtained following 200 mg qd nevirapine for two weeks are summarized in the following table:

	Females N = 10	Males N = 8	All Subjects N = 18	P Value Male vs. Female
Cmax μg/mL	5.1 ± 0.8	4.8 ± 1.0	5.0 ± 0.9	0.450
Cmin μg/mL	3.0 ± 1.0	3.1 ± 0.6	3.0 ± 0.8	0.625
AUC μg*h/mL	86.4 ± 20.3	84.9 ± 16.0	85.7 ± 18.0	0.894
CL/F (L/h)	2.4 ± 0.5	2.4 ± 0.5	2.4 ± 0.5	0.894
CL/F mL/Kg/h	40.5 ± 12.2	29.4 ± 5.5	35.5 ± 11.1	0.030
Half-Life (h)	27.7 (N = 5)	34.4 (N = 4)	30.3 ± 5.9	0.111

No statistically significant differences in Cmax, Cmin, CL/F, $T_{1/2}$, or AUC were noted based on gender following multiple doses (Day 20). Body weight adjusted apparent clearance for females was about 38% higher compared to males. As observed following single dose of nevirapine, the higher weight adjusted oral clearance in females was offset by greater weight in males, resulting in no significant differences in the overall exposure, i.e. AUC.

Pharmacokinetic parameters following a single dose 200 mg of nevirapine and 200

mg qd for 14 days are compared in the following table:

	Single 200 mg Dose (Day 0)	200 mg qd (Day 20)	P Value
AUC μg*h/mL (N = 18)	147.2 ± 39.9	85.7 ± 18.0	p<0.01
CL/F (L/h) (N = 18)	1.45 ± 0.36	2.42 ± 0.47	p<0.01
CL/F mL/Kg/h (N = 18)	21.0 ± 6.4	35.5 ± 11.1	p<0.01
Half-Life (h) (N = 9)	41.2	30.3	p<0.01

On average, apparent oral clearance increased by 70%, AUC decreased by 42% and the mean half-life decreased from 41 hours to 30 hours. These results are consistent when looking at the data for individual subjects. These results are indicative of autoinduction of nevirapine following multiple doses.

Pharmacokinetic parameters following single and multiple doses of nevirapine (Days 0, 20 and 35) are presented in the following table:

N = 4	Single 200 mg Dose (Day 0)	200 mg qd (Day 20)	200 mg bid (Day 35)
AUC μg*h/mL	112.81 ± 15.17	72.74 ± 3.79	63.06 ± 11.10
CL/F (L/h)	1.8 ± 0.23	2.76 ± 0.15	3.24 ± 0.49
CL/F mL/Kg/h	21.18 ± 4.05	32.96 ± 8.02	37.7 ± 5.00
Half-Life (h)	45.9	-	32.6

Based on data in a limited number of subjects (N = 4), a further increase in clearance and decrease in AUC were noted on Day 35 compared to Day 20, indicating further induction of enzyme occurring between Days 20 to 35. However, significant autoinduction was noted in first two weeks.

Estimated nevirapine renal clearance on Day 20 was about 15% higher on Day 20 which was not statistically significant. Renal clearance represented a small fraction of the overall systemic clearance (see table below).

	Single 200 mg Dose (Day 0); N = 25	200 mg qd (Day 20); N = 18	P* value	200 mg bid (Day 35); N = 4
CL_R (mL/h)	18.9 ± 7.3	21.9 ± 11.0	0.326	23.5 ± 9.8
CL_R (mL/Kg/h)	0.27 ± 0.09	0.31 ± 0.15	0.217	0.27 ± 0.07
Ratio (%)**	1.3 ± 0.5	1.0 ± 0.6	0.002	0.7 ± 0.3

* Day 0 versus Day 20

** Ratio = (CL_R divided by CL/F*100)

A total of 75 adverse events were reported, 44 of which were attributed to the drug by the investigators. Two of the three female volunteers experiencing toxic hepatitis were in the upper quartile of AUC however, their concentrations do not appear to be unusually high.

In conclusion, on multiple dosing, nevirapine undergoes autoinduction which appears to continue beyond 14 days of dosing. Autoinduction increases the clearance and decrease the AUC and half-life by about 50%. No significant gender differences in nevirapine concentrations following single or multiple doses were noted. Toxic hepatitis led to early termination of the study and the adverse events appear to be related to higher dose and duration of administration.

Title: A single Rising Dose Pharmacokinetics Phase I Evaluation of Nevirapine Tablets by Oral Administration in Individuals with HIV Infection (CD4+ Cell Count <400 mm^3), (Study # 1100.742, Volume 54, Page 217)

The objectives were (1) To generate initial information on the pharmacokinetics, dose proportionality, sensitivity and specificity of the analytical methodology for monitoring nevirapine plasma levels; (2) To assess safety and tolerance of single rising oral doses of nevirapine in subjects with HIV infection; (3) To determine dose to achieve trough plasma levels of 10-20 X IC_{50} which can serve as the initial dosage level, as well as to determine a preliminary dosing interval and dosage escalation schedule for a multiple dose clinical study in HIV infected subjects.

Twenty one HIV-infected patients received (N=3 for each dose) 2.5, 12.5, 25, 50, 100, 200 and 400 mg single dose nevirapine (tablets used 2.5 mg, 12.5 mg and 50 mg). Blood samples were collected up to 48 hours for 2.5 and 12.5 mg dose and up to 168 hours for doses ≥ 25 mg.

Results:
Mean plasma concentrations of nevirapine are presented in Figure 3 and pharmacokinetic parameters presented in Tables 5 and 6. Plots of Cmax and AUC versus dose are presented as Figures 4 and 5.

The mean half-life, apparent volume of distribution and clearance were 40 hours (range 22 to 84 hours), 1.37 L/kg (range 1.13 to 1.86 L/kg) and 1.37 ± 0.17 mL/kg/min, respectively. Figures 4 and 5 and Tables 7 - 9 indicate (a) When AUC or Cmax is plotted against dose there is deviation from linearity; (b) When dose is adjusted for weight, Cmax and AUC appear to show linearity with dose; (c) higher doses resulted in shorter half-life; (d) patients with history of intravenous drug use had longer half-life (median of 65 compared to 34.5 for those patients with no history of i.v. drug use); (e) patients taking concomitant antifungal had the highest AUC for their doses.

In conclusion, AUC and Cmax tended to be non-linear at the highest dose. Dose adjustment for weight tended to show linearity in Cmax and AUC, suggesting that with a fixed dose, patients lighter in weight may tend to have more toxicity related to high drug concentrations. History of I.V. drug use appears to increase half-life. Higher doses tended to result in longer Tmax and shorter $T_{1/2}$. Higher AUC in patients using antifungal medications concomitantly were noted, which is consistent with the knowledge that nevirapine is metabolized by CYP4503A isoenzyme. However, results from this study should be considered preliminary since the study was a single dose (nevirapine undergoes autoinduction), parallel design using several concomitant medications and in very few patients.

Title: An Open-Label, Staggered Rising Dose Cohort Study Assessing Safety, Tolerance, and Activity of Nevirapine (BI-RG-587) in Patients with HIV Infection (CD4+ Cell Count <400 mm^3), (Study # 1100.744, Volume 82, Page 1)

The objectives were to evaluate in HIV-infected adult patients (CD4+ counts < 400/mm^3) the: (1) Safety and tolerance of multiple oral doses of nevirapine for up to 24 weeks; (2) Pharmacokinetics and dose proportionality of multiple doses of nevirapine; (3) Effects of treatment with nevirapine for up to 24 weeks on virological activity (p24 antigen levels) and immunologic response (CD4+ count).

This was a staggered dose-escalation study, evaluating single daily doses of 12.5, 50, 200, and 400 mg, starting with the lowest dose of nevirapine (N= at least 10 patients for each dose level). Originally, it was planned to study doses up to 250 mg however, the protocol was amended to replace 250 mg with 200, 400 and 600 mg dose (data analysis from 600 mg dose is not part of this report). Blood sampling for pharmacokinetics determinations was performed on Days 0 and 14 at 0.5, 1,2, 4, and 8 hours and urine was collected over 24 hours. Further, trough levels were determined on Days 1,3, 4, 7 and 15 and in some patients nevirapine trough levels were periodically determined up to 100 days.

Immune complex dissociation (ICD) p24 antigen levels (virological activity, measured up to 24 weeks of treatment) that declined to less than 50% of their baseline were defined as having a response to nevirapine therapy and if the response was seen on two consecutive visits, patients were defined as responders. Patients showing increase in CD4+ counts of 50 or more on two consecutive visits were defined as having immunologic response.

Results:
Nine of the 52 patients enrolled completed nevirapine treatment for 24 weeks. Median change in baseline of ICD p24 antigen and CD4+ counts are presented in Figure 6 and 7, respectively. The only group showing statistically significant decline in p24 antigen levels at weeks 24, 8, 12 and 16, was the one receiving the

400 mg daily dose. The proportion of responders (p24 antigen levels) in each dose level was 0/9, 2/6(33%), 3/7(43%) and 13/19(68%) at 12.5, 50, 200 and 400 mg daily doses, respectively. The proportion of responders based on 50 units increase in CD4+ counts were 1/11(9%), 2/10(20%), 0/8, and 1/22(5%) at 12.5, 50, 200 and 400 mg daily doses, respectively.

Mean nevirapine concentration profiles for first dose and steady-state are presented in Figures 8 and 9, respectively. Pharmacokinetic parameters are presented in the following tables:

Nevirapine pharmacokinetics following first dose

Dose (mg/day) Number of patients	Cmax1 µg/mL	Cmin1 µg/mL	Tmax1 (median) hours
12.5 (N = 10)	0.11 ± 0.02	0.07 ± 0.01	2.0
50 (N = 10)	0.61 ± 0.14	0.38 ± 0.06	1.5
200 (N = 9)	1.96 ± 0.53	1.48 ± 0.49	4.0
400 (N = 14)	3.43 ± 1.04	2.72 ± 0.72	4.0

Based on single dose data, dose adjusted Cmin (Figure 10) for four different doses was not significantly different and also dose adjusted Cmax for 12.5, 50 and 200 mg doses was not significantly different. However, dose adjusted Cmax for 400 mg dose were significantly higher at 50 mg than at 400 mg (Figure 11). Based on single dose nevirapine Cmax, the pharmacokinetics of nevirapine appear to be proportional between 12.5 to 200 mg dose.

Nevirapine pharmacokinetics following multiple doses:

Dose (mg/day) Number of patients	$Cmax_{ss}$ µg/mL	$Cmin_{ss}$ µg/mL (t = 0)	$Cmin_{ss}$ µg/mL (t = 24)	CL/F (mL/kg/hr)	Accumulation Ratio
12.5 (N = 10)	0.38 ± 0.11	0.25 ± 0.07	0.22 ± 0.07	24.57 ± 6.50	2.97 ± 0.77
50 (N = 10)	1.75 ± 0.56	1.24 ± 0.47	1.16 ± 0.53	24.89 ± 12.06	3.04 ± 1.25
200 (N = 6)	3.60 ± 0.49	2.17 ± 0.53	1.92 ± 0.44	46.55 ± 8.59	1.51 ± 0.30
400 (N = 10)	7.24 ± 1.41	4.36 ± 0.84	3.68 ± 1.37	44.00 ± 11.68	1.46 ± 0.63

Significantly (2 fold) lower accumulation ratios and higher CL/F values (Figure 12), were obtained for higher doses (200 and 400 mg) compared to the lower dose (12.5 and 50 mg) group. There were no significant differences in any of the parameters between 12.5 and 50 mg doses; and between 200 and 400 mg doses. Boxplots of average nevirapine trough concentrations over time (14 to 99 days) for 200 mg and 400 mg group are presented in Figures 13 and 14, respectively.

Dose-Response/Blood Level Response:
Eleven patients in the 400 mg daily dose group had p24 antigen levels (Table 10) over at least six weeks of therapy. The applicant stresses that three of the four patients with the shortest duration of p24 suppression below 50% of their baseline had 3 of the 4 lowest steady state trough concentrations. However, this reviewer dose not see any association of lower trough concentration and lack of p24 suppression. Patient 1105 with the lowest mean Cmin had a response and Patients 120 and 127 had mean Cmin of 3.6 μg/mL; one showed activity and the other did not).

In conclusion, based on steady state conditions, the pharmacokinetics of 12.5 and 50 mg doses appear to be proportional and further, the pharmacokinetics of the 400 mg dose appear to be proportional to the 200 mg dose of nevirapine. Apparent oral clearance of the higher doses (200 and 400 mg) was double that of the lower doses (12.5 and 50 mg) and the accumulation ratio of the lower doses was 2 fold higher than the high dose. Results indicate that a 400 mg/day dose of nevirapine reduced ICD p24 antigen levels in 68% of the patients (13/19). Immunologic activity (increase in CD4+ counts) was not impressive for any of the doses tested. Although CD4+ and ICD p24 were the surrogate markers used at the time the study was conducted, recent trend is to monitor the viral load and genotype.

Title: An Open-Label, Staggered Rising Dose Cohort Study Assessing the Pharmacokinetics, Safety, and Tolerance of BI-RG-587 in Combination with Zidovudine in Patients with HIV Infection (CD4+ Cell Count <400 mm^3), (Study # 1100.834, Volume 90, Page 1)

The primary objectives of this study were: (1) Pharmacokinetics and dose proportionality of multiple doses of nevirapine in combination with zidovudine (ZDV); (2) To assess the safety and tolerance of multiple oral doses of nevirapine in combination with ZDV; (3) Effect of nevirapine on ZDV pharmacokinetics; (4) Plasma levels of nevirapine when co-administered with ZDV and to compare these levels with those obtained in study 1100.744 in which nevirapine was administered alone.

Secondary objectives were to obtain virologic and immunologic activity data on the combination of nevirapine and ZDV, and to examine whether resistance to either drug is slowed by using the combination therapy.

The study design was similar to study 744 (described earlier in the review), except patients were administered ZDV 600 mg/day (200 mg tid) and nevirapine (12.5, 50, 200, 400 or 600 mg/day) was added to their regimen on Day 7 of the study. All nevirapine doses (except 600 mg given as 300 mg bid) were administered once

daily. If toxicities associated with ZDV or nevirapine were reported in any patient, the relevant (ZDV or nevirapine) drug dose was reduced until toxicities resolved. Eighty three patients enrolled (10, 10, 20, 3, 22 and 18 patients in 12.5 mg/day, 50 mg/day, 200 mg/day, 400 mg/day, 400 mg/day after 200 mg lead-in dose for 2 weeks, 600 mg/day after 4 weeks of 200 mg/day lead-in dose, respectively) and 63 patients completed the study. Most patients were white (88%) males (93%).

Blood sample for pharmacokinetics determinations were drawn on Days -7, 0 and 14 (Day 0 refers to when nevirapine was added to the regimen), subsequently the protocol was amended to take blood samples only on Day -7 and 14 (or Day 14 after the lead-in dose).

Results:
Median changes from baseline of ICD p24 antigen and CD4+ counts are presented in Figures 15 and 16, respectively. The proportion of responders (p24 antigen levels, defined in study 744) in each dose level were 25%, 75%, 58%, 89% and 89% in 12.5, 50, 200, 400 and 600 mg daily dose, respectively. The proportion of responders based on 50 units increase in CD4+ counts ranged between 10% at lower doses to 28% at the 400 mg dose.

Nevirapine Pharmacokinetic Parameters:
Mean nevirapine concentration profiles for first dose and/or steady-state are presented in Figures 18 (panel A to E). Pharmacokinetic parameters are presented in the following tables:

Nevirapine pharmacokinetics following first dose

Dose (mg/day) Number of patients	Cmax1 µg/mL	Cmin1 µg/mL	Tmax1 (median) hours
12.5 (N=6)	0.17 ± 0.03	0.10 ± 0.04	1.5
50 (N=6)	0.53 ± 0.10	0.35 ± 0.06	2.8
200 (N=9)	1.89 ± 0.40	1.22 ± 0.22	2.5

Based on single dose data dose adjusted Cmin or Cmax values were not significantly different for 12.5, 50 and 200 mg doses. First dose mean nevirapine Cmax and Cmin observed in this study were comparable to those obtained in study 744 (Nevirapine administered alone), suggesting coadministration with ZDV did not significantly affect nevirapine pharmacokinetics.

The multiple dose pharmacokinetics were characterized on different days depending on the dose administered (because of lead in 200 mg/day for 400 and 600 mg/day dose). Mean study day of multiple dose pharmacokinetics was Day 14, 28 and 42 for doses of 200 mg, 400 mg and 600 mg/day, respectively.

Nevirapine pharmacokinetics following multiple doses:

Dose (mg/day) Number of patients	$Cmax_{ss}$ µg/mL	$Cmin_{ss}$ µg/mL (t = 0)	CL/F L/hr	CL/F (mL/kg/hr)	Accumulation Ratio
12.5 (N = 6)	0.5 ± 0.17	0.33 ± 0.10	1.51 ± 0.43	21.97 ± 4.57	3.16 ± 0.35
50 (N = 6)	1.50 ± 0.15	1.03 ± 0.13	1.79 ± 0.12	23.16 ± 1.86	2.92 ± 0.31
200 (N = 6)	4.01 ± 0.94	2.48 ± 0.86	3.22 ± 0.66	45.33 ± 12.86	1.83 ± 0.56
400 (N = 13)	6.91 ± 1.91	4.47 ± 0.79	3.64 ± 1.16	42.43 ± 14.98	
600 (N = 9)	8.42 ± 3.25	6.25 ± 2.32	4.16 ± 1.28	52.74 ± 17.68	

There were no statistically significant differences between pharmacokinetic parameters (dose corrected where appropriate) of lower doses (12.5 and 50 mg/day) and between higher doses (200, 400 and 600 mg/day). However, a significantly (about 2 fold) lower accumulation ratio and higher CL/F for high dose (200, 400 mg) were obtained compared to the low dose (12.5 and 50 mg) group. Further, the increase in mean CL/F was 2.5 fold for the 600 mg/day dose compared to low dose (12.5 and 50 mg). Apparent oral clearance adjusted for body weight is presented in Figure 18, this figure suggests that CL/F for daily doses of <2 mg/kg, <7mg/kg and >7 mg/kg were 2L/hr, 2 to 4 L/hr (for most subjects) and between 4 to 6 L/hr.

Nevirapine dose-adjusted Cmin, Cmax, and apparent oral clearance versus dose are presented in Figures 19-23 were consistent with the findings noted in the earlier study.

ZDV Pharmacokinetic Parameters:
Percent change in ZDV Cmax and AUC are presented in the following table:

Nevirapine Dose mg/day (Number of patients)	12.5 (N = 6)	50 mg (N = 6)	200 (N = 6)	400 mg (N = 8)	600 mg (N = 8)
Cmax(%)Median	24	2	19	0	30
Mean	23 ± 45	-8 ± 26	69 ± 133	2 ± 51	46 ± 94
Range	-41, 78	-44, 30	37, 316	-64, 74	-50, 185
p-value	0.270	0.495	0.258	0.907	0.208
AUC_{ss}(%)Median	-5	-21	5	-13	-13
Mean	5 ± 32	-17 ± 16	5 ± 23	3 ± 59	-8 ± 38
Range	-33, 57	-31, 13	-26, 36	-56, 128	-46, 63
p-value	0.705	0.048	0.630	0.888	0.557

The ZDV concentration time profiles taken alone and concomitantly with nevirapine are presented in Figure 24. The percent change in ZDV Cmax (Figure 25) was highly variable (range of median -2% to +30%) among treatment groups. The ZDV

AUC was significantly reduced in 50 mg treatment group and in other treatment groups no significant differences were noted. Review of individual data (Table 11) showed that following autoinduction by nevirapine, the percent change in ZDV AUC or Cmax was highly variable and at clinically relevant doses (200 - 400 mg/day) the mean change in AUC was -13% (range -56, 128).

Dose-Response/Blood Level Response:
Percent change in ICD-p24 plotted against dose is presented in Figures 26 and 27, dose dependent decrease in ICD-p24 was observed. Doses higher than 200 mg/day did not indicate any further decrease in ICD p-24 although variability at 600 mg/day dose was much lower compared to lower doses.

The concentration-effect relationship was assessed by the applicant by plotting (Figure 28) median steady state trough concentrations for each patient against change in p24 on Day 84 of the treatment. Applicant indicates that trough concentrations greater than 2.5 μg/mL may be needed to obtain a desirable decrease in ICD p24 antigen levels. However, some of the limitations of this analysis are: Very few patients in the lower dose level, other co-factors are not taken into consideration and ZDV concentrations are not taken into account.

In summary, addition of nevirapine 12.5, 200, 400 or 600 mg/day did not significantly affect ZDV AUC, while, addition of 50 mg/day significantly (-21%) reduced ZDV AUC. At clinically relevant doses of nevirapine, mean steady state ZDV pharmacokinetics (Cmax and AUC4) did not appear to be significantly affected. The nevirapine pharmacokinetic parameters were consistent with those observed in study 744. Nevirapine apparent oral clearance was about 2 fold greater at 200 - 400 mg/day dose and about 2.5 fold higher at 600 mg/day dose than at the lower doses. Further, the pharmacokinetics of higher doses 200 - 400 mg/day appear to be linear.

Title: Report on the First 28 Days of Trial 1100.1009: Evaluation of the pharmacokinetics interaction and safety/tolerance of nevirapine, zidovudine (ZDV), dideoxyinosine (ddi) and dideoxycytidine (ddc) in HIV-1 infected adults when nevirapine is given in combination with ddi, ZDV + ddi, and ZDV + ddc in the clinic of (Study # 1100.1009, volume 72, page 79)

The study objectives were (1) to characterize the effects of concurrent nevirapine administration at 200 mg q12h on steady state pharmacokinetics of zidovudine (ZDV), dideoxycytidine (ddc) and dideoxyinosine (ddi); (2) to assess safety and tolerance of nevirapine in combination with ddi, ZDV + ddi, or ZDV + ddc over a 24 week period; and (3) to evaluate the virologic and immunologic activity of the above therapeutic regimens. The pharmacokinetics were evaluated during the first 28 day period of the study and this report only covers that period (objective 3 is

not covered in this report).

This study was conducted in 24 patients with CD4+ cell counts ≤400 mm^3 and ≥50 mm^3. Six patients (power to detect 50% difference) each were assigned to the following four treatment groups (each treatment group received nevirapine (NEV) 100 mg q12h for the first two weeks followed by 200 q12h for 22 weeks):

A 200 to 300 mg ddi in divided doses q12h (NEV given concurrently)
B 200 to 300 mg ddi in divided doses q12h (NEV given 2 hours prior to ddi)
C 200 to 300 mg ddi q12h + 300 - 600 mg ZDV given tid + NEV given 2 hours prior to nucleoside administration.
D 0.375 to 0.75 mg ddc given tid, + 300 - 600 mg ZDV given tid + NEV given concurrently.

All patients were on these nucleoside regimen without any dose change, for at least 6 weeks prior to the study. On Day -1, blood samples were collected for measuring nucleoside concentrations, using validated assays. On day 0, 100 mg q12h of nevirapine was added and on Day 14, the nevirapine dose was increased to 200 q12h. On Day 28 blood samples were collected to evaluate effect of nevirapine, on steady state pharmacokinetics of nucleoside (comparing to Day -1, paired t-test).

Results:
The applicant had only reported the mean differences in AUC and Cmax. At the request of this reviewer, the applicant submitted (dated 5/13/96) the mean AUC, Cmax, percent differences and a plot comparing apparent oral clearance CL/F (L/hr and mL/kg/hr) in this study and other studies.

Pharmacokinetic parameters for individual subjects can be found in Tables 12-15. Mean parameters are summarized in the following table:

Nucleoside	AUC (ng*h/mL)				Cmax ng/mL			
	- Nev	+ Nev	% Change (range)	p-value	- Nev	+ Ne	% Change (range)	p-value
ZDV (+ DDC) (N=5)	729 ± 132	742 ± 289	0 (-29 to +36)	0.89	672 ± 218	589 ± 215	+4 (-77 to +97)	0.66
ZDV (+ DDI) (N=6)	1528 ± 361	1021 ± 458	-33 (-74 to -3)	0.03	1677 ± 743	1223 ± 712	-15 (-83 to +84)	0.30
DDI (N=18)	1964 ± 674	2150 ± 836	+15 (-65 to +75)	0.29	1398 ± 526	1461 ± 907	+9 (-52 to +263)	0.79
DDC (N=5)	36 ± 22	36 ± 19	+21 (-29 to +76)	0.96	12 ± 5	11 ± 4	+1 (-26 to +68)	0.09

Note that ZDV AUC and Cmax were much lower in group taking DDC compared to group taking DDI.

Effect of Staggered versus Concurrent Nevirapine on ddi:
Dideoxyinosine contains an alkaline buffering agent, p-values (two sample t-test) for AUC and Cmax for nevirapine indicate no significant differences. However, when nevirapine was taken 2 hours prior to DDI administration, mean nevirapine AUC and Cmax was 22% and 29% higher, respectively. The sponsor has only reported that the means were not significantly different without reporting the percent increase. The results indicate that taking nevirapine 2 hours prior to DDI may increase the bioavailability of nevirapine by about 20%.

Effect of Nevirapine on ddi PK:
No significant differences were found in the mean steady state ddi AUC (mean difference 187 ± 724 μg*h/mL) and Cmax (mean difference 62 ± 962 μg/mL). Individual data revealed that patients 1603 and 1610 had substantial increases in ddi concentrations.

Effect of Nevirapine on ddc PK:
No significant differences were found in the mean steady state ddc AUC (mean difference 4.2 ± 12.7 ng*h/mL) and Cmax (mean difference 0.45 ± 5.4 ng/mL). Individual data revealed that patient 1631 had substantial increases in ddc concentrations (although this patient had vomited on the day of treatment).

Effect of Nevirapine on ZDV PK:
(ZDV + ddc group):
No significant differences were found the in mean steady state ZDV AUC (mean difference 13 ± 209 ng*h/mL) and Cmax (mean difference -84 ± 394 ng/mL). Individual data revealed that patient 1631 had substantial increases in ZDV concentrations.

(ZDV + ddi group)
A significant decrease (32%, p=0.03) in the mean steady-state ZDV AUC (mean difference -507 ± 409 ng*h/mL) was noted in this treatment group. A statistically non significant decline in Cmax (percent decrease 27%, mean difference -454 ± 967 ng/mL) was observed. Individual data revealed that patients 1624 had a substantial increase in ZDV levels.

Effect of Triple Therapy on Nevirapine PK:
The applicant reports no significant differences (p=0.07, permutation t-test) in nevirapine clearance (CL/F) when administered with ddi+ZDV (2.32 ± 1.34 L/h) compared to when administered with ddc+ZDV (4.21 ± 2.22 L/h). However, the mean clearance in the ddc group is about twice that in the ddi group. The mean nevirapine clearance reported from historic data is about 3.5 to 4.0 L/hr for 400

mg per day doses of nevirapine. The nevirapine apparent oral clearance in the ddi group is about half of that observed in the other studies whereas, CL/F for the ddc group is comparable to the reported clearance. The clearance for individual subjects has been provided in Figures 29-30. Three patients had CL/F of 0.55, 1.15 and 1.39 producing Cminss of greater than 10 μg/mL who may benefit from reduction in nevirapine dose. These two groups (ddi and ddc) differed even in ZDV PK. The reason for this difference is unknown.

The results indicate that nevirapine clearance in the ddi group was much lower compared to that for the ddc group or to other studies. Reason for this decreased clearance is not known. It appears that patients taking ddi concomitantly may benefit from reduction in dose if they are experiencing adverse events related to nevirapine.

In conclusion, a 32% decline in ZDV AUC was noted in the group taking NEV + ddi + ZDV. Oral nevirapine clearance was about one half in the group taking ddi + NEV + ZDV. It appears that patients on ddi + ZDV + NEV triple therapy may benefit (reduced toxicity) by reducing nevirapine dose.

Nevirapine Sub-group Analyses:
Comparing nevirapine clearance for different group (see below) showed no significant differences (or trends) in clearance: Difference in CD4 counts, age (18 to 47 years), weight, prior nucleoside use, gender (F = 3), race (n = 2 blacks).

Title: Double Blind, Rising Single-Dose to investigate the Pharmacokinetics and Tolerance of Nevirapine (BIRG-587) following Intravenous Infusion to Normal Male Volunteers (Study # 1100. Volume 65, page 1)

This study was not reviewed (recommended dosing regimen in the labeling is 200 mg qd for two weeks and 200 mg bid oral administration after two weeks).

BIOAVAILABILITY/BIOEQUIVALENCE:
Title: Absolute Bioavailability of Nevirapine From Tablets and Solution Following Single-Dose Oral and Intravenous Administrations to Normal Adult Volunteers (Study # 1100.815, Volume 69, Page 50)

In a randomized , open-label, three way cross-over (3 week wash-out) study, the absolute bioavailability (15 mg i.v. infusion over 37.5 minutes) of nevirapine from tablets (50 mg) and solution (50 mg) was determined in 12 healthy male volunteers.

Results:
The mean plasma concentration time profiles are presented in Figure 31. Pharmacokinetic parameters for individual subjects are provided in Table 16. Mean pharmacokinetic parameters are summarized below:

Mean ± S.D.	Cmax ng/mL	AUC (0-inf) μg * h/mL	Tmax h	$T_{1/2}$ h	F - Absolute bio.	Compartmental Parameters I.V.	
50mg Sol.	554 ± 112	33.7 ± 7.1	1.4	48.3 ± 10.6	91.0 ± 8.3	CL mL/kg/h	$T_{1/2}\beta$
50mg Tablet	651 ± 119	34.5 ± 7.3	1.0	51.3 ± 11.0	93.0 ± 9.2	18.0 ± 4.5	52.8 ± 14.8

The mean absolute bioavailability of 50 mg solution or tablet doses of nevirapine was greater than 90% (range 77 to 112). Mean Vdss following i.v. infusion, oral solution and oral tablets was 1.34 ± 0.12, 1.33 ± 0.11 and 1.37 ± 0.14 L/kg, respectively (Table 16). The i.v. data were best described by a two compartment model (Boxenbaum F test) compared to a one compartment model. Mean Cmax and Tmax indicate that tablets had slightly better absorption compared to solution.

Title: Open Label, Randomized, Four-way Crossover Study to Assess the Relative Bioavailability of Nevirapine in Fifteen Healthy Male Volunteers (Study # 1100.896, Volume 63)

This was an open label, randomized, four-way crossover (14 day washout period) study in 15 healthy male (14 completed) volunteers to assess the relative bioavailability, pharmacokinetics and tolerance of nevirapine (four formulations): 100 mg of solution nevirapine powder 0.25 mg/mL in 1.25% citric acid solution; 100 mg of the pediatric suspension (5 mg/mL); and 100 mg doses of two different tablet formulations, 50 mg (2x50 mg) and 100 mg (1x100 mg).

Results:
Mean concentration time profile for the four formulations are provided in Figure 32. Individual pharmacokinetic parameters are provided in table 17 and the mean data are summarized below:

Formulation Mean ± SD	AUCinf ng*h/mL	Cmax ng/mL	Tmax h	Relative Bio	90% CI for AUC	90% CI for Cmax
Suspension 100 mg	74932 ± 23679	1184 ± 182	2.0 ± 2.9	1.01 ± 0.09	95.5, 106.3	71.0, 84.9
1*100 mg tablet	75462 ± 20670	1050 ± 203	3.0 ± 3.9	1.04 ± 0.17	97.3, 108.3	69.1, 82.6
2*50 mg tablet	71433 ± 19918	1103 ± 271	2.0 ± 2.2	0.98 ± 0.11	92.0, 102.4	66.3, 79.3
Solution 100 mg	71864 ± 17740	1498 ± 2432	1.0 ± 1.1	---	---	---

90% CI based on ln(AUC) and ln(Cmax).

The relative bioavailability of the suspension and two tablet formulations were all within 90-110% of the solution (Table above). There was no significant difference in Cmax among the suspension and tablet formulations. However, Cmax was significantly lower and mean Tmax higher for the suspension and two tablet formulations compared to the solution.

The mean apparent oral clearance (Cl/F), Vdss, $T_{1/2}$ and MRT were 18.98 ± 4.0 mL/kg/hr, 0.98 ± 0.12 L/kg, 45.3 ± 9.2 hr and 52.9 ± 7.7 h, respectively. No significant differences in Cl/F, Vss/F, $T_{1/2}$ or MRT between blacks (n=4) and white (n=9) volunteers were noted. No significant period effect was noted for CL/F, Vdss, $T_{1/2}$ and MRT.

Title: An In-Vivo Bioequivalence Assessment of Nevirapine 200 mg Tablets Manufactured in the Production facility of Boehringer Ingelheim Pharmaceuticals, Inc., Danbury, CT (Study # 1100.934, Volume 87)

The objectives of this study were to assess the bioequivalence of an NDA batch (NDA3; Lot PD-1462) of nevirapine 200 mg tablets relative to a clinical batch (Lot PD-1229) of 200 mg tablets; to assess the bioavailability of nevirapine tablets relative to an oral solution; and, to further assess safety and tolerance of single dose nevirapine 200 mg.

Twenty four (18 completed) healthy male volunteers received single doses of four treatments (wash out three weeks between treatments) in a random assignment to a 4X4 Latin Square design. Blood samples were collected for 168 hours following treatment for bioequivalence and bioavailability assessments. Following are the four treatments administered:

Treatment A: One 200 mg milled tablet (clinical batch)
Treatment B: One 200 mg milled tablet (NDA batch)
Treatment C: Four 50 mg unmilled tablets (clinical batch)
Treatment D: 400 mL of citric acid solution containing 200 mg nevirapine.

Results:
The mean plasma concentration time profiles are presented in Figure 33 and pharmacokinetic parameters for individuals are presented in Tables 18 - 21. Point estimates and 90% C.I are presented in the following table:

Parameter	Point estimate B/A	90% CI B/A	Point estimate A/D	90% CI A/D	Point estimate B/D	90% CI B/D	Point estimate C/D	90% CI C/D
AUC	0.96	0.93-1.00	1.08	1.04-1.11	1.03	1.00-1.07	1.03	0.99-1.07
Ln AUC	0.96	0.92-0.99	1.08	1.04-1.12	1.04	1.00-1.07	1.04	1.00-1.07
AUCinf	0.97	0.93-1.00	1.06	1.02-1.09	1.02	0.99-1.06	1.04	1.00-1.07
Ln AUCinf	0.96	0.93-0.99	1.06	1.02-1.09	1.02	0.98-1.05	1.03	1.00-1.07
Cmax	1.06	0.96-1.15	0.85	0.77-0.93	0.9	0.82-0.98	0.92	0.84-1.00
Ln Cmax	1.06	0.98-1.15	0.84	0.77-0.92	0.9	0.82-0.97	0.90	0.82-0.97
Tmax	0.39	--	2.93	--	1.03	--	1.19	

The data indicate that the 200 mg milled tablets (production lot) are bioequivalent (90% CI for Ln AUC and Ln Cmax within 0.80 to 1.25) to the 200 mg tablets used in the pivotal clinical trial. The results also indicate that oral tablet formulations were completely bioavailable relative to the solution formulation however, as would be expected the tablet formulations had a lower Cmax compared to solution. It should be noted that Tmax for the clinical trial 200 mg tablets was longer compared to other tablet formulations.

Effect of Food:

Title: Randomized, Open Label, Four Period, Three Treatment Crossover Study to Assess the Effects of Food or Antacid on the Bioavailability of Nevirapine in Normal Adult Volunteers (Study # 1100.1055, Volume 75)

The objectives were to: (1) determine the effect of concurrent administration of antacid (Maalox) or food (high fat) on the bioavailability of nevirapine; (2) assess the effects of gender on nevirapine pharmacokinetics and (3) further assess safety/tolerance of nevirapine 200 mg single dose.

Twenty four (12M, 12F) healthy volunteers received three treatments: A = 200 mg nevirapine fasting; B = 200 mg nevirapine following high fat meal; C = 200 mg

nevirapine followed by 20 mL Maalox. The sequence for crossover administration (n=4 per group, three week washout period) was ABCA, BCAB, CABA, CBAC, BACB, ACBA.

Results:
The mean plasma concentration time profiles are presented in Figure 34 and pharmacokinetic parameters for individuals are presented in Tables 23 - 28.

The mean pharmacokinetic parameters are presented in the following table:

Mean ± SD	Fasted	Fed	Antacid
AUCinf µg*h/mL Mean 90% CI P-value	134.6 ± 32.7	140.5 ± 32.4 98.5, 108.2 0.201	140.8 ± 42.1 99.0, 108.7 0.148
AUC0-t µg*h/mL Mean 90% CI P-value	121.1 ± 24.7	124.7 ± 21.9 96.9, 105.1 0.246	123.9 ± 30.1 96.1, 104.3 0.426
Cmax µg/mL Mean 90% CI P-value	2.1 ± 0.7	1.9 ± 0.3 86.5, 104.5 0.329	1.7 ± 0.3 75.0, 93.0 < 0.01
Tmax L Mean ± SEM Range P-value	4.4 ± 1.0 1 - 10	6.3 ± 1.0 1 - 24 < 0.01	8.4 ± 1.0 2 - 24 < 0.01

The results indicate that both food and antacid significantly affect the rate (Tmax) of absorption and further, antacid significantly reduces the nevirapine Cmax. However, extent (AUC) of bioavailability is not affected by either food or antacid.

As shown in the following table, gender does not have any significant effect on CL/F, $T_{1/2}$ and MRT. Vdss was statistically significantly reduced in males however, the mean difference was about 10%.

Mean ± SD (P-Value)	Females (N = 12)	Males (N = 11)	All
CL/F L/h	1.48 ± 0.31 (0.45)	1.68 ± 0.45	1.57 ± 0.39
CL/F mL/kg/h	23.0 ± 5.5 (0.61)	22.0 ± 5.1	22.5 ± 5.2
MRT h	73.0 ± 15.7 (0.41)	68.1 ± 12.6	70.6 ± 14.2
T1/2 h (harmonic Mean)	47.3 (0.61)	44.6	45.9
Vdss L/kg	1.60 ± 0.18 (0.04)	1.45 ± 0.11	1.52 ± 0.17

In conclusion, food and antacid significantly affect rate of absorption of a 200 mg single dose of nevirapine without significantly affecting the extent. Since extent of bioavailability is not significantly affected by food or antacid no dose adjustments are needed. Gender does not significantly affect the pharmacokinetics of a single dose 200 mg nevirapine. There was one reported spontaneous abortion which was not attributed to the drug by the investigators.

PEDIATRICS:

Title: A single Rising Dose Pharmacokinetic Phase I Evaluation of Nevirapine Suspension by Oral Administration in Children with HIV-1 Infection (Study 0853, Study # 1100.853, Volume 56, Page 61)

The objectives of the study were (1) To generate initial information on the pharmacokinetics and dose proportionality in HIV-infected children; (2) To assess the safety and tolerance of single rising doses of nevirapine in HIV infected children; (3) To confirm that single doses which achieve trough plasma levels of about 10-30 X IC_{50} and 100-200 X IC_{50} in adults achieve similar trough levels in HIV-infected children and to determine a preliminary dosing interval and dose escalation schedule for a multiple dose clinical study.

Nine HIV-1 positive children (9 mo. to 14 years) were administered 7.5, 30, or 120 mg/m^2 (N = 3 for each dose) of nevirapine as pediatric suspension in a parallel design study.

Results:
The mean plasma concentration time profiles are presented in Figure 35. The pharmacokinetic parameters obtained are summarized in the following table:

Mean ± SD	7.5 mg/m² Dose (N = 3)	30 mg/m² Dose (N = 3)	120 mg/m² Dose (N = 3)	Overall Combined for all doses
Cmax ng/mL	265 ± 70	724 ± 169	2870 ± 230	
X - IC_{50} **	25	68	271	
Tmax (median h)	2	2	4	
AUC ng*h/mL	9151 ± 1687	30233 ± 5912	135137 ± 4214	
CL/F mL/Kg/h	33.5 ± 9.4	40.6 ± 13.2	36.3 ± 2.3	36.8 ± 10.5
Half-Life (h) Range	18 to 49	16 to 39	27 to 24	24.8 (harmonic Mean)

** - Cmax expressed as multiple of in-vitro IC_{50}

Looking at the relationship between dose and Cmax and AUC, the pharmacokinetics appear to be dose proportional (Figure 36 and 37). Mean apparent clearance and half-life appear to be similar for all three doses.

The nevirapine clearance decreases with increasing age (figure 5 and 6 of the report). Age related clearance was also reflected by correlation between age and half-life (Figures 38). One 9 month old subject who had active hepatitis during the trial had a longer half-life (35.5 hours) . Apparent clearance adjusted for body weight correlated well with age and was about 42.64 mL/kg/hr for under 6 year old children compared to 29.5 for children between the ages of 7 to 14.

Comparison of pharmacokinetic parameters (single dose data) between HIV infected children and adults (Figures 39 and 40) reveal that children had higher clearance (36.8 ± 10.5 versus 18.9 ± 4.0 in adults) and shorter half-life (24.8 versus 45.3 hours). However, since it is known that nevirapine undergoes autoinduction, clinical relevance of single dose data in pediatric patients is unknown. Further, pharmacokinetic data in pediatric patients is limited to 3 patients at each dose level.

In conclusion, doses of 7.5 to 120 mg/m² appear to be dose proportional. Apparent nevirapine clearance appears to decrease with age. Assuming similar bioavailability in children and adults (pediatric suspension was bioequivalent to tablets in adults), children appear to have higher clearance and lower AUC and half-life. Since nevirapine undergoes autoinduction, clinical relevance of single dose data is difficult to interpret and is unknown.

SUBPOPULATION ANALYSIS:

Nevirapine steady state trough concentrations were monitored in studies 1100.744, 1100.834, 1100.1037, 1100.1031 (ACTG 241). The individual

patient median Cminss (N = 387 patients) was utilized for determination of the relationship of Cminss to factors such as demographics (age, weight, gender, ethnicity) and disease state (HIV classification, history of Hepatitis B). Results are summarized in Figures 41 to 55 and the following table summarizes the data:

Mean ± SD of Median Cminss computed by the applicant: The numbers reported in the body of the report were slightly different than what was found in the SAS output. The following table has been compiled from the data found in the SAS output:

Variable Doses reported are mg/day	Median Cminss
200 mg dose (N = 127)	2.84 ± 1.33
400 mg dose (N = 242)	4.47 ± 1.86
600 mg dose (N = 18)	5.90 ± 1.96
Trial 744, 400 mg N = 18	4.06 ± 1.71
Trial 834, 400 mg N = 25	4.13 ± 1.70
Trial 1031, 400 mg N = 156	4.6 ± 1.95
Trial 1037, 400 mg N = 43	4.34 ± 1.68
Females, 400 mg, N = 37	4.98 ± 2.97
Male, 400 mg, N = 205	4.38 ± 1.58
Blacks, 400 mg, N = 27	5.53 ± 3.31
Hispanics, 400 mg, N = 24	3.90 ± 1.76
Whites, 400 mg, N = 189	4.40 ± 1.53
AIDS or ARC, 400 mg, N = 64	4.58 ± 1.83
HIV Asymptomatic, 400 mg, N = 178	4.43 ± 1.88
No Hepatitis B, 400 mg, N = 196	4.50 ± 1.87
Yes Hepatitis B, 400 mg, N = 46	4.33 ± 1.84

Dose Proportionality: Median Cminss for 200, 400 and 600 mg/day were 2.84 ± 1.33 μg/mL (N = 127), 4.46 ± 1.86 μg/mL (N = 242), and 5.9 ± 1.95 μg/mL (N = 18), respectively. The median Cminss appears to increase linearly.

None of the nevirapine treatment regimens in combination with ZDV (n = 185) or ZDV + ddI (n = 176) had an effect on the nevirapine Cminss compared to nevirapine monotherapy (n = 26). However, in study 1009 decreased steady state apparent clearance was noted for patients receiving ddi as compared to that of monotherapy. This finding questions the validity of using median Cminss to look for differences in pharmacokinetics.

No trend in increased or decreased median Cminss was noted based on age, gender, weight, ethnicity baseline disease factors (AIDS, ARC, Hepatitis B). However, as discussed earlier in the review, the single dose pharmacokinetics of nevirapine in pediatric patients differ between children and adults. This is possibly due to metabolic differences (hepatic metabolic systems are more active in younger children than in adults) and not due to formulation differences (suspension and tablets were equally bioavailable in adults).

Subpopulation Analysis for Drug Interactions:
(Fax submission 5/10/96)

Median Cminss (n = 311) from studies 1100.744, 1100.834, 1100.1010, 1100.1011, 1100.854, 1100.1037 and 1100.1031 (ACTG 241) were used for assessment of drug interactions. Boxplots (Figures 56 to 59) compare median Cminss for all the patients to the median Cminss for patients who were also concomitantly taking rifampin (n = 2), rifabutin (n = 18, Figure 56), cimetidine (n = 12), ranitidine (n = 18, Figure 57), erythromycin (n = 3), clarithromycin (n = 14), azithromycin (n = 7, Figure 58), ketoconazole (n = 11, Figure 59).

It can be seen that cimetidine and macrolides, known inhibitors, elevated nevirapine median Cminss whereas, rifampin and rifabutin, know inducers of CYP3A slightly lowered median Cminss compared to overall median Cminss. It appears that cimetidine, macrolides and rifampin would have a significant interaction because most of the median Cminss in these groups were outside the range of 75% (upper or lower limit of the boxplots) of Cminss for the overall patients.

Based on the finding that Cminss comparisons for patients taking ddI did not appear to be different (to overall patient population, Figure 43); whereas, the mean nevirapine apparent clearance (Figure 30) for the group taking ddI was reduced to 50%. This finding indicates that the reliability of Cminss as a surrogate marker for interactions is questionable. Median Cminss may give a preliminary indication of interaction, but the extent of drug interaction cannot be based on comparing the median Cminss. Further, nevirapine's effect on the metabolism of other drugs is unknown.

In vitro Drug Interaction Studies

This section describes assessment of in vitro interaction between nevirapine and commonly used medications in patients with the HIV-1 infection: dapsone, rifabutin, rifampin, ketoconazole, trimethoprim, sulfamethoxazole

Nevirapine is metabolized in vitro to 2-hydroxy-nevirapine (2HNVP),

3-hydroxy-nevirapine (3HNVP), 8-hydroxy-nevirapine (8HNVP), and hydroxymethyl-nevirapine (HMNVP), hereafter referred to as 12-hydroxy-nevirapine (12HNVP). 2HNVP and 12HNVP were the major metabolites. 8HNVP, a minor metabolite, was not detected in all of the analyses and, therefore, was not included in the applicant's data analysis for all the in-vitro drug interaction studied.

Title: Potential for Metabolic Drug Interaction with Nevirapine as Determined in vitro with Human-Liver Microsomes (Dapsone, Report # U95-3023, Volume 70): The potential for drug interactions between nevirapine (0, 25 and 100 μM) and dapsone (0, 0.8 and 8 μM) was examined in vitro using human liver microsomes.

The rates of formation of 2HNVP, 12HNVP, and 3HNVP in the 25 μM nevirapine incubations containing no dapsone were 1.0, 1.3, and 0.2 picomoles/minute/milligram of microsomal protein, respectively; in the 100 μM nevirapine incubates with no dapsone, the rates of formation were 5.2, 6.2, and 0.5 picomoles/minute/milligram of microsomal protein, respectively. Adding 0.8 or 8.0 μM dapsone to the incubations with nevirapine gave similar rates (Table 29 and 30) of metabolic formation which were not statistically significant.

In Conclusion, the data suggests that the drug interactions between nevirapine and dapsone may be less likely in patients receiving these concomitant medications. However, the effect of nevirapine on dapsone is unknown.

Title: Potential for Metabolic Drug Interaction with Nevirapine as Determined in vitro with Human-Liver Microsomes (Rifabutin, Report # U95 3150), Volume 70):

The primary objective of this study was to determine the effect of rifabutin on the in vitro metabolism of nevirapine by human liver microsomes. As a secondary objective, the effect of nevirapine on the metabolism of rifabutin in these incubations was monitored. The potential for drug interactions between nevirapine (0, 25 and 100 μM) and Rifabutin (0, 0.6 and 6 μM) was examined in vitro using human liver microsomes.

The rates of formation of 2HNVP, 12HNVP, and 3HNVP in the 25 μM nevirapine incubations containing no rifabutin were 1.23, 1.23 and 0.369 picomoles/minute/milligram of microsomal protein, respectively; in the 100 μM nevirapine incubates with no rifabutin, the rates of formation were 5.85, 5.43 and 1.68 picomoles/minute/milligram of microsomal protein, respectively.

Statistically significant reductions in the rates of nevirapine metabolite formation were observed in the presence of rifabutin (Tables 31 and 32). The highest (31 %) reduction was observed in the rate of 2HNVP formation by 6.0 μM of rifabutin. The apparent Ki's of rifabutin on the in vitro formation of the two major metabolites, 2HNVP and 12HNVP, by human-liver microsomes were 22 and 44

μM, respectively. The applicant indicates that these concentrations are well above the concentration of rifabutin that would be expected to occur at the site of metabolism in the liver (about 6.0 μM). 8HNVP, a minor metabolite, was not included in the data analysis due to an analytical problem (co-eluting peak in the chromatogram).

As a secondary objective, the effect of nevirapine 25 or 100 μM concentrations on rifabutin metabolism was studied in the incubations containing either 0.6 or 6.0 μM rifabutin. A trend of dose-dependent inhibition of rifabutin by nevirapine and a statistically significant reduction in rate of metabolism of 0.6 μM rifabutin in presence of 100 μM nevirapine was observed. The apparent Ki of nevirapine for the inhibition of rifabutin metabolism by human liver microsomes was 26 μM.

In summary, 6.0 μM rifabutin inhibited the rate of metabolism of 12HNVP and 2HNVP by 25% and 31%, respectively in 25 and 100 μM nevirapine incubations, respectively. Inhibition of HMNVP or 3HNVP was less than 20%. Apparent Ki values for the inhibitions were 22 and 44 μM for 2HNVP and 12HNVP, respectively. A trend of dose-dependent inhibition of rifabutin by nevirapine was noted. Nevirapine had an apparent Ki of 26 μM for the inhibition of rifabutin metabolism in human liver microsomes. This value is about the plasma Cmax achieved in patients receiving nevirapine. Based on the in-vitro data, rifabutin inhibition by nevirapine is more likely than inhibition of nevirapine by rifabutin.

Title: Potential for Metabolic Drug Interaction with Nevirapine as Determined in vitro with Human-Liver Microsomes (Rifampin, Report # U95-3024, Volume 70):

The objective of this study was to determine the effect of rifampin on the in vitro metabolism of nevirapine by human liver microsomes. The potential for drug interactions between nevirapine (0, 25 and 100 μM) and rifampin (0, 10 and 30 μM) was examined in vitro using human liver microsomes.

The rates of formation of 2HNVP, HMNVP, 3HNVP and 8HNVP in the 25 μM nevirapine incubations containing no rifampin were 1.76, 1.37, 1.24 and 0.267 picomoles/minute/milligram of microsomal protein, respectively; in the 100 μM nevirapine incubates with no rifampin, the rates of formation were 10.48, 6.22, 4.91 and 1.34 picomoles/minute/milligram of microsomal protein, respectively (Tables 33 and 34). 2HNVP formation was significantly reduced (19%) in the incubation containing 30 μM rifampin and 25 μM nevirapine. An other difference, although not statistically significant, was a reduction by 21% in the formation of 2HNVP (incubation of 30 μM rifampin and 100 μM nevirapine). The apparent Ki for this reduction was 120 μM which is above the reported Cmax (30 μM) of rifabutin.

Inhibition of nevirapine metabolism to 2HNVP by rifampin is possible for the

patients taking these two drugs concomitantly. Further, the effect of nevirapine on rifam[illegible] [illegible]nknown.

T[illegible]ial for Metabolic Drug Interaction with Nevirapine as Determined in vit[illegible] [illegible] Human-Liver Microsomes (Ketoco[illegible]azole, Report # U95 3011, Volume 70):

The objective of this study was to determine the effect of ketoconazole on the in vitro metabolism of nevirapine by human liver microsomes. The potential for drug interactions between nevirapine (0, 25 and 100 μM) and ketoconazole (0.1, 1.0 and 10 μM) was examined in vitro using human liver microsomes.

The rates of formation of 2HNVP, HMNVP, 3HNVP and 8HNVP in the 25 μM nevirapine incubations containing no ketoconazole were 0.66, 1.46, 0.12 and 0.08 picomoles/minute/milligram of microsomal protein, respectively (Tables 35 and 36); in the 100 μM nevirapine incubates with no rifampin, the rates of formation were 5.7, 6.2, 0.56 and 0.25 picomoles/minute/milligram of microsomal protein, respectively (Tables 35 and 36). In the presence of 1.0 μM ketoconazole and 100 μM nevirapine, the rates of formation of 2HNVP, HMNVP and 3HNVP were inhibited by 70%, 53% and 38%, respectively. Whereas, in 25 μM nevirapine and 0.1 μM ketoconazole the rates of formation were inhibited by 36%, 21%, and 50%, respectively. The apparent Ki's for 2HNVP, HMNVP and 3HNVP were 0.8, 3.3 and 0.4 μM, respectively. 8HNVP, a minor metabolite, was detected in the incubation with no ketoconazole however, in other incubations it was not detectable.

In summary, the in-vitro rate of metabolism of nevirapine was reduced for all the four metabolites. The apparent Ki's of ketoconazole for inhibition are within the range of expected concentrations of 200 mg daily dose of ketoconazole. Results indicate that there is a significant potential of drug interaction when these two drug are taken concomitantly by the patients. It should also be noted that effect of nevirapine on the in-vitro metabolism of ketoconazole is unknown.

Title: Potential for Metabolic Drug Interaction with Nevirapine as Determined in vitro with Human-Liver Microsomes (Trimethoprim, Report # U95-3060, Volume 75):

The objective of this study was to determine the effect of trimethoprim on the in vitro metabolism of nevirapine by human liver microsomes. The Secondary objective was, to monitor the effect of nevirapine on the in vitro metabolism of trimethoprim in these incubations. The potential for drug interactions between nevirapine (0, 25 and 100 μM) and trimethoprim (0, 25, 100 μM) was examined in vitro using human liver microsomes.

The rates of metabolite formation are provided in Tables 37 to 39. The rates of formation of 2HNVP, HMNVP, 3HNVP and 8HNVP in the 25 μM nevirapine incubations containing no trimethoprim were 1.49, 1.21, 0.6 and 0.34, respectively picomoles/minute/milligram of microsomal protein, respectively; in the 100 μM nevirapine incubates with no trimethoprim, the rates of formation were 8.89, 6.66, 2.45 and 1.33 picomoles/minute/milligram of microsomal protein, respectively.

Statistically significant increases (about 3 and 1.5 fold in presence of 25 and 100 μM nevirapine, respectively) in the rates of 3HNVP formation were observed in the presence of 100 μM trimethoprim. Whereas, the presence of 100μM trimethoprim produced a 20% reduction in the rate of formation of 8HNVP in the 100 μM nevirapine incubations. The applicant reports (data not shown in the report) apparent Ki's of trimethoprim for the formation of 2HNVP, HMNVP and 8HNVP were 384, 305 and 153 μM, respectively and further indicate that these concentrations are well above that would be above expected in patients.

The addition of nevirapine to the incubations of trimethoprim had no statistically significant effect on the rates of formation of trimethoprim metabolites. It should be noted that trimethoprim metabolites were not identified, but were designated as M1, M2 and M3. Based on no effect on the rates of formation of M1, M2 and M3, it appears that nevirapine is not likely to affect trimethoprim metabolism.

In summary, 100 μM trimethoprim inhibited the rate of metabolism of 8HNVP by 20% in 100 μM nevirapine incubations and increased the rate of formation of 3HNVP by about 3 and 1.5 fold in the 25 and 100 μM nevirapine incubations, respectively. The presence of nevirapine had no statistically significant effect on the production of trimethoprim metabolites. The in-vitro data suggests that drug interaction between trimethoprim and nevirapine are less likely.

Title: Potential for Metabolic Drug Interaction with Nevirapine as Determined in vitro with Human-Liver Microsomes (Sulfamethoxazole, report # U95-3081, Volume 75):

The objective of this study was to determine the effect of sulfamethoxazole on the in vitro metabolism of nevirapine by human liver microsomes. Secondarily, the effect of nevirapine on the in vitro metabolism of 1.25 nM sulfamethoxazole to sulfamethoxazole hydroxylamine (HA-SMX) in theses incubations was monitored. The potential for drug interactions between nevirapine (0, 25 and 100 μM) and sulfamethoxazole (0, 0.2, 125 mM) was examined in vitro using human liver microsomes.

The rates of metabolite formation are provided in Tables 40 to 42. The rates of formation of 2HNVP, HMNVP, 3HNVP and 8HNVP in the 25 μM nevirapine

incubations containing no sulfamethoxazole were 1.045, 0.992, 0.363 and 0,129 picomoles/minute/milligram of microsomal protein, respectively; in the 100 μM nevirapine incubates with no sulfamethoxazole, the rates of formation were 5.679, 4.663, 1.456 and 0,800 picomoles/minute/milligram of microsomal protein, respectively. The addition of 0.2 or 1.25 mM sulfamethoxazole resulted in statistically significant reductions in the formation rates of 2HNVP, HMNVP and 8HNVP with greatest being 80% reduction in 8HNVP in the presence of 1.25 nM sulfamethoxazole. The formation rate of 3HNVP was only reduced by 1.25 mM sulfamethoxazole. The apparent Ki's of sulfamethoxazole for the in vitro formation of 2HNVP, HMNVP, 3HNVP and 8HNVP were reported to be 2.92, 1.42, 6.63 and 0.19 nM, respectively (data not shown by the applicant). The applicant reports, that these with the exception of ki for 3HNVP, ki values are within those reported in plasma or serum of patients that were treated with trimethoprim and sulfamethoxazole. The data suggest that sulfamethoxazole may inhibit metabolism of nevirapine when these drugs are taken concomitantly.

The effect of nevirapine on the in vitro metabolism of sulfamethoxazole HA-SMX was studied indirectly by monitoring the presence of nitro-sulfamethoxazole (NO2-SMX) in the extract of the microsomal incubations. The HA-SMX was converted to NO2-SMX with 90% efficiency during the extraction process. Based on the areas of the NO2-SMX peaks in the chromatogram, the presence of nevirapine 25 or 100 μM concentrations had no statistically significant effect on the appearance of NO2-SMX.

In summary, the presence of 0.2 and 1.25 mM sulfamethoxazole decreased the rate of formation of all nevirapine metabolites, with the exception that the rate of formation of 3HNVP was not significantly affected at 0.2 mM. Ki values obtained for all (except 3HNVP) nevirapine metabolites were within the range of plasma or serum concentrations expected in AIDS patients receiving trimethoprim-sulfamethoxazole Based on in-vitro data sulfamethoxazole has the potential to inhibit the formation of all nevirapine hydroxylated metabolites. The presence of nevirapine did not appear to affect the in vitro metabolism of sulfamethoxazole.

ANALYTICAL METHODS:

Analytical methods utilized for determination of nevirapine concentrations were reviewed and found to have acceptable variability. Details of analytical methods will be included prior to finalization of review. methods were used for ddi, AZT and ddc determinations.

DISSOLUTION:

The applicant had only submitted dissolution using phosphate buffer at pH2.0. At the request of this reviewer the applicant submitted dissolution data in water and 0.1N HCl. Nevirapine has better solubility at lower pH, the dissolution is much faster when using 0.1N HCL as the dissolution media. The applicant has requested

phosphate buffer at pH of 2.0 be used as dissolution media, as it gives better discrimination ability. The dissolution method and specifications proposed by the applicant are acceptable.

PHARMACOLOGIST'S REVIEW

NDA: 20-636
Date Submitted: November 0, 1995
Date Assigned: November 15, 1995
Date Review Completed: January 26, 1996
Assigned Reviewer: Pritam S. Verma, Ph.D.
HFD-530

SPONSOR: Boehringer Ingelheim Pharmaceuticals, Inc.
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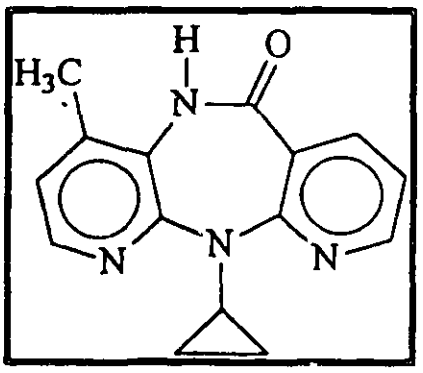
DRUG: VIRAMUNE[R]



USAN Names: Nevirapine
Code Name: BIRG 587 BS
Other Names: NVP, BIRG 587
Chemical Name (USAN): A: 6H-Dipyrido [3,2-b:2'3'-e][1,4]diazepin-6-one, 11-cyclopropyl-5,11-dihydro-4-methyl
B: 11-Cyclopropyl-5,11-dihydro-4-methyl -6H-dipyrido[3,2-b:2'3'-e][1,4] diazepin-6-one

CAS Registry:	129618-40-2
Molecular Formula:	$C_{15}H_{14}N_4O$
Molecular Weight:	266.31
Solubility:	0.1 mg/ml in water at 25°C
Melting Point:	247 - 249°C
Physical Description:	an off-white crystalline powder
pKa:	2.9

INDICATION: Combination of Nevirapine with ddI and ZDV in previously treated HIV patients for whom current therapy is deemed inadequate.

RELATED IND:

ROUTE OF ADMINISTRATION: Oral

PROPOSED TREATMENT REGIMEN: 400 mg/day

INTRODUCTION:

Viramune® (nevirapine) represents a class of non-nucleoside compounds that inhibits reverse transcriptase (RT) activity of human immunodeficiency virus-type 1 (HIV-1). Nevirapine was chosen from a series of dipyridodiazepinone inhibitors of HIV-1 RT which were identified from a synthetic program of muscarinic receptor antagonists by random screening. Nevirapine does not inhibit human DNA polymerases α, ß, δ or γ to any great extent. The compound is now in development as a therapeutic agent for the treatment of HIV-1 infection in various patient populations. Tentatively, this NDA is for nevirapine as a non-competitive inhibitor for HIV-1 RT in combination with ddI and ZDV in previously treated HIV patients for whom current therapy is deemed inadequate. Presently, the sponsor has submitted a Pre-NDA Submission of the NONCLINICAL PHARMACOLOGY AND TOXICOLOGY TECHNICAL SECTION of this NDA.

BACKGROUND:

Nevirapine was characterized adequately in nonclinical toxicological studies carried out during the course of its development. Acute oral toxicity studies conducted in mouse, rat, dog and monkey revealed that the No-Observable-Effect-Level (NOEL) was in the range of 100 to 200 mg/kg. These studies indicated that the sensitivity of the species tested was: rat > dog > mouse > monkey. In the chronic one-year rat and dog studies, principal target organs included liver, esophagus, intestines, bone marrow, lymph nodes, spleen, thymus, thyroid, adrenal, tonsils, testes, epididymides, prostate and skin. In the dog study, the NOEL for nevirapine was considered to be 50 mg/kg/day and the maximum tolerated dose was 200 mg/kg/day.

Nevirapine was adequately absorbed following oral administration, and systemic bioavailability ranged from 30% in dogs to 75% in chimpanzees. Nevirapine was cleared by hepatic oxidative metabolism to metabolites hydroxylated in the 2-,3-,8- and 12 positions, which underwent phase II metabolism to the corresponding glucuronides in most species. In addition, 12-hydroxynevirapine was further oxidized to form 4-carboxynevirapine. The pathways of biotransformation in animals reflected those occurring in humans. The rate at which nevirapine was cleared from the systemic circulation varied with the species and was ranked in the following order: mouse > dog > monkey > chimpanzee. Nevirapine caused autoinduction of liver metabolizing enzymes, therefore the potential exists in humans for drug interactions with concomitant medications which were metabolized by the same cytochrome(s) P450 as nevirapine. Nevirapine also crossed the placental barrier and was excreted into breast milk, and therefore the fetus and nursing baby would be exposed to

nevirapine in mothers taking the drug.

SUMMARY:

The review of the individual nonclinical toxicity, pharmacokinetics and general pharmacology studies can be found in Appendix # 1.

Acute toxicity studies: results and equivalent dose in humans are summarized in Appendix # 2 (Table 1). The acute studies were conducted in mice, rats and dogs. The approximate oral LD_{50} of nevirapine was shown to be > 2000 mg/kg in mice, > 500 mg/kg in rats and > 3200 in dogs. The NOELs were shown to be 200 mg/kg (male) and 400 mg/kg (female) in rats, 100 mg/kg (female) and 200 mg/kg (male) in mice, and 150 mg/kg in dogs.

Multiple-dose toxicity studies: results and comparison of animal NOELs with the human therapeutic dose are summarized in Appendix # 2 (Table 2 & 3) and Appendix # 6 (Table 1), respectively. Mice: in a 13-week diet study, nevirapine induced target organ toxicity in the liver, thyroid gland, hematopoietic-reticuloendothelial system, heart, intestine and kidney. Based on clinical signs and microscopic finding, the MTD was 1500 mg/kg/day. The NOEL was 50 mg/kg/day. Based on a body surface area conversion factor, an equivalent dose in humans would be 16.7 mg/kg/day. Rats: in a series of 4 separate studies, nevirapine was orally administered to rats in doses ranging between 1-150 mg/kg/day for a period of 4-13 weeks. Doses of > 50 mg/kg/day produced signs of toxicity that were dose-related in severity, were generally more severe in females than males, and induced liver enzymes. Liver and thyroid changes observed were consistent with altered T4 and TSH levels secondary to hepatic enzyme induction. The major effects included: decreased motor activity, lacrimation, skin lesions, decreased food consumption and body weights; decreased erythrocyte parameters, reticulocytosis and increased APTT; increased serum cholesterol, GGT levels and urine proteins; and hepatocellular, thyroid-follicular and adrenal cortical hypertrophy. Doses of > 100 mg/kg/day caused mortality in female rats and were associated with pronounced gross pathology and histopathology. The NOELs ranged between 5-10 mg/kg/day in these studies. Based on a body surface area conversion factor, an equivalent doses in humans would be 0.71-1.42 mg/kg/day. 52-week rat toxicity study: clinical signs of toxicity included skin lesions with scratching and biting. A few rats were killed *in extremis*. Changes in clinical pathological parameters included reduced erythrocyte parameters, WBC, TPT values and dose-dependent increases in cholesterol, AST, ALT, BUN, creatinine and proteinuria. Histopathological changes included ulcerative dermatitis accompanied by glossitis, hepatocellular hypertrophy, thyroid follicular cell hypertrophy and hyperplasia, thymic

atrophy and adrenal cortical hypertrophy. The NOEL for male rats was 5 mg/kg/day, a NOEL was not identified for female rats. Dogs: in 3 separate studies (2-wk, 4-wk & 13-wk), clinical signs of toxicity included sedation, reduced activity, ataxia and increased body temperatures. The severity of the changes tended to increase with dose, and at 650-800 mg/kg/day (4-wk) decreases in erythrocyte parameters were also evident. Changes in clinical pathology parameters included relative lymphopenia, decreased in serum potassium, increased in cholesterol, triglycerides, calcium and AP levels. Histopathological changes included bronchopneumonia, lymphoid depletion/necrosis and deceased spermatogenesis, and epithelial atrophy in the prostrate and epididymides at 700 mg/kg/day level (13-wk). The NOELs ranged between 20-200 mg/kg/day. Based on a body surface area conversion factor, an equivalent doses in humans would be 10-100 mg/kg/day. The approximate MTD was 650 mg/kg/day in 4-week study. 52-week dog toxicity study: clinical signs of toxicity directly related to nevirapine administration included deceased motor activity, tremors, ataxia, ptosis, emaciation, absence of gum (gingiva) capillary refill time, distended abdomen, third eyelid eversion, dehydration, yellow sclera and gums, and labored respiration. The principal target organs were liver, esophagus, intestines, bone marrow, lymph nodes, spleen, thymus, tonsil, testes, epididymides and prostate. The NOEL was 50 mg/kg/day. Based on a body surface area conversion factor, an equivalent dose in humans would be 25 mg/kg/day.

Special toxicity studies: results are summarized in Appendix # 2 (Table 4). Several special toxicity studies were conducted in rats,rabbits, guinea pigs and dogs. Increases in thyroid gland weight, associated with follicular cell hypertrophy and hyperplasia in rats was shown to be caused by increased circulatory TSH levels that were compensatory to increased metabolism and elimination of T4, which in turn was the result of hepatic microsomal enzyme induction. Results from six irritancy studies in rabbits indicated that nevirapine was not a dermal irritant. Nevirapine produced slight conjunctival irritation (with complete recovery within 72 hr) when applied to ocular tissues. Nevirapine was not found to be a dermal sensitizing agent in guinea pigs.

Reproductive and developmental toxicity studies: results and equivalent dose in humans are summarized in Appendix # 2 (Table 5). Segment I study in rats: signs of parental toxicity occurred at doses of 50 mg/kg/day and included reduced body weight gain, lethargy, sedation and scratching/scabby dermal lesions. Doses of < 50 mg/kg/day in males and < 25 mg/kg/day in females did not affect estrous cycles or significantly reduced the fertility index. The fertility index and the gestation index were significantly reduced at 150 mg/kg/day (high) in males and 50 mg/kg/day (mid) in females. Impairment of reproductive

performance significantly increased preimplantation and postimplantation loss and decreased viability rates (mid & high). The NOEL for parental, reproductive and developmental toxicity was considered to be 5 mg/kg/day. Based on a body surface area conversion factor, an equivalent dose in humans would be 0.71 mg/kg/day. Segment II study in rats: body weights, number of corpora lutea, fetuses, implantations and resorption sites were significantly reduced (50 mg/kg/day, high). Three individual skeletal malformations (bifid and missing ribs in one [low]); cleft vertebra in one and cleft palate in another (high) fetuses were observed. In the absence of a dose-response curve, the malformations could not be attributed to the treatment. The maternal and the developmental NOEL was 25 mg/kg/day. Based on a body surface area conversion factor, an equivalent dose in humans would be 3.6 mg/kg/day. Segment II study in rabbits: weight gains were markedly suppressed at 300 mg/kg/day (high) level. There was a statistically significant reduction in the number of corpora lutea, implantation sites and live fetuses, which was paralleled by an increased number of early resorptions (high). Total resorption of the entire litter occurred in 3 (high), 1 (mid) and 2 (low) rabbits. Visceral and skeletal examination of fetuses revealed that malformations were only present in the mid-dose dams, and included one fetus with bifid rib and hemivertebra and one fetus with a cranial hematocele. The number of fetuses with variations (incomplete ossification of skull/limbs, ventricular septal defects, limb flexure and/or malformed sacral vertebrae) was slightly increased in low and mid dose groups when compared to the concurrent controls; however, the changes were not dose-related or statistically significant. In the high dose group, 13.8% of the fetuses (vs 0% in controls) had supernumerary ribs. The maternal and the developmental NOEL was 100 mg/kg/day. Based on a body surface area conversion factor, an equivalent dose in humans would be 32.3 mg/kg/day. Segment III study in rats: offspring (F1) in the 40 mg/kg/day dose group (high) had significantly reduced postpartum viability, retarded maturation and reduced fertility. The maternal and reproductive/developmental NOELs were 40 and 25 mg/kg/day, respectively. Based on a body surface area conversion factor, an equivalent doses in humans would be 5.7 and 3.6 mg/kg/day, respectively.

Genotoxicity studies: results are summarized in Appendix # 2 (Table 6). In the Ames bacterial assay, there was no significant increase in the number of revertant bacterial colonies. In the two in vitro mammalian cell (CHO/HGPRT and CHO chromosome aberration) assays, there was no mutagenic or clastogenic effects, while in the in vivo mouse micronucleus test, no significant increases in micronuclei were observed. Nevirapine was devoid of mutagenic or clastogenic effects.

General pharmacology studies: for the summary, see Appendix # 3 (Table 1).

Nonclinical pharmacokinetic and ADME studies: for the summary, see Appendix # 4 (Table 1-10).

CONCLUSIONS:

In the clinic, the test compound is being administered as an oral formulation at a dose level of 400 mg/day (approximately 6.7 mg/kg/day). The proposed therapeutic dose produced a mean steady-state AUC value of 130 μg*hr/ml. The kinetic data from subchronic/chronic toxicity studies in rats and dogs showed that the mean AUC value at the therapeutic dose was found to be considerably higher than that achieved in the nonclinical toxicity studies at the NOELs/NOAELs. Based on either the body surface area equivalence factors or drug exposure (AUC values), the dosages used in the clinic are higher than the NOELs/NOAELs identified in animal studies (Appendix # 6, Table 1).

Nevirapine can be classified as Pregnancy Category C. Nevirapine should be used during pregnancy only the potential benefit justifies the potential risk to the fetus. Nevirapine also crossed the placental barrier and was excreted into breast milk, and therefore the fetus and nursing baby would be exposed to nevirapine in mothers taking the drug. However, the U.S. Public Health Service Centers for Disease Control and Prevention advises HIV-infected women not to breast-feed to avoid post-natal transmission of HIV to a child who may not be infected.

There are no nonclinical pharmacology and toxicology issues which would preclude the approval of this Pre-NDA. The sponsor submitted protocols, which have been approved by the Executive CAC, to initiate the two-year carcinogenicity studies in mice and rats.

The issue of labelling will be addressed when the NDA is submitted and the review of the labelling appended to this review.

APPENDICES:

Six appendices are attached. These are listed below.

1. **Non-clinical toxicology, pharmacokinetics and pharmacology.**

2. **Tabulated summary of nevirapine animal toxicity studies.**

3. Tabulated summary of nevirapine general pharmacology studies

4. Tabulated summary of nevirapine animal pharmacokinetic studies.

5. Comparison of animal and human pharmacokinetic parameters

6. Comparison of animal NOELs with the human therapeutic dose.

Pritam S. Verma, Ph.D.
Reviewing Pharmacologist

Concurrences:

HFD-530/DFreeman 3/21/96
HFD-530/JFarrelly 3/5/96
HFD-530/PVerma PSV 2/27/96

Disk
HFD-530/JFarrelly

cc
HFD-530/NDA 20-636
HFD-340
HFD-530/AZeccola
HFD-530/PVerma
HFD-530/JMartin
HFD-530/SMiller
HFD-530/NBattula
HFD-345/GJames

Appendix # 1

Non-clinical toxicology, pharmacokinetics and pharmacology.

NON-CLINICAL TOXICOLOGY

Toxicity Studies Summary: The studies marked with an astrict were conducted in accordance with the FDA Good Laboratory Practice Regulations.

Acute Toxicity Studies

1. Nevirapine acute oral (gavage) toxicity study in male and female mice, Batch # 1, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, August 23, 1990, (TX-9011/U91-0019)*

2. Nevirapine preliminary acute oral (gavage) toxicity study in female rats, RM-1152, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, August 14, 1990, (TX-9002/U90-0534)

3. Nevirapine acute oral (gavage) toxicity study in male and female rats, RM-1177, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, December 21, 1990, (TX-9016/U91-0020)*

4. Nevirapine acute toxicity study by inhalation in rats, Batch # 1, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, December 21, 1990, (TX-9113/U91-0507)*

5. Nevirapine preliminary oral (capsule) rising dose tolerance toxicity study in dogs, Lot # RM-1152, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, September 26, 1990, (TX-9004/U90-0599)

6. Oral Rising Dose Toxicity Study of nevirapine (Granulation) in Beagle Dogs, Lot # TX-0412,

   August 27, 1992, (TX-9302/U92-0637)*

Multiple Dose Toxicity Studies

7. A 14-Day Intravenous Toxicity Study of Nevirapine in the Albino Rat, Lot # TX-0493,

   May 28, 1992, (TX-9207/91-096)*

8. Nevirapine 4-week oral (gavage) toxicity study in male albino rats, Lot # RM-1177, Boehringer Ingelheim Pharmaceuticals, Inc., May 31, 1991 (TX-9103/U91-0537)*

9. A 4-week oral (gavage) toxicity study in female rats, Lot # RM-1177, Boehringer Ingelheim Pharmaceuticals, Inc., September 4, 1991, (TX-9102/U91-0404)*

10. A 13-Week Oral (Gavage) Toxicity Study of Nevirapine in the Albino Rat, Lot # RM-1212 . May 3, 1993, (TX-9303/U93-0462)*

11. Thirteen week oral (diet) range-finding toxicity study in the CD-1 mouse with nevirapine, Batch No. RM-1177, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, July 13, 1994, (93-159/TX-9306)*

12. Thirteen week oral (diet) range-finding toxicity study in the rat with nevirapine, Lot # RM-1290, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, May 12, 1995, (U95-3128/TX-9305)*

13. Nevirapine: 14-Day Oral (Capsule) Toxicity Study in the Beagle Dog, Lot # XP-1413-029, Boehringer Ingelheim Pharmaceutical, Inc., Ridgefield, CT, 5 June, 1990, (TX9007/90-034)*

14. Two Week Oral Toxicity Study of nevirapine (Granulation) in Beagle Dogs (Dose Range Finding), Batch # TX-0488, . September 25, 1993, (TX-9301/U92-0643)*

15. A 14-Day Intravenous Infusion Toxicity Study of Nevirapine in the Beagle Dog, Lot # TX-0496, May 28, 1992 (TX-9208/91-097)*

16. A 28-day oral (capsule) toxicity study of nevirapine in the beagle dog, Lot # G, Boehringer Ingelheim Pharmaceuticals, Inc., May 23, 1991, (TX-9105/U91-0552)*

17. A 4-week oral (capsule) toxicity study of nevirapine in the Beagle dog, Lot # G, Boehringer Ingelheim Pharmaceuticals, Inc., May 23, 1991, (TX-9104/U91-0352)*

18. A 13-Week Oral (Capsule) Toxicity Study of Nevirapine in the Beagle Dog, Lot # RM-1230/1243, May 13, 1993, (TX-9116/U93-0463)*

19. 52-Week Oral (Gavage) Toxicity Study of Nevirapine in the Male and Female Rat, Batch No. # 13007, Boehringer

Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, August 6, 1993, (U93-2023/TX-9304)*

20. 52-week oral toxicity study in dogs with nevirapine granulation, Lot # RM-1379, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, October 11, 1995, (U95-3317/93-169)*

Special Toxicity Studies

21. Effect of Oral Dosing of nevirapine on Circulating Levels of Thyroid Hormones in Female Rats, Lot # RM-1177, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, June 4, 1993, (TX-9111/U93-0530)*

22. Assessment of primary eye irritation study in rabbits with nevirapine, Lot # 117, March 28, 1991, (TX-9108/90-049)*

23. A 5-Day Intravenous Irritancy Study in the Albino Rabbit with Nevirapine, Lot # PD-1138, May 28, 1992 (TX-9205/91-092)*

24. A 5-Day Intra-Arterial Irritancy Study in the Albino Rabbit with Nevirapine, Lot # TX-0496, , May 28, 1992 (TX-9206/91-093)*

25. A Single Dose Intramuscular Irritancy Study in the Albino Rabbit with Nevirapine, Lot # TX-0496, May 28, 1992 (TX-9204/91-091)*

26. Assessment of primary dermal irritation study in rabbits with nevirapine, Lot # RM-1177, March 28, 1991, (TX-9109/90-050)*

27. Assessment of acute dermal toxicity study in rabbits with nevirapine, Lot # RM-1177, March 28, 1991, (TX-9107/90-048)*

28. Assessment of dermal sensitization study in guinea pigs with nevirapine-close patch technique, Lot # RM-1177, March 28, 1991, (TX-9110/U91-0417)*

29. Exploratory Dose (IV) Tolerance Study in the Beagle Dog on nevirapine, Batch I, Lot # 1177, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, March 31, 1992, (TX-9201/U92-0254)*

30. Five Day Exploratory I.V. Formulation Study in the Dog, Lot # TX-0493, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, May 19, 1992, (TX-9117/U92-0471)*

Reproductive Toxicity Studies

31. Nevirapine: Study of fertility and general reproductive performance in rats after oral treatment by gavage (segment I), Batch No. # 13007, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, March 25, 1994, (U94-2043/TX-9402/72Q)*

32. Range finding teratology study in rats with nevirapine by oral gavage, Lot # RM-1177, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, March 4, 1991, (TX-9106/U91-0353)*

33. Teratology Study in Rats with nevirapine by Oral Gavage, Lot # RM-1177, Boehringer Ingelheim, Birkendorfer Strabe, December 20, 1991, (TX-9211/U92-0299)*

34. Nevirapine: Study of peri- and postnatal development in rats after oral treatment by gavage (segment III), Batch No. # 13007, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, March 25, 1994, (U94-2083/TX-9403/82Q)*

35. Range finding teratology study in rabbits with nevirapine by oral gavage, Lot # RM-1177, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, March 7, 1991, (TX-9112/U91-0529)*

36. Teratology Study in Rabbits With Nevirapine by Oral Gavage (Segment II), Lot # RM-1177, Boehringer Ingelheim, Birkendorfer Strabe, October 21, 1991, (TX-9210/U92-0312)*

Genotoxicity Studies

37. Nevirapine mutagenicity testing with Salmonella typhimurium TA 1535, TA1537, TA98 and TA100. Pre-incubation reverse mutation assay, Lot # RM-1152, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, December 12, 1989, (U90-0142/TX-8901)*

38. Nevirapine mutagenicity testing with Salmonella typhimurium TA 1535, TA1537, TA98 and TA100 and Escherichia coli WP2 uvr A. Pre-incubation reverse mutation assay, Lot # RM-1152, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, July 16, 1990,

(U90-0479/TX-9001)*

39. BIRG 0106 (metabolite of nevirapine) mutagenicity testing with Salmonella typhimurium TA 1535, TA1537, TA1538, TA98 and TA100 and Escherichia coli WP2 uvr A. Pre-incubation reverse mutation assay, Lot # 1303/64, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, August 28, 1990, (U90-0544/TX-9001)*

40. Nevirapine: CHO/GPRT mutation assay, Lot # RM-1177, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, November 14, 1990, (U90-0676/TX-9009)*

41. Chromosome aberrations in Chinese hamster ovary (CHO) cells, Lot # RM-1177, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, December 26, 1990, (TX-9101/90-040)*

42. Nevirapine: Micronucleus test in mice, Batch # I, Boehringer Ingelheim Pharmaceutical, Inc., Federal Republic of Germany, August 19, 1992, (TX9202/MUT0198/U92-0175)*

43. Nevirapine: Mutagenicity testing with Salmonella typhimurium and Escherichia coli. Plate incorporation reverse mutation assay with and without metabolic activation, Lot # 2439-98-1, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, April 11, 1995, (U95-3056/40025-02)*

44. CAPIC: Mutagenicity testing with L5178Y TK+/- mouse lymphoma cells, forward mutation assay, Lot # IVT-0105, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, August 23, 1995, (U95-3316)*

45. BIRH 414 BS: Mutagenicity testing with Salmonella typhimurium and Escherichia coli. Plate incorporation reverse mutation assay with or without metabolic activation, Lot # 2589-25-3, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, August 24, 1995, (U95-3319)*

46. BIRG 616 BS: Mutagenicity testing with Salmonella typhimurium and Escherichia coli. Plate incorporation reverse mutation assay with or without metabolic activation, Lot # 2589-21-2, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, August 24, 1995, (U95-3321)*

Exploratory Toxicity Studies

47. Exploratory (IV) tolerance study in the female rat with 30% propylene glycol, Lot # I, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, June 20, 1995, (U95-3162/TX-9203)*

48. Preliminary Rising Dose Oral (Capsule) Toxicity Study in the Beagle Dog, Lot # RM-1162/1169, Boehringer Ingelheim Pharmaceutical, Inc., Ridgefield, CT, 22 May, 1990, (TX9007/90-030)

Review of Toxicity Studies:

Acute Toxicity Studies

1. Nevirapine acute oral (gavage) toxicity study in male and female mice, Batch # 1, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, August 23, 1990, (TX-9011/U91-0019)*

Groups of male and female mice (5/sex/group) were given via gavage single oral doses of nevirapine at dose levels of 0, 50, 100, 200, 400, 800, 1600 or 2000 mg/kg. The animals were observed for 14 day after dosing. Results: drug-related signs of toxicity were evident at doses of 400 mg/kg (males) or 600 mg/kg (females) or higher. These effects included decreased motor activity and ptosis. Slow/labored breathing was noted at doses of 1200 mg/kg and higher. In general, clinical signs first appeared approximately 2-3 hr after dosing and disappeared within 48 hr. Fecal output was affected, with a decrease noted for some females at 1200 mg/kg and above and males at 1600 mg/kg and above. One female mouse (1600 mg/kg) was euthanized due to its moribund condition. The only drug-related macroscopic finding for this mouse was white material in the lumen of the ileum and jejunum. The NOEL for this study was 200 mg/kg (♂) and 400 mg/kg (♀). No target organs were identified macroscopically. The MTD was 1200 mg/kg, and the median lethal dose exceeded 2000 mg/kg.

Comments: Doses of 200 mg/kg (♂) and 400 mg/kg (♀) may be considered NOELs in this study. Based on a body surface area conversion factor, equivalent doses in humans would be 16.7 mg/kg (♂) and 33.3 mg/kg (♀).

2. Nevirapine preliminary acute oral (gavage) toxicity study in female rats, RM-1152, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, August 14, 1990, (TX-9002/U90-0534)*

Groups of female rats (2/group) were given single oral doses of nevirapine via gavage at dose levels of 50, 100, 250, 500, 750 or 1000 mg/kg. The animals were observed for 7 days after dosing. Results: one rat (500 mg/kg) and all rats (750 or 1000 mg/kg)

were euthanized in extremis approximately 24 hr after dosing. Drug-related signs of toxicity were evident at doses of 500 mg/kg or higher. These effects included viscous clear ocular discharge, evidence of chromodacryorrhea (brown flecks around the eyes, snout and forefeet), decreased motor activity, signs of dehydration, hypothermia (coldness to touch), decreased feces and weight loss. Behavioral/clinical signs of toxicity were first observed approximately 2 hr after dosing and continued to be evident on the day after dosing. Drug-related gross pathological findings were observed only in animals killed in extremis and consisted of pulmonary hemorrhages (500 mg/kg or higher) and green urine (750 mg/kg or higher). There were no drug-related toxicological or gross pathologic effects at doses of 50, 100 or 250 mg/kg.

Comments: The NOEL for female rats in this study was 250 mg/kg. Based on a body surface area conversion factor, an equivalent dose in humans would be 35.7 mg/kg (♀).

3. Nevirapine acute oral (gavage) toxicity study in male and female rats, RM-1177, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, December 21, 1990, (TX-9010/U91-0020)*

Groups of male and female rats (5/sex/group) were given single oral doses of nevirapine via gavage at dose levels of 50, 100, 220, 400, 600 or 800 mg/kg. The animals were observed for 14 days after dosing. Results: two female rats (400 mg/kg), three (600 mg/kg) and five (800 mg/kg) were euthanized in extremis on study days 1 or 2. Probit analysis of mortality data for these animals indicated a median lethal dose of 502 mg/kg. Drug-related signs of toxicity were evident at doses of 200 mg/kg or higher for female rats and 400 mg/kg or higher for male rats. These effects included viscous clear ocular discharge, evidence of chromodacryorrhea (brown flecks around the eyes, snout and forefeet), chromorhinorrhea, slow breathing, greenish or brown urine, greenish or brownish staining of the abdomen, decreased motor activity, signs of dehydration, evidence of hypothermia (coldness to touch), decreased feces and weight loss and moribund condition. Behavioral/clinical signs of toxicity were first observed approximately 3 hr after dosing and some signs continuing for several days. Drug-related macroscopic findings were observed principally in females given doses of 400 mg/kg and higher and killed moribund. These finding included red and/or pale discoloration of the adrenal cortex, focal and/or multifocal red discoloration of the lungs (hemorrhage, thymic atrophy, and green urine.

Comments: Doses of 100 mg/kg for female and 200 mg/kg for male rats may be considered the NOELs. Based on a body surface area conversion factor, equivalent doses in humans would be 14.3 mg/kg (♀) and 28.6 mg/kg (♂). Potential target organs were the lungs,

adrenal glands and thymus. Sex differences in sensitivity to toxic effects of nevirapine may be attributed to a slower rate of metabolism and higher plasma concentration of drug in the female rat.

4. Nevirapine acute toxicity study by inhalation in rats, Batch # 1, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, December 21, 1990, (TX-9113/U91-0507)*

Seven male and female albino rats (strain Chbb:THOM; weight 231 - 280 g; age 11 weeks) were administered nevirapine (dose 161.1 mg/kg; concentration 1.28 mg/L) via nose-only inhalation exposure for 4 hr to study the acute toxicity of the drug as micronized dust. The terminal autopsies were performed after 2 weeks of the treatment. One day after the exposure, one female rat showed reduced motility and piloerection, but recovered on the following day. There were no treatment-induced changes in rectal body temperature, and no death occurred during the study. One male rat showed moderate food reduction and slight weight reduction during the first week of the study. Animals sacrificed after 24 hr (2 male and 2 female) and 2 weeks (5 male and 5 female) exposures showed no gross or histopathological changes. In conclusion, nevirapine has shown a very low acute toxicity in rats when administered as a respiratory dust via inhalation.

5. Nevirapine preliminary oral (capsule) rising dose tolerance toxicity study in dogs, Lot # RM-1152, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, September 26, 1990, (TX-9004/U99-0599)

One male and one female dog were given nevirapine orally in capsules at escalating single doses of 50, 150, 250 or 400 mg/kg, with a 1-3 day washout period between doses. Results: reduced amounts of defecation were observed for several hr after dosing (250 mg/kg) only. No clearly drug-induced gross or histopathologic alterations were identified.

6. Oral Rising Dose Toxicity Study of Nevirapine (Granulation) in Beagle Dogs, Lot # TX-0412, August 27, 1992, (TX-9302/U92-0637)*

Nevirapine (66% pure, a granulation formulation) was administered orally in a single alternating dosages of 100, 200, 400, 800, 1600 and 3200 mg/kg to one male and one female beagle dog (age: 28-45 months; weight: 11-12 kg) as well as in a dosage of 1000 mg/kg to one additional male and female animal consecutively over a period of 7 days. The purpose of this study was to assess the toxicity of a granulated formulation of nevirapine when administered orally to beagle dogs. Clinical Observations: single administration of 100 and 200 mg/kg - no abnormal findings; 400

and 800 mg/kg - white particles in the feces; and 1600 and 3200 mg/kg - white particles in the feces; repeated vomitus and slight ataxia. Repeated (7 days) administration of 1000 mg/kg/day resulted white particles in the feces; the male vomited on day one; the female vomited repeatedly and was slightly sedated. The food intake was not influenced. The female (7 days) showed a mild decrease in the weight gain. Clinical Laboratory Examination: values of GGT, AP and total cholesterol were slightly or moderately increased in both male and female. Both females (single and repeated doses) had a slight decrease in hemoglobin, erythrocyte counts and hematocrit. In the repeated dose animals, the leucocyte number was increased in the female and the percentage of lymphocytes was decreased compared to that of the neutrophils in the differential blood picture in both animals. Pathology: the repeatedly dosed female showed lymphoid depletion of the thymus and lymph nodes as well as reduced erythropoiesis in the bone marrow as a result of nevirapine administration.

Comments: The single and the repeated (7 days) administrations of high dosages of nevirapine to beagle dogs revealed CNS signs, emesis and biochemical evidence of liver toxicity. Based on these findings, the highest dose for repeated dose toxicity studies should not exceed 1000 mg/kg/day.

Multiple Dose Toxicity Studies

7. A 14-Day Intravenous Toxicity Study of Nevirapine in the Albino Rat, Lot # TX-0493,
May 28, 1992, (TX-9207/91-096)*

Groups of male and female Sprague-Dawley rats (weight: 251-292 g for males and 189-222 g for females; age: 8-10 weeks; 10 rats/sex/group) were given nevirapine (1 ml/kg from 0.8 mg/ml solution in 30% propylene glycol/10% alcohol/0.9% sodium chloride) at dose rates (ml/min) of 0.3 once-a-day (low dose), 0.5 once-a-day (mid dose) and 0.5 twice-a-day (high dose) by slow bolus tail vein injection for 14 consecutive days. The control animals received the vehicle (0.5 ml/min twice-a-day) only. The purpose of this study was to determine the toxicity of the intravenous administration of nevirapine in rats.

There were no mortalities. There were no treatment-related effects on body weights and food consumption data. Lesions of the tail such as pale, yellow, red and dark areas (ranging from 1-4 cm in length) and scabs were seen in all groups with the highest incidence in the high dose and control groups (both groups dosed twice daily). One male (control) showed moderate exfoliation on day 14. Dry or wet green material was found on the cage tray paper of three females at 5 different occasions (control and low dose groups).

In females, analysis of mean values compared to controls revealed statistically significant decreases in white blood cell count (mid and high dose) and platelet count (mid dose). Animals [control (4), high dose (2), mid dose (1)] showed marginally to slightly decreased red blood cell counts and statistically significant decreased red cell distribution width (RDW) (low and mid dose). Additionally, all these animals showed a high reticulocyte count and a low hemoglobin and hematocrit. Compared to control animals, there were statistically significant increases in A/G ratios (males in mid dose, females in low and mid doses).

At the routine urinalysis, all groups showed an incidence of blood in urine collected overnight starting at day 1 of the treatment which continued throughout the study period. The incidence in week 2 was slightly higher compared to week 1. The daily urine test for occult blood was positive for the presence of blood for all groups, with a marginally higher incidence in both mid and high dose animals.

Relative to body weight and brain weight, there was a statistically significant reduction in absolute and relative spleen weights in males (high dose) and in females (low and high dose). In addition, without statistical significance, relative spleen weights were also reduced in males (low and mid dose). Absolute and relative (relative to body weight and brain weight), thyroid/parathyroid weights for males (low dose) were statistically significantly higher than those of controls. Gross pathological examination revealed miscellaneous findings in rats from all groups, with the most frequent being pale areas in the liver. Some rats from all groups had scabs or ulcerations on the skin of the tail and one female (high dose) had blue discoloration on the tail. Histopathological evaluation of the injection sites revealed thrombosis, fibrosis, hemorrhage and mixed cell infiltration which were distributed throughout control and all treated groups. A foreign crystalline material was observed within the injection site lesions (one male each in high dose and control).

Comments: The design of this safety study did not permit delineating the role of the test article in the overt toxicities that were seen in the animals. The observed toxicities may have occurred due to a complex function of different dosing regimens of the vehicle, particularly in control and high dose groups, test article itself, toxic nature of the vehicle or the injection procedure. Also, the sponsor has not mentioned the time period between the doses in those instances where the animals received two doses a day. Nevertheless, the intravenous administration of nevirapine solution in 30% propylene glycol/10% alcohol/0.9% sodium chloride to rats at a dose level of 0.8 mg/kg/day at a rate of 0.3 and 0.5 ml/min once-a-day and twice-a-day at a rate

of 0.5 ml/min for 2 weeks, resulted in various overt toxicities. The clinical observations included lesions, scabs or ulcerations and exfoliation on the tail. Histopathological evaluation of the affected tissues demonstrated thrombosis, fibrosis, hemorrhage and mixed cell infiltration in both control and treated groups.

A statistically significant reduction in absolute and relative weights of spleen and an increase in thyroid/parathyroid weights, pale areas in the liver of all groups, although not accompanied by abnormal histopathological changes, were observed. Compared to control, there were significant decreases in red blood cell count and RDW, and platelet count in treated animals; however, each of these value was within expected range for rats of this strain. The increased A/G ratio in both male and female animals (low and mid doses) indicated that the increase, generally speaking, may be due to liver dysfunction [decrease in albumin synthesis] that may be related to the test article. The daily urine test for occult blood was positive for the presence of blood approximately 50% of the time for all groups, with a marginally higher incidence of blood in urine of mid and high dose animals.

8. Nevirapine 4-week oral (gavage) toxicity study in male albino rats, Lot # RM-1177, Boehringer Ingelheim Pharmaceuticals, Inc., May 31, 1991 (TX-9103/U91-0537)*

Groups of male Sprague Dawley VAF/Plus albino rats (weight 229 - 289 g; age 7 weeks; 15 animals/group) were administered nevirapine (1, 10, 50 and 150 mg/kg in 10 ml/kg solution) orally by gavage or vehicle (0.5% Methocel) for a minimum of 28 days. Five animals per group were assigned to a recovery phase of the study and received no treatment during the 4 week recovery period. No death occurred during the study. No drug-induced effects on body weight, food consumption, rectal temperature or ophthalmological examinations were noted. There were no drug-related clinical signs of toxicity at doses of 1 or 10 mg/kg. At 50 mg/kg, one rat had black/brown chromorhinorrhea (on day 7); at the high dose of 150 mg/kg one animal had black/brown chromodacryorrhea (days 8-28) and swelling of nose (days 8-13). Minor hair loss on the neck or forefeet, brown staining on the feet or limbs were observed in a few rats in both control and drug-treated groups. During the recovery phase, one animal (10 mg/kg) developed brown/black chromodacryorrhea and chromorhinorrhea. A dose of 10 mg/kg may be considered as NOEL in the male rats.

With regard to clinical laboratory data, slightly increased mean corpuscular hemoglobin concentration (MCHC) in 50 mg/kg group after 4 weeks of treatment. One animal (10 mg/kg) had decreased total leukocytes, red blood cell counts, hematocrit and hemoglobin; upon autopsy this animal was found to have a subcapsular hematoma of the kidney. One rat (150 mg/kg) had

moderate number of Burr cells (abnormal erythrocytes). At the end of the recovery period, a statistically significant decrease in hematocrit was noted in the high dose group (150 mg/kg) of animals. Activated partial thromboplastin time (APTT) was significantly increased (150 mg/kg) compared to controls after 4 weeks of the treatment, but returned to normal after the recovery phase. A statistically significant increase in serum cholesterol (150 mg/kg) compared to controls was noted which returned to normal after the recovery period. The low dose group (1 mg/kg) had a statistically significant increase in serum cholesterol as well; however, a dose response trend was absent.

Pathology data demonstrated that statistically significant dose-related increases in mean absolute and relative organ weights occurred in liver (50 or 150 mg/kg), thyroid (150 mg/kg) and adrenals (150 mg/kg). These increases were reversible after a 4 week recovery period. Drug-related macroscopic findings were limited to hepatic enlargement in 2 and thyroid gland enlargement in 1 of 10 animals (150 mg/kg). Histopathologic findings showed diffuse centrilobular hepatocellular hypertrophy of the liver in all animals (50 and 150 mg/kg), diffuse bilateral hypertrophy of the zona fasciculata of the adrenal cortex in 1 animal (50 mg/kg) and 5 animals (150 mg/kg), and diffuse follicular cell hypertrophy generally accompanied by follicular cell hyperplasia of the thyroid (50 mg/kg; 8 animals). Cytoplasmic inclusions in the liver (50 or 150 mg/kg) and in the proximal tubule of the kidney (150 mg/kg; 5 of 10 rats) were also considered drug-related. After the 4 weeks of recovery period, hepatocellular cytoplasmic inclusions (150 mg/kg; 3 of 5 animals) were present.

Plasma samples from a concurrent satellite group of 3 animals per dose group were collected via cardiac puncture before the start of dosing, and at 1.5, 5 and 24 hr after dosing on days 1, 14 and 28. The results are summarized in Table 1.

Table 1
Mean pharmacokinetic parameters of nevirapine following 4 weeks of oral dosing in rats

Parameters	Day	Dose levels (mg/kg)			
		1	10	50	150
Cmax (μg/ml)	1	0.05	0.67	5.3	8.9
	14	0.04	0.56	2.9	4.1
	28	0.06	0.67	3.1	3.3
Tmax (hr)	1	1.5	1.5	1.5	1.5
	14	1.5	1.5	1.5	5.0
	28	1.5	1.5	1.5	1.5
AUC (μg*hr/ml)	1	0.23	2.8	21.5	90.6
	14	0.17	2.1	12.5	56.0
	28	0.24	2.6	17.1	39.6

The data indicated that nevirapine was adequately absorbed after doses of 1 to 150 mg/kg. After the first dose, mean peak plasma levels were 0.05, 0.67, 5.3 and 8.9 μg/ml for 1, 10, 50 and 150 mg/kg groups, respectively. Peak concentrations were observed at 1.5 hr after dosing, except on day 14 (150 mg/kg) group when the T_{max} was 5 hr. On day 1 mean AUCs increased with increasing doses, ranging from 0.23 to 90.6 μg.hr/ml after doses of 1 to 150 mg/kg, respectively. However on days 14 and 28, mean AUCs were lower (17.1 and 39.6 μg.hr/ml on day 28 for 50 or 150 mg/kg, respectively) compared to day 1. A similar decrease in AUCs with repeated dose of nevirapine was not observed in rats (1 or 10 mg/kg) dose groups.

Comments: 1) Oral administration of nevirapine at doses of 50 and 150 mg/kg for 4 weeks induced clinical, biochemical, hematological, gross-pathological and histopathological manifestations of toxicity in male rats. A dose of 10 mg/kg may be considered as NOEL in the male rats.

2) As expected based on previous studies in female rats, the target organs were liver, thyroid and adrenal glands, and kidney in the male rats. Other drug-related toxicological meaningful changes observed in the present studies are: decreases in hematocrit and leukocytes, increases in APTT and serum cholesterol.

3) In this study, cytoplasmic inclusions in the liver persisted after the 4 weeks of the recovery period in some high dose group (3/5) animals. This finding indicates that the drug-induced change reverses more slowly than other morphological toxic manifestations.

4) Given, nevirapine causes zona fasciculata hypertrophy in the

adrenal gland. It was possible, the drug induces enzyme synthesis that metabolize adrenal corticosteroids; which in turn, as a compensatory mechanism induces the hypertrophy of cells that produce these hormones. Likewise, the hypertrophy and hyperplasia of thyroid follicular cells may be, at least in part, due to increase synthesis of enzymes that metabolize thyroxine.

5) In general, the degree and multiplicity of target organ changes in the male rats were less than that observed in previous study in female rats at comparable doses for 4 weeks. This increase in sensitivity of the female rat was attributed to reduced hepatic enzyme synthesis in female as compare to male leading to comparable reduction in rate and degree of metabolism of the drug in female rats. However, the present study does not support that view, since the observed hepatic enzymes levels in the male rats were in normal range. Therefore, the increased sensitivity of the female to the drug was due to other sex-related differences.

6) With repeated dosing at 1 or 10 mg/kg, nevirapine plasma concentrations and AUCs did not decrease; however, both of these parameters were found to be reduced at terminal end of the study in 50 and 150 mg/kg groups of rats. In the absence of a hepatic enzyme induction, the results suggest that the absorption of the compound may be limited due to changes in, for example, GI fluid pH, gastric emptying rate, GI blood flow rate, and GI surface area and "membrane" characteristics.

9. A 4-week oral (gavage) toxicity study in female rats, Lot # RM-1177, Boehringer Ingelheim Pharmaceuticals, Inc., September 4, 1991, (TX-9102/U91-0404)*

Six groups of female rats (Sprague Dawley, SD) (147-204 grams, 15 animals/group) were administered nevirapine (1, 3, 10, 50, 125 mg/kg) or vehicle (0.5% aqueous Methocel; total volume 10 ml/kg) by oral gavage once daily for 28-29 consecutive days. Ten animals per group were killed after the treatment; the remaining five animals per group were allowed to recover for four weeks and were sacrificed. One animal (3 mg/kg) was killed in a moribund condition on study Day 2 and was later found to have pyelonephritis, cystitis and alterations in the epithelium (necrosis, hyperplasia) of the kidney or urinary bladder. These findings were incidental to the treatment. Drug related clinical signs were observed at all the doses tested. They included: scab, reduced feces, intermittent tremors, hard area abdomen, hair loss, brown/black chromorhinorrhea, and excessive lacrimation. In the 125 mg/kg group, 47% of the rats had adverse clinical signs, including decreased motor activity, swollen extremities, ptosis, chromorhinorrhea, and chromodacryorrhea. Two rats, one each at 3 and 125 mg/kg were found to have cystic calculi and pyelonephritis, which were considered to be unrelated to the treatment (by the sponsor). However, upon the ophthalmologic

examination, iritis, focal retinopathy, corneal scar, and focal hemorrhage were clearly due to the drug treatment. There were no reported effects of the drug treatment on food consumption, body weight, and rectal temperature.

Statistically significant increases in reticulocytes (50 mg/kg), leukocytes (50 mg/kg), erythrocytes (50 mg/kg), and hemoglobin and hematocrit (10 and 50 mg/kg); decreases in eosinophils (50 mg/kg) were observed during the four weeks of treatment. There were statistically significant increases in partial thromboplastin time (125 mg/kg), serum cholesterol (50 and 125 mg/kg); increases in GGT (50 and 125 mg/kg), calcium, inorganic phosphorous, total protein, globulin, glucose, albumin, sorbitol dehydrogenase; and decreases in sodium and chloride. These changes appeared to be subsided during four weeks of a recovery period. There were no reported drug related effects on urinalytical parameters, except that one rat (3 mg/kg) had increased urinary pH, protein, volume, leukocytes, erythrocytes, amorphous crystals, occult blood, and hazy appearance.

Statistically significant or significant drug treatment related increases in mean absolute and/or relative organ weights were found in liver (21-74%, 50 or 125 mg/kg), thyroid (up to 50%, 125 mg/kg), adrenals (25-37%, 50 and 125 mg/kg), heart (14-20%, 50 and 125 mg/kg), kidney (5-10%, 50 and 125 mg/kg), and uterus (up to 34%, 50 and 125 mg/kg). No increase in weight was reported in thymus in the present study. Furthermore, after the four weeks of a recovery period, absolute and/or relative weight for the liver (9%), thyroid (18%), adrenals (19-22%), and ovary (18%)(a 5% initial increase in weight was not significant, 50 mg/kg) remained significantly elevated at high doses. Drug induced macroscopic changes were found, such as diffuse enlargement of the liver (10 of 10 animals), bilateral adrenal enlargement (5 of 10) and small thymus at 125 mg/kg. Drug induced histopathological changes after 4-weeks of treatment at 50 or 125 mg/kg included: centrilobular nepatocellular hypertrophy of the liver, diffuse bilateral hypertrophy of the zona fasciculata of the adrenal, and/or hyperplasia of thyroid follicular cells. In addition, two high dose animals had minimal diffuse cortical lymphoid depletion of the thymus. Moreover, histopathological alterations correlated with increases in the weights of the liver, thyroid, adrenal gland, and with liver enlargement observed macroscopically. However, histopathological alterations after the recovery period were limited to multifocal or diffuse follicular cell hypertrophy in the thyroid of 2 of 5 high dose animals. Lymphoid depletion of the thymus was also observed in two high dose animals. No drug-induced pathological abnormalities were observed at doses of 1 to 10 mg/kg. Histopathological findings of the ovary demonstrated multifocal bi-lateral follicular necrosis 1/10 (125 mg/kg), and corpus luteum cyst 1/10 (1, 3, 125 mg/kg) and 4/10 (50 mg/kg); the corpus luteum cyst were still present 1/5 (10, 125 mg/kg), following the recovery. Results of the uterus histopathology were

normal; a focal endometrial cyst was present in 1/5 (1 mg/kg) following the recovery period. Based on clinical and pathological data, doses of 1 to 10 mg/kg did not produce any adverse effects. A dose of 10 mg/kg was considered the NOEL in female rat.

Nevirapine was adequately absorbed after doses of 1 to 125 mg/kg. After the first dose, mean peak plasma concentrations were 0.29, 0.76, 4.24, 21.27, and 44.48 µg/ml after doses of 1, 3, 10, 50, and 125 mg/kg, respectively. Peak concentrations were measured 1.5 hr after dosing on all days in groups 1, 3, 10 mg/kg. At higher doses, absorption was prolonged, with peak concentrations occurring 5 hours post dose on day 0 and 28 for the 50 mg/kg group. Mean AUCs increased proportionately with increasing dose ranging from 1.54 to 772.23 µg.hr/ml after the first doses of 1 to 125 mg/kg; however, mean AUCs were lower on days 14 and 28, compared to day 0.

Comments: Nevirapine administration has induced a wide range of toxicity to various organs, and changes in coagulation, serum chemistry and hematologic system. The principal target organs after four weeks of the drug treatment were liver, thyroid, and adrenal gland as reflected by increases in their absolute and relative weights. This finding was further supported by histopathological alterations in the respective organs. After four weeks of a recovery period, that is discontinuation of the drug treatment, the absolute weights of these organs remained elevated suggesting that the drug might be capable of inducing long lasting toxicity to these organs. Although not supported by histopathological changes, there was an increase in absolute or relative weights of heart, kidney, ovary, and uterus; suggesting that these organs may be possible target organs for the toxicity. Toxicities were observed above a 10 mg dosage and appeared to be dose related. (In contrast to original studies on the drug, statistically significant differences in absolute and/or relative thymic weights were not noted in the present study); however, lymphoid depletion of the thymus was found in two high dose animals.

Clinical signs after the 4 weeks of a recovery phase were not reported by the sponsor. Based on clinical and pathological observations, doses of 1 to 10 mg/kg do not appear to produce adverse effects in this study. Further, the drug sponsor suggests that the 10 mg/kg dose can produced mean peak plasma levels up to 5.15 µg/ml after 20 days of dosing which was 500 times the in vitro IC50 of 10 ng/ml. Hepatic microsomal enzyme induction may be responsible for the increase cholesterol levels, serum gamma glutamyltransferase and centrilobular hepatocellular hypertrophy of the liver. If enzyme induction occurs, AUCs were likely to be decreased at the terminal end of the study.

10. A 13-Week Oral (Gavage) Toxicity Study of Nevirapine in the Albino Rat, Lot # RM-1212, . May 3, 1993, (TX-9303/U93-0462)*

Groups of male and female Sprague-Dawley rats {strain: Crl:CD (SD)BR VAF; age: 35 days; weight: 241-289 g for males and 170-212 g for females} were administered nevirapine daily via oral dosing (gavage) in a suspension of 0.5% hydroxypropyl methylcellulose (10 ml/kg) for a period of 13 weeks and then terminally sacrificed. The experimental design is shown in Table 2. The purpose of the study was to assess the potential toxicity of nevirapine during daily oral administration in rats.

Table 2
Experimental Design of the Study

Group	Nevirapine (mg/kg/day)	No. of animals/sex/group		
		Toxicity	Toxicokinetics	Recovery*
Control	0	15	5	5
Low	5	15	5	5
Mid	50	15	5	5
High	125	15	5	5

* Maintained untreated for an 8-week period following the 13-week treatment period.

Mortality: seven animals died or were sacrificed for humane reasons during the treatment. There were no deaths in the recovery phase of the study. Clinical Observations: dose-related skin lesions and/or areas of severe scabbing were observed in 2 high dose males and 1 high dose female. A dose-related incidence of excessive salivation was observed in the high dose females and to a lesser extent in the mid dose females. There were no abnormal clinical signs noted during the recovery phase. Body Weights: a treatment-related effect (reduction) on mean body weight of females was noted. In the recovery phase, the majority of mid and high dose females had body weight gains similar to or higher than control. No treatment-related effect of food intake was not noted for either sex. Ophthalmoscopy: no treatment-related changes were noted. Incidental findings included superficial punctate keratopathy, focal nuclear cataract and focal chorioretinal atrophy. These were common findings in rats of this age and strain and there was no difference in the incidence of the findings in the treated animals versus the control animals. Hematology: at week 13, there were significant increases in reticulocyte counts in mid and high dose females

when compared to controls. Bone Marrow Smears: the bone marrow of one female (high) showed an increase in the number of cells from the granulocytic series, a decrease in the number of cells from the erythroid series, an abnormal maturation of erythroid series and an increase M:E ratio which was consistent with histopathological changes observed in this animal. Clinical Biochemistry: at week six, treatment-related changes consisted of significant increases in mean cholesterol, calcium and globulin levels for females (mid and high). Significant reductions were noted in glucose, ALP and chloride levels and significant increases in ALT were noted for high dose females. At week 13, treatment-related changes were noted in high dose males and mid and high dose females. Slightly increased cholesterol levels, significantly decreased mean triglyceride levels and A/G ratio and increased mean globulin values were noted for males (high). Significant increases in mean BUN, cholesterol, total protein, calcium and globulin values and reductions in A/G ratio and potassium values were noted for mid and high dose females. During the recovery phase, there was a resolution of these parameters that were observed in weeks 6 and 13. Urinalyses: there were no changes in the urinary parameters which showed any indication of an adverse effect attributable to the treatment. Drug Metabolism and Pharmacokinetics: data from this group of animals were discussed separately in the following study (DM-9206/U92-0639). Organ Weights: after 13 weeks, treatment-related changes were noted in the mean absolute and/or relative organ weights [elevated liver weights (mid and high), increased thyroid and kidney weights (high, males; mid and high, females) and increased heart and adrenal weights (mid and high, females)]. Slightly reduced thymus weights were noted in high dose animals. Slightly lower testicular weights were noted in the high dose males and slightly higher ovarian weights were noted in the high dose females. At the end of the 8-week recovery period, no significant differences were noted. Gross and Histological Examination: enlarged adrenal and livers were noted in both sexes (high). Histopathological examination revealed treatment-related findings in mid and high dose animals consisting of centrilobular hepatocellular hypertrophy of the liver, thyroid follicular cell hypertrophy and hyperplasia and hypertrophy of the zona fasciculata of the adrenal cortex. Cortical lymphoid depletion of the thymus was noted in a few high dose animals. The histopathological alterations generally correlated with alterations for organ weights and with macroscopical observations and were more marked in females than in males.

Comments: The low dose of 5 mg/kg/day may be considered a NOEL. The primary target organs identified after 13 weeks of dosing were the liver, thyroid and adrenal. Centrilobular hepatocellular hypertrophy of the liver observed at mid and high doses was consistent with microsomal enzyme induction, a known property of nevirapine in the rat. The thyroid follicular cell hypertrophy and hyperplasia at the same doses were attributable to hepatic

induction of enzymes that metabolize thyroxine (a known effect). The pathogenesis of the adrenal cortical hypertrophy was unknown, but may involve hepatic induction of enzymes that hydroxylate corticosteroids produced by the adrenal cortex.

Unscheduled sacrifice of two high dose females late in the study was performed when the animals developed extensive cutaneous ulceration and severe hindlimb edema. These finding were considered drug-induced by the sponsor.

11. Thirteen week oral (diet) range-finding toxicity study in the CD-1 mouse with nevirapine, Batch No. RM-1177, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, July 13, 1994, (93-159 TX-9306)*

Groups of male and female mice {strain: Crl:CD-1(ICRBR) VAF+; 15 animals/sex/group} were given nevirapine in the diet (powdered) at dose levels of 0 (control) 50 (low), 500 (mid) or 1500 mg/kg/day (high) initially. In treatment weeks 5 and 12, the mid dose was escalated to 3000 and 6000 mg/kg/day, respectively. From treatment week 7 until the end of the study, 2% lactose was added to all feed (including controls) to increase the palatability of the drug. Drug-diet concentrations were adjusted weekly. The purpose of the study was to determine the MTD, NOEL and target organ toxicity of nevirapine to support dose levels selection for the 2-year carcinogenicity study in mice. Results: drug consumption ranged from 75-151% of the targeted dose. Drug-related food spillage was observed at >3000 mg/kg/day. Addition of 2% lactose to the food did not reverse the spillage. Mortality: drug-related mortality/moribundity was observed at the escalated mid dose of 6000 mg/kg/day only. Four male mice were found dead in weeks 12 and 13; and 3 female mice were found dead in week 14. Clinical observation: decreased activity, anogenital staining, labored respiration, ptosis, distended abdomen, soft stool and swelling and/or erythema of legs were observed at 3000 and 6000 mg/kg/day. Body weight and food consumption: mean body weights in 3000 mg/kg/day male mice were decreased (8%) in week 5 only and increased in females (7-21%) at 1500 and 3000 mg/kg/day. Mean food consumption was increased (10-48%) in males at >1500 mg/kg/day and in females (13-16%) at 3000 mg/kg/day only. Plasma drug concentrations: mean plasma drug concentrations at the 50, 500, 1500 and 6000 mg/kg/day doses ranged from below quantifiable limits (<0.25 μg/ml) - 0.33, 0.72 - 1.2, 2.7 - 4.2 and 31.8 - 57.3 μg/ml, respectively, and were roughly dose proportional up to the 1500 mg/kg/day dose level. Post-mortem analysis: revealed target organ toxicity at the 1500 and 500-6000 mg/kg/day doses in the liver, thyroid gland, hematopoietic-reticuloendothelial systems, heart, intestine and kidney. At the 1500 mg/kg/day dose, drug-related changes included increased liver weights and size, hepatocellular hypertrophy and necrosis, increased thyroid with thyroid follicular epithelial hypertrophy, thymic cortical and medullary lymphoid depletion, diffuse extramedullary

hematopoiesis in the spleen, cardiac myofiber necrosis and mineralization and increased kidney weights with renal tubular degeneration/necrosis and hemorrhage. The following were observed at the 500-6000 mg/kg/day dose level: liver discoloration and prominence of the lobular architecture macroscopically, thyroid follicular epithelial hyperplasia, decreased thymus weights, diffuse lymphoid depletion in the spleen, mesenteric lymph node cortical lymphoid depletion and subscapular macrophage infiltration, subacute colitis, -typhlitis and -ileitis, acute colitis and subacute mesenteric inflammation in the stomach. Spleen weights were increased in females at 1500 mg/kg/day, but decreased in males at 500-6000 mg/kg/day. These changes were consistent with enlarged spleen in females (2 highest doses) and small spleen in males (500-6000 mg/kg/day). Microscopic abnormalities such as marked diffuse necrotizing hemorrhagic myocarditis, marked multifocal acute necrotizing colitis and very severe multifocal hepatocellular coagulative necrosis were seen in the drug-related mortality/moribundity.

Comments: Administration of nevirapine to mice in the feed induced target organ toxicity in the liver, thyroid gland, hematopoietic-reticuloendothelial systems, heart, intestine and kidney. Liver and thyroid changes were consistent with altered T4 and TSH levels secondary to hepatic enzyme induction. Since weight loss was not seen at the dose level of 1500 mg/kg/day, although the liver and kidney toxicities were noted, the sponsor has selected the dose level of 1500 mg/kg/day a MTD. The dose level of 50 mg/kg/day may be considered a NOEL. The drug exposure values (AUCs) were not submitted.

12. Thirteen week oral (diet) range-finding toxicity study in the rat with nevirapine, Lot # RM-1290, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, May 12, 1995, (U95-3128/TX-9305)*

Groups of male and female rats (15 animals/group) were administered nevirapine daily via oral dosing (diet) at dose levels of 0 (vehicle control), 5 (low), escalated mid dose group: 25 mg/kg/day (week 1-4) and 100 mg/kg/day (week 5 to study termination), or 50 mg/kg/day (high) for a period of 13 weeks. The purpose of the study was to identify target organs, estimate the Maximum Tolerated Dose (MTD) and No Effect Dose Level (NOEL) of the test compound in rats. Results: following drug week 5, six males and one female (mid) and two males and one female (high) showed signs of dermal swelling and erythema of the forelimbs which were considered to be drug-related. A decrease (approximately 8%) in body weight was observed in the female (mid and high) beginning on day 53 and 70, respectively. Food consumption for the female (mid and high) were decreased beginning day 35 and 56, respectively. Hematology: revealed a decrease in RBC, HGB, HCT and MCV (mid) during week 13. A decrease HCT and MCV was also observed in the female (high). A

decrease in the percentage of lymphocytes and an increase in percentage of neutrophils and monocytes were detected in (mid) in weeks 8 and 13. Clinical Chemistry: an increased serum cholesterol was detected in males and females (mid). Urine protein levels were increased in males (mid). Pathology: significant changes were limited to mid and high dose groups and consisted of: swelling of the forelimbs; chronic enteritis accompanied by granulocytic hyperplasia of the bone marrow, splenic extramedullary hematopoiesis and lymphadenitis of draining lymph nodes; centrilobular hepatocellular hypertrophy of the liver; lamellar body formation in liver; follicular cell hypertrophy/hyperplasia of the thyroid; increased anterior lobe secretion of the pituitary gland; accumulation of green pigmentation in the pituitary gland; and, zona fasciculata/reticularis cellular hypertrophy of the adrenal glands. Conclusion: the NOEL for nevirapine under the conditions of this study was 5 mg/kg/day. On the basis of body conversion factor, an equivalent dose in humans would be 0.71 mg/kg/day. The target organs of toxicity include: adrenal, liver, lymphoid tissue, pituitary, small and large intestines, and thyroid glands.

13. Nevirapine: 14-Day Oral (Capsule) Toxicity Study in the Beagle Dog, Lot # XP-1413-029, Boehringer Ingelheim Pharmaceutical, Inc., Ridgefield, CT, 5 June, 1990, (TX9007/90-034)*

Four groups of male and female purebred beagle dogs (weight: 7.4 - 10.0 kg males and 7.4 - 8.2 kg females; age: 9 - 10 months; 2 animal/sex/group) were dosed with either vehicle (gelatin capsule) or nevirapine in capsule(s) at doses of 0, 100, 400 or 800 mg/kg once a day for 14 days. The purpose of this study was to assess the toxicity of nevirapine when given orally to the dogs. Clinical signs of toxicity including decreased motor activity, ataxia, tremors, scleral injection and/or lacrimation were noted. Decreased food consumption and weight loss in the male and female animals in both the mid and high dose groups were observed. One male (400 mg/kg) with the CNS signs also developed diarrhea and dehydration, and was not dosed on day 7 to 9 because of drug intolerance. This animal had a neutrophilic leukocytosis and increased serum ALP and AST levels on day 6 and 14. Transient head tremors also were noted in one female (400 mg/kg). Alterations in organ weights included reduced thymic weight and size in the mid and high dose groups. Discoloration of the lungs was noted in one or more animals in each drug-treated group. Lymphoid depletion of thymus, tonsil and lymph nodes was observed in the mid (#363, male) and high dose groups. The pulmonary lesions of interstitial pneumonitis and bronchopneumonia were more severe in the animals of the high dose group. One male and two females (400 mg/kg) had splenic enlargement with prominent lymphoid follicles in one high dose female. One male (400 mg/kg, #363) that had marked clinical signs and lymphoid depletion in a

variety of tissues also had mild membranoproliferative glomerulonephritis in the kidney and mild diffuse chronic cholangitis in the liver. The cholangitis correlated with increased serum ALP and AST levels on day 6 and 14. Additionally, the dog had increased cholesterol, ALT, LDH, white blood cell count along with lymphopenia and neutrophilia (relative and absolute) and APTT; decreased glucose, total protein (caused by decreased albumin), calcium, RBC, hematocrit and hemoglobin. Other findings in this male were mild granulocytic hyperplasia of the bone marrow and sinusoidal leukocytosis of the liver and spleen. Acute necrosis of crypts was observed in the mucosa of the colon and rectum in one high dose male. There were no drug-related ophthalmologic or electrocardiograms/heart rate effects.

With considerable inter- and intra-animal variability, peak plasma concentrations of the drug tended to increase in females with increasing dose but not in males. For example, after the first dose in the three dose groups, peak concentrations averaged 0.33, 32.30 and 21.4 μg/ml in males and 0.11, 6.13 and 9.5 μg/ml in females. Table 3 shows a correlation between the toxicity of nevirapine, and its peak plasma concentrations and AUCs.

Table 3

Summary of Correlation between the Drug Intensity Exposure and Induced Toxicity

Dog#, Sex & Dose (mg/kg)	Day	C_{max} (μg/ml)	AUC (μg*hr/ml)	Histopath. Alterations	Clinical Signs
#363 (male, 400 mg)	1 6	61.7 43.3	584 545	lymphoid depletion of thymus and other lymphoid tissues, severe interstitial pneumonitis & broncho-pneumonia, cholangitis in the liver & glomerulo-nephritis	ataxia & tremor, decreased motor activity
#375 (male, 800 mg)	1	41.2	821		
#380 (female, 800 mg)	6	22.3	254		
#376 (male, 400 mg), #355 (male, 800 mg), #371 (female, 800 mg)	-	15-20	~200	lung lesions (low incidence)	NONE
all animals (100 mg) and #379 & 382 (female, 400 mg)	-	less than 15	less than 200	NONE	NONE

Comments: 1. Peak plasma concentrations and AUCs were highest in animals that were showing pathological alterations of lymphoid depletion of thymus and other lymphoid tissues, interstitial pneumonitis and bronchopneumonia, liver and kidney lesions. Secondly, it appears that a peak plasma concentration in excess of 20 μg/ml and an AUC greater than 250 μg*hr/ml were required, regardless of dose, to induce significant clinical or pathological changes in dogs (Table 3).

2. Drug-related alterations in liver weight were not observed in this study. Drugs, that induce microsomal enzyme synthesis, are known to cause centrilobular hypertrophy of the liver. However in this study, the increased enzyme induction was not accompanied by the pathological change. Secondly, cholangitis in the liver was correlated with increased AST and ALP levels. However, because of the chronic nature of cholangitis in the liver and since the alteration was known to occur in laboratory bred beagle dogs sporadically, the toxicity may not be related to the treatment.

3. The severity of interstitial pneumonitis and bronchopneumonia in the lung appeared to be correlated with higher plasma concentrations and AUCs. The agent may have caused damage to the alveoli and that could have caused the toxicity.

4. Nevirapine was well-tolerated when given to dogs at repeated doses of 100 mg/kg/day (both males and females) and at 400 mg/kg/day (females only). The peak plasma concentrations in mid and high dose groups on day 6 were found to be lower than on day 1 (Table 3). This may have occurred due to the saturation of absorption process and/or auto-induction of metabolizing enzymes.

14. Dose Range Finding Study: Two Week Oral Toxicity Study of nevirapine (Granulation) in Beagle Dogs, Batch # TX-0488,

September 25, 1993, (TX-9301/U92-0643)*

Groups of male and female dogs (2 animals/sex/group) were orally administered nevirapine at dose levels of 0 (vehicle control), 150 (low), 300 (mid) or 600 mg/kg/day for 14 days to determine dose-range of nevirapine granulated formulation in dogs. Results: one male dog (high) showed inactivity, anorexia, hypothermia and icterus during week 2 of the study. This dog was killed *in extremis*. Body weight loss of up to 2 kg occurred in high dose animals mainly during the 2nd week of the study. Corresponding, significant food consumption was seen in the high dose animals. The number of erythrocytic precursor cells in the bone marrow was significantly decreased (high) compared to the controls. Drug-related alteration in organ weights included decreased thymus weight in all high dose animals and one mid dose female and increased spleen weight in two females (high). Histopathology: the principal drug-related findings (high) were hepatocellular enlargement, lymphoid depletion and gastric mucosal ulcers or

erosions. The high dose male killed in moribund condition was icteric and had endocardial hemorrhage, testicular degeneration, conjunctival erosions, proximal tubular swelling and degenerating of the kidneys and hepatic changes of multifocal necrosis, anisokaryosis, diffuse fatty change, bile tubular hyperplasia and bile retention.

Comments: A dose 150 mg/kg/day may be considered the NOEL. Based on a body surface area conversion factor, an equivalent dose in humans would be 75 mg/kg/day. The immune system and liver were identified as the principal target organs.

15. A 14-Day Intravenous Infusion Toxicity Study of Nevirapine in the Beagle Dog, Lot # TX-0496,
May 28, 1992 (TX-9208/91-097)*

Groups of male and female beagle dogs (weight: 6.8-8.9 kg for males and 6.3-7.6 kg for females; age: 6-7 months; 3 dogs/sex/group) were given nevirapine (1 ml/kg from 0.8 mg/ml solution in 30% propylene glycol/10% alcohol/0.9% sodium chloride) at dose rates (ml/min) of 0.3 once-a-day (low dose), 0.5 once-a-day (mid dose) and 0.5 twice-a-day (high dose) by slow infusion into either the left/right cephalic or saphenous veins for 14 consecutive days. The control animals received the vehicle (0.5 ml/min twice-a-day) only. The study was designed to determine the toxicity of the intravenous administration of nevirapine in dogs. Due to the poor and deteriorating condition of the infusion sites [it became difficult to insert the abbocath into the vein and/or the vein collapsed once the abbocath was inserted], one male (control) did not receive 3 consecutive doses due to his condition and was sacrificed on day 14. Clinical observations for this animal included slight contusions on the left hindlimb and both forelimbs from day 8 through day 10 and vocalizing during dosing on day 9 and 10. In other animals, treatment-related observations included vocalizing during dosing (one animal, control), contusions on the forelimb(s) and/or hindlimb(s) (one animal each in control and high dose), severe alopecia on a small area of the right forelimb (one animal in low dose), reddish brown colored urine (two animals in control on day 12 and 13), slight salivation and/or biting of the sling (one animal in control, one in mid dose and two in high dose) and a small amount of emesis (one animal in low dose on day 14) during dosing. There were no mortalities. There were no treatment-related effects on body weights and food consumption data. There were no significant differences in the absolute and relative organ weights.

There were no toxicological effects on the hematological parameters. Males and females (all groups) showed an increase in serum creatinine compared to pretreatment values. The daily pre- and post-dose tests for occult blood in the urine did not reveal any significant inter-group differences. The incidence of blood

in the urine tended to be higher in males from all groups and females from control and high dose groups after the daily dose.

Dark areas were frequently noted at the infusion sites along with raised and/or discolored areas in the lungs in animals from all groups. Histopathological finding at the infusion sites included fibrosis, hemorrhage, thrombosis, eosinophilic material (probably fibrin) and mixed cell infiltration. A foreign crystalline material was found in the infusion site lesions of several animals in all groups. Similar foreign crystalline material was seen within pulmonary granulomas in two dogs (one each in control and high dose).

Comments: The intravenous administration of nevirapine solution in 30% propylene glycol/10% alcohol/0.9% sodium chloride to dogs at a dose level of 0.8 mg/kg/day at a rate of 0.3 and 0.5 ml/min once-a-day and twice-a-day at a rate of 0.5 ml/min for 2 weeks resulted in irritation of the injection sites as demonstrated by thrombosis, fibrosis, hemorrhage and mixed cell infiltration in the affected areas of both control and treated groups. Both the incidence of occult blood in the urine after dosing and the presence of a foreign crystalline material [possibly corresponding to the vehicle] in the infusion sites of animals in each group does suggest an adverse effect of the vehicle. The vehicle may also be responsible for causing pulmonary granulomas in two dogs (one each in control and high dose). The lung lesions observed in dogs from both control and treatment groups may be due to a lungworm infestation that is known to occur in beagle dogs.

16. A 28-day oral (capsule) toxicity study of nevirapine in the beagle dog, Lot # G, Boehringer Ingelheim Pharmaceuticals, Inc., May 23, 1991, (TX-9105/U91-0552)*

Two groups of acclimatized and fasted, male and female beagle dogs (weight 7.9 - 9.8 kg; age 5 to 7 months; 5 animals/sex/group) were administered nevirapine orally 800/650 mg/kg or vehicle (gelatin capsule) for 4 weeks. Due to a severe toxicity observed in the animals (800 mg/kg) after 10 days of the treatment, the daily dosage was reduced to 650 mg/kg during rest of the course of study. Following the 4-week treatment period, 2 males and 2 females of each group were maintained untreated for a 5-week recovery period. The purpose of the study was to assess toxic effects in dogs at a dose level approximate to a maximum tolerated dose (MTD). No death occurred during the 4-week treatment period or during the recovery period. Drug induced clinical signs observed during the treatment phase consisted of sedation, tremors, elevated body temperatures, swollen or irritated foot pads, redness of gums and/or eyes, and swelling of the plantar aspect of the limbs. Also, there was a slightly higher incidence of soft unformed or liquid feces in treated animals when compared to control animals.

During the first 10 days of treatment, mild to moderate sedation, slight to moderate tremors, often accompanied by an elevated body temperature (>40 C) were the most significant toxic effects noted in the animals. Once the dose was lowered from 800 to 650 mg/kg on the 11th day, tremors were less severe and body temperature returned to normal; however, some of the animals continued to show mild to moderate sedation accompanied by an abnormal gait and occasional loss of balance for the remainder of the treatment. The daily occurrence of white particles in feces was observed in all treated animals, and blood was also found in feces of one dog. Sporadic occurrences of emesis were noted among animals in both control and treated animals. During the recovery phase however, sedation, tremor, elevated body temperature, redness of the gums and/or eyes, swollen or irritated foot pads, and presence of white particles in feces were no longer seen in any of the treated animals.

Treatment with nevirapine at 800 mg/kg resulted in weight loss and reduced food intake; immediately following the reduction of dose to 650 mg/kg, the treated animals continued to lose weight, however, in the later part of the treatment phase weight gains were observed. The food intake of treated animals was comparable to the controls during the recovery phase. Ophthalmological and cardiovascular examinations (EKG, heart rate and blood pressure) were found to be normal during the treatment and recovery periods.

A trend to neutrophilia and lymphopenia was noted in treated animals. One animal had decreased white blood cell counts. Statistically significant ($p < 0.05$; Dunnett's test) decreases in red blood cell counts, hemoglobin and percent hematocrit were also noted in the treated animals. At the end of the recovery phase, the hematological parameters of the treated animals were comparable to controls except for the red blood cells which remained slightly depressed in the males. The bone marrow of one female showed a slight increase in the number of cells from the eosinophilic series but the animal returned to normal after the recovery phase.

Statistically significant ($p < 0.05$; Dunnett's test) increases in total bilirubin, ALT, GGT, ALP, cholesterol and triglyceride levels, and a reduction in phosphorus levels were found in treated animals. Also noted was an increase in BUN in all the animals; a slight increase in AST in few dogs and elevated LDH in one female. During the recovery phase, the ALP levels continued to be slightly elevated in males, whereas other clinical biochemistry parameters returned to normal range in both male and female dogs. Urinalytical parameters revealed no changes indicative of the treatment-related effect.

Three females had slightly lower thymus weight (absolute and relative to body weight and to brain weight); one male and a

female had higher liver weight (absolute and relative), and slightly lower spleen weights. Furthermore, one male also had a low testes weight (absolute and relative), and one female had low thyroid, parathyroid and adrenal weights (absolute and relative). After the completion of recovery phase, higher thymus weights (absolute and relative) were noted in two treated males. No gross or histopathological abnormalities were reportedly found to be associated in any of the animals examined.

Comments: 1) Administration of nevirapine in dogs (800 mg/kg/day) causes sedation, decreased motor activity, tremors, elevated body temperature, significant body weight loss and inappetence, lymphopenia and neutrophilia and increases in ALT, AST, ALP, GGT, cholesterol and triglyceride levels. Then due to these signs and biochemical changes, it was considered that some of the animals would not have survived if the dosing continued at 800 mg/kg. Therefore, the daily dose was reduced from 800 to 650 mg/kg on day 11 of the treatment for rest of the experiment. This resulted in a milder response in the behavioral changes and a gradual gain in body weight and food intake, that is to say that the animals recovered sufficiently to conclude that the MTD was 650 mg/kg/day.

2) Although no gross or histopathological abnormalities of liver were observed in the treated group of animals, the increase in clinical biochemistry parameters, such as, ALT, AST, GGT, triglycerides and cholesterol suggest the significance of the drug treatment on liver and point to the fact that the liver toxicity may be hidden in the study.

3) The presence of white particles in feces of all the treated animals suggests that there may be a problem in the absorption of the drug which should be further investigated; for example, the feces should be analyzed for parent drug and its metabolites.

4) Nevirapine was toxic to the thyroid gland, it was suggested that the sponsor incorporates assays of T_3, T_4 and reverse T_3 as part of the clinical biochemistry profile.

17. A 4-week oral (capsule) toxicity study of nevirapine in the Beagle dog, Lot # G, Boehringer Ingelheim Pharmaceuticals, Inc., May 23, 1991, (TX-9104/U91-0352)*

Groups of acclimatized and fasted, male and female Beagle dogs (4.7-8.0 kg, age 5-6 months; 3 animals/sex/drug group) were administered orally nevirapine (20, 50, 100, 400/500 mg/kg/day) or vehicle (gelatin capsule) for minimum of 28 days. The group receiving a dose level of 400/500 mg/kg, for the first 14 days received 400 mg/kg, then the dose was increased to 500 mg/kg as of day 15 for the next two weeks. The recovery animals were maintained untreated for a 5-week period following the 4-week treatment period. No deaths were reported due to the treatment;

however, one female dog died in week 2 due to a revealed bronchopneumonia. There were no clinical signs of toxicity which could be attributed to the drug treatment. The presence of white particles in feces was not uncommon in treated animals, but not found in controls, suggesting a problem in drug absorption. There were no reported significant differences in the group mean body weights and food consumption of the treated animals (20, 50, and 100 mg/kg/day groups) in comparison to the controls. The highest dose group (400/500 mg/kg/day), however, lost 18% weight and consumed 5% less food than the control group. Upon the ophthalmological examination, no abnormalities were noted other than those observed at the pretreatment examination. There were no reported changes related to treatment in EKG tracings, heart rates or blood pressures. However, slightly decreased heart rates were found in 4/5 males and 1/5 females at the high dose in the last week of the study. Results of the hemograms showed statistically significantly higher mean neutrophil count, lower lymphocyte count, and decreased platelets in the highest dose male group. A slight trend to neutrophilia and lymphopenia was observed in individual male and female rats. At the end of the recovery period, however, the high dose group continued to have a slightly elevated neutrophil count and depressed lymphocyte count. Bone marrow smears were normal. Clinical biochemistry: significantly lower potassium levels in males; increases in mean cholesterol, calcium and triglyceride levels in females (400/500 mg/kg); significantly higher mean total protein values (20 mg/kg) and elevated mean calcium levels (50 mg/kg) were found when compared to control values. Slightly elevated alkaline phosphatase levels were found at lower doses as well. One male (400/500 mg/kg), which had shown reduced food intake and body weight loss had high serum alkaline phosphatase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma glutamyl transferase (GGT) values. After the recovery phase, potassium levels were low in males; alkaline phosphatase value were high in females. Marginally lower pH values and incidence of ammonium urate were noted in urine of males, which returned to normal after the recovery phase. After 4 weeks of drug treatment, female dogs (20, 50, 100, and 400/500 mg/kg) had significantly lower lung weights relative to body weight and significantly higher kidney weights (100 mg/kg) One female had a slightly increased spleen (100 mg/kg); one female had slightly reduced thymus (400/500 mg/kg). Two males dogs had high spleen weights (400/500 mg/kg); and one male dog had lower thymus weights (absolute and relative to body weight/brain weight, 50 mg/kg group). However, no gross or histopathological findings were seen due to the drug treatment. Although, various inflammatory lesions in the lungs were scattered throughout all the groups, including the controls. Thymic atrophy was noted in 2 animals one each (400/500 and 50 mg/kg males).

Comments: Administration of nevirapine resulted in neutrophilia and lymphopenia in both male and female dogs. A slight trend to

both of these disorders was continued even after the 5-weeks of recovery period. Together with the alterations in the weights of thymus and spleen at higher dosages, it was explicitly clear that the drug was toxic to the lymphoid system. In addition, elevated serum liver enzymes (ALT, GGT, AST and alkaline phosphatase) coupled with reduced food intake, body weight loss, increased cholesterol and triglycerides, clearly suggested that the liver was going to be the target organ for this drug. Yet, there was an absence of gross or histopathological changes due to the drug treatment. Toxicities were dose related, and a dose of 100 mg/kg was considered NOEL in dogs. Plasma drug levels were not determined in the animals. Further, detailed analysis of white particles in feces of the drug treated animals should be performed. Although significantly higher mean total protein values (proteinemia) were found in one group of females (20 mg/kg), proteinemia was present during the pretreatment period and reflects elevated levels of serum globulins. The urinalytical parameters were normal.

18. A 13-Week Oral (Capsule) Toxicity Study of Nevirapine in the Beagle Dog, Lot # RM-1230/1243, May 13, 1993, (TX-9116/U93-0463)*

Groups of fasting male and female dogs (strain: beagle; age: 5-6 months; weight: 7.7-10 kg for males and 5.9-8.5 kg for females) were administered nevirapine orally approximately 2 hr prior to feeding via gelatin capsule once daily for a period of 13 weeks and then terminally sacrificed. The experimental design is shown in Table 4. Males and females were treated separately. Blood was collected from the jugular vein of each animal into heparinized tubes. The purpose of the study was to assess the potential toxicity of nevirapine during daily oral administration in dogs.

Table 4
Experimental Design of the Study

Groups	Dose (mg/kg/day)	Males	Females	Recovery*
Control	0	3	3	2M+2F
Low	75	3	3	-
Mid	200	3	3	-
High	500	3	3	-
MTD	700/650**	3	3	2M+2F

* Maintained untreated for a 5-week period following the 13-week treatment period.

** Dose level decreased 700 to 650 mg/kg/day as of week 9.

Mortality: three animals died or were sacrificed for humane reasons during the treatment. During week 8, one male was weak, mucous membranes were pale and chest auscultation revealed rales from the lungs and cardiac arrhythmias. The animal was subsequently sacrificed. One male was found dead in week 7. No abnormal clinical signs were observed prior to its death. One male was sacrificed in week 4 after showing signs of lethargy, incoordination, pallor of mucous membranes and abnormal respiration. There were no deaths in the recovery phase of the study. Clinical Observations: treatment-related clinical signs were observed among males and females (MTD) and for 1 male (high). These clinical signs consisted of sedation, reduced activity, incoordination and elevated body temperature. The signs were generally transient and the onset, severity and/or the nature of the behavioral changes were variable among the animals. For the MTD group, following reduction in dose from 700 to 650 mg/kg/day in week 9 [due to signs of overt toxicity], the severity of the clinical signs previously observed diminished significantly. The presence of white particles in the feces was noted among animals in all treated groups. This was not noted during the recovery phase nor in any control animals. Body Weights: treatment-related effect (reduction) on mean body weight of males and females was noted. In the recovery phase (MTD), weight losses were recorded in recovery week 15 for females but their body weight returned to normal thereafter. Food Consumption: the weekly mean food consumption values of males and females in the MTD group were generally lower than control values. A tendency to slightly lower total food intake was also noted for females in the high dose and males in the mid dose groups. Ophthalmological & Cardiovascular Examinations: no treatment-related changes were noted. Hematology: during the treatment phase, a slight depression in the erythrocyte parameters and a trend to neutrophilia and lymphopenia was observed in males and 1 female (MTD). The investigations performed during the recovery phase revealed similar values for treated and control animals. Bone Marrow Smears: the bone marrow of one male (MTD, sacrificed in week 8) showed a severe increase in the number of cells from the granulocytic series, a decrease in the number of cells from the erythroid series and an increased M:E ratio. These changes were considered to be treatment-related. No abnormalities were noted in the bone marrow of animals examined at the end of the treatment or recovery phases. Clinical Biochemistry: one male (MTD) that was sacrificed in week 8 showed elevated liver enzymes during that week. One female (MTD) showed increased liver enzymes prior to dose level reduction in week 9. Urinalyses: there were no changes in the urinary parameters which showed any indication of an adverse effect attributable to the treatment. Drug Metabolism and Pharmacokinetics: data from this group of animals are discussed separately in the following study (D-9205/U91-072). Organ Weights: females (MTD) showed slightly higher liver weights. The weights of the remaining organs for this group of females and for females in the other treated groups

were similar to the control group. The organ weights of the recovery females were similar to controls. Gross and Histological Examination: for the two moribund sacrificed males, clinical illness was attributed to bronchopneumonia with secondary toxemia and/or septicemia. In addition, lymphocytic depletion and/or necrosis of follicles and/or paracortical-equivalent areas were observed for a variety of hematopoietic-lymphoreticular tissues including thymus, spleen, tonsil, lymph nodes, Peyer's patches of the intestinal tract and lymphoid follicles of the stomach. Histopathological examination of the tissues for the male found dead, revealed that the cause of death was attributed primarily to toxemia/septicemia resulting from a perforation in the left cervical region. Treatment-related changes were observed in the reproductive system for males (high and MTD). These changes included decreases in spermatogenesis for testes and epithelial atrophy for prostate and epididymides. One female (MTD) showed marked diffuse granulomatous bronchopneumonia correlated with pale green discoloration of the lung.

Comments: Daily administration of nevirapine at a dose level of 700 mg/kg/day resulted in the death of 3 male dogs. Nevirapine caused sedation, decreased activity, incoordination, elevated body temperature, reduction in body weight and food intake, lymphopenia and neutrophilia. As result of these observations, the daily dose was reduced from 700 to 650 mg/kg/day in week 9 of the treatment. Exacerbation of spontaneously occurring inflammatory disease in the lung and other tissues (causing the death of 3 males) was found to be related to the administration of nevirapine at 700 mg/kg/day. It was likely that these changes were secondary effects due to the impairment of immunity by the test article. Nevirapine was found to have the deleterious effects on the male reproductive system.

The doses of 200 and 650 mg/kg/day may be considered a NOEL and a MTD, respectively.

19. A 52-Week Oral (Gavage) Toxicity Study of Nevirapine in the Male and Female Rat, Batch No. # 13007, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, August 6, 1993, (U93-2023/TX-9304)*

Groups of male and female rats {strain: Chbb:THOM(SPF); age: 53 days; weight: 227.5-279.5 g for males and 172.1-205 g for females; 20 animals/sex/group} were administered nevirapine daily via oral dosing (gavage) in a suspension of 0.5% hydroxyethylcellulose (5 ml/kg) at dose levels of 0 (vehicle control), 5 (low), 50 (mid) or 100 mg/kg/day (high) for a period of 52 weeks and then terminally sacrificed. The purpose of the study was to assess the spectrum of undesirable side effects, identify target organs and estimate the Maximum Tolerated Dose (MTD) and No Effect Dose Level (NOEL) of the drug in rats. Mortality: one male (mid) was sacrificed for humane reasons

during the treatment. One female (mid) died during anesthesia for blood sampling after having dyspnea and foreleg edema. One male and female (high) were found dead after having poor clinical condition. Two male and nine females (high) were killed prematurely because of an esophagus-perforation due to gavage application error and multifocal skin injuries. Clinical Observations: transient dose-dependent sedation occurred in males (high) and females (mid or high) that disappeared after 6 weeks of dosing in males and after 36 weeks of dosing in females. Multifocal ulcerative skin lesions associated with scratching and biting occurred in one female (mid) and 2 males and 10 females (high). Body Weights: a treatment-related significant effect (reduction) on mean body weight of males (high, -17%) and females (mid, -21%; high, -41%) was noted. Food and Water Consumptions: food intake tended to increase in animals of both sexes (mid and high). Water consumption was dose-dependently increased in males (mid and high) and females (high) and slightly reduced in males (low). Ophthalmoscopy: no treatment-related changes were noted. Hematology: reduced RBC count, hemoglobin and hematocrit were noted in females (high), with increased reticulocyte count in both sexes and increased normocyte count in females. Total WBC count was reduced in mid and high dose males. Decreased lymphocyte to neutrophil ratio was noted at mid and high doses in both sexes. TPT was reduced in mid and high doses females Bone Marrow Smears: numbers and distribution of myeloid and erythroid series including erythroblasts, were within normal limits. Clinical Biochemistry: the main biochemical changes were dose-dependent increases in AST[1], ALT[1], GGT[1], total cholesterol, urea nitrogen and creatinine in both sexes at mid and high doses. Urinalyses: urine volume tended to decrease and specific gravity increased in females with increased protein excretion in females and to a lesser extent in males at mid and high doses. Feces Investigation: determination of occult blood was consistently negative. Drug Metabolism and Pharmacokinetics: nevirapine was adequately absorbed at all doses and peak plasma concentrations and AUCs increased with increasing doses (Table 5). Steady state plasma nevirapine levels were reached by week 8. C_{max} and AUC were consistently higher in females compared to males given the same dose. Peak plasma concentrations occurred at the earliest time point measured, 1.5 hr postdose, in males (mid and high) and in animals of both sexes (low). Organ Weights: dose-dependent increases in relative and/or absolute weight were noted for the following organs: liver and thyroid in males (mid and high); females thyroid at all doses and liver (mid and high); heart of high dose males and females at all doses; kidney in both sexes (mid and high); adrenals in females (mid and high) and males (high), and ovary and pituitary of females (mid and high). Gross and Histological Examination: the principal macroscopical finding

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[1] * statistically significant $p = <0.05$
** statistically significant $p = <0.01$

was ulcerative skin lesions in one female (mid) and 12 animals of both sexes (high). Edematous swelling of the subcutis of the legs or tail region was noted in one female in each mid and high dose groups. The main drug-induced histopathological findings were centrilobular hepatocellular hypertrophy and diffuse thyroid follicular hypertrophy and hyperplasia in both sexes (mid and high). Focal proliferative lesions of the thyroid follicular cells (cystic hyperplasia and adenoma) occurred at an increased incidence in males (high) and females (mid and high). Other findings were bilateral diffuse adrenal cortical hypertrophy mainly in females (high) and increased incidence and severity of thymic atrophy (mid and high). Ulcerative dermatitis in males (3 high) and females (2 mid and 13 high), sometimes accompanied by glossitis was observed.

Table 5

Mean Peak Plasma Concentrations and AUC_{0-24} of Nevirapine in Rats in the 52-Week Study

Dose (mg/kg)	Week	C_{max} (µg/ml)				AUC (µg*hr/ml)			
		Male		Female		Male		Female	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
5	Day 1	0.4	0.1	1.3	0.2	1.0	0.3	4.6	0.55
50	Day 1	5.0	0.8	13	2.0	16.2	3.1	54.6	7.3
100	Day 1	9.5	1.6	23.7	2.6	33.4	10.9	100.7	12.7
5	8	0.4	0.1	1.8	0.3	1.7	0.8	18.8	2.8
50	8	3.9	1.3	21.9	3.0	29.5	13.9	372.1	82.7
100	8	11.8	4.2	49.1	5.3	83.1	43.6	892.6	131.9
5	13	0.5	0.1	1.9	0.2	2.0	0.4	19.8	2.0
50	13	4.7	1.6	22.5	3.7	37.1	17.5	344.8	85.9
100	13	12.8	4.3	43.3	5.7	110.9	51.4	707.3	139.7
5	26	0.6	0.2	2.1	0.3	3.1	0.9	20.7	3.9
50	26	5.1	1.5	20.7	3.4	40.1	14.6	335.3	63.3
100	26	11.7	3.9	44.8	5.2	114.1	39.7	810.5	94.8
5	39	0.7	0.2	2.1	0.6	4.4	1.5	20.1	5.5
50	39	6.1	1.9	24.8	5.6	58.5	28.3	412.1	133.8
100	39	16.3	9.3	42.9	4.2	204.2	174.7	712.5	173.8
5	52	0.7	0.1	2.0	0.6	3.9	1.3	19.5	5.9
50	52	6.4	0.9	18.2	3.4	54.7	12.4	287.3	61.5
100	52	12.2	3.6	45.9	-	136.6	50.2	737.1	-

Comments: The main drug-induced clinical findings were cutaneous lesions in the form of ulcerative wounds and/or edema, which were observed particularly in high dose females. The primary target organs identified after 52 weeks of dosing were the liver, thyroid and adrenal. Nevirapine administration resulted in centrilobular hypertrophy and hyperplasia of the liver, with focal benign thyroid follicular cell tumors [presumably due to

hepatic microsomal induction] at mid and high doses. The thyroid follicular cell hypertrophy and hyperplasia at the same doses were attributable to hepatic induction of enzymes that metabolize thyroxine (a known effect). The pathogenesis of the adrenal cortical hypertrophy was unknown, but may involve hepatic induction of enzymes that hydroxylate corticosteroids produced by the adrenal cortex. The high dose in males and mid and high doses in females were poorly tolerated clinically, perhaps due to development of mucocutaneous lesions, with marked body weight gain suppression. The MTD was judged to be 50 mg/kg/day for males, and between 5 and 50 mg/kg/day for females. The NOEL for males (C_{max}, 0.7 μg/ml; AUC, 3.9 μg*hr/ml) was 5 mg/kg/day. The NOEL for females could not be identified; the low dose of 5 mg/kg/day resulted in C_{max} and AUC value of 2.0 μg/ml and 19.5 μg*hr/ml, respectively.

The plasma concentrations and the pharmacokinetic analysis demonstrated that nevirapine as a suspension was adequately absorbed in both male and female rats and dose related increases in plasma concentrations were observed in both sexes in this study. No unusual accumulation of the test compound in either sex was observed during the course of the study. As expected from earlier studies with nevirapine, C_{max} and AUCs were consistently higher in females which have been shown to metabolize the drug more slowly than males. Accordingly, incidence and severity of all toxic changes were more pronounced in females.

20. 52-week oral toxicity study in dogs with nevirapine granulation, Lot # RM-1379, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, October 11, 1995, (U95-3317/93-169)*

Groups of male and female dogs (strain: beagle; age: 7-9 months; weight: 7.4-12.5 kg; 4 dogs/sex/group) were administered nevirapine at initial dose levels of 0 (vehicle control), 50 (low), 150 (mid) or 400 mg/kg/day (high) orally via gelatin capsule once daily approximately 2 hr prior to feeding for a period of 52 weeks and then terminally sacrificed. Due to signs of excessive toxicity, the high dose was decreased in week 3 and 5 to 300 and 200 mg/kg/day, respectively. Correspondingly, the mid dose was decreased to 100 mg/kg/day in week 5. During weeks 4-7 of the study, 1/8 mid dose and 3/8 high dose animals were removed from the drug-dosing regimen due to extreme toxicity. These animals were readmitted to drug-dosing by week 8. Males and females were treated separately. Blood was collected from the jugular vein of each animal into heparinized tubes. The purpose of the study was to assess the potential toxicity of nevirapine during daily oral administration in dogs. Mortality: three animals (high) were sacrificed moribund prior to the termination of the treatment. One male was sacrificed on day 20 with signs of fulminant gastroenteritis; one male was sacrificed on day 115 with generalized bacterial folliculitis; and one female was sacrificed on day 325 with generalized cutaneous acariasis. One

female (high) displayed tremors, decreased motor activity and ataxia. The two other animals were sacrificed due to debilitated condition and severe skin changes. Clinical Observations: treatment-related clinical signs were observed among males and females (mid and high) included decreased motor activity, tremors, ataxia, ptosis, emaciation, absence of gum capillary refill time, distended abdomen, third eyelid eversion, dehydration, yellow sclera and gums, and labored respiration. Other drug-related clinical signs consisted of skin changes (lesions, scabs, erythema, hair loss and swelling), yellow, green or white discharge from the eyes, greenish discharge from the nostrils and mouth ulceration. Body Weights and Food Consumption: body weight loss due to drug toxicity was so severe in selected high dose animals in weeks 3-7 that diets were changed to increase appetite. Significant decreases in mean body weights were seen in females (high) compared to the controls. Body weight gains were decreased in male and female dogs (mid and high). Decreases in food consumption occurred in selected animals in weeks 3-9 (high). Ophthalmological & Cardiovascular Examinations: signs consistent with the presence of Horner's syndrome (bilateral protrusion of the nictitating membrane and conjunctival injection) were observed during week 4; in week 9 these changes were judged due to inflammation probably caused by nevirapine. In weeks 9, 13, 18, 38 and 51, there was no indication of primary ocular disease associated with nevirapine. No drug-related changes occurred in ECGs. Hematology: changes in selected animals (high) consisted of decreases in WBC count, RBC count, hemoglobin, hematocrit, MCHC, platelet count and absolute numbers of lymphocytes. Nucleated RBC and/or band neutrophils were observed in differential count of peripheral blood from selected animals (high). Clinical Biochemistry: drug-related changes included increased AST, ALT, ALP, GGT and total bilirubin in selected mid and high dose animals starting at week 5. Changes in triglycerides, total protein, albumin/globin ratios were noted in selected animals (mid and high). Drug Metabolism and Pharmacokinetics: the observed mean peak plasma concentrations and AUCs in male and female dogs are summarized in Tables 6-9. The peak plasma concentrations were generally observed at 1.5 hr after the low dose; however, in the mid and high dose groups, peak concentrations were observed either at 5 or 8 hr samples in both sexes. This may indicate that with increase in dose, prolonged absorption results.

Table 6
The mean peak plasma concentrations (μg/ml) in male dogs

Dose	Day 0	Wk 7	Wk 14	Wk 26	Wk 39	Wk 52
Low	4.7	6.1	6.3	7.4	3.6	4.6
Mid	26.7	8.9	5.5	10.8	14.3	9.3
High	50.7	20.9	17	25.9	28.7	21.1

Table 7
The mean peak plasma concentrations (μg/ml) in female dogs

Dose	Day 0	Wk 7	Wk 14	Wk 26	Wk 39	Wk 52
Low	3.1	6.1	6.1	2.3	2.1	5.2
Mid	15.7	10	13.6	5.1	10	10.5
High	30.3	20.9	25.9	11.9	25.6	20.2

Table 8
The mean $AUC_{0\text{-}24\ hr}$ (μg*hr/ml) in male dogs

Dose	Day 0	Wk 7	Wk 14	Wk 26	Wk 39	Wk 52
Low	33	31	42	28	23	27
Mid	345	89	68	112	198	134
High	794	288	201	284	270	253

Table 9
The mean $AUC_{0\text{-}24\ hr}$ (μg*hr/ml) in female dogs

Dose	Day 0	Wk 7	Wk 14	Wk 26	Wk 39	Wk 52
Low	12	29	36	17	14	45
Mid	165	53	112	47	82	116
High	456	294	359	173	357	274

Organ Weights: drug-related organ weight changes were present for the livers (mid and high). There was a statistically significant increase in the liver mean absolute weight, body weight ratio and brain weight ratio when compared to the controls. Macroscopic Observations: moribund animals: drug-related findings were seen in the tissues of the liver and gastrointestinal tract, hematopoietic-lymphoreticular and male reproductive systems of one male (high). These changes include pale discoloration of the liver and red discoloration of the cecum, colon and rectum, with

red luminal contents present in the lumen of the colon and rectum. Reduction in size of the thymus, prostate and testes were seen in one male (high). Liver enlargement was observed in the female (high). Enlargement of lymph nodes from several locations was seen in a high dose male and female, and both animals had skin changes consisting of multifocal papules and crusting. Terminal sacrifice animals: treatment-related findings were seen in the tissues of the liver and hematopoietic-lymphoreticular systems. Enlargement of the liver in one high dose male and splenic enlargement in one female (high) were seen. Microscopic Observations: correlations macroscopic/microscopic-moribund animals: drug-related hepatic enlargement correlated with hepatocellular hypertrophy in one female (high). In one male (high), pale yellow parenchymal discoloration of the liver correlated with treatment related diffuse hepatocellular vacuolation, and mucosal red discoloration in the cecum, colon and rectum correlated with typhlitis, colitis, and terminal colitis (proctitis), respectively. In the same animal, drug-related marked reduction in the size of the thymus correlated with corticomedullary lymphoid depletion and bilateral reduction in size of the testes and prostate corresponded to bilateral seminiferous tubular degeneration and prostate atrophy, respectively. Correlations macroscopic/microscopic-terminal animals: drug-related hepatic enlargement correlated with hepatocellular hypertrophy in one male (high). Lobular architectural prominence correlated with drug-related hepatocellular hypertrophy in several animals (mid and high). In the tissues of the hematopoietic-lymphoreticular system, drug-related splenic and lymph node enlargement (high, female) correlated with extramedullary hematopoiesis in the organs. Treatment-related changes-moribund animals: treatment-related findings were seen in the tissues of the liver, gastrointestinal tract, hematopoietic-lymphoreticular, and male reproductive systems. In the liver of three animals (high), treatment-related subacute, cholangiohepatitis, hepatocellular vacuolation, centrilobular hepatocellular hypertrophy, hepatocellular necrosis (1-4 cells) and biliary ductular increases were all seen. In the gastrointestinal tract, drug-related necrotizing ulcerative esophagitis, neutrophil infiltration in the duodenum, ileitis, typhlitis (cecum), colitis, and terminal colitis (in the rectum) were observed in one male (high); lymphoid depletion of the gut-associated lymphoid tissue (GALT) of the intestinal tract was seen in the other two animals. In the hematopoietic-lymphoreticular tissues, treatment-related focal pulp necrosis in the spleen, decreased bone marrow hematopoiesis, and corticomedullary lymphoid depletion in the thymus were seen in one male (high) and tonsillar lymphoid follicular depletion was seen in a female (high). Males (high) had diffuse lesions in the tissues of the male reproductive system. Bilateral seminiferous tubule degeneration in the testes, bilateral decreased spermatozoa/tubular luminal debris in the epididymides, bilateral atrophy of the epididymides and atrophy of the prostrate were

seen. Treatment-related changes-terminal sacrifice animals: in the liver, centrilobular hepatocellular hypertrophy was seen in males and females (mid and high) with a dose-related increase in severity, and hepatocellular necrosis (1-4 cells) was observe in males (high). Lymphoid depletion was observed in the GALT of the intestinal tract of males and females (high). Changes in the hematopoietic-lymphoreticular system included lymphoid depletion in the lymph nodes of males and females (mid and high), and lymphoid depletion in the splenic pulp (mid and high) with increases in severity by increasing dose.

Comments: Daily administration of nevirapine at a dose level of 400/300/200 mg/kg/day led to the death of 3 male and female dogs. Nevirapine was found to have deleterious effects on the male reproductive system. Principal target organs included liver, esophagus, intestines, bone marrow, lymph nodes, spleen, thymus, tonsil, testes, epididymides and prostate. The MTD was 200 mg/kg/day. The NOEL under the conditions of this study was considered to be 50 mg/kg/day. Based on body surface area conversion factor, an equivalent dose in humans would be 25 mg/kg/day.

Special Toxicity Studies

21. Effect of Oral Dosing of Nevirapine on Circulating Levels of Thyroid Hormones in Female Rats, Lot # RM-1177, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, June 4, 1993, (TX-9111/U93-0530)*

Female albino rats (weight: 167-225 g; age: 7 weeks; strain: Charles River Crl: CD BR VAF/plus) were randomly distributed into 12 groups of 10 females each. Six groups received 125 mg/kg suspended in 0.5% aqueous Methocel orally by gavage at a dosing volume of 10 ml/kg. Six groups were given 10 ml/kg of the vehicle control. Three drug-treated and three vehicle control groups received a single dose; the remaining groups received either drug or vehicle for 15 consecutive days. Serum levels of the thyroid hormones were measured by using appropriate validated radioimmunoassays. The purpose of the study was to assess the effect of nevirapine on serum levels of thyroid hormones (T3, T4, TSH and Reverse T3) in female rats when given orally for 15 consecutive days at a dose known to cause hepatic microsomal enzyme induction, centrilobular hepatocellular hypertrophy and thyroid follicular cell hypertrophy and hyperplasia.

Clinical Signs: drug-related signs were observed in few rats and included increased lacrimation, decreased motor activity and evidence of chromodacryorrhea. Thyroid Hormones Levels: immediately after the first dose of nevirapine, there were slight increases in serum T4 levels and a slight decrease in T4 levels after 2 and 24 hr ($p<0.05$) after dosing, respectively. After the 15th dose, T4 levels were significantly decreased relative to

controls at all post dose time points evaluated. These changes in T4 were accompanied by significant ($p<0.01$) increases in serum TSH levels and increase in T3 levels. Organ Weights: statistically significant increases in mean and relative organ weights were noted for liver, thyroid and adrenal. Histopathology: enlarged liver observed macroscopically showed mild to moderate centrilobular hepatocellular hypertrophy. Diffuse bilateral hypertrophy of the zona fasciculata of the adrenal cortex and diffuse thyroid follicular cell hypertrophy and hyperplasia also were observed.

Comments: Nevirapine elicited the expected (based on the results of previous studies) decreases in serum T4 and increases in serum TSH levels when given to female rats at daily oral doses of 125 mg/kg/day for 15 days. Changes in thyroid hormone levels were consistent with increased metabolism/elimination of T4 due to microsomal enzyme induction followed by compensatory increases in TSH

22. Assessment of primary eye irritation study in rabbits with nevirapine, Lot # 117, March 28, 1991, (TX-9108/90-049)*

Nevirapine was evaluated for its primary eye irritation potential in three male and three female acclimated albino rabbits (2.0 - 2.1 kg) following a single ocular administration of 0.03 g (0.1 ml weight equivalent) placed into the conjunctival sac of the right eye, with the left eye serving as the untreated control. The treated eyes were observed for ocular irritation at 24, 48 and 72 hr after the treatment. The test material produced slight conjunctival irritation in 4 of the 6 rabbits during the study. All eyes had returned to a normal appearance by 72 hr after the treatment. In conclusion, nevirapine was considered to be a strong eye irritant.

23. A 5-Day Intravenous Irritancy Study in the Albino Rabbit with Nevirapine, Lot # PD-1138, May 28, 1992 (TX-9205/91-092)*

Two groups of male New Zealand White rabbits (weight: 2.46-3.0 kg; age: 20 weeks; 5 animals/group) received either 0.5 ml of nevirapine (0.8 mg/ml in 30% propylene glycol/10% alcohol/0.9% sodium chloride) or vehicle control injected into the marginal ear vein of one ear by infusion (0.5 ml/min) once-a-day for 5 consecutive days. The purpose of this study was to determine the irritancy of nevirapine following intravenous infusion in the rabbit. In the nevirapine treated group, one animal was found dead on day 2 before administration of drug. A moderate amount of red liquid on the cage tray and red dry material on the nose of the animal were present. Upon gross pathological examination of the animal, the lungs were dark and uncollapsed with dark frothy fluid at their cut surface and in the trachea. In both the

groups, small contusions were observed at the infusion sites from day 1 until the day of sacrifice. Very slight to slight edema and very slight to severe erythema were noticed at the sites throughout the study. Microscopically, slight to severe, subacute perivascular inflammation characterized mainly by edema, hemorrhage, eosinophilic material suggestive of fibrin, mixed cell infiltration and fibroblast proliferation was observed in the ears of all animals of both groups. In addition, there was phlebitis and necrosis in the venous wall in animals of both groups. There were no nevirapine related effects on body weights.

Comments: The intravenous administration of 0.5 ml nevirapine (0.8 mg/ml in 30% propylene glycol/10% alcohol/0.9% sodium chloride) by infusion to the rabbit marginal ear vein once-a-day for 5 days was associated with perivascular inflammation, phlebitis and necrosis of the wall.

Based upon the results of gross pathological examination, it was unlikely that the animal died due to the toxicity of the test article administration. However, the sponsor did not perform a histopathological examination to determine cause of the death.

24. A 5-Day Intra-Arterial Irritancy Study in the Albino Rabbit with Nevirapine, Lot # TX-0496,
May 28, 1992 (TX-9206/91-093)*

Two groups of male New Zealand White rabbits (weight: 2.44-2.98 kg; age: 20 weeks; 5 animals/group) received either 0.5 ml of nevirapine (0.8 mg/ml in 30% propylene glycol/10% alcohol/0.9% sodium chloride) or vehicle control injected into the left auricular artery of one ear by infusion (0.5 ml/min) once-a-day for 5 consecutive days. The objective of the study was to determine the irritancy of nevirapine following the infusion in the rabbit. There were no deaths during the study. There were no test article related effects on body weights. Small to large contusions were noticed at the infusion sites in animals of both groups throughout the treatment period. In addition, on days 4-6, severe erythema and/or moderate edema were observed with similar incidence in both treated as well as control groups. Microscopic examination revealed changes in the treated ear of all animals of both groups. Perivascular inflammation in the ears was subacute, moderate or severe. The inflammation extended into the surrounding tissues and was associated with epidermal ulceration and crust. In addition, necrosis of the arterial wall with or without thrombosis and a slight acute arteritis were seen in both groups of animals. Hence, the daily intra-arterial administration by infusion of 0.5 ml nevirapine (0.8 mg/ml) to the rabbit auricular artery, for 5 consecutive days, has caused intra-arterial irritancy.

25. A Single Dose Intramuscular Irritancy Study in the Albino Rabbit with Nevirapine, Lot # TX-0496, May 28, 1992 (TX-9204/91-091)*

Two groups of male New Zealand White rabbits (weight: 2.48-2.81 kg; age: 20 weeks; 12 animals/group) were given a single im bolus injection (0.5 ml) of nevirapine (0.8 mg/ml in 30% propylene glycol/10% alcohol/0.9% sodium chloride) in the right thigh and the vehicle in the left thigh (Group I) or only sterile water in the right thigh (Group II). The purpose of the study was to determine the intramuscular irritancy of nevirapine in rabbits for 24 hr, 72 hr and 7 days after the injection. There were no deaths or nevirapine related clinical signs observed during the study. During the observation period, very slight erythema and very slight or slight edema were noticed in both nevirapine as well as control sites. Gross and histopathological changes observed at both the control and nevirapine injection sites were considered to result from irritation produced by the intramuscular administration of the vehicle. Hence, a single intramuscular bolus dose of 0.5 ml nevirapine (0.8 mg/ml) caused irritation at the injection sites.

Comments: A single im bolus dose of 0.5 ml nevirapine (0.8 mg/ml in 30% propylene glycol/10% alcohol/0.9% sodium chloride) to rabbits caused irritation at the injection sites. However, the incidence was similar in both control as well as treated animals. Thus, these findings suggest that the irritation at the injection sites may be related to the injection procedure and/or the irritant nature of the vehicle or the test article. However most likely, it was the vehicle which was responsible for the observed insults in the animals

26. Assessment of primary dermal irritation study in rabbits with nevirapine, Lot # RM-1177, March 28, 1991, (TX-9109/90-050)*

Three male and three female acclimated New Zealand White rabbits (2 - 2.3 kg) were tested for primary dermal irritation potential of nevirapine (0.5 g in 0.9% saline per site) on skin (abraded and non-abraded) under 24 occluded conditions. Thirty min after removal of the test material, dermal irritation readings were taken. A second reading was taken at 72 hr after the patch removal. The application of the test material did not result in any dermal irritation. Based on these results, nevirapine was considered to be non-irritant to the skin of rabbits and was not a primary skin irritant.

27. Assessment of acute dermal toxicity study in rabbits with nevirapine, Lot # RM-1177, March 28, 1991, (TX-9107/90-048)*

Six male and six female acclimated (2 - 2.3 kg) rabbits were

equally divided into two groups to study the acute dermal toxicity of nevirapine. The test compound (2.0 g/kg in 0.9% saline) was applied to the skin of both groups animals, except the skin of one group was abraded whereas the other one's remained intact. The area of application was covered with a 10 cm * 10 cm gauze patch secured with paper tape, and over wrapped with Saran Wrap and Elastoplast tape which remained in place for a period of 24 hr. The initial dermal irritation reading was made 30 min after removal of the test material on day 1 and subsequent readings were made on days 2, 3, 7, 10 and 14. There was a very slight dermal irritation on both abraded and intact skin of all the animals on day 1 only. Other than that, all animals appeared clinically normal and exhibited weigh gain throughout the study. In conclusion, nevirapine was not considered to be toxic by dermal route of exposure.

28. Assessment of dermal sensitization study in guinea pigs with nevirapine-close patch technique, Lot # RM-1177, , March 28, 1991, (TX-9110/U91-0417)*

Nevirapine was evaluated for a delayed contact hypersensitivity potential in 12 male and 12 female acclimated albino guinea pigs (360 - 548 g). The drug (0.2 g, moistened with 0.9% saline) was applied to an area along the anterior left flank on each of the 10 test animals. The sites were occluded for 6 hr, wiped clean and examined for dermal irritation 24 and 48 hr after the application. Four positive control animals received (0.3% w/v 2,4-dinitrochlorobenzene in 80% ethanol/deionized water) in the same manner as the test group. The animal received one application per week for a total of 3 applications. The naive control animals (10) were not treated in the induction phase. Two weeks after the third induction dose, a challenge dose of 0.2 g of the drug was administered along the anterior right flank of the treated and the naive control animals. The positive group of animals received the positive test material as during the induction phase.

The drug elicited no sensitization responses in any of the test or naive control animals. All four animals receiving the positive control material, 2,4-dinitrochlorobenzene exhibited dermal sensitization response at challenge. In conclusion based on these results, nevirapine was not considered a skin sensitizer in guinea pigs.

29. Exploratory Dose (IV) Tolerance Study in the Beagle Dog on Nevirapine, Batch I, Lot # 1177, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, March 31, 1992, (TX-9201/U92-0254)*

One male and one female dog were administered a single iv dose of vehicle (50% PG) in a pretest. On study day 0, the male received a single iv dose of nevirapine (4 mg/kg). On study day 1, both

animals received a single iv dose of nevirapine (4 mg/kg, bid). From study 2 through to termination on study day 6, both animals received nevirapine at 4 mg/kg, bid. Results: discolored urine was observed in both animals in pretest and during the dose phase of the study. The discoloration was confirmed to be blood. Conclusions: a maximum achievable dose of 4 mg/kg, bid was tolerated in dogs.

30. Five Day Exploratory I.V. Formulation Study in the Dog on Nevirapine, Lot # TX-0493, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, May 19, 1992, (TX-9117/U92-0471)*

Groups of male dogs (2/group) received iv doses of PG at dose levels of 0 (2 ml saline), 2 ml of 30%, 2 ml of 50% or 1 ml of 50%. Results: discolored urine (tested positive for blood) was observed in all groups receiving PG. A significant decrease in hematocrit (11-21%) was observed in the 50% PG groups but not in the 30% PG group. Conclusions: 30% PG would be utilized in nevirapine iv formulations.

Reproductive Toxicity Studies

31. Nevirapine: Study of fertility and general reproductive performance in rats after oral treatment by gavage (segment I), Batch No. # 13007, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, March 25, 1994, (U94-2043/TX-9402/72Q)*

Groups of male and female rats {strain: Chbb:THOM(SPF); age: 8 weeks for males and 14 weeks for females; weight: 236.5-266.9 g for males and 207.4-269.2 g for females; 24 animals/sex/group} were administered nevirapine via oral dosing (gavage) in a suspension of 0.5% hydroxyethylcellulose (10 ml/kg) at dose levels of 0 (vehicle control) 5 (low), 25 (mid) or 50 mg/kg/day (high) for females and 0 (vehicle control) 5 (low), 50 (mid) or 150 mg/kg/day (high) for males. The treatment of males started 10 weeks and of females 2 weeks before mating and lasted in the females through pregnancy and lactation. Hysterectomy was performed on half of the dams on day 22 of pregnancy, the other half was allowed to litter and rear their progeny. The purpose of the study was to evaluate general reproductive performance and fertility of rats. Parental toxicity: clinical signs of toxicity as lethargy was observed in all and scratching and skin scores in a few males (high). Body weight gain of males and females was reduced (15-42%) dose-dependently (mid and high) with concurrently decreased (31-38%) food consumption in females only. Estrous cycle: two dams (control), three (low), seven (mid) and five (high) showed an estrous cycle longer than 4 days. Reproductive toxicity: compared to the controls, the fertility and gestation indices were markedly decreased (high). Hysterectomy group: the number of implantation sites was significantly lower (high) compared to the controls. The preimplantation loss was significantly higher (mid and high)

compared to the controls. The resorption rate was significantly higher (high) than the controls. Correspondingly the number of viable fetuses was significantly lower (high). The sex ratio shifted in favor of females. The mean fetal weight was significantly decreased (22.3%, high) and (13.1%, mid) compared to the controls. One malformed fetus (low) showed hemivertebra and missing ribs, and one (mid) showed cleft vertebra and fused vertebrae. The frequency of delayed ossification was slight (mid) and significantly increased (high) compared to the controls. Littering group: a delay of delivery of one day appeared in one litter (low), in five litters (mid) and in four litters (high). A delay of two days was obvious in one litter (high). There was a significant and dose dependent decreased in the mean number of implantations and new-borns (mid and high). Effect on F1 offspring: one pup (mid) of a female and two pups (high) of a female were born dead. After weaning, there was a significant reduction in the mean body weight gain of the pups (mid) during lactation at days 14-21. Until week 10, a significant weight reduction was noticed in pups (high) compared to the controls. Fertility of F1-generation: the reproductive performance of one male and one female weanling from each litter was studied from week 10 p.p. on. One male each in control, low and high dose groups did not inseminate their partners. One female (low) did not become pregnant.

Comments: nevirapine induced lethargy, sedation, scabby wounds and scratching in the F0 males (high), reduced body weight gain in males and females of the F0 (mid and high), and impairment of reproductive performance (mid and high) males and females. Effects of nevirapine in the F1 were reduced fetal weight (mid and high) and reduced body weight gains (high). A NOEL for parental as well as reproductive and developmental toxicity was 5 mg/kg/day.

No plasma concentrations were measured in this study. In a previous teratology study, the plasma peak concentrations measured after the tenth dose ranged 15.7 to 19.4 µg/ml after 25 mg/kg/day, from 37.8 to 54.7 µg/ml after 50 mg/kg/day and from 62.2 to 75.4 µg/ml after 150 mg/kg/day nevirapine.

32. Range finding teratology study in rats with nevirapine by oral gavage, Lot # RM-1177, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, March 4, 1991, (TX-9106/U91-0353)*

Groups of nulliparous, sexually mature, inseminated, female, Chbb:Thom (strain) rats (ca. 200 g, 10 weeks old, 6 animals/drug group) were administered orally nevirapine (25, 50, 150 mg/kg/day) or vehicle (0.5% hydroxymethylcellulose) from early to late organogenesis: days 7 to 16 of gestation for a total of 10 days. The blood samples were taken at 0, 1.5 and 5 hr after the treatment. On gestation day (GD) 22, the animals were sacrificed and parameters with respect to maternal, embryo or fetal toxicity

were recorded. In the high dose of 150 mg/kg, marked clinical signs of toxicity were observed for five hr after dosing and included decreased motor activity, chromorhinorrhea, chromodacryorrhea and edema of the paws. Two dams one each died on GD 13 and 15 post treatment. No remarkable clinical signs were noted in 25 mg/kg group. In 150 mg/kg group, there was a clear reduction of food intake (62%) during the treatment period compared with the starting-point; which returned to normal after the treatment ended. Mean food consumption and body weight gain values exhibited no significant changes in 25 and 50 mg/kg groups compared to the controls.

The pregnancy rate was 66.7, 83.3 and 66.7% for the 25, 50 and 150 mg/kg groups, respectively (control group 66.7%). In the high dose group, all the pregnant animals resorbed their implantation completely. The low and mid dose groups showed no changes in the resorption rate compared to controls. All the pregnant female of the control and the low dose groups had viable fetuses. Percentages of early, late and total resorptions and sex ratio were not affected by the treatment. However, the mean fetal weight at the mid-dose was significantly reduced by 15% and there was a tendency for a decline of mean fetal weight in the low dose (5.1 g) vs control group (5.4 g). In the mid-dose group, three fetuses were classified as runts, e.g, fetus weighing less than 65% of the control fetuses mean weight. There were no external variations or malformations present in any of the dose groups. No pathological findings were seen in the dams at necropsy, and treatment related effects on the reproductive organs were not evident in any of the dose groups. The maternal NOEL in this study may be considered in the order of 50 mg/kg, and fetal NOEL was 25 mg/kg.

Comments: The present study was a dose range finding study. Another teratology study that is definitive should be conducted. In any case, nevirapine when administered during 7 - 16 days of gestation, resulted in maternal, fetal and embryo toxicities at 150 mg/kg, as indicated by the intercurrent deaths, marked clinical signs, reduction of food consumption and body weights, and complete resorption of the implantations in the early stage of organogenesis. Therefore, nevirapine should be considered highly embryotoxic drug. Since all the animals of this group had total resorptions, the reduced body weight gain may be attributed partly to the absence of fetal growth. On the other hand, maternal toxicity may have contributed to the embryolethality, as reflected by the resorption rate in this dose group.

2) Even at the mid dose level, the decrease of the fetal weights coupled with increase number of runts, reflects toxicity not only on the fetus but on maternal health as well. Teratogenicity could not be determined from this study.

3) The gross pathological findings were not observed in the study

probably because of the short duration of the treatment (10 days).

4) The sponsor has not submitted the toxicokinetic data from the study which should be made available with future submissions.

5) Based on the findings of this study, the sponsor will use a dose of 12.5, 25 and 50 mg/kg as a low, middle and high doses, respectively, in future teratogenicity studies.

33. Teratology Study in Rats With nevirapine by Oral Gavage, Lot # RM-1177, Boehringer Ingelheim, Birkendorfer Strabe, December 20, 1991, (TX-9211/U92-0299)*

Four groups of nulliparous, sexually mature and presumed pregnant female rats {strain: Chbb:Thom (SPF); age: 10 weeks; weight: 200-230 g; 23 animals/group} were administered via gavage during gestation days 7 to 16 at dose levels of 0 (vehicle control), 12.5 (low), 25 (mid) and 50 (high) mg/kg nevirapine. The data from the last 10 studies performed with this strain of rats were combined to yield historical values. The purpose of this study was to assess the maternal and embryo/fetal toxicity of the drug when administered by oral gavage to pregnant rats during the period of organogenesis. There were no deaths or abortions in any of the dose groups. No clinical signs or changes in behavior were noted. In the high dose group, there was a slightly lower food intake during mid gestation; however, the difference did not reach statistical significance. Body weight gain in the high dose was significantly lower. The pregnancy rates were 56.5, 82.6, 82.6 and 87% for the control, low, mid and high dose groups, respectively. There were no significant differences in the mean number of corpora lutea, implantation sites or the resorption rate per dam among the treated groups.

All pregnant animals of the different dose groups had viable fetuses. No dead fetuses were noted. The number of fetuses per litter and sex ratio were comparable between the dose groups. Percentages of early, late and total resorption were not affected by the treatment. In the high dose group there was significantly reduced mean fetal weight (5.0 g) compared to control (5.4 g). Two fetuses of the low and mid dose groups were classified as runts (offspring weighing less than 65% of the control). In total, 3 fetuses from three different litters had malformations. One fetus of the low dose showed bifid and missing ribs. Two fetuses of the high dose had a cleft vertebra or a cleft palate. Nevirapine was maternotoxic and embryotoxic at the 50 mg/kg level. The maternal and the developmental NOEL was 25 mg/kg.

Comments: This toxicological investigation was designed to pick up various adverse effects on embryo/fetal development. Under the condition of this study, nevirapine treatment resulted in mild maternal and fetal toxicity. Additionally, the administration of

nevirapine to pregnant rats during the period of organogenesis resulted in a moderate incidence of fetal abnormalities. However, there was no dose-response relationship observed; moreover, these abnormalities are commonly found in the rat strain in this laboratory. Hence, it was not conclusive whether nevirapine provokes teratogenic effects in rats.

The pregnancy rate in concurrent control vehicle group (0.5% hydroxyethylcellulose) was significantly lower compared to the treated groups or the historical values for this rat strain (88.7%) obtained in the laboratory. However, it was not possible to determine from the study which factors were interfering in normal pregnancy.

34. Nevirapine: Study of peri- and postnatal development in rats after oral treatment by gavage (segment III), Batch No. # 13007, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, March 25, 1994, (U94-2083/TX-9403/82Q)*

Groups of pregnant rats {strain: Chbb:THOM(SPF); age: 10 weeks; weight: 196-266 g; 23 animals/group} were administered nevirapine via oral dosing (gavage) in a suspension of 0.5% hydroxyethylcellulose (10 ml/kg) at dose levels of 0 (vehicle control), 5 (G1), 25 (G2), 40 (G3) or 100 mg/kg/day (G4) during late gestation (days 16-21) and through weaning. Results: effects on F0 dams were characterized by sedation after the treatment, chromodacryorrhea, enlargement of adrenals and deaths (G4). Sixteen dams died or were sacrifice in extremis after 6-12 applications (G4). Eight dams had bilateral adrenal enlargement (G4). Because of severe maternal toxicities, G4 was eliminated from the study. Effects on F1 offsprings were characterized by the littering of some animals one day early, a marked reduction in viability rate (day 4) and a slight lowering of weaning rate at day 21 (G3). In G3 animals, some litters exhibited group delays in eruption of incisors, growth of fur and opening of auditory and vaginal orifices. In males (G3), relearning ability (water maze test) was slightly decreased in week 7. Fertility of the F1 generation was characterized by a reduction in pregnancy rate, a decrease in number of corpora lutea and an increase in pre-implantation loss and resultant reduced number of viable fetuses (G3).

Comments: Based on these findings, the no toxic effect level of nevirapine for both maternal and reproductive, and developmental toxicities was considered to be 25 mg/kg/day. No plasma concentrations were measured in this study. In a previous teratology study, the plasma peak concentrations measured after the tenth dose ranged from 15.7-19.4 μg/ml after 25 mg/kg/day, to 37.8-54.7 μg/ml after 50 mg/kg/day and to 62.2-75.4 μg/ml after 150 mg/kg/day nevirapine.

35. Range finding teratology study in rabbits with nevirapine by oral gavage, Lot # RM-1177, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, March 7, 1991, (TX-9112/U91-0529)*

Groups of female, nulliparous, sexually mature and naturally mated rabbits (strain Chbb:HM; weight 2.5 kg; age 20 weeks; 6/group) were administered nevirapine (100, 250, 500 mg/kg in a dose volume of 5 ml/kg) or vehicle (Natrosol) via oral gavage during the period of organogenesis (gestation day 6 - 18, total 13 days) to determine maternal and embryo/fetal toxicity of the drug. In the high dose of 500 mg/kg, marked clinical signs of toxicity were observed for approximately 8 hr after the dosing in all animals and included: decreased motor activity, lethargy, miosis and suppressed respiration. No remarkable clinical signs were noted at the low and mid doses throughout the study period. A clear decline (12%) and a transient decrease in body weights were noted in high and low dose groups, respectively. The pregnancy rates were 83.3%, 66.7% and 66.7% for low, mid and high dose groups, respectively (control 83.3%). In the high dose group, all pregnant animals absorbed their implantations sites completely. So, too, in the mid dose group there was an increase rate of resorption (22.8%) compare to the control (5.6%). Low dose group showed no changes in the resorption rate. Treatment-related effects on the reproductive organs were not evident, and no pathological findings were seen at necropsy in any dose group. All pregnant animals of the control, low and mid dose groups had viable fetuses. Number of viable fetuses per litter and sex ratios were comparable among the groups. There was no influence on mean fetal weight by the treatment. No runts (offspring weighing less than 65% of the control mean 23.6 g) were noted. One fetus of the low dose group showed a hematocele frontalis. No other congenital anomalies were evident by external examination.

Comments: 1) Although the aim of the present study was to select appropriate dose levels for a definitive teratogenicity study in the future; nevertheless under the conditions of this study in rabbits, nevirapine was highly maternotoxic and embryolethal at 500 mg/kg level. Major adverse effects were decreased motor activity, lethargy, miosis and body weight loss. Embryolethality, as reflected in increased resorption rate was probably due to maternal toxicity. In the mid dose also, there was a mildly embryotoxic effect as indicated by an increase in resorption sites.

2) The hematocele frontalis in one fetus of the low dose group may be classified as a spontaneous anomaly; since there is no dose-dependency found.

3) Food intake and water consumption were not decermine in the study.

4) Results of the toxicokinetics were not included in the present

study and should be made available by the sponsor with future submissions.

5) On the basis of this study, the maternal NOEL in rabbits was in the order of 250 mg/kg, and the embryo/fetal NOEL was 100 mg/kg.

6) Granted, the toxicokinetics data parallels with the observed dose relationship in the present study, the sponsor has suggested following dosages (mg/kg) for the definitive teratogenicity study in rabbits: (low 30), (mid 100) and (high 300).

36. Teratology Study in Rabbits With nevirapine by Oral Gavage (Segment II), Lot # RM-1177, Boebringer Ingelheim, Birkendorfer Strabe, October 21, 1991, (TX-9210/U92-0312)*

Four groups of nulliparous, sexually mature and presumed pregnant female rabbits {strain: Chbb:HM (SPF); age: 20 weeks; weight: 2.5 kg; 18 animals/group} were administered via gavage during gestation days 6 to 18 at dose levels of 0 (vehicle control), 30 (low), 100 (mid) and 300 (high) mg/kg nevirapine. The data from the last 10 studies performed with this strain of rabbits were combined to yield historical values. The purpose of this study was to assess the maternal and embryo/fetal toxicity of the drug when administered by oral gavage to pregnant rabbits during the period of organogenesis. There were no deaths. Marked clinical signs of toxicity were noted for 4 hr after dosing (high) in all animals, and included decreased motor activity and lethargy. Abortions occurred in one rabbit (low dose) and 3 animals (high dose). In the high dose group, there was a body weight decrease (4%) after onset of the treatment, and body weight suppression persisted to the end of dosing and gestation. The pregnancy rates were 66.7, 94.4, 77.8 and 77.8% for the control, low, mid and high dose groups, respectively. In the high dose group, there was statistically significant decrease in the number of corpora lutea, implantation sites and live fetuses paralleled by an increase in the resorption rate (early) to 40.7% (control, 9.4%). Late resorption were noted in 3 dams (high), 1 (mid) and 2 (low) dose groups.

All pregnant animals in the control, low and mid dose groups had viable fetuses. In the high dose group, the number of fetuses per litter were halved (3.6) compared to control (7.8) and there was a numerical decrease in live fetuses as well. One runt (control) and two runts (low) dose groups were found. The number of fetuses with variations (incomplete ossification of skull and limbs, ventricular septal defects, flexure of limbs and misshaped sacral vertebrae) were slightly increased, in low and mid dose groups compared to concurrent controls. The number of fetuses with supernumerary ribs were increased to 13.8% (high dose) vs controls (0%). In the mid dose group, 4 fetuses from three different litters had malformations: two fetuses with fused

sternebrae and the other two fetuses with hematocele frontalis or bifid rib and hemivertebra.

Nevirapine, when administered to pregnant rabbits during days 6-18 of gestation, resulted in maternal toxicity in the high dose group, as indicated by the marked clinical signs and body weight reduction. Three abortions in this group were also treatment related; although, it was not clear whether the abortions were due to decreased food consumption or were a direct result of treatment. However, no gross pathological findings related to treatment were noted in the dams.

In the low and mid dose groups, there were no distinct maternotoxic effects; also, no influence on fetal growth or other embryo/fetal toxicity was evident. In the high dose group, however, the resorption rate was clearly increased; particularly, early resorptions were found, indicating damage in an early stage of organogenesis. The decrease in mean corpora lutea and implantation sites were also treatment related.

Exposure of rabbits to nevirapine during the period of major organogenesis affected postimplantation. At high dose, the drug was maternal/fetal toxic and caused fetal malformations. NOAEL for maternal toxicity appeared to be 100 mg/kg/day and NOAEL for developmental toxicity may also be 100 mg/kg/day.

Comments: The incidence of malformed fetuses was similar in treated and vehicle controls and was within the historical range values obtained from this strain of rabbit in the laboratory. Moreover, there was no dose-response relationship observed. Thus, it was inconclusive whether nevirapine was teratogenic.

The studies in the rats and rabbits were confounded by the low pregnancy rates in the vehicle controls as compared to the treated groups. The low rate of pregnancy should be investigated by the sponsor.

Genotoxicity Studies

37. Nevirapine mutagenicity testing with Salmonella typhimurium TA1535, TA1537, TA98 and TA100. Pre-incubation reverse mutation assay, Lot # RM-1152, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, December 12, 1989, (090-0142/TX-8901)*

In an in vitro assay, nevirapine was evaluated, either with or without S9 mix, at concentrations of 0, (vehicle control), 1.7, 5, 16.7, 50, 166.7 or 500 µg/plate using 4 different strains of Salmonella typhimurium: TA1535, TA1537, TA98 and TA100 for its mutagenic potential. Results: nevirapine did not induce an increase in colony number in the 4 Salmonella typhimurium strains either in the presence or absence of S9 mix. Conclusion: nevirapine was not mutagenic under the conditions of this assay.

38. Nevirapine mutagenicity testing with Salmonella typhimurium TA1535, TA1537, TA98 and TA100 and Escherichia coli WP2 uvr A. Pre-incubation reverse mutation assay, Lot # RM-1152, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, July 16, 1990, (U90-0479/TX-9001)*

In an in vitro assay, nevirapine was evaluated, either with or without S9 mix, at concentrations of 0. (vehicle control), 1.7, 5, 16.7, 50, 166.7 or 500 µg/plate using 4 different strains of Salmonella typhimurium: TA1535, TA1537, TA98 and TA100, and WP2 uvr A of Escherichia coli for nevirapine mutagenic potential. Results: nevirapine did not induce an increase in colony number in the Salmonella typhimurium or Escherichia coli strains either in the presence or absence of S9 mix. Conclusion: nevirapine was not mutagenic under the conditions of this assay.

39. BIRG 0106 (metabolite of nevirapine) mutagenicity testing with Salmonella typhimurium TA1535, TA1537, TA1538, TA98 and TA100 and Escherichia coli WP2 uvr A. Pre-incubation reverse mutation assay, Lot # 1303/64, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, August 28, 1990, (U90-0544/TX-9001)*

In an in vitro assay, BIRG 0106 (a metabolite of nevirapine) was evaluated, either with or without S9 mix, at concentrations of 0, (vehicle control), 1.7, 5, 16.7, 50, 166.7 or 500 µg/plate using 4 different strains of Salmonella typhimurium: TA1535, TA1537, TA98 and TA100, and one strain of Escherichia coli: WP2 uvr A for its mutagenic potential. Results: BIRG 0106 did not induce an increase in colony number in the Salmonella typhimurium or Escherichia coli strains either in the presence or absence of S9 mix. Conclusion: BIRG 0106 was not mutagenic under the conditions of this assay.

40. Nevirapine: CHO/GPRT mutation assay, Lot # RM-1177, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, November 14, 1990, (U90-0676/TX-9009)*

Nevirapine was evaluated, either with or without S9 mix, at concentrations of 0 (vehicle control), 51, 102, 204, 408 or 816 µg/ml in the CHO/HGPRT mutation assay. Results: cytotoxicities and mutant frequencies with or without activation (highest to lowest concentration) were not significantly different from the vehicle controls. Conclusions: under the conditions of the assay, nevirapine was found to be negative in the CHO/HGPRT mutation assay both with and without exogenous metabolic activation.

41. Chromosome aberrations in Chinese hamster ovary (CHO) cells, Lot # RM-1177, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, December 26, 1990, (TX-9101/90-040)*

Nevirapine was evaluated for chromosome aberrations using CHO

cells. The drug was tested at concentrations between 100 and 800 µg/ml, and the assay was conducted both in the absence and presence of an Aroclor-induced S-9 activation system. There was no observable increase in chromosome aberrations in either the non-activated or S-9 activated test system; however, the mitotic index was slightly reduced relative to the solvent control at dose level 800 µg/ml at the 20 hour harvest. Levels of drug higher than 800 mg/ml could not be tested due to the solubility limit in the test media. In conclusion, nevirapine causes no chromosomal aberration in the CHO cytogenetics assay.

42. Nevirapine: Micronucleus test in mice, Batch # I, Boehringer Ingelheim Pharmaceutical, Inc., Federal Republic of Germany, August 19, 1992, (TX9202/MUT0198/U92-0175)*

Groups of male and female mice, Chbb:NMRI, (weight: 28 - 50 g; age: 10 weeks; 1 animal/sex/group) were administered nevirapine (2000 mg/kg) orally by gavage in a dose volume of <20 ml/kg. Sampling was performed at 24, 48 and 72 hr. Vehicle (0.5% Methocel solution) and positive (cyclophosphamide, 50 mg/kg) control groups were included in the protocol. The purpose of the study was to determine the mutagenic potential of nevirapine in bone marrow cells of mice using the micronucleus test. At least 1000 polychromatic erythrocytes from each animal were evaluated for the presence of micronuclei and the ratio of polychromatic to normochromatic erythrocytes was determined. One male and a female mouse showed an increase in the number of micronucleated polychromatic erythrocytes frequency in 72 hr- and 24 hr-treatment groups, respectively. None of the other mice in any treatment group showed any biologically relevant or statistically significant increase in the frequency of micronucleated polychromatic erythrocytes. In contrast, cyclophosphamide (positive control) significantly increased the frequency of micronucleated polychromatic erythrocytes. In conclusion, there was no evidence that nevirapine induces chromosome damage in bone marrow cells of mice.

43. Nevirapine: Mutagenicity testing with Salmonella typhimurium and Escherichia coli. Plate incorporation reverse mutation assay with and without metabolic activation, Lot # 2439-98-1, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, April 11, 1995, (U95-3056/40025-02)*

Nevirapine (concentrations ranging from 156 to 5000 µg/plate) was tested with Salmonella typhimurium (strains: TA1535, TA1537, TA98 and TA100) or Escherichia coli (pKM101) in the presence or absence of rat liver preparation (S9) using the plate incorporation method of the bacterial mutation assay. Results: in the presence of an S9 mixture, there was a reproducible, dose related increase in E. coli revertants at dose levels greater than or equal to 3000 µg/plate. In none of the Salmonella strains tested, there was a significant increase in the number of

colonies after treatment with nevirapine either in the presence or absence of an S9 mix. Conclusion: nevirapine was mutagenic under the conditions of this assay.

44. CAPIC: Mutagenicity testing with L5178Y TK+/- mouse lymphoma cells, forward mutation assay, Lot # IVT-0105, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, August 23, 1995, (U95-3318)*

CAPIC, an intermediate in the synthesis of nevirapine, was tested with L5178Y TK+/- mouse lymphoma cells in vitro over a dose range of 200-2000 µg/ml in the absence of an activation system. In the presence of an Aroclor 1254-induced rat liver preparation and cofactors (S9 mix), CAPIC was more toxic and a dose range of 1-7 µg/ml was evaluated. After approximately 48 hr to permit expression of induced mutations, the cells were plated in soft agar to determine the frequency of trifluorothymidine resistant (mutant) cells. Results: in the absence of an activation system, there was a dose related decrease in total relative growth over the entire dose range that reduced the growth to ~10-20% of concurrent controls. There was a dose-related increase in mutant frequency at dose levels greater than 400 µg/ml. In the presence of an activation system, there was a dose-related increase in mutant frequency that reached significant levels at doses of greater than 3 µg/ml. Conclusion: CAPIC was mutagenic under the conditions of this study.

45. BIRH 414 BS: Mutagenicity testing with *Salmonella typhimurium* and *Escherichia coli*. Plate incorporation reverse mutation assay with or without metabolic activation, Lot # 2589-25-3, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, August 24, 1995, (U95-3319)*

BIRH 414 BS, an impurity in nevirapine, was tested with *Salmonella typhimurium* strains: TA1535, TA1537, TA98 and TA100 and *Escherichia coli* WP2 uvrA using the plate incorporation method of the bacterial mutation assay at a dose range of 156-5000 µg/plate in the presence and absence of an S9 mixture. Results: toxicity was evident in TA100 at dose levels greater than or equal to 1250 µg/plate in the absence of an activation system and greater than or equal to 313 µg/plate in the presence of an activation system. The test compound was toxic to WP2 uvrA at a dose level of 5000 µg/plate in the presence of an activation system. In none of the bacterial strains tested was there a significant increase in the number of colonies after treatment with the test compound in either the presence or absence of an S9 mix. Conclusion: BIRH 414 BS was non-mutagenic under the conditions of the study.

46. BIRG 616 BS: Mutagenicity testing with Salmonella typhimurium and Escherichia coli. Plate incorporation reverse mutation assay with or without metabolic activation, Lot # 2589-21-2, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, August 24, 1995, (U95-3321)*

BIRH 616 BS, an impurity in nevirapine, was tested with Salmonella typhimurium strains: TA1535, TA1537, TA98 and TA100 and Escherichia coli WP2 uvrA using the plate incorporation method of the bacterial mutation assay at a dose range of 2.44-78.00 µg/plate in the presence and absence of an S9 mixture. Results: in none of the bacterial strains tested was there a toxicity or significant increase in the number of colonies after treatment with the test compound in either the presence or absence of an S9 mix. Conclusion: BIRH 616 BS was non-mutagenic under the conditions of the study.

Exploratory studies

47. Exploratory (IV) tolerance study in the female rat with 30% propylene glycol, Lot # I, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, June 20, 1995, (U95-3162/TX-9203)*

Four groups of female rats (3/group) received iv administrations: G1 (9% saline), G2 (30% Propylene Glycol; 2 ml/kg; 0.5 ml/min infusion), G3 (30% Propylene Glycol; 1 ml/kg; 0.5 ml/min infusion) or G4 (30% Propylene Glycol; 2 ml/kg; 0.3 ml/min infusion) for 5 consecutive days. The purpose of this study was to determine the effects of total dose volume and rate of administration on the hemolyzing potential of 30% Propylene Glycol (PG). Results: discolored urine (red or green) was seen on all the animals treated with 30% PG. No significant decreases in hematocrit were observed in any of the animals on this study. On test day 4, 6/9 animals treated with 30% PG exhibited moderate hematuria. Microscopically, animals treated with 30% PG were observed with vascular thrombosis accompanied by perivascular fibrinous exudate and edema. Several treated animals were observed to have suppurative vasculitis accompanied by vascular degeneration. Conclusion: these data demonstrated that rats tolerated administration of 30% PG at dose volumes up to 2 ml/kg at a dose rate of up to 0.5 ml/min.

48. Preliminary Rising Dose Oral (Capsule) Toxicity Study in the Beagle Dog, Lot # RM-1162/1169, Boehringer Ingelheim Pharmaceutical, Inc., Ridgefield, CT, 22 May, 1990, (TX9007/90-030)

Groups of male and female purebred beagle dogs (weight: 6.7 - 7.2 kg; age: 9 months; 1 animal/sex/group) were given nevirapine orally in capsules at escalating doses of 200, 400, 800 and 1200 mg/kg in an alternate day dosing regimen. In the second phase, one animal of each sex was given 800 mg/kg/day for 5 days. The

purpose of this preliminary dose range finding study was to assess the toxicity of nevirapine when given orally to the dogs and to select doses for the two-week repeated dose study. No drug-related clinical signs of toxicity occurred at doses of 200 and 400 mg/kg. Single doses of 800 and 1200 mg/kg and repeated doses of 800 mg/kg caused slight to moderate decreases in motor activity, ataxia and head tremors. Slight to mild increases in serum ALP and cholesterol levels were observed in the treated groups. In the escalating dose phase, mild thymic lymphoid depletion was observed in the females. In the repeated dose phase, pathological abnormalities included small thymus in the males, multifocal red or pink foci in the lungs of both the animals, thymic depletion or lymphocytolysis in both the animals, and lymphoid depletion of the tonsil, spleen, gut-associated lymphoid tissue and lymph nodes in the males. The pulmonary changes were multifocal and consisted of interstitial pneumonitis, subacute and/or chronic bronchopneumonia, mononuclear arteritis and periarteritis and acute alveolar hemorrhage.

The peak plasma concentrations at doses of 200 to 1200 mg/kg in the rising dose phase ranged from 1.4 - 82.3 μg/ml. The AUCs showed a roughly linear relationship with the dose. In the repeated dose phase on day 1, peak plasma concentrations were 76.1 μg/ml for the male and 32.4 μg/ml for the female at 24 hr post dose. On day 5, peak concentrations were still high in the male (46.0 μg/ml) vs female (15.9 μg/ml) at 6 hr post dose. In addition, there was a 58 to 85% reduction in AUC from day 1 to day 5 in both the animals.

Comments: Based on the data acquired from this study, the sponsor selected doses of the test compound: 100, 400 and 800 mg/kg/day for the 14-day oral toxicity study in the dogs. The observed difference in the peak plasma concentrations between male and female animals were consistent with the previous observations in dogs and other animals. The observed locomotor toxicity in dogs was a shared property of the benzodiazepines and the degree of toxicity varies for different compounds in the class.

NONCLINICAL PHARMACOKINETICS & ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION (ADME) STUDIES

Summary of ADME Studies:

1. **Whole-body autoradiographical investigations with nevirapine in rats after iv and oral administrations, Boehringer Ingelheim Pharmaceutical, Inc., Ridgefield, CT, September 19, 1990, (U90-0542)**

2. **Induction of drug-metabolizing enzymes in rat liver by nevirapine, Boehringer Ingelheim Pharmaceutical, Inc.,**

Ridgefield, CT, October 17, 1990, (DM-9002)

3. Nevirapine plasma protein binding in several species, Boehringer Ingelheim Pharmaceutical, Inc., Ridgefield, CT, October 24, 1990, (DM-9013)

4. Bioavailability and pharmacokinetics of nevirapine in dogs, Boehringer Ingelheim Pharmaceutical, Inc., Ridgefield, CT, October 26, 1990, (DM-9007)

5. Absorption, distribution and excretion of ^{14}C-nevirapine in rats, Boehringer Ingelheim Pharmaceutical, Inc., Ridgefield, CT, October 29, 1990, (DM-9008)

6. Nevirapine oral rising dose tolerance study in the male and female monkeys: Plasma concentration results, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, September 21, 1990, (DM-9004)

7. Nevirapine oral rising dose tolerance study in the male and female dogs: Plasma concentration results, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, September 21, 1990, (DM-9005)

8. Nevirapine bioavailability and dose linearity in the rat, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, November 28, 1990, (DM-9011)

9. Nevirapine plasma concentrations in male and female rats during 14-day toxicology study, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, October 18, 1990, (DM-9010)

10. Nevirapine pharmacokinetics in chimpanzee after single iv and oral doses, multiple oral doses and coadministration with ^{14}C-AZT, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, December 5, 1990, (DM-9014)

11. Preliminary absorption, distribution and metabolism of nevirapine in chimpanzee, monkey, dog, rat and mouse Boehringer Ingelheim Pharmaceutical, Inc., Ridgefield, CT, December 7, 1990, (DM-9001)

12. Pharmacokinetics of nevirapine in chimpanzees: dosing, sampling and monitoring, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, November 27, 1990, (DM-9015/U91-0189)

13. Nevirapine plasma concentrations in male and female dogs during 14-day toxicology study, Boehringer

Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, December 26, 1990, (DM-9009)

14. Preliminary oral rising dose toxicity study in dogs: Plasma concentrations, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, February 12, 1991, (DM-9103)

15. Co-administration of nevirapine and AZT to cynomolgus monkeys: Blood chemistry & hematology, urinalysis, and EKG, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT (DM-9104/U91-0235)

16. Relative Bioavailability of Two Formulations of nevirapine in Dogs, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT (DM-9105/nevirapine)

17. Nevirapine concentration in plasma samples from satellite groups of male and female dogs during the 4-week oral toxicity study, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT (DM-9101)

18. One week oral (diet) bioavailability study comparing nevirapine bulk powder to nevirapine granulation in the rat, Lot # RM-1167, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, May 26, 1994, (U94-3067/TX-9401)

19. Nevirapine concentration in plasma samples from satellite groups of male and female rats during the 4-week oral (gavage) toxicity study, Boehringer Ingelheim maceuticals, Inc., Ridgefield, CT, (DM-9102/90-054)

20. Nevirapine concentration in plasma samples from male and female dogs after 800 mg/kg oral dose in a 28-day toxicity study, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, (DM-9106)

21. Supplementary studies of ^{14}C-Nevirapine in rats: Excretion balance after iv and oral dosing, Boehringer Ingelheim Pharmaceutical, Inc., Ridgefield, CT, March 5, 1992, (DM-9108)

22. Plasma AZT Levels in Chimpanzees after Oral Administration of ^{14}C-AZT Alone and in Combination with Nevirapine, AZT Lot # 5499, Nevirapine Batch # H, Boehringer Ingelheim Pharmaceutical, Inc., Ridgefield, CT, March 20, 1992, (DM-9110/U92-0198)

23. Whole-body Autoradiographical Investigations With Nevirapine in Pregnant Rats After Intravenous and Oral

Administration, Batch/Lot not provided, Boehringer Ingelheim KG, Binger Strabe, March 3, 1992, (DM-9209/U92-0220)

24. Plasma Concentration of Nevirapine in Female Rats During a Range Finding Teratology Study, Batch # RM-1177, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, February 24, 1992, (DM9109/U92-0237)

25. Plasma Concentration of Nevirapine in Female Rabbits During a Range Finding Teratology Study, Batch # I, RM-1177, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, March 2, 1992, (DM-9111/U92-0238)

26. Plasma Concentrations of Nevirapine in Beagle Dogs During a Two Week Oral Dose Range Finding Toxicity Study of Nevirapine Granulation, Batch # TX-0412, RM-1231, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, April 11, 1992, (DM-9204/U92-0582)

27. Plasma Concentration of Nevirapine in Female Rabbits During a Segment II Teratology Study After Oral Administration (Gavage) of Nevirapine, Batch I, Lot # RM 1177, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, September 1, 1992, (DM-9203/U92-0583)*

28. Concentration in Plasma Samples from Albino Rats During a 13-Week Oral (Gavage) Toxicity Study of Nevirapine, Lot # RM-1212, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, September 21,1992, (DM-9206/U92-0639)

29. Concentration in Plasma Samples from Beagle Dogs During a 13-Week Oral (Gavage) Toxicity Study of Nevirapine as Powder in Gelatine Capsule, Lot # RM-1230/1231, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, January 26, 1993, (D-9205/U91-072)

30. Concentration in Plasma Samples from Albino Rats During a 13-Week Oral (Gavage) Toxicity Study of Nevirapine, Lot # RM-1212, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, September 21,1992, (DM-9206/U92-0639)

31. Nevirapine Concentration in Plasma Samples From Albino Rats During a 52-Week Oral (Gavage) Toxicity Study of Nevirapine in the Male and Female Rat, Batch No. # 13007, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, June 4, 1993, (U93-0537/DM-9303)

32. The comparative metabolite pattern of ^{14}C-nevirapine in the urine of the male rat and beagle dog, Lot # G,

Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, December 20, 1993, (DM-9319/U94-0008)

33. In vitro human red blood cell partitioning of ^{14}C-Nevirapine, Lot # A1126-90, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, May 6, 1994, (U94-3038/DM-9406)

34. Plasma analysis for one week oral (diet) bioavailability study comparing nevirapine bulk powder to nevirapine granulation in rat, Lot # RM-1167, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, May 5, 1994, (U94-3053/DM-9318)

35. Nevirapine plasma protein binding in several species: a review of the original data plus previously unreported data, Batch # GJV 1407:2, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, October 31, 1994, (U94-3128/DM-9419)

36. Excretion balance studies following oral administration of ^{14}C-Nevirapine to mouse, rat, rabbit, dog and monkey, Batch # GJV 1407:175, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, October 31, 1994, (U94-3153/DM-9410)

37. Lacteal (milk) transfer of ^{14}C-nevirapine after single oral (gavage) administration in rats, Lot # RM-1167, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, December 29, 1994, (U95-3043/DM-9505)

38. In vitro metabolism of nevirapine by liver microsomes from the chimpanzee, Lot # K, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, March 3, 1995, (U95-3063/DM-9402)

39. The biotransformation of ^{14}C-nevirapine in the mouse, rat, rabbit, dog and monkey, Lot # K, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, March 24, 1995, (U95-3083/DM-9507)

40. The comparative metabolic pattern of ^{14}C-nevirapine in the mouse, rat, rabbit, dog and monkey, Lot # K, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, March 28, 1995, (U95-3090/DM-9506)

41. The pharmacokinetics of nevirapine in male and female dog plasma, Lot # I, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, May 31, 1995, (U95-3161/DM-9515)

42. The comparative metabolic pattern of ^{14}C-nevirapine in

rat milk and plasma, Lot # A, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, June 6, 1995, (U95-3167/DM-9511)

43. Pharmacokinetic report of 52-week oral toxicity study in the beagle dog with nevirapine granulation, Lot # TX-0579, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, July 14, 1995, (U95-3308/DM-9502)*

44. In vitro metabolism of nevirapine by liver microsomes from male rat, female rat, dog and monkey, Lot # K, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, July 28, 1995, (U95-3312/DM-9510)

45. The pharmacokinetics of nevirapine in male chimpanzee plasma, Lot # XP-1413-140, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, July 28, 1995, (U95-3313/DM-9516)

46. Plasma analysis for thirteen week oral (diet) range-finding toxicity study in the CD-1 mouse with nevirapine, Lot # RM-1167, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, August 1, 1994, (U94-3068/DM-9315)

47. Potential for metabolic drug interaction with nevirapine as determined in vitro with human liver microsomes: III. Rifampicin, Lot # K, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, January 16, 1995, (U95-3024/DM-9422)

48. The pharmacokinetics of nevirapine in male and female mouse plasma, Lot # I, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, May 31, 1995, (U95-3160/DM-9514)

49. Potential for metabolic drug interaction with nevirapine as determined in vitro with human liver microsomes: II. Rifabutin, Lot # K, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, January 12, 1995, (U95-3023/DM-9421)

50. Potential for metabolic drug interaction with nevirapine as determined in vitro with human liver microsomes: V. Trimethoprim, Lot # K, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, February 24, 1995, (U95-3060/DM-9503)

51. The in vitro metabolism of nevirapine by human-liver microsomes, Lot # K, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, August 25, 1995, (U95-3327)

Review of ADME Studies:

1. Whole-body autoradiographical investigations with nevirapine in rats after iv and oral administrations, Boehringer Ingelheim Pharmaceutical, Inc., Ridgefield, CT, September 19, 1990, (U90-0542)

Groups of male rats were administered a single dose of ^{14}C-nevirapine via oral (19 mg/kg) or iv (1.05 mg/kg) routes. The animals were sacrificed after 5 min, 30 min, 3 hr, 8 hr and 24 hr to investigate the distribution of the test compound in the organs and tissues by whole-body autoradiography. Results: after the iv administration, nevirapine was distributed over the whole animal, with higher concentrations in the adrenal grands, liver, kidneys and gastric and intestinal contents. At 8 hr, radioactivity can be detected only in liver, kidneys and GI tract. After the oral administration, the compound was adequately absorbed. Higher radioactivity concentrations were found in the liver, kidneys and GI tract.

2. Induction of drug-metabolizing enzymes in rat liver by nevirapine, Boehringer Ingelheim Pharmaceutical, Inc., Ridgefield, CT, October 17, 1990, (DM-9002)

Groups of male rats were dosed with nevirapine via gavage at 0 (vehicle control) or 100 mg/kg for four days to study induction of drug-metabolizing enzymes in the liver and were compared to those produced by treatment with ß-naphthoflavone (BNF) and phenobarbital (PB). Results: the livers from rats treated with nevirapine (130%), BNF (119%) or PB (142%) had significantly higher (p=0.01) relative weights (with respect to total body weights) than did livers from control rats. The levels of microsomal protein (mg/g liver weight) were greater (p=0.01) in nevirapine and PB-treated rats than in control rats, while protein levels in microsomes from BNF-treated rats were significantly lower (p=0.05) than those of control rats. The activity of 7-ethoxycoumarin-o-deethylase was elevated in nevirapine, BP and BNF treated rats with respect to the controls; however, the 7-ethoxyresorfin-o-deethylase activity was elevated only in liver microsomes from rats treated with BNF. The activity of d-benzphetamine-N-demethylase in microsomes from PB and nevirapine treated animals was significantly elevated over the activity of microsomes from control and BNF-treated rats. Conclusions: these data indicate that the induction of drug-metabolizing enzymes resulting from treatment with nevirapine was similar to that resulting from PB treatment.

3. Nevirapine plasma protein binding in several species, Boehringer Ingelheim Pharmaceutical, Inc., Ridgefield, CT, October 24, 1990, (DM-9013)

Plasma protein binding of ^{14}C-nevirapine was studied by

equilibrium dialysis at 37°C in rats, dogs, monkeys, chimpanzees and humans. Results: at non-saturating concentrations, binding averaged 52.2% for rat, 60.9% for dog, 46% for monkey, 45.6% for chimpanzee and 61.5% for human. Saturation of binding was not observed at nevirapine concentrations up to 19 μg/ml in rat, 0.24 μg/ml in dog, 0.91 μg/ml in monkey 0.89 μg/ml in chimpanzee and 4.9 μg/ml in human. Conclusions: nevirapine protein binding was found to be moderate in all species studied.

4. Bioavailability and pharmacokinetics of nevirapine in dogs, Boehringer Ingelheim Pharmaceutical, Inc., Ridgefield, CT, October 26, 1990, (DM-9007)

Four male dogs received a 1 mg/kg (iv) dose, a 20 mg/kg oral suspension and two different tablet formulations, each consisting of 200 mg of nevirapine. Dogs were allowed a washout period of two weeks between doses. Results: nevirapine pharmacokinetic parameters after the 1 mg/kg iv dose are described in Table 1. After oral dosing, bioavailability averaged 29.5%, 24.6% and 33.3% for the oral suspension, tablet # 1 and tablet # 2, respectively.

Table 1
Mean nevirapine pharmacokinetic parameters after 1 mg/kg iv dose

Parameters	Mean ± SD
Elimination half-life (hr)	0.397 (Harmonic mean)
Clearance (ml/min/kg)	44.7 ± 5.1
Vss (l/kg)	1.78 ± 0.47
AUC (ng*hr/ml)	377 ± 41.7

5. Absorption, distribution and excretion of ^{14}C-nevirapine in rats, Boehringer Ingelheim Pharmaceutical, Inc., Ridgefield, CT, October 29, 1990, (DM-9008)

Groups of rats received either a single iv (1 mg/kg) or single or multiple oral (20 mg/kg) doses (for 7 days) of ^{14}C-nevirapine. Results: are summarized in Table 2 and 3. In separate experiments, biliary excretion of ^{14}C-nevirapine after iv or intraduodenal (id) dosing was studied. The biliary excretion was a major pathway during the first 24 hr after dosing, with 33% to 55% of the dose being recovered in bile during that period. The kinetics of biliary ^{14}C-nevirapine elimination in bile was nearly twice as high in males as in females. Enterohepatic recirculation of ^{14}C-nevirapine was also found with 46% and 39% of id dosed biliary ^{14}C being recovered within 24 hr in males and females,

respectively. Uptake of ^{14}C-nevirapine into 11 selected tissues was studied after oral dosing. Moderate levels of ^{14}C were detected in all tissues including brain at all time point studied (2, 4 and 8 hr). Elimination of ^{14}C from tissues paralleled that from plasma, and no target tissues for accumulation of drug and/or metabolites were identified.

Table 2
Plasma ^{14}C-nevirapine AUCs and MRTs in male and female rats

Parameters	Route of dosing		
	iv	single dose	multiple dose
AUC (µg eq.*hr/ml)			
♂	3.5	64.3	39.0
♀	5.9	205.1	90.5
MRT (hr)			
♂	5.2	7.4	6.8
♀	7.3	10.6	8.1

Table 3
Percentage of dose recovered after ^{14}C-nevirapine dosing in rats

Route	Renal	Fecal	Carcass	Total
iv: 0-120 hr				
♂	43.0	40.1	0.2	83.3
♀	51.8	30.3	0.4	82.5
oral: 0-96 hr				
♂	51.6	39.8	0.3	91.7
♀	50.3	26.1	0.5	76.9

Comments: Sex differences were observed in the pharmacokinetics of ^{14}C-nevirapine in rats. After single iv, single oral and multiple oral dosing, blood and plasma levels, AUCs and MRTs were significantly higher in females than in males. A higher peak rate of biliary elimination of ^{14}C in males may account for these findings.

6. Nevirapine oral rising dose tolerance study in the male and female monkeys: Plasma concentration results, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, September 21, 1990, (DM-9004)

Two male and female monkeys were dosed single oral doses of nevirapine (20 to 600 mg/kg) and plasma samples were collected approximately 2 hr after dosing and were analyzed by a validated HPLC assay method. Results: plasma concentrations of nevirapine

ranged from 1.93 to 15.2 μg/ml after the various doses. The concentration were similar in both the male and female monkeys, with increase in the dose administered, increase in the plasma concentration were observed.

7. Nevirapine oral rising dose tolerance study in the male and female dogs: Plasma concentration results, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, September 21, 1990, (DM-9005)

One male and female dogs were dosed single oral doses of nevirapine (200 to 1200 mg/kg); in addition, a fixed dose of 800 mg/kg/day was administered for 5 day to one male and female dog. Plasma samples were collected and were analyzed by a validated HPLC assay method. Results: plasma concentrations of nevirapine ranged from 23 to 82 μg/ml after the various doses. The AUCs of nevirapine demonstrated a roughly linear relationship to the doses administered. After multiple doses of 800 mg/kg/day for 5 days, there was a 60-80% reduction in the AUC in both sexes.

Comments: Multiple dose administration of nevirapine resulted in decrease in plasma concentrations in both sexes, which may indicate induction of liver metabolizing enzymes in dogs.

8. Nevirapine bioavailability and dose linearity in the rat, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, November 28, 1990, (DM-9011)

The time course of plasma nevirapine was followed after administration of 1 mg/kg iv and 5, 20 or 100 mg/kg orally in rats. Results: after iv administration, nevirapine was immediately distributed throughout the body. The mean pharmacokinetic parameters of nevirapine via both the routes are described in Table 4. Conclusions: nevirapine was rapidly absorbed and evenly distributed in the rat. It was rapidly eliminated after both iv and oral administrations.

Table 4
Mean model-independent pharmacokinetic parameters of nevirapine in rats

Parameters	1 mg/kg, iv	5 mg/kg, po	20 mg/kg, po	100 mg/kg, po
Cmax (µg/ml)	-	2.04	4.55	15.6
Tmax (hr)	-	1	1.3	4
MRT (hr)	1.9	2.4	3.5	5
T½ (hr)	1.1	1	-	-
F (%)	100	53	49	54
Vss (ml/kg)	984	-	-	-
AUC (µg*hr/ml)	1.97	5.27	19.18	106
Cl (ml/kg/min	8.5	-	-	-

9. Nevirapine plasma concentrations in male and female rats during 14-day toxicology study, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, October 18, 1990, (DM-9010)

Groups of male and female rats were administered nevirapine orally at dose levels of 10, 50, 100 or 200 mg/kg/day for 14 days. Plasma samples were collected and were analyzed by a validated HPLC method. Results: are described in Table 5.

Table 5
Mean pharmacokinetic parameters of nevirapine in male and female rats

Parameters	Dose (mg/kg)	Male			Female		
		day 0	day 4	day 11	day 0	day 4	day 11
Cmax (µg/ml) Tmax (hr) AUC (µg*hr/ml)	10	0.84 1.5 3.66	0.85 1.5 3.43	0.98 1.5 3.89	4.22 1.5 50.4	2.67 1.5 30.6	3.98 1.5 41.6
Cmax (µg/ml) Tmax (hr) AUC (µg*hr/ml)	50	6.27 1.5 36.74	1.54 1.5 15.52	1.6 1.5 13.2	21.9 5 360	22.3 5 322	17.1 1.5 237.4
Cmax (µg/ml) Tmax (hr) AUC (µg*hr/ml)	100	11.07 1.5 96.8	7.27 1.5 39.36	5.94 1.5 48.9	29.9 5 534	36.78 5 605.4	20.5 1.5 563
Cmax (µg/ml) Tmax (hr) AUC (µg*hr/ml)	200	19.99 5 264.2	19.84 5 261.4	23.78 5 310.8	77.8 5 999	69.5 5 1394	82.2 5 1448

10. Nevirapine pharmacokinetics in chimpanzee after single iv and oral doses, multiple oral doses and coadministration with ^{14}C-AZT, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, December 5, 1990, (DM-9014)

Three female chimpanzees were given single iv or oral doses of nevirapine at dose levels of 1 and 25 mg/kg, respectively, with a washout period of three weeks between doses. Results: are described in Table 6. Ten weeks later, further studies were carried out in the same chimpanzees with and without AZT. Plasma concentrations of nevirapine were observed throughout the time-course of each leg of the study with little difference seen after single nevirapine dosing, multiple nevirapine dosing or coadministration of nevirapine with 2 mg/kg AZT. Conclusions: coadministration of AZT and nevirapine did not change the pharmacokinetics of nevirapine.

Table 6
Mean pharmacokinetic parameters of single and repeated doses (6) of nevirapine in female chimpanzees

Parameters	single 1 mg/kg, iv	single 5 mg/kg, po	repeated 5 mg/kg, po	single 25 mg/kg, po
Cmax (µg/ml)	-	3	3.8	9.48
Tmax (hr)	-	5.7	6	20
MRT (hr)	-	4.1	0.4	-
T½ (hr)	15.5	-	-	-
F (%)	100	75	76	64
Vss (ml/kg)	797	-	-	-
AUC (µg*hr/ml)	22.06	80.25	80.58	332.6
Cl (ml/kg/min	0.85	-	-	-

11. Preliminary absorption, distribution and metabolism of nevirapine in chimpanzee, monkey, dog, rat and mouse Boehringer Ingelheim Pharmaceutical, Inc., Ridgefield, CT, December 7, 1990, (DM-9001)

Nevirapine was administered as single iv (1 mg/kg) and oral (20 mg/kg) doses to rats, monkeys, dogs, chimpanzees and mice. Serial plasma samples were obtained and were analyzed by a validated HPLC method. Results: are summarized in Tables 7 and 8.

Table 7
Mean pharmacokinetic parameters after 1 mg/kg iv dose of nevirapine in rat, monkey, dog and chimpanzee.

Parameters	Rat	Monkey	Dog	Chimp
Cl (ml/kg/min)	8.5	15.9	43	0.85
Vss (ml/kg)	984	1645	1530	797
MRT (hr)	1.9	1.7	0.59	17.2
AUC (µg*hr/ml)	1.97	1.06	0.39	22.06
T½ (hr)	1.1	1.5	0.41	15.5

Table 8
Mean pharmacokinetic parameters after 20 mg/kg oral dose of nevirapine in rat, monkey, dog, chimpanzee and mouse.

Parameters	Rat	Monkey	Dog	Chimp	Mouse
Cmax (µg/ml)	4.55	1.45	1	12.28	4.1
Tmax (hr)	1.3	1.2	1.1	5.7	1.2
MRT (hr)	3.5	6.4	3.8	19.6	2
F (%)	49	73	30	75	-
AUC (µg*hr/ml)	19.18	14.9	2.26	357	8.48
Metabolite	BIRJ-106	BIRJ-106	BIRJ-106	BIRJ-106	BIRJ-106

12. Pharmacokinetics of nevirapine in chimpanzees: dosing, sampling and monitoring, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, November 27, 1990, (DM-9015/U91-0189)

Three female chimpanzees (age 8 - 16 yr, weight 35 - 43 kg) were administered: 1) single i.v. (1 mg/kg in 20% ethanol/80% saline solution) or oral dose of nevirapine (26 mg/kg) with intensive 24 hr monitoring and one week follow-up, 2) single oral dose administration of C^{14}-AZT (2 mg/kg) with intensive 24 hr monitoring followed by a single dose of nevirapine (5 mg/kg) with intensive 24 hr monitoring and then 7 consecutive daily oral doses of nevirapine (5 mg/kg) and a single dose of C^{14}-AZT on the last day of the study followed by intensive monitoring for 24 hr. This study was designed to study the pharmacokinetics of nevirapine in combination with AZT; the results were reported previously in the sponsor's report #DM-9014/nevirapine. The

animals did not display any abnormal behavior during the course of the study. The hematocrit and the hemoglobin levels decreased slightly, but returned to normal within few weeks after completion of the study. The EKG and urinalytical parameters were normal. Modest to moderate increases were observed in serum ALT, AST, LDH, GGT and alkaline phosphatase at the end of the multi-dose study.

13. Nevirapine plasma concentrations in male and female dogs during 14-day toxicology study, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, December 26, 1990, (DM-9009)

Groups of male and female dogs were administered nevirapine orally as powder in gelatin capsule at dose levels of 10, 50, 100 or 200 mg/kg/day for 14 days. Plasma samples were collected and were analyzed by a validated HPLC method. Results: are described in Table 9.

Table 9

Mean pharmacokinetic parameters of nevirapine in male and female dogs

Parameters	Dose (mg/kg)	Male			Female		
		day 0	day 6	day 12	day 0	day 6	day 12
Cmax (µg/ml)	100	0.33	3.3	4.1	0.11	1.0	1.5
Tmax (hr)		2	2	2	2	2	2
AUC (µg*hr/ml)		311	26.7	25.5	2.04	5.2	8.1
Cmax (µg/ml)	400	32.2	30.1	7.0	6.1	5.3	0.9
Tmax (hr)		24	6	2	2	2	2
AUC (µg*hr/ml)		361	372	53.3	44.5	33.2	7.3
Cmax (µg/ml)	800	21.4	18.7	4.7	9.4	12.6	5.0
Tmax (hr)		6	6	2	6	6	6
AUC (µg*hr/ml)		435	238	19.7	121.6	149	63.6

Comments: Prolongation in the absorption was observed with increase in dose, especially at 400 and 800 mg/kg doses. Multiple administration of nevirapine at higher doses resulted in a decrease in concentrations perhaps due to liver enzyme induction.

14. Preliminary oral rising dose toxicity study in dogs: Plasma concentrations, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, February 12, 1991, (DM-9103)

One male and female dogs were administered escalating doses of 50, 100, 150, 200 and 400 mg/kg of nevirapine powder in a capsule. Plasma samples were collected and were analyzed by a validated HPLC assay method. Results: plasma concentrations of

nevirapine ranged from 0.16 to 54.2 μg/ml after the various doses.

15. Co-administration of nevirapine and AZT to cynomolgus monkeys: blood chemistry & hematology, urinalysis, and EKG, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT (DM-9104/U91-0235)

Three adult male cynomolgus monkeys (4 - 6 kg) were administered once daily for 15 consecutive days nevirapine (5 mg/kg, in 0.5% methylcellulose) by gavage followed immediately by 2 mg/kg of AZT and then 5 ml of water to rinse the tubing. The animals were sedated during the course of the study with ketamine HCl (10 - 15 mg/kg) and atropine (0.27 mg) given im. This study was conducted as a prelude to a pharmacokinetic study of nevirapine alone and co-administration with AZT to chimpanzees. The animals were monitored and observed closely for abnormal behavior for several hours immediately after drug administration and daily thereafter. No deviation from normal attitude or behavior in the animals was noted, other than the expected effects of ketamine anesthesia. The EKG and urine analysis parameters were normal. The hematocrit and hemoglobin levels were decreased slightly, but reverted to normal within few weeks after completion of the study. In comparison to the upper normal range of the enzymes, co-administration of nevirapine and AZT resulted 4 - 6 fold increase in alkaline phosphatase and modest to moderate increases were noted in ALT, AST and LDH also. Contribution of AZT towards the enzyme induction was not delineated.

Comments: 1. There were no findings in the monkey study that would preclude the simultaneous administration of nevirapine and AZT in chimpanzees.

2. The slight decrease in the hematocrit and hemoglobin levels may be due to the intensive blood sampling since the parameters returned to normal within few weeks of the completion of the study.

3. The observed increased enzyme synthesis was consistent with all the previous studies submitted so far; however, it was not clear whether AZT may play a role, if any, in the enzyme induction.

16. Relative Bioavailability of Two Formulations of nevirapine in Dogs, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT (DM-9105)

Groups of fasted, anesthetized, fitted with an indwelling jugular catheter, male and female beagle dogs (9 - 13 kg, age 2 - 4 yr, 2 animals/sex/drug formulation) were administered orally nevirapine (100 mg/kg dose active ingredient only or granulated formulation). Blood samples were drawn from the catheter at 0,

20, 40, 60 min and 1.5, 2, 3, 4, 6, 8, 12, 24 and 30 hr after dosing. Plasma samples were analyzed by a HPLC method. Model independent pharmacokinetic parameters (AUC and AUMC) were calculated from the plasma concentrations; in addition, C_{max}, T_{max} and MRT were also determined. Table 10 compares the parameters (mean) for the two formulations.

Table 10

Mean pharmacokinetic parameters of two different formulations in dogs

Drug	C_{max} ng/ml	T_{max} hr	AUC ng.hr/ml	AUMC $ng.hr^2.ml$	MRT hr	F_{rel} median
Active	3469	3.2	22,459	226,520	6.7	_
Granule	6893	4.0	61,900	678,579	9.6	2.8

Peak plasma levels of nevirapine tended to be substantially greater in dogs receiving the granulated formulation compared to dogs administered active ingredient only; where as, T_{max} was similar for both the formulations. The mean AUC for dogs receiving the granulated formulation was significantly greater ($p<0.02$) than the mean AUC of dogs administered the active ingredient only. Thus, the results suggest that the granulated formulation was approximately 2.8 times more bioavailable and provided a longer duration of exposure that was 3 hr longer when compared to the active ingredient alone.

Comments: 1. Based on the extent of absorption, the granulated formulation was approximately 2.8 times more bioavailable than the active ingredient; the rate of absorption was also about two times faster in the granulated formulation.

2. The purpose of the present study was to provide information on the relative bioavailability of two oral formulations of nevirapine; the information obtained may assist in the selection of a formulation for use in future toxicological studies.

17. Nevirapine concentration in plasma samples from satellite groups of male and female dogs during the 4-week oral toxicity study, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT (DM-9101)

Different groups (3 male and 3 female/group) of Beagle dogs were dosed with either 20, 50, 100 or 400/500 mg/kg and were bled at 2, 6, 24 hr after the dosing on days 1, 14, and 28. The oral

administration of nevirapine (gelatin capsule) in the dog yielded measurable drug levels in plasma from both male and female animals. Table 11 compares mean peak plasma concentration, Tmax, and AUC for different dose groups on day 1.

Table 11
Mean pharmacokinetic parameters of nevirapine after 4 weeks of oral dosing in dogs

Dose mg/kg	ConcMax µg/ml	TimeMax hr	AUC µg.hr/ml
20	1.02	2	4.8
50	0.51	2	3.2
100	1.53	2	7.8
400/500	10.61	6	145
800	14.77	6	233

The data of male dogs demonstrated that after the 1st dose of either 20, 50 or 100 mg/kg no directly proportional increase in the peak plasma concentration was observed. However after the administration of 400 mg/kg, approximately a 7 fold increase in peak plasma concentration was found on day 1. A similar trend was also seen in the mean peak plasma concentrations in the female dog. For the two intermediate doses (i.e., 50 and 100 mg/kg), the plasma concentrations were observed to be similar to that of the 20 mg/kg dose. The mean peak plasma levels did not demonstrate any dose proportional increases in either sex studied. However, the mean peak plasma concentrations after the administration of the 14th or the 28th dose of 400/500 mg/kg in the male dog resulted in a decrease in the mean peak plasma concentrations (6.7 µg/ml, day 14). A similar trend was also seen in the mean peak plasma levels in the female dog in this dose group; the mean peak plasma level in the female dog was 18.48 µg/ml.

TimeMax: Generally, the highest concentration was measured at 2 hr after dosing; however, absorption was prolonged after the administration of the highest dose. In the 400 mg/kg dose group, TimeMax was 6 hr in the male rat on days 1 and 14 and in the female rat on day 28.

At the three lower doses, the mean AUCs ranged from 3.2 to 10.1 µg.hr/ml in the male dog, which were considerably higher than the

mean AUCs observed in the female dog (i.e., range 1.6 to 5.8 µg.hr/ml). Multiple doses of the three lower doses in both sexes did not result in significant increases in AUC, indicating an absence of any accumulation of the drug. After the first dose of 400 mg/kg, the mean AUCs observed were 145 in the male and 64 in the female. In both sexes, at the highest dose studied, there was roughly a dose proportional increase in AUCs.

18. One week oral (diet) bioavailability study comparing nevirapine bulk powder to nevirapine granulation in the rat, Lot # RM-1167, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, May 26, 1994, (U94-3067/TX-9401)

In a one week oral (diet) bioavailability study, nevirapine bulk powder formulation (100 mg/kg/day) was compared to nevirapine granulation formulation (100 mg/kg/day) in groups of male and female rats (25/sex/group). Over the course of the 12 hr sampling period on drug day 7, plasma sample were analyzed for nevirapine concentration by a validated HPLC method. Results: no animal died prior to the termination of the study. There were no drug-related clinical observations. Body weight gain and food consumption appeared normal for all the animals. The results of plasma analysis indicated that there were no significant differences in the bioavailability profiles of animals administered nevirapine bulk powder or nevirapine granulation. Peak plasma concentrations were generally observed at 9 hr, except for nevirapine bulk powder females where the peak appeared at 6 hr. Plasma concentrations ran between 0.24-7.4 µg/ml and 0.22-4.16 µg/ml for the males and 1.96-21.4 µg/ml and 1.4-19.9 µg/ml at the various timepoints for the females administered nevirapine bulk powder and nevirapine granulation, respectively. AUCs values were 15.8 and 9.16 µg*hr/ml for the males and 97.7 and 100.3 µg*hr/ml for the females administered nevirapine bulk powder and nevirapine granulation, respectively. Conclusion: there were no significant differences in bioavailability between nevirapine bulk powder and nevirapine granulation. The sponsor has decided to use Nevirapine bulk powder formulation in rodent range finding toxicity and carcinogenicity studies.

19. Nevirapine concentration in plasma samples from satellite groups of male and female rats during the 4-week oral (gavage) toxicity study, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, (DM-9102)

Briefly, 3 male and 3 female rats in each dose group were bled at 1.5, 5, and 24 hr after dosing the 1st (0 day), 15th (14 day) and the 29th (28 day). Plasma samples were analyzed by a HPLC assay method. Model independent pharmacokinetic parameters of ConcMax, TimeMax and AUCs were computed for the animals on the above mentioned days. Oral administration of nevirapine (5% Methocel) indicates that the drug was absorbed giving measurable concentrations in the rat plasma. Table 12 compares mean peak

plasma concentration time and AUC for male and female rats on day 0 following a 1 mg/kg oral dose.

Table 12
Mean pharmacokinetic parameters of nevirapine after 4 weeks of oral dosing in rats

Rat 1 mg/kg	ConcMax µg/ml	TimeMax hr	AUC µg.hr/ml
Male	0.05	1.5	0.226
Female	0.29	1.5	1.540

The results also demonstrate that, when sex was included as a factor, highly statistically significant differences were observed between male and female. Further, inability of the female to metabolized the drug at par with male animal, rendered them susceptible to the drug. In male animals on day 1, the peak plasma levels were 0.66, 5.3, and 8.9 µg/ml for 10, 50, and 150 mg/kg doses, respectively. On the other hand, female animals' peak plasma levels were 0.8, 4.2, 21.3, 44.5 µg/ml for 3, 10, 50, 125 mg/kg doses, respectively. However, there was a decrease in the peak plasma concentrations in the male rats on day 14 and day 28 after the administration of either 50 or 150 mg/kg dose. For example at the 50 mg/kg dose, the mean peak plasma levels on day 14 (2.92 µg/ml) and day 28 (3.1 µg/ml) were lower than the peak plasma levels on day 1 (5.3 mg/ml). In female animals, this trend towards a decrease in the peak plasma levels was more obvious on day 14 than on day 28. After the 50 mg/kg dose, the peak plasma level of 12.2 µg/ml on day 14 was lower than the peak plasma level of 21.3 µg/ml on day 1. A similar trend was observed in both male and female animals at other higher doses.
TimeMax: Generally, plasma peak levels were measured at 1.5 hr after dosing; however, absorption seemed prolonged after higher doses. For example in the male animals after the administration of repeated doses of 150 mg/kg, TimeMax was 5 hr on day 14. In the female animals, delayed absorption was observed at doses of 50 and 125 mg/kg.

The mean AUCs were considerably higher in the female rat than in the male and ranged between 1.54 to 772.53 µg.hr/ml after the administration of 1 to 125 mg/kg. Both in the male and female rat, AUCs after the administration of the first dose (day 1) were increased with larger doses; for instance in male rat, the AUCs were 0.23, 2.80, 21.53, and 90.56 after administration of 1, 10, 50, and 150 mg/kg, respectively. Multiple doses of 50 or 150

mg/kg in male rat resulted in a decrease in the mean AUCs on days 14 and 28 as compared to day 1. For instance after the administration of 50 mg/kg dose, the mean AUCs of 12.5 on day 14 and 17.14 on day 28 were lower than the AUC of 21.53 observed on day 1. This trend, although not as pronounced as in the male rat, was also observed in the female rat in the 50 and 125 mg/kg dose groups.

20. Nevirapine concentration in plasma samples from male and female dogs after 800 mg/kg oral dose in a 28-day toxicity study, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, (DM-9106)

The dose of the test compound was reduced to 650 from 800 mg/kg on the 11th day of treatment due to signs of excessive toxicity in both sexes of animals. The mean peak plasma levels after the first dose were 14.77 and 18.48 μg/ml in the male and female dogs, respectively. After the multiple dose administration, the mean peak plasma levels on days 14 and 28 were 1.45 to 41.54 μg/ml in the male, and between 2.03 - 35.81 μg/ml in the female dog. The TimeMax was 6 hr for majority of the dogs. After the first dose, the mean AUCs (μg.hr/ml) were 233 (range 59 - 340) in the male and 241 (range 49 - 416) in the female dog. Multiple doses of 800 mg/kg (or 650 mg/kg from day 11) resulted in a decrease in plasma levels (male=9.0 μg/ml, female=14 μg/ml on day 14) indicating that repeated administration of the dose might have induced the liver metabolizing enzymes, thus resulting in decreased bioavailability of nevirapine.

Comments: 1. These studies indicate that nevirapine administered as powder in gelatin capsule was adequately absorbed in both rats and dogs, and was measurable in all the plasma samples even at 5 hr at any given dose after the oral administration on day 1. Because samples were taken at only a few time points, and there were only 3 animals per sex in each dose group, a detailed characterization of the drug pharmacokinetics was not appropriate.

2. The following findings suggest that an accumulation may occur at repeated high doses: a) in the dog, a thirty to forty fold increase in the extent of absorption coupled with prolonged time to maximum plasma concentration (6 hr) in the two highest dose groups (400 and 800 mg/kg), b) no linear dose proportional increase in either the peak plasma concentration or the extent of absorption, and c) a relatively short time to maximum plasma concentration (2 hr) in the low or intermediate dose groups(20, 50, 100 mg/kg) as oppose to the high dose groups (6 hr).
c) Peak plasma levels and AUCs were consistently lower in male than female rat at each dose level. This finding was most likely due to the greater enzyme induction in the male than female.
d) Furthermore, the observed hepatic enzyme synthesis suggests that there was going to be a decrease in bioavailability of the

drug and a increased duration of drug activity. e) Multiple administration of the drug at the two highest doses in both sexes, resulted in a decrease in plasma concentration, again, indicating liver enzyme induction may have occurred and lead to decreased bioavailability.

3. Although the assay employed to study the pharmacokinetics was satisfactory, it was requested that following parameters should be determined and reported in the validation package of the assay: lower detection limit, upper and lower range of quantification, intraassay precision, and the three quality control samples utilized in the assay should represent the 20%, 50%, and 80% slope of the standard curve, respectively.

21. Supplementary studies of ^{14}C-Nevirapine in rats: Excretion balance after iv and oral dosing, Boehringer Ingelheim Pharmaceutical, Inc., Ridgefield, CT, March 5, 1992, (DM-9108)

This report was submitted as a supplement to experiments conducted and reviewed previously (DM-9008). In the present report, hydroxymethylnevirapine was identified as a major metabolite by HPLC following the oral and iv administrations in rats.

22. Plasma AZT Levels in Chimpanzees after Oral Administration of ^{14}C-AZT Alone and in Combination with Nevirapine, AZT Lot # 5499, Nevirapine Batch # H, Boehringer Ingelheim Pharmaceutical, Inc., Ridgefield, CT, March 20, 1992, (DM-9110/U92-0198)

Three female chimpanzees (weight: 36.4 - 42.6 kg; age: 8 - 16 yr) were used in this 4-arm study. In the first arm, chimps received a single oral dose of ^{14}C-AZT (2 mg/kg). In the second one, after a 3-day washout period, chimps received a single oral dose of nevirapine (5 mg/kg). In the third arm, after a 10-day washout period, the chimps received six oral daily 5 mg/kg doses of nevirapine. In the fourth arm[2], the chimps received single oral doses of both nevirapine (5 mg/kg) and ^{14}C-AZT (2 mg/kg). The purpose of this study was to ascertain whether repeat doses of nevirapine would affect the pharmacokinetics of AZT since nevirapine will most likely be administered concomitantly with AZT. Results: the average plasma levels of AZT are shown in Table 13. The results showed that the co-administration of nevirapine with AZT did not elevate plasma AZT levels between 2 to 12 hr after the co-administration.

[2] 48 hr after the third arm.

Table 13
Mean Plasma AZT levels After Oral Administration of AZT Alone and in Combination with Nevirapine

TIME (hr)	AZT ALONE	AZT AND NEVIRAPINE COMBINATION
	AZT PLASMA LEVELS (ng/ml)	AZT PLASMA LEVELS (ng/ml)
2	216	350
2.5	178	215
3	152	146
5	130	93
5.5	102	73
6	81	54
8	66	61
10	60	68
12	less than 50	less than 50

Comments: The AUCs for the administration of AZT alone and in co-administration with nevirapine were similar (AZT alone = 950 and AZT + nevirapine = 906 ng*hr/ml). Thus, it appears that the daily administration of nevirapine followed by co-administration of nevirapine and AZT in chimps does not elevate plasma levels of AZT between 2 and 12 hr. Secondly, since the time-points chosen for blood sampling were apparently at the time (2 hr) at which maximum plasma AZT levels were observed, the sponsor was proposing to conduct additional experiments to determine the effects, if any, of AZT and nevirapine co-administration prior to 2 hr post-dose. However, as to the co-administration, the effect on AZT metabolites was not known. Thus, there was a possibility that the co-administration may elevate plasma levels of AZT metabolites.

23. Whole-body Autoradiographical Investigations With Nevirapine in Pregnant Rats After Intravenous and Oral Administration, Batch/Lot not provided, Boehringer Ingelheim KG, Binger Strabe, March 3, 1992, (DM-9209/U92-0220)

Four groups of pregnant female albino rats {strain: Chbb:Thom (SPF); age: 10 weeks; weight: 260 - 273 g; 4 animals/group} were administered nevirapine either via gavage (20 mg/kg/day) or iv (1 mg/kg/day) in the tail vein, during two different stages of pregnancy: early stage (day 6 - day 13) and late stage (day 6 - day 18). On day 14 and day 19 respectively, the animals were given the same dose (0.86 mg/kg or 37.6 μCi/animal) of ^{14}C-nevirapine; subsequently, the distribution of radioactivity in the animals' bodies was investigated by whole body autoradiography after various intervals of time (for iv

administration, the animals were killed at 30 min, 3 hr, 8 hr and 24 hr; and for po administration, the animals were killed at 1, 3, 8 and 24 hr). The aim of this study was to investigate the transfer of ^{14}C-nevirapine and its metabolites from the maternal to the fetal system. Distribution after iv administration: on day 14 of pregnancy 30 min and 3 hr after the last administration of nevirapine, the radioactivity was distributed very evenly over the whole animal. Higher concentrations of radioactivity were detected in adrenals, liver, kidney and the intestinal contents. Radioactivity was clearly detected in the placentas and fetuses; at 30 min, the concentrations in the placentas and fetuses were equally high; however at 3 hr, the concentrations in these two tissues were already decreased and were slightly higher in the placentas than the fetuses. At 8 hr, the radioactivity was decreased in almost all organs; however, it remained about equally high in fetus and placenta. At 24 hr, the radioactivity was still detected in the liver, kidney and lung; in the fetus and placenta, it was no longer possible to detect. The main amount of radioactivity not yet excreted was to be found in the intestinal contents. On day 19 of pregnancy, the radioactivity distribution patterns found were identical with those found on day 14. However, at 24 hr on day 19, traces of radioactivity were detected in the placenta and fetus. Distribution after po administration: at 1 and 3 hr, the radioactivity was distributed very evenly over the whole animal and it was also equally distributed in the organs. At 1 hr, the radioactivity concentration in the placenta was as high as that in the fetus; from 3 hr onward, it was always higher in the placenta than in the fetus. At 8 and 24 hr, the concentration of radioactivity in the organs and tissues of animals had decreased. On day 19 of pregnancy, the distribution of radioactivity in the animal bodies was similar to that found on day 14 of pregnancy. The crossing of the blood-brain barrier by the radioactivity was detected up to 24 hr after po administration of the substance.

Comments: Nevirapine and/or its metabolites have crossed the placental barrier. The concentrations of radioactivity in the placenta were as high or slightly higher than in the fetus. The radioactivity was distributed evenly in the fetus. No differences could be seen in the radioactivity distribution patterns that were obtained from the early and late pregnancies. After multiple oral administration, the radioactivity was excreted more slowly than after multiple iv administration of the substance.

24. Plasma Concentration of Nevirapine in Female Rats During a Range Finding Teratology Study, Batch # RM-1177, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, February 24, 1992, (DM9109/U92-0237)

Four groups of pregnant female albino rats {strain: Chbb:Thom (SPF); age: 10 weeks; weight: 200 g; 3 animals/group} were administered nevirapine via gavage in 0.5% Natrosol 250HX (0.5%

hydroxymethylcellulose, 5 ml/kg) during gestation days 7 to 16 at dose levels of 0 (vehicle control), 10 (low), 50 (mid) or 150 (high) mg/kg. Blood samples (0.5 ml) were collected on the last day of treatment (gestation day 16) at times 0 (predose) and at 1.5 and 5 hr after dosing, and assayed by a validated HPLC-UV assay method to determine plasma nevirapine concentrations. Model independent pharmacokinetic parameters are computed and are shown in Table 14.

Table 14
Model Independent Pharmacokinetic Parameters of Nevirapine in Pregnant Female Rats (Day 16)

Dose (mg/kg)	Peak Conc. (µg/ml)	Peak Time (hr)	Mean AUC (µg*hr/ml)	Dose Linearity (AUC/Dose)
0	0.037	1.5	0.115	0.0
25	17.07	5	74.91	3
50	45.62	5	208.31	4.2
150	64.58	5	303.03	2.02

As part of a teratology study, these results demonstrate that nevirapine was adequately absorbed, giving measurable concentrations in rat plasma after oral administration as a suspension.

Comments: First, there was a negative deviation from dose proportionality (AUC/Dose) for the 150 mg/kg/day dose. Second, trace levels of nevirapine were seen in the animals dosed with vehicle. However, HPLC experiments show that there was no carryover of the drug taking place, but GC/MS experiments confirmed the presence of nevirapine in plasma samples from the control group. This suggests that contamination may have taken place during sample handling and/or plasma collection.

25. Plasma Concentration of Nevirapine in Female Rabbits During a Range Finding Teratology Study, Batch # I, RM-1177, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, March 2, 1992, (DM-9111/U92-0238)

Four groups of nulliparous, sexually mature and presumed pregnant female rabbits (strain: Chbb:HM (SPF); age: 20 weeks; weight: 2.5 kg; 3 animals/group) were administered nevirapine via gavage in 0.5% Natrosol 250HX (0.5% hydroxymethylcellulose, 5 ml/kg) during gestation days 6 to 18 at dose levels of 0 (vehicle control), 100 (low), 250 (mid) and 500 (high) mg/kg. Blood samples (0.5 ml) were collected on the last day of treatment (gestation day 18) at

times 0 (predose) and at 2 and 4 hr after dosing, and assayed by a validated HPLC-UV assay method to determine plasma nevirapine concentrations. Model independent pharmacokinetic parameters are computed and are shown in Table 15.

Table 15
Model Independent Pharmacokinetic Parameters of Nevirapine in Pregnant Female Rabbits (Day 18)

Dose (mg/kg)	Peak Conc. (µg/ml)	Peak Time (hr)	Mean AUC (µg*hr/ml)	Dose Linearity (AUC/Dose)
0	0.054	0	0.207	0.0
100	17.28	2	45.05	0.45
250	36.16	2	103.57	0.41
500	23.01	4	66.93	0.13

As part of a teratology study, these results demonstrate that nevirapine was adequately absorbed, giving measurable concentrations in rabbit plasma after oral administration as a suspension. There is a negative deviation from dose proportionality for the 500 mg/kg dose as the observed ratio (AUC/Dose) was less than either of the doses. There was once again, some contamination of nevirapine in the control.

26. Plasma Concentrations of Nevirapine in Beagle Dogs During a Two Week Oral Dose Range Finding Toxicity Study of nevirapine Granulation, Batch # TX-0412, RM-1231, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, April 11, 1992, (DM-9204/U92-0582)

Groups of purebred beagle dogs (weight: 10 - 12 kg; age: 12 - 15 months; 2 animals/sex/group) were dosed with 0 (vehicle), 150, 300 or 600 mg/kg/day nevirapine, as a granulated formulation to evaluate toxicokinetics during a 14 day study. Blood samples were collected from each dog prior to and at 2, 4, 7 and 24 hr after dosing on day 1 (first dose), day 7 (7th dose) and on day 14 (14th dose). Plasma samples were assayed for the determination of nevirapine concentration by a validated HPLC-UV assay method. Model independent pharmacokinetic parameters are computed and are shown in Table 16.

Table 16
Model Independent Pharmacokinetic Parameters of Nevirapine in Male and Female Dogs After Oral Administration of Granulated Formulation

Parameters	Dose (mg/kg/day)	Male			Female		
		Days					
		1	7	14	1	7	14
Peak Conc. (µg/ml)	150	31.1	5.6	7.6	10.6	16.4	7.3
Peak Time (hr)		24	24	24	4	4	2
AUC (µg*hr/ml)		277.1	67.1	80.9	153	260	37.6
Peak Conc. (µg/ml)	300	22.4	21.7	18.8	29.7	10.6	6.1
Peak Time (hr)		4	2	4	24	24	2
AUC (µg*hr/ml)		458	189	198	335	110	38
Peak Conc. (µg/ml)	600	37.5	17.7	33.4	26.6	14.6	24.7
Peak Time (hr)		24	4	4	4	2	7
AUC (µg*hr/ml)		528	159	438	508	191	362

The plasma concentrations and pharmacokinetic analysis demonstrated that nevirapine as granulated formulation was adequately absorbed in both male and female dogs. Measurable concentrations were present at the 24 hr samples, indicating adequate exposure to the test compound at all doses. When sex was included as a factor, statistical significant differences were observed between days (p=0.01), but not between doses (p=0.3).

Comments: Multiple doses resulted in decrease in plasma concentrations and AUCs values, indicating induction of liver metabolizing enzymes. In general, dose linearity was observed with AUCs values in both males and females; however, a negative deviation from dose proportionality at the highest dose was more pronounced in the male dogs than in the female dogs.

27. Plasma Concentration of Nevirapine in Female Rabbits During a Segment II Teratology Study After Oral Administration (Gavage) of Nevirapine, Batch I, Lot # RM 1177, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, September 1, 1992, (DM-9203/U92-0583)*

Three groups of nulliparous, sexually mature and presumed pregnant female rabbits {strain: Chbb:HM (SPF); age: 20 weeks; weight: 2.5 kg; 3 animals/group} were administered nevirapine via gavage in 0.5% Natrosol 250HX (0.5% hydroxymethylcellulose, 5 ml/kg) during gestation days 6 to 18 at dose levels of 30 (low), 100 (mid) or 300 (high) mg/kg. Blood samples (0.5 ml) were collected on the last day of treatment (gestation day 18) at times 0 (predose) and at 2 and 4 hr after dosing, and assayed by a validated HPLC-UV assay method to determine plasma nevirapine concentrations. Model independent pharmacokinetic parameters are computed and are shown in Table 17.

Table 17
Model Independent Pharmacokinetic Parameters of Nevirapine in Pregnant Female Rabbits (Day 18)

Dose (mg/kg)	Peak Conc. (µg/ml)	Peak Time (hr)	Mean AUC (µg*hr/ml)	Dose Linearity (AUC/Dose)
30	2.93	2	8.1	0.27
100	16.19	2	46.5	0.46
300	22.87	2	66.99	0.22

The results of this study demonstrated that 30, 100 or 300 mg/kg doses of nevirapine as a suspension was adequately absorbed when administered to pregnant rabbits. Plasma peak concentrations of nevirapine and computed AUCs increased with increase in dose. Generally, a peak plasma concentration was observed at 2 hr post dose.

28. Concentration in Plasma Samples from Albino Rats During a 13-Week Oral (Gavage) Toxicity Study of Nevirapine, Lot # RM-1212, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, September 21,1992, (DM-9206/U92-0639)

This report consists of the plasma concentration results from male and female albino rats (5 animals/sex/group) that were included as a metabolic group of animals in the toxicology study that was conducted to investigate the potential toxicity of nevirapine during daily oral (gavage) administration of nevirapine for 13 weeks. Blood samples were collected from the orbital sinus in heparinized tubes at 0, 1.5, 5 and 24 hr after

dosing on day 1 and weeks 4, 8 and 13. The plasma was analyzed for nevirapine by a validated HPLC method.

Plasma Concentrations: samples from the predose rats including the control groups did not contain any measurable quantities of nevirapine. Model independent pharmacokinetic parameters of peak mean concentration (μg/ml), peak time (hr) and $AUC_{0-24\ hr}$ were computed after the administration of the first dose, and after 4, 8 and 13 weeks of consecutive administration of nevirapine in the males and females. These results are presented in Table 18. These results indicated that there was a dose related increase in the plasma concentration of the drug. The plasma concentrations in the female rats were significantly higher compared to the levels in the male rats at equal doses. Peak concentrations were observed at 1.5 hr after all doses in the males, whereas a prolongation in absorption was observed in the female rats (5 hr after 50 and 125 mg/kg/day dose). Dose Linearity: in the male and female rats, a positive deviation from dose proportionality occurred, ie, at higher doses, plasma concentrations were higher than expected from the extrapolation of the concentrations at lower doses. A linear regression analysis of the observed AUCs versus doses (not shown) suggested either accumulation at the mid-dose, or decrease in bioavailability at the high-dose. Analysis of Variance: in order to test for statistical significance of differences between the males and females, day of dosing and deviation from dose proportionality, analyses of variance of AUCs (AUC normalized to 5 mg/kg/day dose) in all the treatment groups using dose, day and sex as factors (class variables) were done. Analyses were done separately for each sex. In males, the results demonstrated that significant difference was observed between doses ($p=0.01$), but only a borderline statistically significant differences were observed between days ($p<0.06$). Both T-test and Tukey's multiple comparisons demonstrated that low dose was significantly different from mid ($p<0.05$) and high ($p<0.05$) doses. No statistical significant differences were observed between mid and high doses. The results demonstrated that week 13 was statistically significantly different ($p<.05$) from day 1, week 4 and week 8, but no differences between day 1 or weeks 4 and 8 was observed. When sex was included as a factor, highly statistically significant differences were observed between the sexes ($p<0.0001$).

Table 18
Model Independent Mean Pharmacokinetic Parameters of Nevirapine in Rats After Oral the Oral Administration as Suspension for 13-Week Study

Parameter	Dose (mg/kg/day)	Male Rats				Female Rats			
		Day 1	Wk 4	Wk 8	Wk 13	Day 1	Wk 4	Wk 8	Wk 13
C_{max}	5	0.231	0.22	0.43	0.4	1.23	1.8	3.4	2.9
T_{max}		.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
AUC		1.4	0.63	1.53	1.9	11.0	19.1	20.4	30.9
C_{max}	50	4.9	2.9	4.1	5.6	21.5	13.9	14.5	29.4
T_{max}		1.5	1.5	1.5	1.5	5	5	1.5	1.5
AUC		26.8	18.6	23.7	47.2	296	187	166	348
C_{max}	125	11.4	4.7	4.8	5.6	36.5	35.6	40.9	44.6
T_{max}		1.5	1.5	1.5	1.5	5	5	5	5
AUC		123	43.7	71.3	48.4	639	505	579	877

C_{max} = μg/ml, T_{max} = hr, AUC = μg*hr/ml

Comments: The plasma concentrations and the pharmacokinetic analysis demonstrated that nevirapine as suspension was adequately absorbed in both male and female rats and dose related increase in plasma concentrations were observed in both sexes in this study. No unusual accumulation of the test compound in either sex was observed during the course of the study.

29. Concentration in Plasma Samples from Beagle Dogs During a 13-Week Oral (Gavage) Toxicity Study of Nevirapine as Powder in Gelatine Capsule, Lot # RM-1230/1231, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, January 26, 1993, (D-9205/U91-072)

This report consists of the plasma concentration results from male and female dogs that were included in the toxicology study conducted to investigate the potential toxicity of nevirapine during daily oral administration of nevirapine for 13 weeks. Blood was collected from the jugular vein of each animal into heparinized tubes at 0, 2, 6 and 24 hr on day 1 and weeks 3, 7 and 13 after dosing. The plasma was analyzed for nevirapine by a validated HPLC method. Plasma Concentrations: samples from the dogs included in the control groups did not contain any measurable quantities of nevirapine. Model independent pharmacokinetic parameters of peak mean concentration (μg/ml), peak time (hr) and $AUC_{0-24\ hr}$ were computed after the administration of the first dose, and after 3, 7 and 13 weeks of consecutive administration of nevirapine in the males and females. These results presented in Table 19 indicated that there was a dose related increase in the plasma concentration of the drug. Generally, peak concentrations were observed at 2 hr after a dose in the majority of dogs; however, after the administration

of the higher doses (500 and 700/650 mg/kg/day), in one male and in three females, peak times were observed at 6 hr after dosing. Dose Linearity: the observed ratios of AUCs versus the doses of 75, 200, 500 or 700/650 mg/kg/day were: 1:2:41:39 for the males, and 1:4:53:40 for the female dogs, compared to the expected ratios of 1:3:7:9. Dose proportional increases in the bioavailability of nevirapine in both sexes were observed between 75 and 200 mg/kg/day doses. However, a positive deviation (a 6 to 7 fold higher levels than expected) from dose proportionality occurred at 500 and 700/650 mg/kg/day doses in both sexes. Analysis of Variance: in order to test for statistical significance of differences between the males and females, day of dosing and deviation from dose proportionality, analyses of variance of AUCs (AUC normalized to 75 mg/kg/day dose) in all the treatment groups using dose, day and sex as factors (class variables) were done. Analyses were done separately for each sex. In males, the results demonstrated that significant differences were observed between doses ($p=0.0006$, in males, and $p=0.0126$ in females), but no statistically significant differences were observed between days. Both T-test and Tukey's multiple comparisons demonstrated that statistically significant differences were observed between dose groups 75-700 ($p<0.05$) in both sexes. The results of the T-test (at $p<0.05$) demonstrated statistically significant differences in the day effect, ie, between weeks 7-13, and 1-13 in the males. When sex was included as a factor, no statistically significant differences were observed between the sexes.

Table 19

Model Independent Mean Pharmacokinetic Parameters of Nevirapine in Dogs After Oral the Oral Administration as Suspension for 13-Week Study

Parameter	Dose (mg/kg/day)	Male Dogs				Female Dogs			
		Day 1	Wk 3	Wk 7	Wk 13	Day 1	Wk 3	Wk 7	Wk 13
C_{max}	75	0.89	0.8	1.0	0.8	0.4	0.5	0.5	0.7
T_{max}		2	2	2	2	2	2	2	0
AUC		4.4	3.7	4.6	3.5	2.1	3.3	3.7	8.0
C_{max}	200	1.2	1.0	2.1	1.5	0.9	1.3	0.9	2.6
T_{max}		2	2	2	0	2	2	2	0
AUC		8.4	7.2	9.5	9.3	8.4	9.8	4.4	11.5
C_{max}	500	15.1	4.3	7.7	5.9	6.7	8.7	6.5	4.5
T_{max}		2	2	6	2	2	2	2	2
AUC		179	26.4	107	39.5	111	88	31.4	25.1
C_{max}	700/650	11.0	21.3	19.8	4.7	9.9	12.3	14.4	5.7
T_{max}		6	6	6	2	2	6	6	2
AUC		172	259	289	19.4	84.8	141	195	77.6

C_{max} = μg/ml, T_{max} = hr, AUC = μg*hr/ml

Comments: The plasma concentrations and the pharmacokinetic analysis demonstrated that nevirapine as powder in gelatin capsule was adequately absorbed in both male and female dogs. Measurable concentrations of the drug in plasma were observed 24 hr after single or multiple doses, indicating exposure to the test compound in dogs during the study.

30. Concentration in Plasma Samples from Albino Rats During a 13-Week Oral (Gavage) Toxicity Study of Nevirapine, Lot # RM-1212, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, September 21,1992, (DM-9206/U92-0639)

This report consists of the plasma concentration results from male and female albino rats (5 animals/sex/group) that were included as a metabolic group of animals in the toxicology study that was conducted to investigate the potential toxicity of nevirapine during daily oral (gavage) administration of nevirapine for 13 weeks. Blood samples were collected from the orbital sinus in heparinized tubes at 0, 1.5, 5 and 24 hr after dosing on day 1 and weeks 4, 8 and 13. The plasma was analyzed for nevirapine by a validated HPLC method. Plasma Concentrations: samples from the predose rats including the control groups did not contain any measurable quantities of nevirapine. Model independent pharmacokinetic parameters of peak mean concentration (μg/ml), peak time (hr) and $AUC_{0-24\ hr}$ were computed after the administration of the first dose, and after 4, 8 and 13 weeks of consecutive administration of nevirapine in the males and females. These results are presented in Table 20. These results indicated that there was a dose related increase in the plasma concentration of the drug. The plasma concentrations in the female rats were significantly higher compared to the levels in the male rats at equal doses. Peak concentrations were observed at 1.5 hr after all doses in the males, whereas a prolongation in absorption was observed in the female rats (5 hr after 50 and 125 mg/kg/day dose). Dose Linearity: in the male and female rats, a positive deviation from dose proportionality occurred, ie, at higher doses, plasma concentrations were higher than expected from the extrapolation of the concentrations at lower doses. A linear regression analysis of the observed AUCs versus doses (not shown) suggested either accumulation at the mid-dose, or decrease in bioavailability at the high-dose. Analysis of Variance: in order to test for statistical significance of differences between the males and females, day of dosing and deviation from dose proportionality, analyses of variance of AUCN (AUC normalized to 5 mg/kg/day dose) in all the treatment groups using dose, day and sex as factors (class variables) were done. Analyses were done separately for each sex. In males, the results demonstrated that significant difference was observed between doses ($p=0.01$), but only a borderline statistically significant differences were observed between days ($p<0.06$). Both T-test and Tukey's multiple comparisons demonstrated that low dose was significantly different from mid ($p<0.05$) and high ($p<0.05$) doses. No

statistical significant differences were observed between mid and high doses. The results demonstrated that week 13 was statistically significantly different ($p<.05$) from day 1, week 4 and week 8, but no differences between day 1 or weeks 4 and 8 was observed. When sex was included as a factor, highly statistically significant differences were observed between the sexes ($p<0.0001$).

Table 20

Model Independent Mean Pharmacokinetic Parameters of Nevirapine in Rats After Oral the Oral Administration as Suspension for 13-Week Study

Parameter	Dose (mg/kg/day)	Male Rats				Female Rats			
		Day 1	Wk 4	Wk 8	Wk 13	Day 1	Wk 4	Wk 8	Wk 13
C_{max}	5	0.231.5	0.22	0.43	0.4	1.23	1.8	3.4	2.9
T_{max}		1.4	1.5	1.5	1.5	1.5	1.5	1.5	1.5
AUC			0.63	1.53	1.9	11.0	19.1	20.4	30.9
C_{max}	50	4.9	2.9	4.1	5.6	21.5	13.9	14.5	29.4
T_{max}		1.5	1.5	1.5	1.5	5	5	1.5	1.5
AUC		26.8	18.6	23.7	47.2	296	187	166	348
C_{max}	125	11.4	4.7	4.8	5.6	36.5	35.6	40.9	44.6
T_{max}		1.5	1.5	1.5	1.5	5	5	5	5
AUC		123	43.7	71.3	48.4	639	505	579	877

C_{max} = μg/ml, T_{max} = hr, AUC = μg*hr/ml

Comments: The plasma concentrations and the pharmacokinetic analysis demonstrated that nevirapine as suspension was adequately absorbed in both male and female rats and dose related increase in plasma concentrations were observed in both sexes in this study. No unusual accumulation of the test compound in either sex was observed during the course of the study.

31. Nevirapine Concentration in Plasma Samples From Albino Rats During a 52-Week Oral (Gavage) Toxicity Study of Nevirapine in the Male and Female Rat, Batch No. # 13007, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, June 4, 1993, (U93-0537/DM-9303)

This report included the plasma concentration results from male and female rats (satellite group of animals) in the toxicity study conducted to investigate the potential toxicity of nevirapine during daily oral (gavage) administration of nevirapine for 52 weeks ((U93-2023/TX-9304). Drug Metabolism and Pharmacokinetics: nevirapine was adequately absorbed at all doses and peak plasma concentrations and AUCs increased with increasing doses. Toxicokinetic parameters are shown in Table 21. Steady state plasma nevirapine levels were reached by week 8. C_{max} and AUC were consistently higher in females compared to males given

the same dose. Peak plasma concentrations occurred at the earliest time point measured, 1.5 hr postdose, in males (mid and high) and in animals of both sexes (low).

Table 23
Mean Peak Plasma Concentrations and AUC_{0-24} of Nevirapine in Rats in the 52-Week Study

Dose (mg/kg)	Week	C_{max} (µg/ml)				AUC (µg*hr/ml)			
		Male		Female		Male		Female	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
5	Day 1	0.4	0.1	1.3	0.2	1.0	0.3	4.6	0.55
50	Day 1	5.0	0.8	13	2.0	16.2	3.1	54.6	7.3
100	Day 1	9.5	1.6	23.7	2.6	33.4	10.9	100.7	12.7
5	8	0.4	0.1	1.8	0.3	1.7	0.8	18.8	2.8
50	8	3.9	1.3	21.9	3.0	29.5	13.9	372.1	82.7
100	8	11.8	4.2	49.1	5.3	83.1	43.6	892.6	131.9
5	13	0.5	0.1	1.9	0.2	2.0	0.4	19.8	2.0
50	13	4.7	1.6	22.5	3.7	37.1	17.5	344.8	85.9
100	13	12.8	4.3	43.3	5.7	110.9	51.4	707.3	139.7
5	26	0.6	0.2	2.1	0.3	3.1	0.9	20.7	3.9
50	26	5.1	1.5	20.7	3.4	40.1	14.6	335.3	63.3
100	26	11.7	3.9	44.8	5.2	114.1	39.7	810.5	94.8
5	39	0.7	0.2	2.1	0.6	4.4	1.5	20.1	5.5
50	39	6.1	1.9	24.8	5.6	58.5	28.3	412.1	133.8
100	39	15.3	9.3	42.9	4.2	204.2	174.7	712.5	173.8
5	52	0.7	0.1	2.0	0.6	3.9	1.3	19.5	5.9
50	52	6.4	0.9	18.2	3.4	54.7	12.4	287.3	61.5
100	52	12.2	3.6	45.9		136.6	50.2	737.1	-

Comments: The plasma concentrations and the toxicokinetics analysis demonstrated that nevirapine as a suspension was adequately absorbed in both male and female rats and dose related increases in plasma concentrations were observed in both sexes in this study. No unusual accumulation of the test compound in either sex was observed during the course of the study. As expected from earlier studies with nevirapine, C_{max} and AUCs were consistently higher in females which have been shown to metabolize the drug more slowly than males.

32. The comparative metabolite pattern of ^{14}C-nevirapine in the urine of the male rat and beagle dog, Lot # G, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, December 20, 1993, (DM-9319/U94-0008)

The comparative metabolite pattern of ^{14}C-nevirapine was

d d in the 0-24 hr urine pools of the male rat and beagle d owing a single oral administration of a ^{14}C-nevirapine 0.5% Methocel suspension at 20 mg/kg for both the species. Each species demonstrated extensive metabolism of the parent compound. ^{14}C-nevirapine was barely present in the rat (0.8%) and not detected in the dog urine samples. The metabolites identified in the dog urine were the glucuronides of hydroxymethyl-nevirapine (39.2%), 3-hydroxy-nevirapine (22.7%) and metabolite A (22.3%). The metabolites identified in the male rat urine were metabolite A (72.5%), hydroxymethyl-nevirapine (1.4%), 3-hydroxy-nevirapine (2.3%) and the glucuronide conjugates of these two hydroxylated compounds (9.9% and 1.1%, respectively).

33. In vitro human red blood cell partitioning of ^{14}C-Nevirapine, Lot # A1126-90, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, May 6, 1994, (U94-3038/DM-9406)

In vitro, the extent to which ^{14}C-nevirapine is taken up into human red blood cells was determined at concentrations of 1 and 10 μg/ml [these concentrations were chosen because they were in the range of peak and trough plasma concentrations of nevirapine observed in patients treated with 200 mg bid] at three different time points: 20, 40 and 60 min. Results: human red blood cell partitioning of ^{14}C-nevirapine reached equilibrium by the first time point [20 min] and was consistent throughout the experimental period within each concentration. Uptake of ^{14}C-nevirapine was greater at 1 μg/ml concentration; the uptake averaged (mean ± SD) 1.56 ± 0.11 and 1.27 ± 0.11 for 1 and 10 μg/ml concentrations, respectively.

Comments: The results suggested a saturable mechanism for uptake of ^{14}C-nevirapine into human red blood cells. Although the uptake may have been saturable at higher drug concentrations, the results were in contrast to earlier observations in the rat which showed that ^{14}C-nevirapine uptake into rat erythrocytes was not saturable over a concentration range of 0.20 to 0.97 μg/ml.

34. Plasma analysis for one week oral (diet) bioavailability study comparing nevirapine bulk powder to nevirapine granulation in rat, Lot # RM-1167, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, May 5, 1994, (U94-3053/DM-9318)

In a one week oral (diet) bioavailability study, nevirapine bulk powder formulation (100 mg/kg/day) was compared to nevirapine granulation formulation (100 mg/kg/day) in groups of male and female rats (25/sex/group). Over the course of the 12 hr sampling period on drug day 7, plasma samples were analyzed for nevirapine concentration by a validated method. Results: In males, the AUC_{0-12} for dosing with nevirapine bulk powder (15.8 μg*hr/ml) was 73% greater than the AUC for dosing with nevirapine granulation (9.16 μg*hr/ml); in females, the AUCs were 97.76 μg*hr/ml and 100.1 μg*hr/ml for the bulk and granulation formulations,

respectively. Conclusion: there was a great degree of variability in plasma concentrations at individual time-points; hence, there were no significant differences between nevirapine plasma levels from animals receiving powder versus granulation. Due to the ease of formulation, nevirapine powder was selected for use in a 13-week range finding study in rats.

35. Nevirapine plasma protein binding in several species: a review of the original data plus previously unreported data, Batch # GJV 1407:2, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, October 31, 1994, (U94-3128/DM-9419)

This report presented previously reported as well as unreported data on nevirapine plasma protein binding in several species. These results are combined in one report for ease of reference. Results: Nevirapine plasma protein binding, measured by equilibrium dialysis at 37°C, was found to be moderate in all species studied. At non-saturating nevirapine concentrations, binding averaged 43.7% for mouse, 52.2% for rat, 68.2% for rabbit, 69.9% for dog, 46% for monkey, 45.6% for chimpanzee and 61.5% for human. Saturation of binding was not observed at nevirapine concentrations up to 0.88 μg/ml in mouse, 19 μg/ml in rat, 1.1 μg/ml in rabbit, 0.24 μg/ml in dog, 0.91 μg/ml in monkey, 0.89 μg/ml in chimpanzee and 4.9 μg/ml in humans. Saturation of binding was detected at concentrations of 85.2 μg/ml in mouse, 90.2 μg/ml in rat, 20.4 μg/ml in rabbit, 0.95 μg/ml in dog, 4.3 μg/ml in monkey, 81.4 μg/ml in chimpanzee and 20.1 μg/ml in humans. Nevirapine binding to human serum albumin, α1-acid glycoprotein and fetal calf serum was 4%, 0.07% and 10%, respectively. Conclusions: at the level of plasma protein binding observed in the study, the potential for drug interactions which might seriously affect the pharmacokinetics of nevirapine should be low.

36. Excretion balance studies following oral administration of ^{14}C-Nevirapine to mouse, rat, rabbit, dog and monkey, Batch # GJV 1407:175, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, October 31, 1994, (U94-3153/DM-9410)

Excretion balance studies were conducted after oral administration of ^{14}C-nevirapine in mouse, rat, rabbit, dog and monkey. Results: are summarized in Table 22 For all species, most of the administered radioactivity was recovered during the first 24 hr collection interval. For mouse, rat, rabbit and monkey, total elimination (urine plus feces) during the first day after dosing ranged from 78-89%. For dog, total elimination during the first day averaged only 70% of the final cumulative recovery in urine plus feces. For all species except dog, the predominant route of elimination was the urine. In the dog, this pattern was reversed with the predominant route of elimination being the feces. A comparison of results for males versus females suggested that there was no important sex differences of the rate or route

of elimination.

Table 22

Summary of mean excretion balance data after oral dosing of ^{14}C-nevirapine in mouse, rat, rabbit, dog and monkey

Species	Sex	N	Dose (mg/kg)	Collection period (hr)	Percent of Dose Recovered			
					Urine	Feces	Cage rinse	Total
Mouse	♂	10	20	0-96	64.2	38.4	-	102.5
	♀	10	20	0-96	68.6	35.3	-	103.9
Rat	♂	2	20.6	0-96	50	38.4	1.9	90.2
	♀	2	21.5	0-96	59.2	31.3	1.1	91.5
Rabbit	♀	3	3o	0-96	67.7	29.4	-	97.1
Dog	♂	2	19.8	0-96	36.2	70.9	1.3	108.3
	♀	1	20.3	0-96	32.8	72.7	2	107.5
Monkey	♂	2	19.6	0-96	78	9	7.9	94.9
	♀	1	19.9	0-96	66.3	19.2	8	93.5

37. Lacteal (milk) transfer of ^{14}C-nevirapine after single oral (gavage) administration in rats, Lot # RM-1167, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, December 29, 1994, (U95-3043/DM-9505)

Two groups of rats (15-day postpartum) received a single oral gavage dose of 21.49 mg/kg ^{14}C-nevirapine (160 μCi/kg) to evaluate the plasma and milk concentrations of ^{14}C-nevirapine. At various times over a one-day period, blood and milk samples were collected. Results: are summarized in Table 23.

Table 23

^{14}C-Nevirapine concentrations in plasma and milk following the administration of ^{14}C-nevirapine (21.49 mg/kg; 160 μCi/kg) to rats

Time (hr)	Concentration (μg equiv/ml)		Milk/Plasma ratio
	Plasma	Milk	
3			
8			
24			
48			

Comments: Overall, the results from this study indicated that the transfer of nevirapine did occur from the plasma to the milk. A substantial concentration of nevirapine radioactivity was noted in the milk at the earlier time points and the milk to plasma ratio would indicate some accumulation in the milk in comparison to plasma concentrations.

38. In vitro metabolism of nevirapine by liver microsomes from the chimpanzee, Lot # K, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, March 3, 1995, (U95-3063/DM-9402)

The rate of metabolism of nevirapine (30 μM) was determined in vitro using liver microsomes (prepared from two different female chimpanzees) over a 30 min period at 37°C. Results: the rates of total in vitro metabolism of nevirapine were calculated to be 14.9 and 7.6 pmoles/min/mg of microsomal protein (mean = 11.3 pmoles/min/mg protein) for chimp A and chimp B, respectively.

Comments: The sponsor stated that the rate of metabolism of nevirapine by the chimp was similar to the one seen in human-liver microsomes. Thus, the females chimp was likely to be a better animal model for predicting the metabolism and pharmacokinetics of nevirapine in humans.

39. The biotransformation of ^{14}C-nevirapine in the mouse, rat, rabbit, dog and monkey, Lot # K, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, March 24, 1995, (U95-3083/DM-9507)

The biotransformation of ^{14}C-nevirapine after oral administration (20 mg/kg) was determined from the 0-24 hr urine metabolite pool of the male and female rat, mouse (male only), beagle dog and cynomolgus monkey. Results: the ß-glucuronidase incubations of the 0-24 hr urine from the rat, mouse, dog and monkey hydrolyzed the known major and minor glucuronide metabolites. The resultant aglycon metabolites were the 2-hydroxy-nevirapine, 3-hydroxy-nevirapine, hydroxymethyl-nevirapine and 8-hydroxy-nevirapine. At least two other unknown minor glucuronide metabolites were confirmed in all the urine samples examined. Prior addition of D-saccharic acid 1,4-lactone (a known inhibitor of ß-glucuronidase, inhibited the enzymatic hydrolysis of the urinary glucuronides. Finally, sulfuric acid hydrolysis and sulfatase incubations demonstrated the absence of sulfate conjugates.

40. The comparative metabolic pattern of ^{14}C-nevirapine in the mouse, rat, rabbit, dog and monkey, Lot # K, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, March 28, 1995, (U95-3090/DM-9506)

The comparative metabolite pattern of ^{14}C-nevirapine after oral (20 mg/kg) and iv (1 mg/kg) administration were determined from 0-24 hr urine and fecal pools (0-48 hr) of male and female mice,

rat, rabbits (female only), dogs and monkeys. The bile (0-24, rats only) and plasma (rats and dogs) were also analyzed after the oral administration of ^{14}C-nevirapine (20 mg/kg). Results: demonstrated that ^{14}C-nevirapine was extensively metabolized in both sexes of all the species examined. The metabolic patterns for both sexes of all the animals examined demonstrated that ^{14}C-nevirapine underwent extensive oxidative metabolism (Phase I) followed by glucuronidation (Phase II) of hydroxylated metabolites before being excreted into the urine and feces. The known ^{14}C-nevirapine Phase I metabolites which appeared as major and/or minor metabolites in the urine, feces, bile (rat only) and/or plasma (rat and dog only) were 2-hydroxy-nevirapine, 3-hydroxy-nevirapine, 8-hydroxy-nevirapine, hydroxymethyl-nevirapine and 4-carboxylic acid-nevirapine. The identified Phase II glucuronide conjugates which appeared as major and/or minor metabolites in all animals were 2- and 3-hydroxy-nevirapine glucuronide and hydroxymethyl-nevirapine glucuronide. The 8-hydroxy-nevirapine glucuronide always appeared as a minor metabolite. The urine and fecal metabolite patterns after the oral administration of nevirapine, were highly qualitative (having identical metabolites) and quantitative (having the same amounts of the identical metabolites) between the sexes in the mouse and dog. After iv administration, the male and female rat profile were also qualitative in nature. When the urine and fecal metabolite patterns following the oral and iv administrations were compared within each sex, the results demonstrated the pattern to be neither qualitative nor qualitative. Following either the oral or iv administration, a metabolite with a percentage greater than or equal to 10 was defined as a major metabolite and a metabolite with a percentage less than 10 was defined as a minor metabolite. The results are summarized in Tables 24-27.

Table 24 (Urine)

Summary of nevirapine and metabolites expressed as percentage of total radioactivity in the urine following oral (20 mg/kg) and iv (1 mg/kg) administrations of ^{14}C-nevirapine

Species	NVP	4-CA	HOMe	3-OH	8-OH	HOMegluc	8-HO-gluc	2&3-OH gluc	Total
♂ rat po	0.3	70.8	8.7	1.7	-	2.2	-	3.7	87.4
♀ rat po	5.6	53.2	17.1	1.6	-	3.2	-	7.8	88.5
♂ rat iv	1	73.6	8.9	-	-	1.9	-	3.5	88.9
♀ rat iv	2.3	53.7	16.6	1.9	-	4.8	-	9.8	89.1
♂ dog po	-	18.4	-	-	-	33.5	1.8	23.9	75.8
♀ dog po	0.9	19.9	-	-	-	36.2	2.4	26.9	83.9
♂ cyno po	-	16.9	0.7	0.9	-	11.7	3.9	55.2	89.3
♀ cyno po	0.1	35	-	0.6	0.2	7.2	4.2	41.2	88.5
♂ mice po	1.2	54.5	2.6	2.2	-	-	-	20.2	80.7
♀ mice po	1	49.2	2.9	1.6	-	-	-	25.8	80.5
♀ rbt po	0.8	5.8	1.3	-	-	36.2	3.9	38.4	86.4

NVP = nevirapine; HOMe = hydroxymethyl-nevirapine; 8-OH = 8-hydroxy-nevirapine; 8-OHgluc = 8-hydroxy-nevirapine glucuronide; 4-CA = 4-carboxylic acid-nevirapine; 3-OH = 3-hydroxy-nevirapine; HOMe-gluc = hydroxymethyl-nevirapine glucuronide

Table 25 (Feces)

Summary of nevirapine and metabolites expressed as percentage of total radioactivity in the feces following oral (20 mg/kg) and iv (1 mg/kg) administrations of ^{14}C-nevirapine

Species	NVP	4-CA	HOMe	3-OH	8-OH	HOMegluc	8-HO-gluc	2&3-OH gluc	Total
♂ rat po	1.7	62.2	9.5	6.9	2.2	-	-	-	82.5
♀ rat po	5.1	54.9	8.3	11.8	3.1	-	-	-	83.2
♂ rat iv	-	82.2	4	3.7	1.4	-	-	-	91.3
♀ rat iv	2.3	51.8	12.3	10.6	3	-	-	-	80
♂ dog po	41	15.4	5.3	20.9	2.4	-	-	-	85
♀ dog po	46	14	4.6	19.6	2.8	-	-	-	87
♂ cyno po	4.7	28	4.2	32.6	5.1	-	-	-	74.6
♀ cyno po	1.6	49.1	6	20.9	3.3	-	-	-	80.9
♂ mice po	-	40.8	2.6	25.4	2	-	-	-	7.8
♀ mice po	-	27.9	-	26.8	-	-	-	-	54.7
♀ rbt po	3.6	12.3	11.1	32	3.8	-	-	-	62.8

Table 26 (Bile)
Summary of nevirapine and metabolites expressed as percentage of total radioactivity in the bile following iv (1 mg/kg) administration of ^{14}C-nevirapine

Species	NVP	4-CA	HOMe	3-OH	8-OH	HOMegluc	8-HO-gluc	2&3-OH gluc	Total
♂ rat iv	0.8	25.9	701	-	-	44.2	2.9	7.6	88.5
♀ rat iv	3.5	7.4	3.2	-	-	35.5	4.6	21.4	75.6

Table 27 (Plasma)
Summary of nevirapine and metabolites expressed as percentage of total radioactivity in the plasma following oral (20 mg/kg) administration of ^{14}C-nevirapine

Species	NVP	4-CA	HOMe	3-OH	8-OH	HOMegluc	8-HO-gluc	2&3-OH gluc	Total
♂ rat po 2h	35	11.7	47.5	-	-	1.6	-	1.7	97.3
♂ rat po 4h	22	11.2	58.7	-	-	3	-	1.5	96.5
♀ rat po 4h	82	2.1	10.5	-	-	1.1	-	1.4	97.1
♀ rat po 8h	78	2.4	13.1	-	-	0.8	-	1.3	95.9
♂ dog po 1h	16	14	12.6	1.6	-	28.5	0.5	21.6	95
♂ dog po 3h	6	7.5	4.6	1.1	-	47.5	0.8	22.3	90
♀ dog po 1h	10	10.1	14.1	10.6	1.4	-	24.40.6	33.8	95
♀ dog po 3h	1.4	7	2.8	0.7	-	47.4	0.6	29	88.9

41. The pharmacokinetics of nevirapine in male and female dog plasma, Lot # I, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, May 31, 1995, (U95-3161/DM-9515)

The pharmacokinetics were determined for ^{14}C-nevirapine in dog plasma after an oral administration (20 mg/kg; 5.42 μCi/kg) Results: are summarized in Table 28. Conclusion: the female dog tended toward higher nevirapine plasma levels than male dogs after the oral administration.

Table 28
Comparison of pharmacokinetic parameters after oral (20 mg/kg) administration in dogs

Parameters	Male	Female
$AUC_{0-24\,hr}$ (µg*hr/ml)	47.2	49.7
Tmax (hr)	1-2	1
Cmax (µg/ml)	6.7	13.3
MRT (hr)	11.2	5.9

42. The comparative metabolic pattern of ^{14}C-nevirapine in rat milk and plasma, Lot # A, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, June 6, 1995, (U95-3167/DM-9511)

Groups of rats (15-day postpartum) received a single oral gavage dose of 20 mg/kg ^{14}C-nevirapine (160 µCi/kg) to determine the ^{14}C-nevirapine rat milk and plasma metabolite patterns and to determine whether ^{14}C-nevirapine and metabolites transferred and/or accumulated into the milk. At various times over a one-day period, blood and milk samples were collected. Results: the ^{14}C-nevirapine levels (µg equiv/g) were greater in the milk (3 hr = 15.7; 8 hr = 14.5 and 24 hr = 4.8) than plasma (3 hr = 9.1, 8 hr = 7.5 and 24 hr = 2.4). These results demonstrated that a rapid transfer and accumulation of the drug and metabolites (ie, hydroxymethyl-nevirapine and 2-hydroxy-nevirapine) occurred up to the 3 hr time point. The majority of the accumulation was attributed to the parent drug and hydroxymethyl-nevirapine. At 24 hr, the metabolites represented approximately 33% of the milk accumulation as compared to 13% at 3 hr. The summary of the ^{14}C-nevirapine and metabolite milk to plasma ratios are shown in Table 29.

Table 29
Summary of ^{14}C-Nevirapine and metabolite milk to plasma ratios following the administration of ^{14}C-nevirapine (21.49 mg/kg; 160 µCi/kg) to rats

Sample	NVP	HOMe	2-OH	Total Metabolite	NVP + Metabolite	Total radioactivity
po ♀ rat 3 hr	1.7	1.7	0	1.9	1.7	1.7
po ♀ rat 8 hr	2.1	1.8	1	1.6	2	1.9
po ♀ rat 24 hr	2.6	2.1	0.5	1.5	2	2
Mean	2.1	1.9	0.5	1.7	1.9	1.9

NVP = nevirapine; HOMe = hydroxymethyl-nevirapine; 2-OH = 2-hydroxy-nevirapine

Comments: Overall, the results from this study indicated that if nevirapine was orally administered to nursing human females, nevirapine and its metabolites would rapidly transfer and accumulate in the mother's milk,

43. Pharmacokinetic report of 52-week oral toxicity study in the beagle dog with nevirapine granulation, Lot # TX-0579, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, July 14, 1995, (U95-3308/DM-9502)

Groups of male and female dogs received nevirapine daily via diet dosing at dose levels of 0 (vehicle control), 50 (low), 150 mg/kg/day for the first 4 weeks and 100 mg/kg/day for rest of the study (mid), or 400 mg/kg/day for first 2 weeks and 300 mg/kg/day for weeks 3 & 4, and 200 mg/kg/day for rest of the study (high) consecutively for a period of 52 weeks. Plasma samples were assayed by a validated HPLC method. Results: the observed mean peak plasma concentrations and AUCs in male and female dogs are summarized in Table 30-33. The peak plasma concentrations were generally observed at 1.5 hr after the low dose; however, in the mid and high dose groups, peak concentrations were observed either at 5 or 8 hr samples in both sexes. This may indicate that with increase in dose, prolonged absorption results.

Table 30

The mean peak plasma concentrations (μg/ml) in male dogs

Dose	Day 0	Wk 7	Wk 14	Wk 26	Wk 39	Wk 52
Low	4.7	6.1	6.3	7.4	3.6	4.6
Mid	26.7	8.9	5.5	10.8	14.3	9.3
High	50.7	20.9	17	25.9	28.7	21.1

Table 31

The mean peak plasma concentrations (μg/ml) in female dogs

Dose	Day 0	Wk 7	Wk 14	Wk 26	Wk 39	Wk 52
Low	3.1	6.1	6.1	2.3	2.1	5.2
Mid	15.7	10	13.6	5.1	10	10.5
High	30.3	20.9	25.9	11.9	25.6	20.2

Table 32
The mean $AUC_{0-24\ hr}$ (μg*hr/ml) in male dogs

Dose	Day 0	Wk 7	Wk 14	Wk 26	Wk 39	Wk 52
Low	33	31	42	28	23	27
Mid	345	89	68	112	198	134
High	794	288	201	284	270	253

Table 33
The mean $AUC_{0-24\ hr}$ (μg*hr/ml) in female dogs

Dose	Day 0	Wk 7	Wk 14	Wk 26	Wk 39	Wk 52
Low	12	29	36	17	14	45
Mid	165	53	112	47	82	116
High	456	294	359	173	357	274

44. In vitro metabolism of nevirapine by liver microsomes from male rat, female rat, dog and monkey, Lot # K, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, July 28, 1995, (U95-3312/DM-9510)

The rate of metabolism of nevirapine (30 μM) was determined in vitro using liver microsomes prepared from male rats, female rats, dogs and monkeys. Using 1 mg of total microsomal protein in incubations, the in vitro rates of metabolism of nevirapine to the metabolites: 2-hydroxynevirapine (2-HN), 3-hydroxynevirapine (3-HN), 8-hydroxynevirapine (8-HN) and 12-hydroxynevirapine (12-HN, in earlier report it was referred as hydroxymethylnevirapine) were determined over a 15 min period. Results: are summarized in Table 34. In both male and female rat-liver microsome incubations, the major in vitro metabolite was 12-HN. Both 12-HN and 3-HN were major metabolites in dog and monkey. A currently unidentified metabolite was also present in all of the microsomal incubations. However, the unidentified metabolite was the most minor of the metabolites (based on relative areas of the peaks in the HPLC chromatograms) in the in vitro incubations. Conclusion: liver microsomes from male rats, female rats, dogs and monkeys produced the same in vitro metabolites that were found in in vitro incubation of nevirapine with human-liver microsomal incubations.

Table 34
In vitro metabolism of 30 μM nevirapine by liver microsome from various species. The values are given as mean ± standard deviation.

Species	The rate of Metabolites (pmole/min/mg of microsomal protein) by liver microsomes			
	2-HN	12-HN	3-HN	8-HN
♂ Rat	70±15	238±60	33±5	24±4
♀ Rat	8±1	18±2	6±1	10±1
Dog	69±8	268±24	265±27	68±13
Monkey	32±4	175±36	218±36	97±26

45. The pharmacokinetics of nevirapine in male chimpanzee plasma, Lot # XP-1413-140, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, July 28, 1995, (U95-3313/DM-9516)

Four male chimpanzees were used to study the pharmacokinetics of nevirapine (preliminary study) following a single 800 mg gavage oral dose. Serial blood samples were taken over a period of 72 hrs after dosing. The plasma was obtained and analyzed by HPLC. Results: nevirapine was detected in all samples at 72 hr after the dosing. The mean pharmacokinetic parameters that were calculated from the plasma concentrations were AUC (367.5 μg*hr/ml), Tmax (14.5 hr), Cmax (10.17 μg/ml) and MRT (26.7 hr).

46. Plasma analysis for thirteen week oral (diet) range-finding toxicity study in the CD-1 mouse with nevirapine, Lot # RM-1167, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, August 1, 1994, (U94-3068/DM-9315)

Groups of male and female mice {strain: Crl:CD-1(ICRBR) VAF+; 15 animals/sex/group} were given nevirapine in the diet (powdered) at dose levels of 0 (control) 50 (low), 500 (mid) or 1500 mg/kg/day (high) initially. In treatment weeks 5 and 12, the mid dose was escalated to 3000 and 6000 mg/kg/day, respectively. From treatment week 7 until the end of the study, 2% lactose was added to all feed (including controls) to increase the palatability of the drug. Drug-diet concentrations were adjusted weekly. The purpose of the study was to determine the MTD, NOEL and target organ toxicity of nevirapine to support dose levels selection for the 2-year carcinogenicity study in mice [the study has been reviewed previously]. The plasma concentrations which were determined are reported here. Results: mean plasma drug concentrations at the 50, 500, 1500 and 6000 mg/kg/day doses ranged from below quantifiable limits (<0.25 μg/ml) - 0.33, 0.72 - 1.2, 2.7 - 4.2 and 31.8 - 57.3 μg/ml, respectively, and were

roughly dose proportional up to the 1500 mg/kg/day dose level.

47. Potential for metabolic drug interaction with nevirapine as determined in vitro with human liver microsomes: III. Rifampicin, Lot # K, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, January 16, 1995, (U95-3024/DM-9422)

In vitro, using human-liver microsomes, the effect of rifampicin (concentrations: 10 or 30 μM) was studied in incubations containing either 25 or 100 μM nevirapine to determine the potential drug interaction between nevirapine and rifampicin at the metabolic level. Nevirapine was metabolized in vitro to 2-hydroxynevirapine (2-HN), 3-hydroxynevirapine (3-HN), 8-hydroxynevirapine (8-HN) and hydroxymethylnevirapine (HMN) comprising the major metabolites. Results: the rates of formation of 2-HN, HMN and 3-HN and 8-HN in the 25 μM nevirapine in vitro incubations (absence of rifampicin) were 1.76, 1.37, 1.24 and 0.267 pmoles/min/mg of microsomal protein, respectively; in the 100 μM nevirapine (absence of rifampicin), they were 10.48, 6.22 4.91 and 1.34 pmoles/min/mg of microsomal protein, respectively. Statistically significant reductions in the rate of metabolite formation was observed only in the incubation containing 30 μM rifampicin and 25 μM nevirapine, in which 2-HN formation was reduced by 19% (p=0.01). No other statistically significant inhibitory effects were observed, although the presence of 30 μM rifampicin in the 100 μM nevirapine incubations reduced the rate of formation of 2-HN formation by 21%. The apparent Ki of rifampicin for the inhibition of 2-HN formation was 120 μM; this value was well above the potential plasma Cmax of about 30 μM rifampicin that has been reported in pharmacokinetic studies with patients receiving daily doses of 600 mg rifampicin. Conclusion: the data suggested there was a potential for an inhibition of nevirapine metabolism to 2-HN by rifampicin.

48. The pharmacokinetics of nevirapine in male and female mouse plasma, Lot # I, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, May 31, 1995, (U95-3160/DM-9514)

The pharmacokinetics were determined for nevirapine in mice plasma after oral (20 mg/kg) and iv (1 mg/kg) administrations. Results: are summarized in Table 35.

Table 35
Comparison of pharmacokinetic parameters after iv (1 mg/kg) and oral (20 mg/kg) administration in mice

Parameters	iv (♂ & ♀)	oral (♂)	oral (♀)
$AUC_{0-\infty}$ (µg*hr/ml)	195 (0-1.5 hr)	8529	8590
Tmax (hr)	-	0.25	0.5
Cmax (µg/ml)	-	7.3	5.6
MRT (hr)	-	0.96	1.11

49. Potential for metabolic drug interaction with nevirapine as determined in vitro with human liver microsomes: II. Rifabutin, Lot # K, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, January 12, 1995, (U95-3023/DM-9421)

In vitro, using human-liver microsomes, the effect of rifabutin (concentrations: 0.6 or 6.0 µM) was studied in incubations containing either 25 or 100 µM nevirapine to determine the potential drug interaction between nevirapine and rifabutin at the metabolic level. Nevirapine was metabolized in vitro to 2-hydroxynevirapine (2-HN), 3-hydroxynevirapine (3-HN), 8-hydroxynevirapine (8-HN) and hydroxymethylnevirapine (HMN) comprising the major metabolites. Results: the rates of formation of 2-HN, HMN and 3-HN in the 25 µM nevirapine in vitro incubations in the absence of rifabutin were 1.23, 1.23 and 0.369 pmoles/min/mg of microsomal protein, respectively; in the 100 µM nevirapine (absence of rifabutin), they were 5.85, 5.43 and 1.68 pmoles/min/mg of microsomal protein, respectively. Statistically significant reductions in the rate of metabolism of nevirapine to 2-HN, HMN and 3-HN resulted in the presence of rifabutin in the vitro incubations. The presence of 6 µM rifabutin resulted in an inhibition of the rate of metabolism of nevirapine to 2-HN by 25% and 31% in the 25 and 100 µM nevirapine incubations, respectively. Inhibition by rifabutin of the rates of metabolism of nevirapine to either HMN or 3-HN was less than 20%. The apparent Ki values for the inhibition of 2-HN and HMN formation by rifabutin were 22 and 44 µM, respectively. Conclusion: these values were well above the potential maximal tissue concentration of 6 µM rifabutin; therefore, an inhibition of nevirapine metabolism by rifabutin was unlikely. Secondly, an apparent Ki value of 26 µM nevirapine for the inhibition of rifabutin metabolism was calculated from these experiments. This value approximates a plasma Cmax that could be achieved in patients receiving nevirapine. Thus, the inhibition of rifabutin metabolism by nevirapine may be likely, particularly in those patients in whom plasma concentrations of nevirapine approach or exceed 25 µM.

50. Potential for metabolic drug interaction with nevirapine as determined in vitro with human liver microsomes: V. Trimethoprim, Lot # K, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, February 24, 1995, (U95-3060/DM-9503)

In vitro, using human-liver microsomes, the effect of trimethoprim (at concentrations: 25 or 100 μM) was studied in incubations containing either 25 or 100 μM nevirapine to determine the potential drug interaction between nevirapine and trimethoprim at the metabolic level. Nevirapine was metabolized in vitro to 2-hydroxynevirapine (2-HN), 3-hydroxynevirapine (3-HN), 8-hydroxynevirapine (8-HN) and hydroxymethylnevirapine (HMN) comprising the major metabolites. Results: the rates of formation of 2-HN, HMN and 3-HN and 8-HN in the 25 μM nevirapine in vitro incubations (absence of trimethoprim) were 1.49, 1.21, 0.60 and 0.34 pmoles/min/mg of microsomal protein, respectively; in the 100 μM nevirapine (absence of trimethoprim), they were 8.89, 6.66 2.45 and 1.33 pmoles/min/mg of microsomal protein, respectively. The addition of 25 μM trimethoprim to the in vitro incubations with nevirapine had no statistically significant effect on the rates of formation of these metabolites. The presence of 100 μM trimethoprim increased the rate of 3-HN formation about 3 and 1.5 folds in the 25 and 100 μM nevirapine incubations, respectively. Oppositely, the presence of 100 μM trimethoprim produced a modest reduction (about 20%) in the rate of 8-HN formation in the 100 μM nevirapine incubations. As a secondary focus of this study, the effect of nevirapine at either 25 or 100 μM in the incubation containing either 25 or 100 μM trimethoprim was examined. The presence of nevirapine in the incubations had no statistically significant effect on the metabolism of trimethoprim. Conclusion: the data suggested that a metabolic drug interaction may be unlikely to occur in patients receiving trimethoprim and nevirapine concomitantly.

51. The in vitro metabolism of nevirapine by human-liver microsomes, Lot # K, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, August 25, 1995, (U95-3327)

The in vitro metabolism of nevirapine was characterized using human-liver microsomes from different donors and from microsomes containing cDNA-expressed human cytochrome P-450 isozymes. Results: nevirapine was metabolized in vitro by human-liver microsomes to four hydroxylated products, ie, 2-hydroxynevirapine (2-HNVP), 3-hydroxynevirapine (3-HNVP), 8-hydroxynevirapine (8-HNVP) and 12-hydroxynevirapine (12-HNVP). Typically, the in vitro rates of formation of the four nevirapine metabolites by human-liver microsomes were observed to follow the rank order: 2-HNVP >12-HNVP>3-HNVP>8-HNVP. The same four metabolites were observed in patients receiving an oral dose of ^{14}C-nevirapine. Simple linear correlations of the rates of cytochrome P-450 isozyme-specific biotransformation associated CYP3A with the formation of the above mentioned metabolites. In vitro metabolism studies

using microsomes containing separate cDNA-expressed human cytochrome P-450 indicated that CYP3A4 and CYP2D6 were the isozymes that had the greatest capacity to metabolize nevirapine. The presence of troleandomycin (a specific inhibitor of CYP3A activity) in the in vitro incubations reduced the rates of formation of all four nevirapine metabolites by 41-93%, with the rates of 3HNVP formation being the least inhibited. Conclusion: CYP3A isozyme was primarily responsible for the in vitro metabolism of nevirapine to 2-HNVP, 12-HNVP, 3-HNVP and 8-HNVP in human-liver microsomal incubations. The metabolism of nevirapine by human-liver microsomes appeared to be primarily affected by enzymes belonging to the CYP3A family.

ANCILLARY PHARMACOLOGY

Summary of Ancillary Pharmacology Studies:

1. **EEG-experiments with nevirapine, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, January 19, 1990, (PH-9001)**

2. **Effects of nevirapine on the nocturnal locomotor activity in mice, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, January 25, 1990, (PH-9012)**

3. **Effects of nevirapine on sleep wakefulness pattern in cats, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, March 15, 1990, (PH-9011)**

4. **Effect of nevirapine on the explorative locomotor activity of mice, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, May 17, 1990, (PH-9013)**

5. **Immunological profile of nevirapine, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, June 18, 1990, (IM-9001)**

6. **In vitro characterization of nevirapine, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, July 3, 1990, (BC-9008)**

7. **Nevirapine: The evaluation for potential interaction with nucleic acids and human DNA polymerases, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, January 16, 1990, (BC-9004)**

8. **Nevirapine: CNS effects in mice, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, June 10, 1990, (PH-9002)**

9. **Nevirapine: Autonomic profile and calcium entry blocker**

activity in vitro in guinea pig trachea and aorta, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, February 16, 1990, (PH-9003)

10. Nevirapine: Ancillary cardiovascular profile in anesthetized dogs, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, August 1, 1990, (PH-9006)

11. Nevirapine: Effect on human platelet aggregation, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, June 18, 1990, (PH-9007)

12. Nevirapine: Effect on Gastrointestinal propulsion in mice, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, June 18, 1990, (PH-9008)

13. Nevirapine: Pulmonary specificity testing in anesthetized guinea pigs, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, February 8, 1990, (PH-9010)

14. Nevirapine: The effect on renal function in anesthetized rats, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, July 30, 1990, (PH-9005)

15. Nevirapine: The effect on renal function in conscious rats, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, July 30, 1990, (PH-9004)

Review of Ancillary Pharmacology Studies:

1. EEG-experiments with nevirapine, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, January 19, 1990, (PH-9001)

Rabbits implanted with silver electrodes in the calvarium were used for EEG-recording from frontal and occipital cortices. Nevirapine was administered at dose levels of 0 (vehicle control) or 0.1 mg/kg via the iv route and at 3 (low), 10 (mid) or 30 mg/kg (high) via a stomach tube. EEG was recorded for 2 hr. Results: the circadian rhythmicity of the changes between wakefulness and relaxation or sleep became more pronounced (0.1 mg/kg; iv). Biphasic reactions were observed at low, mid and high doses. A short lasting slight increase of the power in the theta-band for approximately 15 min, indicated a short lasting increase of vigilance. Then, a decrease of the theta power and increase of the delta power between 70 and 85 min indicated relaxation and decreased vigilance. Conclusions: the experiments with nevirapine clearly showed effects on the CNS.

2. Effects of nevirapine on the nocturnal locomotor activity in mice, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, January 25, 1990, (PH-9012)

Groups of female mice were given nevirapine via oral gavage at dose levels of 0 (vehicle control), 20 (low), 80 (mid) or 320 mg/kg (high) to assess the activity profile. The motility of individual mice was recorded in test chambers with 5 horizontal light beams. Results: nevirapine, in a dose dependent manner caused statistically significant reduction (p=0.01) of motility.

3. Effects of nevirapine on sleep wakefulness pattern in cats, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, March 15, 1990, (PH-9011)

Groups of male and female cats received nevirapine via stomach tubing at dose levels of 0 (vehicle control), 3 (low), 10 (mid) or 30 mg/kg (high). At least 6 months before this experiment, electrodes had been implanted in the animals for the EEG recording. On the day of the experiments, the cats were placed in a Farady cage and connected to the EEG-recorder. The EEG was recorded for 6 hr; behavior of the animals was observed by a TV-camera. Results: showed CNS effects of the test compound in the dose range tested. The effects were biphasic. First, there was an initial slight increase of vigilance which was followed by an increase in relaxation.

4. Effect of nevirapine on the explorative locomotor activity of mice, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, May 17, 1990, (PH-9013)

Groups of female mice were given nevirapine via oral gavage at dose levels of 0 (vehicle control), 2.5 (low), 10 (mid) or 40 mg/kg (high) to evaluate the locomotor activity. Results: at one hr after the administration, nevirapine had no effect on the locomotor activity of mice as compared to the controls. At 2 hr, the locomotor activity was significantly inhibited (p=0.01) at the mid and high dose levels. A slight reduction of the mean activity was seen at the low dose level which was not statistically different from the controls.

Comments: These experiments further confirmed the finding of delayed motor activity inhibition in mice tested during the night (PH-9012).

5. Immunological profile of nevirapine, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, June 18, 1990, (IM-9001)

Nevirapine (concentrations ranging from 3.75 to 187 μM) was tested in the human mixed lymphocyte reaction (MLR). Significant inhibition (70%) of the human MLR was observed at 187 μM. In the concentration range of 0.1 to 1.0 μM, nevirapine showed

significant stimulation of the in vitro murine anti-sheep red blood cell IgM plaque forming response; inhibition was observed at the 10 to 100 μM concentration range.

The effect of nevirapine and AZT were compared for toxic effects on myelo/erythropoiesis in human bone marrow cell culture. AZT showed significant suppressive effects in both myelo and erythropoietic components at 0.4 μM as compared to nevirapine in which significant suppressive effects were noted at 187 μM.

Nevirapine (dose range of 1 to 100 mg/kg) had no demonstrable effect on the Delayed Type Hypersensitivity response in the rat.

6. In vitro characterization of nevirapine, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, July 3, 1990, (BC-9008)

In various in vitro receptor binding assays, nevirapine did not interact with dopamine, serotonin, histamine, benzodiazepine adenosine, muscarinic, opiate and nicotinic receptors.

7. Nevirapine: The evaluation for potential interaction with nucleic acids and human DNA polymerases, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, January 16, 1990, (BC-9004)

Nevirapine was evaluated for interactions with nucleic acids and human DNA polymerases. Using a fluorometric method employing the probe ethidium bromide, no fluorescence changes were noted up to a nevirapine concentration of 1.2 μM with ssDNA, dsDNA and poly (rC)/oligo (dG). Conclusion: Nevirapine did not interact with nucleic acids. Nevirapine (up to 24 μM) did not inhibit alfa, beta, delta and the mitochondrial gamma-DNA polymerases.

8. Nevirapine: CNS effects in mice, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, June 10, 1990, (PH-9002)

Neuropharmacological profile test in mice: groups of male and female mice (6/sex/group) received single doses of nevirapine either orally or intraperitonealy at dose levels of 30 or 100 mg/kg; 3, 10, 30, 50, 100 or 200 mg/kg to evaluate the neuropharmacological profile in mice. Results: nevirapine exhibited hypothermia, slight mydriasis, reduced spontaneous motor activity, reduced reactivity to transfer and reduced touch escape in both male and female mice when administered intraperitonealy at 100 mg/kg. Hexobarbital sleep time test in mice: groups of male and female mice (10/sex/group) were treated with oral nevirapine either acutely at single dose levels of 0 (vehicle control), 3, 10, 50, 100 or 200 mg/kg or chronically at dose levels of 3, 10, 30 or 50 mg/kg/day for 5 days. One hr following the administration, the animals were challenged with a single ip dose of hexobarbital (75 mg/kg). Results: acutely, nevirapine demonstrated a dose dependent increase in the mean

duration of hexobarbital-induced sleep time. Doses of 30 mg/kg or higher produced significant (p=0.01) increases of 77-247%. An oral dose of 10 mg/kg could be considered the NOEL. Following multiple doses (3-50 mg/kg/day) over 5 days, nevirapine failed to show any significant potentiation of hexobarbital-induced sleep time as compared to vehicle controls. Ethanol sleep time test in mice: groups of male mice (10/group) were orally gavaged with nevirapine at dose levels of 0 (vehicle control), 10, 30, 50, 100, or 200 mg/kg; one hr following the administration, the animals were challenged with an ip dose of ethanol (3000 mg/kg). Results: a dose dependent increase in the mean duration of ethanol-induced sleep time was demonstrated by nevirapine following oral doses of 10-200 mg/kg. Doses of 10, 30 or 50 did not produce significant increase in the mean duration of sleep time, which were apparent with the 100 (p=0.02) and 200 mg/kg (p=0.001). An oral dose of 50 mg/kg could be considered the NOEL. Rotored test in mice: groups of male mice (10/group) were trained to balance on a rod rotating at 5 rpm. Following the training, nevirapine was orally administered via gavage at dose levels of 0 (vehicle control), 30, 100 or 300 mg/kg. Motor performance was evaluated 30, 60 and 120 min post-administration. That dose which effectively cause 50% of the animals to fall from the rotating rod more than once during a one min test period was designated the neurotoxic dose 50 (NTD_{50}). Results: nevirapine demonstrated a time dependent, significant effect on the rotored performance of mice. The greatest effect was observed at 300 mg/kg following 120 min post-administration. After both 30 and 60 min at dose of 300 mg/kg, less than 50% of the animals fell from the rod and no NTD_{50} could not be calculated. A dose of 30 mg/kg produced no motor impairment at any of the time studied and may be considered a NOEL. Night motility test in mice: groups of male mice (5/group) were first acclimated to an altered day/night cycle in test room for 10 days. On the test day, the animals were orally treated with nevirapine at dose levels of 0 (vehicle control), 30, 100 or 300 mg/kg and were immediately placed into a motor activity chamber. Reference compounds tested included: d-amphetamine sulfate (5 mg/kg, po), haloperidol (1 or 3 mg/kg), po), diazepam (10 or 30 mg/kg, po). Motor activity counts were recorded at hourly intervals over an 8 hr period. Results: nevirapine demonstrated a dose dependent reduction in the night motility of mice with an inhibitory dose 50 (ID_{50}) of 160 mg/kg. Nevirapine-induced reduction in night motility was similar to that observed with haloperidol and diazepam.

9. Nevirapine: Autonomic profile and calcium entry blocker activity in vitro in guinea pig trachea and aorta, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, February 16, 1990, (PH-9003)

In an in vitro assay using isolated guinea pig trachea, nevirapine (100 μM) did not exhibit any significant anti-cholinergic, anti-histaminergic or anti-serotonergic activity.

Nevirapine exhibited calcium channel blocker activity at 250 μM.

10. Nevirapine: Ancillary cardiovascular profile in anesthetized dogs, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, August 1, 1990, (PH-9006)

Seven anesthetized (sodium pentobarbital, 35 mg/kg, iv) dogs per sex received iv nevirapine at a dose levels of 3 mg/kg. Results: following drug administration, there were no changes in the baseline cardiovascular parameters such as blood pressure, heart rate or ECG.

11. Nevirapine: Effect on human platelet aggregation, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, June 18, 1990, (PH-9007)

In an in vitro assay, nevirapine was investigated for its inhibitory effects on collagen or ADP-induced platelet aggregation in human platelet rich plasma. Results: nevirapine, at a final concentration of 376 μM, reached mean inhibitory levels of 58% in collagen-induced platelet aggregation, and 72% in ADP-induced platelet aggregation. No significant inhibitory effects of nevirapine were noted at concentrations of 113 μM or under.

12. Nevirapine: Effect on Gastrointestinal propulsion in mice, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, June 18, 1990, (PH-9008)

Groups of male mice (6-7/groups) received single ip injections of nevirapine at dose levels of 0 (vehicle control), 30 or 100 mg/kg, or atropine (5 mg/kg) as a reference. Results: atropine inhibited gastrointestinal propulsion (67-71%), while nevirapine exhibited no significant activity. Incomplete absorption of drug was observed at the 100 mg/kg treatment group.

13. Nevirapine: Pulmonary specificity testing in anesthetized guinea pigs, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, February 8, 1990, (PH-9010)

Seven anesthetized guinea pigs received a single ip dose of nevirapine (300 mg/kg). Results: bronchoconstriction and increased mean arterial pressure in response to platelet activating factor were reduced by nevirapine. Nevirapine did not affect the bronchoconstriction or hemodynamic effects induced by histamine or leukotriene C4.

14. Nevirapine: The effect on renal function in anesthetized rats, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, July 30, 1990, (PH-9005)

Groups of anesthetized male rats (6/group) received an iv dose of

nevirapine at 0 (vehicle control, 70% PEG 400) or 3 mg/kg. Results: nevirapine had no significant effect on natriuresis, diuresis or mean arterial pressure when compared to the vehicle controls.

15. Nevirapine: The effect on renal function in conscious rats, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, July 30, 1990, (PH-9004)

Groups of male rats (6/group) were orally gavaged with nevirapine at dose levels of 0 (vehicle control), 20 (low) or 100 mg/kg (high). Results: nevirapine significantly increased Na excretion (51.7%, p=0.05) but did not cause any significant effect on excretion of urine and K (high). Nevirapine showed no significant effect on diuresis or natriuresis (low) when compared to the controls.

Appendix # 2

Tabulated summary of nevirapine animal toxicity studies.

Table 1
Summary of nevirapine acute oral toxicity studies

Species	Dose level (mg/kg)	Approximate LD_{50} (mg/kg)	Deaths	Approx. NOAEL (mg/kg)	BSA equiv dose in man (mg/kg)	Major toxic signs
Mice	50	>2000	1 @ 1600	200 ♂ 400 ♀	16.7 ♂ 33.3 ♀	decreased motor activity, ptosis, white feces
	100					
	200					
	400					
	800					
	1600					
	2000					
Rats	50	>800 ♂ >502 ♀	2 @ 400 3 @ 600 5 @ 800	100 ♀ 200 ♂	14.3 ♀ 28.6 ♂	white feces, decreased motor activity and weight, hemorrhages in lungs, hypothermia, dehydration
	100					
	220					
	400					
	600					
	800					
Dogs	50	>400	None	150	75	white particles in feces, emesis, slight sedation
	150					
	250					
	400					

Table 2

Summary of nevirapine subchronic/chronic oral toxicity studies in mice and rats

Study	Dose levels (mg/kg/day	Major toxic signs & laboratory findings	Gross/Micro Pathology NOEL/NOAEL
Mice 3-month (diet)	50	Moribundity, mortality, decreased activity, labored respiration, distended abdomen, ptosis swelling and/or erythema of legs	Liver-hypertrophy and necrosis; thyroid-hypertrophy; thymus-lymphoid depletion; kidney tubule degeneration; heart necrosis and mineralization NOEL = 50 mg/kg/day
	500>3000 wk #5>6000 wk# 12		
	1500		
♂ Rats 1-month (gavage)	1	No toxic signs. Significant but reversible increases in APTT & serum cholesterol levels (150 mg/kg/day)	Liver, thyroid grossly enlarged (150 mg/kg/day). Hepatocellular, thyroid follicular and adrenal cortical hypertrophy (50 & 150 mg/kg/day) NOEL = 10 mg/kg/day
	10		
	50		
	150		
♀ Rats 1-month (gavage)	1	Lacrimation, ptosis, edema, chromodacryorrhea and decreased activity (125 mg/kg/day). Increased serum cholesterol (>50 mg/kg/day); GGT and APTT significantly increased (125 mg/kg/day)	Grossly enlarged liver, adrenals and small thymus (125 mg/kg/day). Hepatocellular, thyroid follicular cell and adrenal cortical hypertrophy (>50 mg/kg/day NOEL = 10 mg/kg/day
	3		
	10		
	50		
	125		
Rats 3-month (gavage)	5 (low)	Drug-related mortality (mid & high). Skin lesions; decreased body weights (mid & high). Reticulocytosis; decreased RBC parameters, clearance, A/G ratio; increased Ca, TP & cholesterol (mid & high)	Grossly enlarged livers and adrenals (high). Hepatocellular, thyroid follicular and adrenocortical hypertrophy (mid & high) NOEL = 5 mg/kg/day
	50 (mid)		
	125 (high)		
Rats 3-month (diet)	5 (low)	Erythema, edema, skin lesions, decreased body weight (mid & high). Hematology changes; increased serum cholesterol, urine protein (mid & high)	Histopathology showed target organs were: intestines, bone marrow, lymph nodes, spleen, liver, thyroid, pituitary and adrenals NOEL = 5 mg/kg/day
	25(wk1-4) >100 (wk 5-13) (mid)		
	50 (high)		
Rats 12-month (gavage)	5 (low)	Drug-related mortality (mid & high). Sedation, skin lesions, foreleg edema, ptosis, chromodacryorrhea, decreased weight & food consumption. Decreased WBC & RBC parameters, lymphocytes, and TPT. Increased AST, ALT, BUN, creatinine & cholesterol. Proteinuria	Skin lesions, edema of extremities. Organ wt changes that correlated with histopathology. Dermatitis; glossitis; hepatocellular, thyroid-follicular hyperplasia; thyroid adenoma; thymus atrophy; adrenocortical hypertrophy (mid & high) NOEL for males = 5 mg/kg/day NOEL could not be identified in females
	50 (mid)		
	100 (high)		

Table 3
Summary of nevirapine subchronic/chronic oral toxicity studies in dogs

Study	Dose levels (mg/kg/day	Major toxic signs & laboratory findings	Gross/Micro Pathology NOEL/NOAEL
Dogs 2-week (Capsules)	150 (low) 300 (mid) 600 (high)	Emesis, decreased in food consumption and body weight (mid & high). Increased in liver enzymes (mid & high)	Thymus lymphoid decreased (mid & high); GI ulcers and erosions, and hepatic hypertrophy (high) NOEL = 150 mg/kg/day
Dogs 1-month (Capsules)	20 50 100 400 (2 wk)/ 500 (2 wk)	White particles in the feces. Relative lymphopenia (400 mg/kg/day). Decreased K, increased Ca, TG, AP & cholesterol (400 mg/kg/day)	Decreased thymus weight in 2♂ (400 mg/kg/day) and in 1♂ (50 mg/kg/day). No drug-related gross or histopathology. NOEL = 20 mg/kg/day
	800 for 10 days then reduced to 650	Sedation, reduced activity, ataxia, increased body temperature, swelling of limbs, redness of eyes & gums; decreased body weight and food consumption; all reversible. Decreased RBC, Hb, HCT, % lymphocytes and PMN. Increased total bilirubin, ALT, AP, TG & cholesterol.	No change in organ weights, gross or histopathology. NOEL = could not be identified
Dogs 3-month (Capsules)	75 (low) 200 (mid) 500 (high) 700 (8 wk)→650 (5 wk) (MTD)	3/5 ♂ died/killed (MTD). Decreased feed intake, decreased body weights (MTD). Sedation, reduced activity, ataxia & increased body temperature (>High). Decreased RBC, Hb & Hct; increased neutrophils & decreased lymphocytes (MTD)	Secondary pneumonia and lymphoid depletion (MTD). Decreased spermatogenesis in the testes and epithelial atrophy in the prostrate and epididymides. NOEL = 200 mg/kg/day
Dogs 1-year (Capsules)	50 (low)	Mortality: 2♂, 1♀ (high). Body weight loss, decreased BW gain, decreased food consumption, decreased motor activity, tremors, ataxia, ptosis, emaciation, absence of capillary refill time, distended abdomen, 3rd eyelid eversion, dehydration, yellow sclera & gums, labored respiration. Decreased RBC, Hb, Hct, MCHC, platelets. Nucleated RBCs & band cells in peripheral blood. Increased AST, ALT, AP, GGT, total bilirubin.	Liver-hepatocytic hypertrophy, vacuolation, subacute cholangiohepatitis, hepatocellular necrosis. Lymph node, spleen, thymus, tonsil, gut associated lymphoid tissue-lymphoid depletion. Bone marrow-decreased poiesis. Spleen-focal necrosis. Testes-atrophy/degeneration. Prostrate-atrophy. GI tract, esophagus-inflammation. NOEL = 50 mg/kg/day
	150→100 (wk 5) (mid)		
	400→300 (wk 3)→200 (wk 5) (high)		

Table 4

Summary of nevirapine special toxicity studies

Study	Dose levels (mg/kg/day	Major toxic signs & laboratory findings	Gross/Micro Pathology
Rats: thyroid hormone levels 15-day (gavage)	125	Motor activity decreased; lacrimation & chromodacryorrhea increased. Increased TSH, T3 & decreased T4	Liver, adrenal, thyroid weights increased; hepatocellular and adrenal hypertrophy; thyroid hypertrophy & hyperplasia.
Rabbits: eye irritation, 1 application. score at 24,48 & 72 hr	0.03 g	Slight conjunctival irritation in 4/6. Normal at 72 hr	None
Rabbits: primary dermal irritation. 1 application occluded for 24 hr	0.5 g/site	No irritation after 24 and 72 hr. Primary dermal irritation index = 0.0	None
Rabbits: acute dermal toxicity. 1 application occluded for 24 hr. Score on day 1, 3, 7 or 14	2 g/kg	Slight erythema in 3/6 ♂ & 5/6 ♀ on day 1.	None
Guinea pigs: dermal sensitization, 1 application + 1 dermal challenge at 2 wk	0.2 g/kg	No sensitization response in any animal	None
Rabbits: im irritancy, single injection	0.8 mg/kg	Discoloration at some injection sites	Inflammatory changes and myofiber degeneration at some sites.

Table 5

Summary of nevirapine reproductive toxicity studies in rats and rabbits

Study	Dose level (mg/kg/weekly)	Clinical signs & food consumption/body weight gains	Other findings & NOEL (Body surface equivalent dose in humans)
Segment I (♂ rats)	5 (low) 50 (mid) 150 (high)	Lethargy/sedation & self mutilation: 2♂; 1 death due to dosing error. Body weight gain significantly decreased (mid, high)	Fertility index: low, mid & high=95.5%,91.7%,79.2% Gestation index:100%,100%, 79% NOEL = 5 mg/kg/day (humans = 0.71 mg/kg/day ♂)
Segment I (♀ rats) hysterectomy group (day 22)	5 (low) 25 (mid)	Body weight significantly decreased (mid) and food consumption decreased (high)	Implants mean/gp: 16.8, 13, 8.9 Mean preimplant loss(%):3.3,25.5,40.7 Mean resorption rate(%):8.4,10.4,40.5 Viable fetuses(mean/gp); 15.3,12.1,5.6 NOEL = 5 mg/kg/day (humans=0.71 mg/kg/day)
Segment I (♀ rats) littering group	50 (high)	Body weight gain decreased; during lactation food consumption and body weight gains decreased (mid,high)	Implants mean/gp: 15.6,12.4,8.7 Newborns mean/gp: 15.1,11.1,5.7 Resorption rate mean %: 3.5,12.9,39.3 NOEL = 5 mg/kg/day (humans=0.71 mg/kg/day)
Segment II (pregnant rats)	12.5 (low) 25 (mid) 50 (high)	Food consumption & body weight decreased (high)	Pregnancy rate %: 82.6,82.6,87 Implant sites mean/gp: 14.7,14.5,14 Resorption rate %: 3.9,8.2,5.1 Fetal weight(g): 5.4,5.3,5 (sig) 1 fetus with bifid & missing ribs (low) 1 fetus with cleft vertebra; 1 fetus with cleft palate (high) NOEL = 25 mg/kg/day Humans = 3.6 mg/kg/day
Segment II (pregnant rabbits)	30 (low) (AUC=8.1 µg*hr/ml 100 (mid) AUC=46.0 300 (high) AUC=67.0	Activity decreased,lethargy (high); abortions: 1(low), 3 (high) Transient and marked decreases in body weight and food consumption.	Pregnancy rate %: 94.4,77.8,77.8 Implant sites mean/gp: 7.3,7.5,6 (sig) Resorption sites %: 10.3,7.4,40.7(sig) Fetal weight (g): 36.2,35.5,36 1 fetus with fused sternebrae; 1 fetus with hematocele; 1 fetus with bifid rib, hemivertebra; 1 fetus with multiple anomalies (mid) NOEL = 100 mg/kg/day Humans = 32.3 mg/kg/day (BSA) = 141 mg/day (AUC)
Segment III (rats)	5 (low) 25 (mid) 40 (high)	Food consumption & body weight gains increased	Pregnancy rate: 21/23, 18/23, 21/23 # pups/litter mean/gp:13,13.4(sig),12.8 fetal weight(g): 6.3,6,5.9 (sig) % viability: 97.4,95.5(sig),78.8 (sig) % weaning rate: 100,100,94.2 (sig) NOEL maternal = 40 mg/kg/day Humans = 5.7 mg/kg/day NOEL developmental: 25 mg/kg/day Humans: 3.6 mg/kg/day

Table 6
Summary of nevirapine genotoxicity studies

Test type	Test system	Dose levels	Response
Ames bacterial assay	S. typhimurium: TA1535, TA 1537, TA1538, TA98, TA100 & E. coli: WP2uvrA	1.7-500 µg/plate, w/wo S9 mix	No mutagenic response
Ames bacterial assay BIRG 106 (metabolite)	same as above	same as above	No mutagenic response
Mammalian cell in vitro assay CHO/HGPRT	Chinese hamster ovary cells	51,102,204,408,816 µg/plate, w/wo S9 mix	No mutagenic response
Mammalian cell in vitro chromosome aberrations	Chinese hamster ovary cells	100,200,400,800 µg/ml, w/wo S9 mix	No increase in chromosome aberrations
Micronucleus in vivo assay	mouse	2000 mg/kg, po gavage	No significant increase of micronuclei

Appendix # 3

Tabulated summary of nevirapine general pharmacology studies

Table 1
Summary of nevirapine general pharmacological studies

Functions tested	Species	Dose (mg/kg) & route	Comments
CNS: general overt effects	mice	100, po;	No effect
		30-100 ip	slight/mild hypothermia;slight mydriasis;slight reduction of motor activity
Motor coordination	mice	30-300, po	ED_{50} = 140 mg/kg; effect observed 120 min post administration
Locomotor activity	mice ♂	30-300, po	ED_{50} = 160 mg/kg
	mice ♀	20-320, po	dose dependent reduction nocturnal activity
	mice ♀	2.5-40, po	dose dependent reduction explorative activity
Sleep time: hexobarbital	mice ♂	3-100, po	Significant potentiation; NOEL = 10 mg/kg
ethanol	mice ♂	10-200, po	Significant potentiation at 100-200 mg/kg NOEL = 50 mg/kg
EEG activity	rabbits	3-30, po 0.1, iv	Onset of maintained synchronization & increase in power of delta band; slight sedation; no effect on heart rate
	cats	3-30, po	Slight sedation
Cardiovascular: blood pressure, heart rate, EEG & autonomic stim	dogs	3, iv	no effects
calcium entry blockade	guinea pig aorta	10^{-3} - 10^{-8} M, in vitro	IC_{50} = 250 µM Nifedipine
Pulmonary: bronchopulmonary/bronchuconstriction	guinea pigs	100, ip	50% antagonism of PAF; no effect on acetylcholine & histamine
Gastrointestinal: propulsion	mice	30-100, po	compound observed in cavity of some animals; no effects
Renal: natriuretic & diuretic effects	rats	20-100, po	NOEL = 20 mg/kg natriuresis with no diuresis = 100 mg/kg

Appendix # 4

Tabulated summary of nevirapine pharmacokinetics from animal studies.

Table 1
Mean model-independent pharmacokinetic parameters of nevirapine in rats

Parameters	1 mg/kg, iv	5 mg/kg, po	20 mg/kg, po	100 mg/kg, po
Cmax (µg/ml)	-	2.04	4.55	15.6
Tmax (hr)	-	1	1.3	4
MRT (hr)	1.9	2.4	3.5	5
T½ (hr)	1.1	1	-	-
F (%)	100	53	49	54
Vss (ml/kg)	984	-	-	-
AUC (µg*hr/ml)	1.97	5.27	19.18	106
Cl (ml/kg/min	8.5	-	-	-

Table 2
Mean pharmacokinetic parameters of single and repeated doses (6) of nevirapine in female chimpanzees

Parameters	single 1 mg/kg, iv	single 5 mg/kg, po	repeated 5 mg/kg, po	single 25 mg/kg, po
Cmax (µg/ml)	-	3	3.8	9.48
Tmax (hr)	-	5.7	6	20
MRT (hr)	-	4.1	0.4	-
T½ (hr)	15.5	-	-	-
F (%)	100	75	76	64
Vss (ml/kg)	797	-	-	-
AUC (µg*hr/ml)	22.06	80.25	80.58	332.6
Cl (ml/kg/min	0.85	-	-	-

Table 3
Mean pharmacokinetic parameters after 1 mg/kg iv dose of nevirapine in rat, monkey, dog and chimpanzee.

Parameters	Rat	Monkey	Dog	Chimp
Cl (ml/kg/min)	8.5	15.9	43	0.85
Vss (ml/kg)	984	1645	1530	797
MRT (hr)	1.9	1.7	0.59	17.2
AUC (µg*hr/ml)	1.97	1.06	0.39	22.06
T½ (hr)	1.1	1.5	0.41	15.5

Table 4
Mean pharmacokinetic parameters after 20 mg/kg oral dose of nevirapine in rat, monkey, dog, chimpanzee and mouse.

Parameters	Rat	Monkey	Dog	Chimp	Mouse
Cmax (µg/ml)	4.55	1.45	1	12.28	4.1
Tmax (hr)	1.3	1.2	1.1	5.7	1.2
MRT (hr)	3.5	6.4	3.8	19.6	2
F (%)	49	73	30	75	-
AUC (µg*hr/ml)	19.18	14.9	2.26	357	8.48
Metabolite	BIRJ-106	BIRJ-106	BIRJ-106	BIRJ-106	BIRJ-106

Table 5
Model Independent Mean Pharmacokinetic Parameters of Nevirapine in Dogs After Oral Administration as Suspension for 13-Week Study

Parameter	Dose (mg/kg/day)	Male Dogs				Female Dogs			
		Day 1	Wk 3	Wk 7	Wk 13	Day 1	Wk 3	Wk 7	Wk 13
C_{max}	75	0.89	0.8	1.0	0.8	0.4	0.5	0.5	0.7
T_{max}		2	2	2	2	2	2	2	0
AUC		4.4	3.7	4.6	3.5	2.1	3.3	3.7	8.0
C_{max}	200	1.2	1.0	2.1	1.5	0.9	1.3	0.9	2.6
T_{max}		2	2	2	0	2	2	2	0
AUC		8.4	7.2	9.5	9.3	8.4	9.8	4.4	11.5
C_{max}	500	15.1	4.3	7.7	5.9	6.7	8.7	6.5	4.5
T_{max}		2	2	6	2	2	2	2	2
AUC		179	26.4	107	39.5	111	88	31.4	25.1
C_{max}	700/650	11.0	21.3	19.8	4.7	9.9	12.3	14.4	5.7
T_{max}		6	6	6	2	2	6	6	2
AUC		172	259	289	19.4	84.8	141	195	77.6

C_{max} = μg/ml, T_{max} = hr, AUC = μg*hr/ml

Table 6
Model Independent Mean Pharmacokinetic Parameters of Nevirapine in Rats After Oral Administration as Suspension for 13-Week Study

Parameter	Dose (mg/kg/day)	Male Rats				Female Rats			
		Day 1	Wk 4	Wk 8	Wk 13	Day 1	Wk 4	Wk 8	Wk 13
C_{max}	5	0.231.5	0.22	0.43	0.4	1.23	1.8	3.4	2.9
T_{max}		1.4	1.5	1.5	1.5	1.5	1.5	1.5	1.5
AUC			0.63	1.53	1.9	11.0	19.1	20.4	30.9
C_{max}	50	4.9	2.9	4.1	5.6	21.5	13.9	14.5	29.4
T_{max}		1.5	1.5	1.5	1.5	5	5	1.5	1.5
AUC		26.8	18.6	23.7	47.2	296	187	166	348
C_{max}	125	11.4	4.7	4.8	5.6	36.5	35.6	40.9	44.6
T_{max}		1.5	1.5	1.5	1.5	5	5	5	5
AUC		123	43.7	71.3	48.4	639	505	579	877

C_{max} = μg/ml, T_{max} = hr, AUC = μg*hr/ml

Table 7

Summary of mean excretion balance data after oral dosing of ^{14}C-nevirapine in mouse, rat, rabbit, dog and monkey

Species	Sex	N	Dose (mg/kg)	Collection period (hr)	Percent of Dose Recovered			
					Urine	Feces	Cage rinse	Total
Mouse	♂	10	20	0-96	64.2	38.4	-	102.5
	♀	10	20	0-96	68.6	35.3	-	103.9
Rat	♂	2	20.6	0-96	50	38.4	1.9	90.2
	♀	2	21.5	0-96	59.2	31.3	1.1	91.5
Rabbit	♀	3	36	0-96	67.7	29.4	-	97.1
Dog	♂	2	19.8	0-96	36.2	70.9	1.3	108.3
	♀	1	20.3	0-96	32.8	72.7	2	107.5
Monkey	♂	2	19.6	0-96	78	9	7.9	94.9
	♀	1	19.9	0-96	66.3	19.2	8	93.5

Table 8

Plasma protein binding of nevirapine

Species	Nevirapine plasma concentration (µg/ml)	Percent of drug bound
Mouse	0.034 0.88 85.2	42.8 44.6 32.1
Rat	0.99 19 90.2	54.7 53.7 43.5
Rabbit	0.042 1.1 20.4 95.5	68.4 68 64.2 53.4
dog	0.037 0.95 16.9 79	56.2 53.7 41.3 32.5
Monkey	0.18 0.89 4.3 17	45.4 46.3 44.2 41.1
Chimpanzee	0.037 0.89 81.4	45.7 45.4 33.2
Human	0.038 1.02 20.1 94.1	59.7 61.2 57.6 47.1

Table 9
Major metabolites of nevirapine in different species

Species	Plasma	Urine	Feces	Urine & feces	Bile
Human	3-hydroxyNVP glucuronide (3-HNVPG) 2-hydroxyNVP glucuronide (2-HNVPG) 12-hydroxyNVP glucuronide (12-HNVPG) 12-hydroxyNVP (12-HNVP)	3-HNVPG 2-HNVPG 12-HNVPG	-	-	-
Dog	4-carboxyNVP (4-CNVP) 12-HNVPG 2+3-hydroxyNVP glucuronide (2+3-HNVPG)	4-CNVP 12-HNVPG 2+3-HNVPG	4-CNVP 3-HNVPG	-	-
Rat	12-HNVP	4-CNVP ♂ Unknown ♀	4-CNVP Unknown ♀	-	4-CNVP ♂ 12-NVPG
Rabbit	-	12-HNVPG 2+3-HNVPG			
Mouse	-	-	-	4-CNVP 2+3-HNVPG	
Monkey	-	4-CNVP 2+3-NVPG	-	-	-

Table 10
Rate of metabolism of nevirapine in different species

Species	Dose level (μM)	Rate of metabolism (pmole/min/mg of liver microsomal protein
Rat	30	366 ♂ 42 ♀
Dog	30	671
Monkey	30	522
Chimpanzee	30	11.3

Appendix # 5

Comparison of animal and human pharmacokinetic parameters

Table 1

Comparison of iv single dose pharmacokinetic parameters of animals with man

Parameters	Species & Dose					
	Rat (1 mg/kg	Mouse (1 mg/kg	Dog 1mg/kg	Monkey 1 mg/kg	Chimpanzee 1 mg/kg	Human 15 mg/kg
$AUC_{0-\infty}$ (µg*hr/ml)	2.1	0.195	0.39	1.06	22.06	12.07
MRT (hr)	1.9	-	0.59	1.7	17.2	68
$T_{\frac{1}{2}}$ β (hr)	1.3	-	0.41	1.5	15.5	41.2
Cl_T(ml/hr/kg)	480	-	2,604	954	51	17.1
Vss (l/kg)	0.924	-	1.53	1.64	0.797	1.06

Appendix # 6

Comparison of animal NOELs with the human therapeutic dose.

Table 1

Comparison of kinetic data from subchronic/chronic rat, dog and mice toxicity studies with the human therapeutic dose [400 mg/day; AUC = 130 µg*hr/ml]

Study	Dose level (mg/kg/day	Cmax (µg/ml)	AUC (µg*hr/ml)	NOEL (mg/kg/day)	BSA: Equivalent dose in man (mg/kg/day)	AUC: Equivalent dose in man (mg/day)
Rat 1-month (gavage)	1	0.04-0.3	0.24♂,2♀	10	1.42	7.3♂ 149♀
	10	0.55-5.1	2.4♂,48.5♀			
	50	2.9-15	15♂,177♀			
	125/150	3.3-39	48♂,450♀			
Rat 3-month (gavage)	5	0.2-3.4	1.2♂,25♀	5	0.71	3.7♂ 77♀
	50	2.9-29	33♂,257♀			
	125	4.7-45	57♂,691♀			
Rat 12-month (gavage)	5	0.4-2.1	3♂,20♀	5 ♂	0.71 ♂	9.2♂ 62♀
	50	4-25	44♂,350♀			
	100	12-49	143♂,800♀			
Dog 2-week (capsules)	150	5.6-16	74♂,145♀	150	75	228♂ 446♀
	300	6.1-22	193♂,74♀			
	600	15-33	299♂,276♀			
Dog 1-month (capsules)	20	0.2-0.93	4.6♂,2♀	20	10	14♂ 6.1♀
	50	0.3-1	6.7♂,2.5♀			
	100	0.5-1.5	5.6♂,3.8♀			
	400/500	3.3-7.7	64.5♂,73♀			
Dog 3-month (capsules)	75	0.3-1	4♂,5.6♀	200	100	26♂ 25♀
	200	0.9-2.6	8.3♂,8.2♀			
	500	4.3-8.7	67♂,57♀			
	700/650	4.7-21	154♂,137♀			
Dog 12-month (capsules)	50	2.1-7.4	33♂,30♀	50	25	102♂ 92♀
	150/100	5.1-14	133♂,81♀			
	400/300	12-29	245♂,266♀			
Mouse 3-month (diet)	50	-	-	50	4.16	-
	500	-	-			
	1500	-	-			

Consult #565 (HFD-530)

VIRAMUNE nevirapine tablets, 200 mg

The LNC noted two potential look alike/sound alike conflicts with the proposed proprietary name: VIRAZOLE and VIROPTIC, but the LNC feels these trademarks have only a slight potential for confusion. Additionally, the proprietary name contains -vir which is the USAN stem for an antiviral agent. However, the LNC recognizes that -vir has been used commonly in antiviral trademarks and is acceptable to the Committee. The LNC has some discomfort with the use of "-amune" as a syllable as this can serve to conjure up the word immune and perhaps convey the impression that this product could immunize against AIDS. However, since the drug product is used for treating Acquired Immune Deficiency Syndrome, this is not a misleading utilization of a syllable.

The LNC has no reason to find the proposed proprietary name unacceptable.

DCBoring 4/24/96, Chair,
CDER Labeling and Nomenclature Committee

DIVISION OF ANTIVIRAL DRUG PRODUCTS
Review of Chemistry, Manufacturing and Controls

NDA #: **20-636**

CHEMISTRY REVIEW #: 1 **DATE REVIEWED:** 18-Jun-96

SUBMISSION TYPE	DOCUMENT DATE	CDER DATE	ASSIGNED DATE
Original	23-Feb-96	23-Feb-96	26-Feb-96
Amendment (BC)	28-Mar-96	1-Apr-96	5-Apr-96
Amendment (BC)	9-Apr-96	11-Apr-96	16-Apr-96
Amendment (BC)	24-Apr-96	25-Apr-96	2-May-96
Amendment (BC)	31-May-96	3-Jun-96	12-Jun-96
Amendment (BC)	18-Jun-96	NA	NA

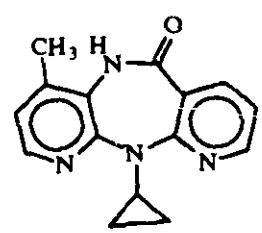
NAME / ADDRESS OF APPLICANT: Boehringer Ingelheim Pharmaceuticals, Inc.
900 Ridgebury Rd.
P.O. Box 368
Ridgefield, CT, 06877

DRUG PRODUCT NAME
Proprietary: Viramune® Tablets, 200 mg
Nonproprietary: Nevirapine
Code Name/#: BIRG 587 BS

PHARMACOLOGICAL CATEGORY: Antiviral
INDICATION: Anti-HIV
DOSAGE FORM/STRENGTH: Tablets, 200 mg , bottles & blister packs of 100
ROUTE OF ADMINISTRATION: Oral

CHEMICAL NAME / STRUCTURAL FORMULA:

CAS Name 11-Cyclopropyl-5, 11-dihydro-4-methyl-6H-dipyrido [3, 2-b:2',3'-e][1,4] diazepin-6-one



Registry Number [129618-40-2]

$C_{15}H_{14}N_4O$ Formula Weight: 266.3

SUPPORTING DOCUMENTS:

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RELATED DOCUMENTS:

8-Apr-96 Teleconference (Discussion of issues related to inspection of
BC dated 9-Apr-96 (Resubmission of EA revised t
NC dated 3-May-96 (Addendum to confidentiality statement in EA)
Facsimile of 3-May-96 (Applicant's corrections to CMC section of NDA)
Facsimile of 6-May-96 (CMC comments on package insert and carton/container labels)
Facsimile of 8-May-96 (Response regarding package insert and carton/container labels)
Facsimile of 31-May-96 (Additional corrections to CMC section of NDA)
Facsimile of 7-Jun-96 (CMC comments and requests for clarification)
10-Jun-96 Teleconference (CMC question on supportive stability data)
11-Jun-96 Teleconference (Final comments on container labels for tablets)
Facsimile of 12-Jun-96 (Applicant's responses to CMC comments and requests)
13-Jun-96 Teleconference (CMC recommendations on regulatory limits for
Facsimile of 13-Jun-96 (Applicant's modifications to tablet container labels)
Facsimile of 14-Jun-96 (Applicant's update of stability data for bottles and blister packages)
Facsimile of 14-Jun-96 (Applicant's additional response to CMC comments and requests)
Facsimile of 14-Jun-96 (Applicant's counterproposals on regulatory limits for
Facsimile of 17-Jun-96 (Final CMC recommendations on expiry, and regulatory limits)
17-Jun-96 Teleconference (Discussion of labeling for bulk drug substance)
19-Jun-96 Teleconference (Final discussion of labeling for bulk drug substance)
Chemistry Reviews of IND

CONSULT REVIEWS:

Trade name review by CDER Labeling and Nomenclature Committee on Viramune®
Environmental Assessment reviewed by HFD-005.
Product specific inspection of two manufacturing and quality control sites.
Instruction in methodology for evaluation of stability by Daphne Lin, Ph.D., Office of Biometrics.

REMARKS / COMMENTS:

DRUG SUBSTANCE: ***Satisfactory***

Nevirapine is a non-nucleoside inhibitor of HIV-1 reverse transcriptase (RT). It is the first member of this class of anti-HIV agents to reach the NDA stage. Nevirapine binds non-competitively to RT (K_i=200 nM) and is not active against HIV-2 or other retroviruses, which are common features of the non-nucleoside RT inhibitors. The details of the inhibition process are known through a single crystal X-ray study of the nevirapine/RT complex (Ref 1 and 2). In tissue culture studies, laboratory and clinical strains of HIV-1 are inhibited by nevirapine with IC_{50} values of 10-100 nM. A long *in vivo* half-life (1-2 days) and the ability to pass the blood/brain barrier (and through the placenta) are favorable aspects of nevirapine's pharmacology. The normal dose will be 200 mg bid (200 mg daily for the initial 14 days) in combination with other antiviral agents.

Nevirapine is a non-hygroscopic white solid with a melting point of 246°C. It exists in 2 pseudopolymorphic forms: a hemihydrate and the anhydrate. No additional polymorphs were identified in recrystallization studies using nine representative solvents and solvent combinations. The hemihydrate is formed in the final precipitation of the synthesis, and is dehydrated to the anhydrous drug substance by drying at 90-110°C. Adequate evidence is presented to show that the manufacturing method of the drug substance consistently produces the anhydrous form, and that interconversion does not occur during storage at high humidity, . The dissolution of nevirapine tablets is slightly reduced by levels of hemihydrate above in the bulk drug, and a specification for this pseudopolymorph is in place for the drug substanc

The proof of structure is well supported by the synthetic route and data from elemental analysis, .

No options for reprocessing or rework of the drug substance are described in this application. Specifications for the starting materials are adequate, and in-process specifications are provided for the final isolated intermediate nevirapine-crude and nevirapine-DMF.

The proposed attributes, including appearance, identity (IR related substances

The commercial process will produce batches. Six primary registration batches are presented in the application, with scales of manufacture between Batch analyses are provided on a total of 27 lots of drug substance, 14 of which are larger than Cross references to clinical and stability lots of drug product are also supplied, and all stability lots (drug substance and tablets) have batch analyses at release. The batch analyses provide adequate justification for the regulatory limits.

Stability studies show no time-dependent changes in appearance, assay, impurity levels or loss on drying. Seven pilot and production-scale lots (65-240 kg) were studied. A retest period of 12-months was requested in the NDA, and is well justified by the 12-48 months of data. Stability testing of the initial three post-approval batches will be carried out at 25°C/60%RH and 40°C/75%. We requested that the Applicant include a recommended storage statement, including a temperature or range, on the labels for bulk drug substance.

DRUG PRODUCT: ***Satisfactory***

Although nevirapine has low intrinsic solubility in water (0.1 mg/mL), the solubility increases at low pH, and an uncoated tablet has been developed with greater than 90% absolute bioavailability in humans.

colloidal silicon dioxide and magnesium stearate. There are no concerns regarding the quality of the excipients or the compositional formula because all components conform to USP or

The proposed attributes, including appearance, identity (related substances by ure adequate to ensure the quality of the tablets. Because nevirapine drug substance is neither hygroscopic nor moisture sensitive, coupled with (limited) stability data in which tablet moisture was monitored, and in consideration of the arguments presented in the Applicant's facsimile of 12-Jun-96, moisture content need not be included in the regulatory specifications for the tablets. The dissolution

The registration batches are fully representative of the proposed commercial process in terms of both scale and facilities. The commercial scale of manufacture is per lot. Batch analyses are provided on lots, all produced at the commercial tableting site (BI Pharmaceuticals Inc., Danbury CT). Three of the lots were produced at the scale using drug substance which was manufactured at the commercial site The bioequivalence of the final formulation was verified in Protocol 1100.934.

The tablets are packaged in white HDPE bottles which are capped with polypropylene child-resistant closures, or in polyvinyl chloride blister packages. Citations to DMFs are provided for all packaging components, and acceptance specifications are adequately described.

The primary and supportive stability data are outlined below. The primary data were updated in an amendment dated 24-Apr-96. By prior agreement (Pre-NDA meeting 10-Aug-95), tablets prepared from both Scheme 1b and Scheme 2 drug substance make up the primary

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The primary stability data were updated (24-Apr-96) to include one lot at 30 months in bottles and six lots out to 12 months in blister packages. Upon request, additional data were provided on the bottle presentation (a second primary lot out to 30 months, and a supportive lot with 36-month data) and the blister packaged product (three primary lots out to 18 months). None of the primary stability samples shows any time-dependent changes which would indicate decomposition when stored for up to 30 months at 30°C/70%RH and for up to 12 months at 40°C/75%RH in HDPE bottles. Equivalent stability is documented for the blister presentation out to 18-months at 25°C/60%RH and 6-months at 40°C/75%RH. Supportive data of 36 months duration (1 lot in bottles) also show no evidence of decomposition or degradation. These data support a 30-month expiration for the tablets in HDPE bottles, and 24-months for the tablets in blister packaging. This was communicated to the Applicant in a facsimile on 17-Jun-96.

ENVIRONMENTAL ASSESSMENT: ***Satisfactory***
The EA for this new molecular entity was submitted for consultative review to HFD-005 on 5-Mar-96. The Agency advised the Applicant that this product could by covered by Tier Zero documentation, and the EA data (minus the Fate and Effect sections) were resubmitted on 11-Apr-96. The EA review is complete, and a FONSI (signed by Nancy Sager and Roger Williams) was issued on 21-May-96.

METHODS VALIDATION: ***Pending***
The analytical methodology is adequately described including the relevant validation. The Methods Validation package was submitted to the Boston District, and to the Division of Drug Analysis on 30-May-96. Arrangements were made with the Applicant, and with the inspector of the drug product site, so that the MV samples were picked up during that inspection. As of 12-Jun-96, the MV samples had been mailed from the Hartford Resident Post to both testing laboratories. Validation of the analytical methodology is not anticipated prior to the approval decision for this application.

LABELING: ***Satisfactory***
The proprietary name, Viramune® Tablets, was submitted for review to the CDER Labeling and Nomenclature Committee (L&NC) on 11-Mar-96, and was judged to be acceptable (with some reservations) on 24-Apr-96. Division reviewers had no objections to this trademark, and the L&NC review was forwarded to the Applicant on 29-Apr-96. A request to make the product name in the package insert agree with the container labels sent to the Applicant by facsimile on 6-May-96, along with other CMC labeling comments. A commitment to

uniformly use "Viramune® (nevirapine) Tablets" as the product name was received on 8-May-96.

The proposed storage statement "Store between 15°C - 30°C (59°F - 86°F)" is justified by the stability data which show satisfactory performance at 25°C and 30°C. This was conveyed to the Applicant on 7-Jun-96, along with the caution that future CDER policy changes might require alterations in the recommended storage temperature.

The Applicant was encouraged to include a suggested storage temperature on the labels for bulk drug substance drums (see Agency's facsimile of 7-Jun-96). The Applicant responded (facsimile of 12-Jun-96) that no "special storage conditions" exist, and that therefore this labeling is not required (citing the "Guide to Inspection of Bulk Pharmaceutical Chemicals"). After internal discussions, it was agreed that, under current regulations, a storage temperature on the bulk drug label was not needed for a drug that is highly stable under all conditions which are likely to be encountered.

ESTABLISHMENT INSPECTION: ***Satisfactory***
The Establishment Evaluation Request was submitted on 22-Mar-96, and covered

was documented as an alternative site for tablet release and stability testing. Inspection Requests were sent to Field District Offices on 28-Mar-96, and inspections were scheduled to begin 20-May-96, the earliest date allowed by the Applicant's validation programs. The drug substance validation lots, and the first 15 commercial lots, are to be rather than the starting material The Applicant proposed this prior to the Pre-NDA meeting (10-Aug-95) because of had been stockpiled at the Ingelheim plant in anticipation of an expanded access program. The Division agreed to this proposal at the Pre-NDA meeting, documentation is included in the NDA, and both inspection teams were informed in Apr/May 1996. The validation program was also the subject of a meeting between

Briefing packages were faxed to the 2 inspection teams one week prior to the inspections. Positive comments were received (unofficially) from both inspection teams during the first week of June. The completed EER was received on 17-Jun-96 by facsimile, with each of the three sites listed with acceptable status.

CONCLUSIONS & RECOMMENDATIONS:

The NDA submission and accompanying amendments provide adequate information on the chemistry, manufacturing and controls for Viramune® (nevirapine) Tablets. The Environmental Impact Assessment is complete, and the manufacturing facilities have acceptable cGMP status. The NDA, as amended, is therefore recommended for **approval** from the chemistry perspective.

Stephen P. Miller 6/17/96
Stephen P. Miller, Ph.D.
Review Chemist

Concurrence:
HFD-530/CChen CWC 6/18/96

cc:

Orig. NDA 20-636	HFD-530/DFeigal	HFD-530/PVerma
HFD-530/Div. File	HFD-530/CChen	HFD-102/AZeccola
HFD-830/ESheinin	HFD-530/SMiller	
	HFD-530/JMartin	
	HFD-530/LIacono-Connors	File: N 20-636 \ 000CNR01.36i

ENVIRONMENTAL ASSESSMENT

AND

FINDING OF NO SIGNIFICANT IMPACT

FOR

NDA 20-636

VIRAMUNE®

(nevirapine)

Tablets

FOOD AND DRUG ADMINISTRATION

CENTER FOR DRUG EVALUATION AND RESEARCH

DIVISION OF ANTI-VIRAL DRUG PRODUCTS
(HFD-530)

FINDING OF NO SIGNIFICANT IMPACT

NDA 20-636

VIRAMUNE® (nevirapine) Tablets

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 **VIRAMUNE®**, **Boehringer Ingelheim Pharmaceuticals, Inc.** has prepared an environmental assessment in accordance with 21 CFR 25.31a (attached) which evaluates the potential environmental impacts of the manufacture, use and disposal of the product.

Nevirapine is a synthetic drug which will be administered orally in the treatment of patients with acquired immunodeficiency syndrome (AIDS). The drug substance will be manufactured by BI Chemicals, Petersburg, VA and the drug product will be manufactured at Boehringer Ingelheim pharmaceuticals, Inc., Danbury, CT. The finished drug product will be used in hospitals, clinics and homes.

Nevirapine may enter the environment from excretion by patients, from disposal of pharmaceutical waste or from emissions from manufacturing sites. The projected environmental introduction concentration from use is less than 1 ppb. CDER has routinely found that concentrations less than 1 ppb have no effect on relevant standard test organism, therefore the applicant has submitted a Tier 0 EA without format items 7, 8, 9, 10 and 11.

Disposal may result from production waste such as out of specification lots, returned goods and user disposal of empty or partly used product and packaging. Pharmaceutical waste will be disposed of by the manufacturer at a licensed incineration or landfill facility. At U.S. hospitals, pharmacies or clinics, empty or partially empty packages will be disposed of according to standard procedures. 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, although minimal quantities of unused drug may be disposed of in the sewer system.

Precautions taken at the sites of manufacture of the bulk product and its final formulation are expected to minimize occupational exposures and environmental release.

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. 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.

5/21/96
DATE

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

5/21/96
DATE

CONCURRED
Roger L. Williams, M.D.
Deputy Center Director for Pharmaceutical Science
Center for Drug Evaluation and Research

Attachment: Environmental Assessment

ADDENDUM TO

ENVIRONMENTAL ASSESSMENT

REDACTIONS MADE BY APPLICANT

VIRAMUNE® (nevirapine) Tablets

(200 mg)

Although the footer included in the EA document (signed) dated April 1, 1996 indicates the information is confidential, I confirm that pages 4 through 31 can be released to the public.

Patricia L. Watson

Patricia L. Watson
DRA Associate Director
Drug Regulatory Affairs

Date: May 3, 1996

REDACTIONS MADE
BY APPLICANT

ENVIRONMENTAL ASSESSMENT

VIRAMUNE® (Nevirapine) Tablets

(200 mg)

TABLE OF CONTENTS

REDACTIONS MADE BY APPLICANT

TABLE OF CONTENTS
(continued)

REDACTIONS MADE BY APPLICANT

LIST OF TABLES

REDACTIONS MADE BY APPLICANT

LIST OF APPENDICES

REDACTIONS MADE BY APPLICANT

EXECUTIVE SUMMARY

Boehringer Ingelheim Pharmaceuticals, Inc. (BIPI) is seeking approval from the United States Food and Drug Administration (FDA) for the manufacture and commercial distribution, within the United States, of a new drug known as VIRAMUNE® (nevirapine). VIRAMUNE® tablets are intended for use as an orally administered antiviral agent for patients with Acquired Immune Deficiency Syndrome (AIDS).

An Environmental Assessment (EA) is required under 21 CFR Part 25.31a for proposed actions to approve new drugs and drug substances. This proposed action includes synthesis of the drug substance, formulation of the drug product, preparation and packaging of the tablets, and distribution of the final product.

The goal of this Environmental Assessment is to demonstrate that the introduction of VIRAMUNE® (nevirapine) into the natural environment will have little if any impact, and cause no environmental degradation. Both the Maximum Expected Environmental Concentration (MEEC) and the Expected Introduction Concentration (EIC) are below one part per billion. Therefore, we are submitting a limited environmental assessment in accordance with CDER guidelines. Environmental testing data is available. This data indicates the product does not readily biodegrade and is not toxic to aquatic organisms.

REDACTIONS MADE BY APPLICANT

3.4 ENVIRONMENTAL ASSESSMENT FOR VIRAMUNE® (NEVIRAPINE) TABLETS

1. Date

November 2, 1995

2. Name of Applicant

Boehringer Ingelheim Pharmaceuticals, Inc. (BIPI)

3. Address

900 Ridgebury Rd./P.O. Box 368
Ridgefield, CT 06877
Attn.: Drug Regulatory Affairs Department

REDACTIONS MADE BY APPLICANT

4. Description of proposed action

a. Description of requested approval

FDA approval for the manufacture and U.S. commercial distribution of VIRAMUNE® (nevirapine) tablets for use as an orally administered antiviral agent to patients with Acquired Immune Deficiency Syndrome (AIDS).

The proposed action includes synthesis of the drug substance, formulation of the drug product, preparation and packaging of the tablets and use of the product designated in this Environmental Assessment (EA) as VIRAMUNE® (nevirapine) tablets (200 mg).

b. Need for the action (proposed use)

The proposed action will provide a therapeutic agent for use in the treatment of AIDS.

VIRAMUNE® contains the active ingredient nevirapine, a dipyridobenzodiazepinone, which is a potent inhibitor of the reverse transcriptase enzyme of HIV-1.

c. *Locations where drug substance and product will be manufactured and packaged*

1) *Manufacturing of the drug substance (nevirapine)*

Manufacturing of the drug substance, nevirapine will be conducted at the Petersburg, Virginia facility:

BI Chemicals, Inc.
2820 North Normany Drive
Petersburg, VA 23805

REDACTIONS MADE
BY APPLICANT

Nevirapine is manufactured in a multi-step batch process. Two highly substituted pyridine compounds are combined to form a substituted carboxamide that is washed and isolated. The carboxamide is reacted with cyclopropylamine prior to reductive ring closure with sodium hydride in mineral oil. This results in the final structure, a cyclopropyl substituted, three ring, nitrogen containing heterocycle. The product is purified using standard recrystallization techniques from dimethylformamide. Further purification utilizes dissolution in an aqueous acidic medium followed by caustic neutralization and precipitation. The wet product is tumble dried to yield anhydrous nevirapine. Milling of raw nevirapine takes place in the Petersburg facility before shipment. A flow chart explaining the manufacturing process is provided as APPENDIX A.

2) *Manufacturing and packaging of drug product (VIRAMUNE® tablets)*

Manufacturing of VIRAMUNE® tablets will take place at the Danbury, Connecticut facility[1]:

Boehringer Ingelheim Pharmaceuticals, Inc. (BIPI)
175 Briar Ridge Road
Danbury, CT 06810

[1] See Section 4d.

Nevirapine will be shipped from the BI Chemicals, Inc. facility in Petersburg, Virginia to BIPI's manufacturing and packaging facility in Danbury, Connecticut. The BIPI facility in Connecticut is an existing facility that produces pharmaceuticals including: solid dosage forms (tablets and capsules), liquids, aerosols, and suppositories.

REDACTIONS MADE BY APPLICANT

The following procedures, associated with the manufacture of VIRAMUNE® tablets, will be performed at this facility: The active ingredient (nevirapine) is combined in a mill with the drug product excipients, formulated into tablets via a tablet press, and packaged for distribution of the final product. Please refer to NDA Section 3.3.4 for more information on the tablet manufacturing process.

d. The types of environments present at and adjacent to manufacturing locations

The following information is provided regarding the physical environment around the manufacturing locations:

The BI Chemicals, Inc. facility in Virginia. The facility is located on approximately 275 acres on the edge of the city of Petersburg, adjacent to Interstate 95. The plant is located on a flat parcel of land. The surrounding area is zoned for light industrial use.

The Boehringer Ingelheim Pharmaceuticals, Inc. facility in Connecticut. The facility is in a hilly, residential/light industrial zone on a 500 acre campus located on the Danbury/Ridgefield town line.

The administrative portion of this united campus is located at 900 Ridgebury Road in Ridgefield with the adjacent manufacturing and packaging facility located at the 175 Briar Ridge Road address in Danbury.

e. Locations where VIRAMUNE® tablets will be used and distributed

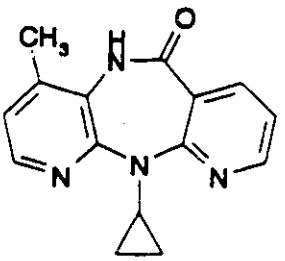
VIRAMUNE® tablets will be distributed by Roxane Laboratories, a Boehringer Ingelheim affiliate, throughout the U.S. to wholesalers, hospitals, and pharmacies. It will be available for use by physician's prescription only, for patients with AIDS.

5. *Identification of chemical substances that are the subject of the proposed action*

Nevirapine is the active ingredient in the antiviral agent VIRAMUNE® tablets with a measured purity of at least 99%. TABLE 3.4:1 provides information on the identity of this compound.

TABLE 3.4:1 Identification of the drug substance in VIRAMUNE® Tablets

	Nevirapine*
Chemical name	11-Cyclopropyl-5,11-dihydro-4-methyl-6H-dipyrido-[3,2-b:2',3'-e][1,4]diazepin-6-one
Manufacturing synonym(s)	BI-RG-587
CAS Reg. No.	129618-40-2
Molecular weight	266.31
Molecular formula	$C_{15}H_{14}N_4O$
Physical description	Off-white, odorless, crystalline powder
Structural formula	



* Material safety data sheet is attached prior to the confidential Appendices

In addition to the active ingredient, VIRAMUNE® tablets contain the following inactive components: microcrystalline cellulose, NF; lactose NF; povidone K25, USP; sodium starch glycolate, NF; colloidal silicon dioxide, NF; magnesium stearate, NF; and purified water, USP.

Although the chemical and physical properties of these excipients may vary, their environmental fate, as a result of VIRAMUNE® tablet production, is not expected to adversely affect the environment based upon the information reviewed. Several of these ingredients are naturally occurring, or derived from nature, and commonly used in pharmaceutical preparations as tablet diluents or suspending, dispersing or

thickening agents (microcrystalline cellulose, lactose, sodium starch glycolate, colloidal silicon dioxide). Magnesium stearate has been listed by the FDA as a generally recognized as safe (GRAS) for use as a direct food additive compound (21 CFR Part 184.1440). Povidone K25, a synthetic polymer which is typically used as a dispersing and suspending agent, and has also been used as a tablet binder, coating agent and viscosity-increasing agent in pharmaceutical preparations, is listed by the FDA as a secondary direct food additive (21 CFR Part 173.55) for use in food supplements and is considered to be essentially non-toxic.

6. ***Introduction of substances into the environment***

The following tables and discussion describe the substances expected to be introduced into the environment during the manufacture and packaging of VIRAMUNE® tablets and as a result of its use by individuals with AIDS.

REDACTIONS MADE BY APPLICANT

a. ***Introduction of substances from the site(s) of production***

1) ***Manufacture of active ingredient***

Nevirapine is manufactured in a small batch process at BI Chemicals, Inc. in Petersburg, Virginia. The batch processing equipment is in a four story building with vertical material flow. All process equipment is serviced by a vapor and dust removal system. This system is serviced by a permitted aqueous scrubber. Wastewater from the facility discharges to the Petersburg, Virginia POTW.

The Petersburg manufacturing plant is in full compliance with all applicable Federal, State and Local environmental permits which have been issued to the facility. APPENDIX B contains statements of compliance from the Petersburg, VA facility. The Environmental permits issued are listed below:

TABLE 3.4:2 Environmental Permits Issued to BI Chemicals Inc., Petersburg, Virginia

Permits Issued	Permit Number	Issuing Authority
Wastewater Discharge	004	City of Petersburg
Hazardous Waste Generation	VAD093561652	Virginia Department of Environmental Quality
Stormwater	VAR240082	Virginia Department of Environmental Quality
Air Discharge	50856	Virginia Department of Environmental Quality

The material balance for the manufacture of nevirapine is provided below in TABLE 3.4:3.

REDACTIONS MADE BY APPLICANT

TABLE 3.4:3 Material Balance for the Manufacture of Nevirapine

Material	Amount Released (per kg of nevirapine produced)
VOCs	0.96 kg emitted during manufacturing process
Organic intermediates and constituents	6.31 kg released in wastewater
Inorganic constituents	3.89 kg released in wastewater
Nevirapine	0.015 kg lost during drug product manufacture

The effluent from the BI Chemical facility passes through a gravity separator to recover any free solvents. It is then given final pH adjustment before being released to the POTW. The City of Petersburg operates an advanced biological wastewater treatment system with nitrification capabilities.

2) ***Manufacture of VIRAMUNE® tablets***

The following substances may be emitted into the environment during the production of VIRAMUNE® tablets: nevirapine; microcrystalline cellulose, NF;

lactose NF; povidone K25, USP; sodium starch glycolate, NF; colloidal silicon dioxide, NF; magnesium stearate, NF; and purified water, USP.

The Danbury, Connecticut manufacturing facility is equipped with both internal and external dust collectors on air emissions points. Wastewater from the facility is discharged to the Danbury, Connecticut POTW. The City of Danbury operates an advanced biological treatment system with nitrification capabilities. Subsequently, the introduction of the inactive ingredients, found in VIRAMUNE® tablets, to the surrounding environment is not expected to result in adverse effects and, as such, is not a consideration for this EA submission.

REDACTIONS MADE BY APPLICANT

The Danbury manufacturing plant is in full compliance with all applicable Federal, State and Local environmental permits which have been issued to the facility. Environmental permits issued are listed below In TABLE 3.4:4:

TABLE 3.4:4 Environmental Permits Issued to BIPI, Danbury, Connecticut

Permits Issued	Permit Number	Issuing Authority
Wastewater Discharge	SP0000021	State of Connecticut Dept. of Environmental Protection
Hazardous Waste Generator	CTD097730709	State of Connecticut Dept. of Environmental Protection

b. Introduction of substance from use and disposal of VIRAMUNE® tablets

The same eight substances listed previously for the manufacture of the drug product may also be emitted into the environment as a result of the use, or disposal of the product by patients.

There are two possible routes by which the components of VIRAMUNE® tablets could be introduced into the environment as a result of its use: 1) elimination of the active ingredient, its metabolites, or the excipients by the patients, and 2) the disposal of unused, or partially used, drug supply by patients, pharmacies, *etc.*

1) Elimination of components following use by patients

APPENDIX C contains a study of the metabolite pattern in urine which was conducted in eight normal volunteers using ^{14}C-radiolabeled nevirapine. The study indicated that the principal pathway for elimination of the drug and its metabolites is via the urine with the majority of the ingested dose (81.3%) excreted by this pathway. Urine analysis for radiolabeled compounds demonstrated that approximately 85.2 of the total radioactivity present in urine was attributable to three glucuronide conjugates of the ^{14}C-nevirapine hydroxy- metabolites. Approximately 5% of the radioactivity is associated with non-glucuronidated urinary metabolites, with only 3.3% identified as the parent compound, nevirapine.

2) Unused material

REDACTIONS MADE BY APPLICANT

Unused material includes any off-spec material, returned product or material that may have passed its expiration date. VIRAMUNE® tablets are tested for conformance to production standards prior to packaging. Tablets which do not meet these standards are generally disposed of by landfill or incineration. Likewise, unused VIRAMUNE® tablets, discarded by consumers would most likely be disposed of by landfill or incineration. Off-spec material and product returned to BIPI as expired material will be disposed of by incineration at a fully licensed and permitted facility (*e.g.*, Rollins Environmental Services, Bridgeport, NJ; EPA ID #NJD053288239).

c. Controls to be used

1) Manufacture of the active ingredient

APPENDIX B contains compliance statements from the BI Chemicals, Inc. Petersburg, VA facility stating that all applicable regulations are being followed during the production of nevirapine at the Boehringer Ingelheim facility.

2) *Manufacture of VIRAMUNE® tablets*

NDA Section 3.3.4 provides details on each of the processes discussed below, the associated waste streams, and the controls used for each at BIPI's Connecticut facility:

a) *Granulation*

Granulation is the process in which the active ingredient (milled nevirapine) is blended with Lactose NF and Povidone K25. The material is then dried for 15 minutes to 40°C.

b) *Milling and Blending*

REDACTIONS MADE BY APPLICANT

The remaining excipients are added to the nevirapine mixture during the milling and blending process. Once blended, the mixture is sent to tablet formulation.

c) *Tablet Formulation*

The material will be formulated into tablets containing 200 mg of the active ingredient (nevirapine) in production batch sizes of [CBI][2].

d) *Packaging*

Following tablet formulation, emission of the drug product is not anticipated. Therefore, no significant dust emissions are expected to occur as a result of the packaging process.

e) *Returned/expired products*

These materials will be incinerated at fully licensed and permitted facilities such as Rollins Environmental Services, Bridgeport, NJ; EPA ID #NJD053288239.

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[2] [CBI] - Confidential Business Information

d. Estimate of quantities and concentrations of substances expected to enter the environment as a result of both manufacturing and use

The BI Chemicals facility in Petersburg, VA and the BIPI facility in Danbury, CT are subject to and in compliance with effluent guidelines and standards for pharmaceutical manufacturing. The standards are contained in the regulations published in 40 CFR Part 439. The manufacturing of the new product will be conducted within these guidelines.

All process and sanitary wastewater is discharged to local treatment plants which are in full compliance with their respective industrial discharge permits. These permits are listed in Sections 6.a.1 and 6.a.2 of this document. Wastewater analyses are performed using USEPA-approved protocols.

REDACTIONS MADE BY APPLICANT

1) Water

The maximum expected environmental concentration (MEEC) and the expected introduction concentration (EIC) of drug substance in wastewater has been estimated using equations found in APPENDIX E. The equations incorporate the total annual production value for the drug substance of [CBI] in five years. The MEEC uses simple dilution based on a per capita estimate of wastewater (in gallons/day), whereas the EIC is calculated by basing dilution on total estimated volume of wastewater entering domestic POTWs. Using the projected total annual (5th year) production of VIRAMUNE® tablets in the U.S., the following estimates are made:

Nevirapine MEEC: approximately [CBI] (wastewater)

Nevirapine EIC: approximately [CBI] (wastewater)

APPENDIX E contains the calculations and assumptions used to derive the MEEC ans EIC value. Both the MEEC and EIC estimate assumes that the entire annual production of the components is released into the environment. Although a majority of the excreted drug is comprised of three glucuronidated metabolites, the

introduction into the environment is calculated as if the nevirapine were being eliminated unchanged due to the structural similarity between the drug substance and its metabolites. There is no reason to believe that there will be significant absorption of Neviripine in activated sludge The values for the MEEC and EIC are very similar. It is expected that the actual environmental introductions would be only slightly less due to metabolism, wastewater treatment, and degradation, in receiving waters.

2) Air

The physical/chemical characteristics (low vapor pressure) and manufacturing controls suggest that the quantities of nevirapine, and associated components, released into the air will be negligible.

3) Terrestrial Environment

REDACTIONS MADE BY APPLICANT

The physical/chemical characteristics (water solubility, low K_{oc}) and manufacturing procedures suggest that the components of nevirapine will not reside in soils and sludges and, as such, introduction into the terrestrial environment is considered to be insignificant.

4) Disposal as Solid Waste

Solid waste will most likely be disposed of by landfill or incineration. Waste discarded by consumers will likely be disposed of in permitted landfills. These landfills are required to meet operating standards which will preclude the release of landfill contents to the surrounding environment. Product returned to BIPI as expired material will be disposed of by incineration at a fully licensed and permitted facility (*e.g.*, Rollins Environmental Services, Bridgeport, NJ; EPA ID #NJD053288239).

5) Occupational Exposure/Removal

Both BI Chemical and BIPI adhere strictly to OSHA policies on worker safety. Safe manufacturing techniques are employed in every step of nevirapine synthesis and formulation of the drug product. Where applicable, employees are required to wear appropriate personal protective equipment. This equipment includes disposable gloves, and air purifying respirators with HEPA filters. In addition, all employees receive training in Good Manufacturing Practices and worker safety. Information regarding the safe handling and disposal of nevirapine is outlined in the Material Safety Data Sheet (MSDS) provided at the end of this document.

e. Summary

REDACTIONS MADE BY APPLICANT

Estimates of the amounts of each substance introduced into the environment are based on the projected 5th year production of VIRAMUNE® tablets. The production figures are confidential and included in APPENDIX F.

Manufacturing of the active ingredient (nevirapine) will take place at the Petersburg, VA facility. APPENDIX B contains statements verifying compliance with applicable regulations.

Manufacturing of the finished product will take place at the Danbury, CT Facility. Emission controls for the Danbury Facility have been described. Overall emissions of the active ingredients plus the excipients (inactive ingredients) is minimal. All solid waste is incinerated at fully licensed and permitted facilities equipped with adequate air pollution control equipment. Wastewater is generated only as a result of equipment washings. All wastewater is disposed of via the City of Danbury POTW in accordance with Permit No. SP0000021.

The largest introduction of the product components into the environment will be as a result of the use of VIRAMUNE® tablets by patients with AIDS. Use, and subsequent elimination, will result in nevirapine and its metabolites, entering the wastewater stream. Nearly all of the active ingredient is excreted via the urine. The excipients include: microcrystalline cellulose, NF; lactose NF; povidone K25, USP; sodium starch

glycolate, NF; colloidal silicon dioxide, NF; magnesium stearate, NF; and purified water, USP.

The maximum expected environmental concentration (MEEC) (or total amounts, if applicable) is presented in APPENDIX E.

7. Fate of emitted substances in the environment

Biological and physiochemical testing to determine the effects, fate and environmental transport of nevirapine has been completed. The Maximum Estimated Environmental Concentration (MEEC) and the Expected Introduction Concentration (EIC) of nevirapine are well below 1 part per billion. There would be no observable environmental effects at a concentration of 1 part per billion. Testing of aquatic organisms (ie-Daphnids, Amphipods and Fathead Minnows) and microbial organisms required significantly higher concentrations (ppm levels) of nevirapine to show an observable effect. In keeping with the November 1995 guidance from the Center for Drug Evaluation and Research (CDER), we have opted for a Tier 0 approach for preparation of this report. However, biological and physiochemical testing data on this substance are available.

REDACTIONS MADE BY APPLICANT

12. List of preparers

Christopher P. D'Alleinne, Ph.D., Principal Health Scientist, ChemRisk® Division of McLaren/Hart Environmental Engineering Corporation. Dr. D'Alleinne has 13 years of post-Ph.D. experience in toxicology and risk assessment.

Alison G. DiPasca, Senior Associate Health Scientist, ChemRisk® Division of McLaren/Hart Environmental Engineering Corporation. Ms. DiPasca has six years of professional experience in public health, environmental science and risk assessment.

Arthur E. Slesinger, Director of Environmental Affairs and Safety at BIPI. Holds Masters degrees in both Chemical Engineering and Environmental Health Sciences and has more than 20 years experience in environmental and regulatory affairs.

David Weinberg, Environmental Specialist at BIPI. Mr. Weinberg has 14 years experience in environmental and regulatory affairs.

13. Certification

The undersigned official certifies that the information presented is true, accurate and complete to the best of the knowledge of the firm or agency responsible for the preparation of the environmental assessment.

Arthur E. Slesinger — 4/1/96

Arthur E. Slesinger
Director, Environmental Affairs and Safety

Date

REDACTIONS MADE BY APPLICANT

14. References

EATAH (Environmental Assessment Technical Assistance Handbook). 1987. Document by the U.S. Food and Drug Administration, Washington, DC. Available through NTIS (PB87-175345).

FDA. 1986. *Procedures for estimating environmental introductions of FDA-regulated chemicals.* Prepared by the Environmental Impact Section of the Center for Food Safety and Applied Nutrition. June 1986. Available upon request.

Material Safety Data Sheet

Nevirapine

MATERIAL SAFETY DATA SHEET

Nevirapine

1. CHEMICAL PRODUCT AND COMPANY IDENTIFICATION

PRODUCT IDENTIFICATION

Product Name: NEVIRAPINE
Chemical Name BI-RG-587; 11-Cyclopropyl-5,11-dihydro-4-methyl-6H-dipyrido-[3,2-b:2',3'-e][1,4]diazepine-6-one

Synonym: NONE
Formula: $C_{15}H_{14}N_4O$

Use: Antiviral

DISTRIBUTED BY:

Boehringer Ingelheim
Pharmaceuticals, Inc.
900 Ridgebury Road
P.O. Box 368
Ridgefield, CT 06877

EMERGENCY TELEPHONE NUMBERS:

Transportation
CHEMTREC (800)424-9300

Other
(203) 798-5521

2. COMPOSITION/INFORMATION ON INGREDIENTS

CHEMICAL NAME	CAS NUMBER	WEIGHT %
11-Cyclopropyl-5 11-dihydro-4-methyl-6H-dipyrido-[3,2-b:2',3'-e][1,4]diazepine-6-one	129618-40-2	100

(Note: See Section 8 of the MSDS for Exposure Guidelines)

3. HAZARDS IDENTIFICATION

EMERGENCY OVERVIEW

White, odorless powder. May be harmful if inhaled or swallowed. May cause irritation to skin and eyes.

Acrid, flammable fumes may develop in a fire. Use water spray, foam, CO_2, or dry chemical agents to extinguish fires.

POTENTIAL HEALTH EFFECTS

PRIMARY ROUTE(S) OF ENTRY

Inhalation:	YES
Eye:	YES
Skin contact:	NO
Ingestion:	YES

SYMPTOMS OF EXPOSURE

Inhalation:	Breathing the powder may cause difficulty in breathing
Eye Contact:	May cause reddening and irritation to develop.
Skin Contact:	May cause reddening and irritation to develop.

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4. FIRST AID MEASURES

INHALATION

REDACTIONS MADE BY APPLICANT

Remove from area to fresh air. Seek medical attention if respiratory irritation develops or if breathing becomes difficult.

EYE CONTACT

Flush eyes with water for 15 minutes. Remove any contact lenses, and continue flushing with plenty of water. Seek medical attention if irritation develops or persists.

SKIN CONTACT

Wash affected areas with plenty of water, and soap if available, for several minutes. Seek medical attention if irritation develops or persists.

INGESTION

Give 3-4 glasses of water, but DO NOT induce vomiting. If vomiting occurs, give fluids again. Get medical attention to determine whether vomiting or evacuation of stomach is necessary. Do not give anything by mouth to an unconscious or convulsing person.

5. FIRE FIGHTING MEASURES

Dust Explosion Hazard....ST2 (dangerous); modified Hartman tube

GENERAL HAZARD

As with all organic powders, this material presents a dust explosion hazard. It can burn in a fire, producing acrid, flammable fumes.

EXTINGUISHING MEDIA

Water Spray. foam, CO_2, or dry chemical.

FIRE FIGHTING EQUIPMENT

As in any fire, wear NIOSH/MSHA approved, pressure-demand self-contained breathing apparatus and full protective gear.

6. ACCIDENTAL RELEASE MEASURES

Wear appropriate protective equipment (See Section 8.) Sweep up and place in a compatible waste container. Avoid creating dust. The very fine particles can cause a fire or explosion; therefore, eliminate all sources of ignition.

REDACTIONS MADE BY APPLICANT

7. HANDLING AND STORAGE

HANDLING

May form flammable dust-air mixtures. Avoid creating dusts. In situations where dusts will be unavoidably generated, ground all equipment and use explosion-proof apparatus.

Avoid breathing dusts; avoid contact with skin or eyes. Wear appropriate protective equipment (See Section 8.) Wash thoroughly after handling.

STORAGE

Store in a cool, dry place. Keep container tightly closed.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

ENGINEERING CONTROLS

Minimize dust generation. Use closed equipment where possible. Use spot ventilation to remove dust from the work area. If operations generate dusts, use explosion-proof ventilation equipment to control airborne levels. Use appropriate respiratory protection based on an industrial hygiene survey.

PERSONAL PROTECTION

Respiratory protection: In operations where dusts are generated, use an appropriate NIOSH/MSHA approved respirator. Respirator should be selected by a qualified professional and based on an industrial hygiene survey.

Eye Protection: Use safety glasses with side shields or goggles.

Hand Protection: Wear durable rubber gloves.

Clothing: Wear long sleeve shirts and long pants to prevent skin contact. In extremely dusty areas protective disposable coveralls should be worn.

COMPONENT	OSHA PEL		ACGIH	ECL (ug/m^3)
	TWA	STEL	TWA	TLV
Nevirapine Particulate	NA	NA	ND	50

ECL stands for Exposure Control Level and is an internal worker safety parameter developed within BIPI. Information concerning these values is available from the EA&S Department.

REDACTIONS MADE BY APPLICANT

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance:	Solid, white powder
Odor:	Odorless
Melting Point °C	247 - 249
Boiling Point °C	n.d.
Vapor Pressure	n.d.
Solubility in Water:	<0.1 mg/mL; soluble in DMSO
Bulk Density:	n.d.
pH	n.a.

10. STABILITY AND REACTIVITY

REACTIVITY

Stable. No evidence of exothermic decomposition under normal manufacturing and handling conditions.

INCOMPATIBLES

None known

HAZARDOUS DECOMPOSITION PRODUCTS

Toxic, flammable fumes

CONDITIONS TO AVOID

None known

11. TOXICOLOGICAL INFORMATION

RTECS: This compound is not listed in RTECS. Data shown is from selected studies conducted during the course of drug development.

Mutagenicity: Nevirapine was not mutagenic in the Ames/Salmonella assay, with or without metabolic activation.

Carcinogenicity: Nevirapine and its hydroxymethyl metabolite, BI-RJ-106, were not mutagenic in the Ames/Salmonella or bacterial mutation assays, with or without metabolic activation.

Man - Single doses of up to 400 mg tablets were given to HIV infected subjects. Nevirapine was well tolerated. There were no adverse events or clearly drug/dose related events reported. The most frequent non-serious event reported was headache (5/21 patients).

Man - Multiple doses in clinical trials in HIV infected subjects of 400 mg/day Nevirapine have resulted in skin rashes in 30-50 % of the patients.

LD_{50} (rat, p.o.): 400 mg/kg body weight

Primary eye irritation studies using 0.03 gms of Nevirapine in rabbits showed a slight potential for conjunctival irritation under the conditions of the study.

The acute dermal toxicity study in rabbits showed Nevirapine to have a very low potential for local or systemic toxicity under the conditions of the study.
The primary irritation study in rabbits showed no erythema or edema. The primary dermal irritation index was 0.0 (lowest).
Nevirapine was not considered a skin sensitizer under the conditions of the study.

REPORTED AS CARCINOGEN OR POTENTIAL CARCINOGEN

X Not Applicable
__ National Toxicology Program
__ OSHA
__ International Agency for Research on Cancer (IARC)

12. ECOLOGICAL INFORMATION

Material may be disposed of via the sanitary sewer

13. DISPOSAL CONSIDERATIONS

RCRA classification .....Not regulated

Preferred disposal is in an approved hazardous waste incinerator.

14. TRANSPORT INFORMATION

This product is not subject to the regulations for the safe transport of hazardous materials

DOT Proper Shipping Name: n.a.

Hazard Class: not hazardous
Identification Number: n.a.
Packing Group n.a.
Label n.a.
Emergency Response Guidebook - n.d.

15. REGULATORY INFORMATION

REDACTIONS MADE BY APPLICANT

This material is not listed on the US TSCA Inventory. Therefore, it can only be used for TSCA exempt purposes such as R&D or drug use.

16. OTHER INFORMATION

ABBREVIATIONS

n.a. - Not applicable
n.d. - Not determined
n.e. - Not established
n.k. - Not known

Date of Issue.....June 15, 1994

APPENDICES

(Confidential, see list in Table of Contents)

The Appendices of this Environmental Assessment contain confidential trade secrets or information from which these trade secrets can be derived. This material could be beneficial to competitors and, therefore, this information should not be duplicated for distribution. All correspondence regarding this Environmental Assessment should be addressed to the Environmental Affairs and Safety Department at 900 Ridgebury Road, P.O. Box 368, Ridgefield, CT 06877.

REDACTIONS MADE
BY APPLICANT

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

NDA#: 20-636

REVIEWERS	: Dempsey
	: Iacono-Connors
CORRESPONDENCE DATE	: 02/23/96
CDER RECEIPT DATE	: 02/26/96
REVIEW ASSIGN DATE	: 02/27/96
REVIEW COMPLETE DATE	: 06/14/96

SPONSOR: Boehringer Ingelheim Pharmaceuticals, Inc.
900 Ridgebury Road
P.O. Box 368
Ridgefield, CT 06877

SUBMISSION REVIEWED: N-000, Original NDA

DRUG CATEGORY: Antiviral

INDICATION: For use in combination with nucleoside analogues for the treatment of patients with advanced HIV-1 infection who current antiretroviral therapy is no longer deemed adequate.

DOSAGE FORM: Tablets (200 mg)

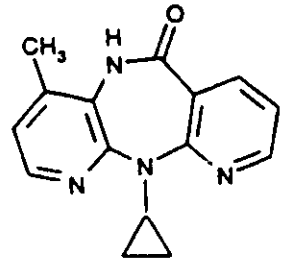
PRODUCT NAMES:

a. **PROPRIETARY:** Viramune

b. **NONPROPRIETARY:** Nevirapine

c. **CHEMICAL:** 11-cyclopropyl-5,11-dyhydro-4-methyl-6H-dipyrido[3,2-b:2'3'-e][1,4]diazepin-6-one.

STRUCTURAL FORMULA:



BACKGROUND

Introduction

Nevirapine is a non-nucleoside dipyridodiazepinone that has been demonstrated to inhibit HIV-1 reverse transcriptase (RT) activity and hence HIV-1 replication. Nevirapine is specific for HIV-1 RT, and does not inhibit other retroviral RTs or human DNA polymerases. The sponsor seeks marketing approval for nevirapine (Viramune), in combination with nucleoside analogs, as a treatment of patients with advanced HIV-1 infection whose current antiretroviral therapy is no longer deemed adequate.

HIV-1 Biology

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

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

Antiviral Therapy Against HIV-1

Clinical treatment of HIV is designed to target certain critical events in the virus infection life-cycle. Therapies under investigation include approaches employing antiviral agents (i.e.; RT inhibitors, viral protease inhibitors (PRIs), and viral integrase inhibitors), virus entry inhibitors (i.e.; neutralizing polyclonal and/monoclonal antibodies), and a broad spectrum of immune-based therapies. Determination of treatment efficacy is assessed primarily by clinical status of the patient and CD4+cell counts, followed by any one of a number of virologic assays aimed at quantifying viral load through the relative measurement

of HIV-infected peripheral blood mononuclear cells (PBMCs) or HIV virions, HIV genomic RNA, HIV proviral DNA, and the HIV antigen p24 in plasma.

Nucleoside analogue inhibitors of HIV-1 RT represent a major class of anti-HIV therapies. Once phosphorylated by cellular enzymes, nucleoside analogues compete for incorporation into viral DNA (as catalyzed by RT) and thus often act as chain terminators for DNA synthesis. One caveat in nucleoside analogue antiviral therapy has been the emergence of drug-resistant HIV strains. The phenomena of nucleoside analogue-resistant HIV development *in vitro* and *in vivo* has been most thoroughly characterized for zidovudine (ZDV). Currently there are two hypotheses which describe the mechanism for the development of resistant HIV under drug pressure. It is thought that a treatment naive HIV(+) individual has a heterogenous population of circulating HIV. Once treatment is initiated, de novo virus-replication cycles are down modulated due, at least in part, to RT inhibition. Over time, genetically altered HIV emerges as a result of low fidelity HIV-RT replication, and/or the selection pressure and passive expansion of agent-resistant HIV present in the heterogenous virus population. The rate at which this occurs clinically is likely to be dependent upon a variety of factors, including the therapeutic agent, dose and schedule, length of therapy, disease stage at treatment initiation, viral load over time, and phenotypic/genotypic HIV characteristics present before and during therapy.

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

SUMMARY

Antiviral Activity of Nevirapine *In Vitro*

The effect of nevirapine on HIV-1 replication has been assessed in several studies utilizing various methodologies. The data from these studies are summarized in Table 1. IC50 values ranged from 10 nM to approximately 375 nM, depending upon the strain of HIV-1 and methodology used. Nevirapine was active against laboratory and clinical isolates of HIV-1, including T cell tropic and monocytotropic laboratory isolates and ZDV-sensitive and resistant clinical isolates. Activity was assessed in T cell lines, human peripheral blood mononuclear cells, and human monocytes and utilized infectious center (or plaque reduction) and p24 antigen production as endpoints.

Table 1. Summary of *In Vitro* Studies on the Anti-HIV activity of Nevirapine

HIV-1 Strain	IC50	Cell Type	Assay Method	Reference
HIV-1 [illegible] HIV-1 [illegible] HIV-1 [illegible]	32.0-42.0 nM	C8166 T Cell Line	[illegible]	1
HIV-1 [illegible]		C8166	[illegible]	2
LAV-1 [illegible]	16nM	CD4-Hela (HT4-6C)		3
LAV-1 [illegible]	39 nM	CEM Cells		3
HIV-1 [illegible]	44 nM	Human Monocytes		3
HIV 1 clinical isolate (A 102b) AZT susceptible	55nM	HT4-6C		3
HIV-1 clinical isolate (A012D) ZDV resistant	95nM	HT4-6C		3
HIV-1 clinical isolate (A1018A) ZDV sensitive)	40nM	HT4-6C		4
HXB2 (derived from infectious molecular clone pHXB2-D)	50-60nM	HT4LacZ-1		5
HIV-1 [illegible]	0.03ug/ml	CEM	[illegible]	6
HIV-1 clinical isolates (pre-nevirapine treatment)	10-75nM	HT4-6C* and PBMC**		7
HIV-1 clinical isolates (2 pre-nevirapine treatment isolates)	10nM	PBMC		8
ClinicalZDV sensitive isolates (G174-6C A036 and H112-6 P026)	35-50 nM	HT4-6C		9
Clinical ZDV resistant isolates G690-1 P026 and G704-2 A036)	65-100 nM	HT4-6C		9
Clinical isolates (strains 3010, 3017, 3028, and 3036)	< 375 nM	PBMC		9

* syncytium inducing isolates

** non-syncytium inducing isolates

Mechanism of Action

Nevirapine is a non-nucleoside, non-competitive inhibitor of the HIV-1 reverse transcriptase activity (1). Both the RNA-dependent and DNA dependent DNA polymerase activities of RT are inhibited by nevirapine. Using four different combinations of template-primers in polymerization assays, however, Tramontano and Cheng (10) demonstrated that inhibition was template dependent (Enclosure 1). In contrast to the polymerase activity, the RNase H activity of HIV-1 RT, which permits the release of viral RNA from the growing DNA strand, is only partially inhibited by nevirapine (IC_{50} = 50nM; I_{max} 54%) (1) (Enclosure 2).

Nevirapine is specific for HIV-1 reverse transcriptase. Activities of human DNA polymerases alpha, beta, gamma or delta are not affected by nevirapine levels up to 24uM (1). In contrast, HIV-RT is inhibited by nevirapine with an IC_{50} of 90 nM; (I_{max} = 100% (Enclosures 2,3). Finally, nevirapine (concentrations up to 200 uM) did not inhibit the simian immunodeficiency virus (SIV), feline immunodeficiency virus (FIV), or human immunodeficiency virus 2 (HIV-2) RT activities.

Inhibition of HIV-1 RT activity by nevirapine is non-competitive with nucleoside triphosphates or template/primer. Steady state kinetic studies (1) utilizing both a homopolymer RNA template and dGTP as substrate, or a heteropolymer RNA template and the four dNTPs demonstrated that V_{max} varies inversely with concentration of nevirapine, but that the K_m remains constant over the tested concentration range. In these studies the K_i for nevirapine was 200-220 nM. Thus, it is postulated that the inhibition is the result of action of nevirapine at a site separate from the nucleoside binding site of the RT molecule.

Additional kinetic studies utilizing from one to all four dNTPs demonstrated that inhibition of RT by nevirapine resulted in a uniform decrease of all lengths of HIV-RT products. A shift in product size from longer to shorter lengths did not occur (11). These data suggest that the activity of nevirapine as an inhibitor of RT was not the result of chain termination or of a change in proscessivity of RT.

Studies employing a photoaffinity analogue of nevirapine (BI-RJ-70) evaluated the binding of nevirapine to HIV-RT. In those experiments, BI-RJ-70 inhibited HIV-1 RT with an IC50 value of 160 nM in the dark. Once activated with uv light, however, BI-RJ-70 bound covalently to HIV-1 RT. Nevirapine was competitive for BI-RJ-70 prior to activation with uv light, but not subsequent to induction of the covalent linkage. The presence of dGTP or a template primer were not competitive for RT binding and subsequent uv inactivation by BI-RJ-70, suggesting that the binding site for BI-RJ-70 (and consequently nevirapine) was separate from the binding site for nucleotides (12).

Several lines of evidence point to the presence of tyrosine residues at the RT binding site for nevirapine. Studies which utilized tryptic and endoproteinase lys C digestion and mapping of the adduct formed by binding of a radiolabelled photoaffinity nevirapine analog BI-RJ-70 to HIV-1RT identified a nevirapine RT binding site at residues 174-199 with the presence of tyrosine residues at positions 181 and 188 (13). Experiments that used chimeric constructs of the HIV-1 RT to evaluate nevirapine binding

demonstrated that both of tyrosine residues are required for the inhibition of RT by nevirapine (14). X-ray crystallography studies showed that nevirapine binds to RT "in a pocket near, but not overlapping with the pol active site" (15). Finally, RT mutations at position 181 are frequent following nevirapine pressure on HIV-1 replication and correlate with high level nevirapine resistant virus.

Antiviral Activity of Nevirapine in Combination With Other Anti-HIV Agents.

The effect of nevirapine, in combination with other antiretroviral drugs, on HIV-1 replication *in vitro* was assessed in human peripheral blood mononuclear cells (PBMCs) using clinically derived HIV-1 isolates (16). The isolates tested were obtained from an individual prior to ZDV therapy and after 26 months of ZDV therapy. Genetic analysis of these isolates indicated that the pre therapy isolate was wild type with respect to RT codons 14, 67, 70, 215, and 219; whereas, the post therapy isolate was resistant to ZDV with mutations at codons 67, 70, 215, and 219. Antiviral activity *in vitro* was assessed by measurement of p24 antigen levels produced in HIV-1 infected, PHA stimulated PBMCs after 4 or 7 days in culture. Combination drug interactions for activity were analyzed by the method of Chou and Talalay (17) and reported as combination index (CI) values. In this system, values <1 indicate synergy; $=1$ indicate additive effects; and >1 indicate antagonism. Nevirapine was synergistic (CI $=0.48$-0.94) in combination with lamivudine (3TC) at ED_{75-95} levels. Interactions at ED_{90} levels were antagonistic. Nevirapine was additive to synergistic (CI $=$ .34-.99) in combination with stavudine (d4T) at all levels tested (ED_{50}-ED_{95}). In combination with ZDV and d4T, nevirapine was also additive to synergistic (CI $=$ .61 -1.02).

Chow, et al. (18) reported that nevirapine in combination with ZDV and ddI was effective in clearing a chronically infected PBMC culture. These results have been contested in the published literature, and some of the results/conclusions have been retracted because one of the recombinant viruses used in this study had an undetected additional mutation that was lethal to the virus. The sponsor has repeated the basic experimental design utilized by Chow et al. (18) to assess combination drug therapy, but utilized HIV-1_{IIIB}, a laboratory strain of HIV-1 that is sensitive to ZDV, ddI and nevirapine. PBMCs were infected with 10^3 $TCID_{50}$ of HIV-1_{IIIB}. After 7 days, the combination of nevirapine (1uM), ZDV (0.3uM) and ddI (10uM) or nevirapine alone (5-25 uM) were added to the cultures, which were maintained for an additional 49 days (Day 56 of culture). Fresh drugs were added to the cultures biweekly with medium changes. After day 56, cultures were maintained for an additional 6 weeks without addition of new drugs. P24 antigen levels in culture supernatants were assayed weekly as a measurement of HIV-1 infection. A DNA PCR analysis was performed on cells removed from culture at day 56 and at the end of the study. The experiment was repeated four times. P24 antigen levels dropped to non-detectable levels in the combination drug cultures between week 5 and 8, and remained undetectable throughout the culture period (enclosure 1). Nevirapine alone was not effective at eliminating p24 production. PCR amplification for HIV-1 was performed on cells from two of the four cultures, and no viral sequences were detected in DNA extracted from cells at week 7 or at week 13 (end of culture). This experiment is limited because no controls which included ZDV and ddI in combination or as single agents were reported. Thus, it cannot be assumed that the apparent elimination HIV-1 from long

term cultures was the result of the triple drug combination (nevirapine, ZDV and ddI). In addition, long term culture of PBMCs is problematic and may select for cells which are not robust and thus not capable of supporting optimal virus production under conditions of any drug pressure. The fact that nevirapine alone did not suppress virus production is not unusual given the rapidity by which resistance occurs in vitro under drug pressure.

These experiments demonstrate that the immunosuppressive potential of nevirapine is no striking at concentration levels which will be utilized clinically. The studies evaluated the effect on both T cell and B cell function *in vitro* and *in vivo*.

Anti-viral Activity of Nevirapine *In Vivo*.

The ability of nevirapine to prevent the development of HIV-infection following exposure was assessed in a chimpanzee model of HIV-1 infection. Four male chimpanzees were infected intravenously with HIV-1 IIIb. One animal received no therapy, one received nevirapine (800 mg) at -6 hours pre-infection and 400 mg/day on days 1-10 post infection; one received 800 mg/day of nevirapine at -36 and -12 hours pre-infection and 800 mg on days 0-20; and one received 800 mg/day at -36, -24 and -6 hours pre-infection and 800 mg on days 0-20. The control animal developed HIV-1 infection as evidenced by the presence of p24 antigen and antibodies to HIV-1 in plasma, infectious virus cultured from PBMCs, and detection of viral RNA and DNA from plasma or PBMCs by branched chain DNA assay or by PCR, respectively. In addition, the induction of virus expression following immunization of the animals with tetanus toxoid was also measured. The control animal was positive for all viral markers tested. For the treated animals, the only evidence of infection was the detection of viral sequences in PBMCs by PCR and intermittent detection of viral RNA in plasma after month 10 post infection. No changes in viral markers were detected in any (control or treated) of the chimpanzees following immune stimulation. These data suggest that prophylaxis with nevirapine may reduce infection rates in the chimpanzee model. Of note is that all of the treated animals received nevirapine prior to infection with HIV-1. It is not known whether post-exposure treatment alone is effective.

Resistance

In vitro antiviral activity against ZDV-sensitive or -resistant clinical isolates.

A plaque reduction assay (20) was performed in HT4-6C (HeLa CD4+) cells to determine nevirapine IC_{50} values for ZDV-resistant and ZDV-sensitive clinical isolates. HT4-6C cells were infected with matched-pair HIV-1 clinical isolates (baseline and ZDV-experienced); A012, P026, A036, and A018. For each matched-pair the ZDV-sensitive strain was isolated prior to ZDV treatment, and the ZDV-resistant strain was isolated post ZDV therapy initiation. The results of this study are presented graphically in enclosure 5. Graph A shows the effect of ZDV on a representative pair of isolates. The ZDV IC_{50}s for the strain A012B (ZDV-sensitive) and the strain A012D (ZDV-resistant) were approximately 0.02 µM and 0.5 µM, respectively revealing a modest 25-fold decrease in ZDV sensitivity. In contrast, the nevirapine IC_{50}s for these paired isolates differed by approximately 0.01 µM with a less than 2-fold decrease in susceptibility. Consistent with this observation, the 3 additional matched pair clinical isolates

measured not more than a 2-fold decrease in susceptibility to nevirapine between the ZDV-sensitive and -resistant strains. Unfortunately, the ZDV susceptibility profiles for matched pair isolates represented in graphs B-D (enclosure 5) were not included in these data. The data demonstrate that for the clinical specimens tested under these assay conditions the ZDV-resistant isolates (1/4 confirmed) retained sensitivity to nevirapine *in vitro*.

In another study Richman et al. (3) determined that a ZDV-resistant HIV-1 isolate, from a patient receiving prolonged ZDV therapy, retained nevirapine-susceptibility *in vitro*. Paired isolates obtained from one patient before and after ZDV therapy were examined. An isolate that had at least a 100-fold decrease in ZDV susceptibility retained baseline susceptibility to nevirapine.

The data provided demonstrate that, for the clinical specimens tested under the assay conditions cited, ZDV-resistant isolates retained sensitivity to nevirapine. Since these antiviral agents have divergent mechanisms of action this result is not surprising. However, the data presented only five ZDV-experienced clinical isolates with no information given regarding genetic changes typically found in the HIV-1 reverse transcriptase gene sequence. In addition, of the five matched-pair clinical isolates only two had ZDV-susceptibility data included. Thus, these data are a limited treatment of the subject. If nevirapine is to be approved for use in combination with or subsequent to therapy with ZDV the sponsor should consider providing all ZDV-susceptibility profiles on the isolates described above to support these data. Finally, the sponsor should consider performing a more thorough analysis of ZDV-resistant HIV isolates for their susceptibility to nevirapine, which would include more unique isolates and possible genetic analysis of the reverse transcriptase gene region.

Development and analysis of nevirapine-resistant HIV-1 variants in vitro.

The rapid emergence of nevirapine-resistant HIV-1 isolates *in vitro* has been well established. This general observation is consistent regardless of whether HIV-1 strains are laboratory derived or low passage ZDV-sensitive clinical isolates. In addition, ZDV-resistant HIV-1 strains, derived by site-directed mutagenesis of an infectious clone, behave similarly when propagated under nevirapine drug pressure *in vitro*; nevirapine resistance emerges rapidly and to a similar degree. The nevirapine resistant phenotype appears to be stable in the absence of drug pressure and, where present, the parental virus' ZDV-resistant phenotype is maintained. The following is a brief description of the pivotal virologic studies which defined the nevirapine-resistant phenotype in HIV-1 when studied *in vitro*.

HIV-1_{BRU} (a laboratory strain) and HIV_{A018A} (a low passage ZDV-sensitive clinical isolate) were passaged in CEM cells in the presence of either 1 μM or 10 μM nevirapine (4). Passaged supernatants were tested for their infectivity and drug-susceptibility phenotype by plaque reduction in HT4-6C cells (20). Nevirapine-resistant HIV-1_{BRU} virus plaques were detected after only 1 passage of 1 μM and 6 passages of 10 μM nevirapine. By passage 20 the variant HIV-1_{BRU} population was approximately 100-fold less susceptible to nevirapine than parent virus (enclosure 6). A nevirapine-resistant strain A018A was detected as early as passage 3 in 1 μM and passage 7 with 10 μM nevirapine. These isolates possessed a 100-fold decrease in nevirapine susceptibility, and by passage 12 the A018A variants were approximately 250-fold less susceptible to nevirapine than the corresponding parent virus (enclosure 7). The passage

12 (10 μM) nevirapine resistant variant of A018A was passaged an additional nineteen times in the absence of nevirapine. The nevirapine-resistant phenotype was not altered in the absence drug pressure. According to Richman et al. (4), the growth rate of the variant virus was identical to that of parent virus.

$HIV1_{HXB41L/215Y}$ and HIV_{RTMC}, both derived by site-directed mutagenesis of the wild type laboratory strain $HIV\text{-}1_{HXB2}$ (21), are highly ZDV-resistant laboratory strains (for references see 5). Virus was passaged in MT2 cells (human T-lymphoblastoid cell line) in the presence of subinhibitory concentrations (1 μM) of nevirapine. By passage 3 both resultant virus populations were resistant to nevirapine, differing in susceptibility by 356-fold (HXB41L/215Y) and 93-fold (RTMC) when compared to parent virus *in vitro* (5) (enclosure 8). Interestingly, both nevirapine resistant-HIVRTMC and -HIV1HX retained their parental phenotype for ZDV-resistance (enclosure 8).

A third study measured nevirapine-resistance development *in vitro* using the laboratory strain HIV_{IIIB} and using a different phenotypic assessment assay; a syncytium formation reduction assay in CEM cells (6). In addition to a monotherapy analysis, nevirapine was combined with either BHAP, TIBO R82913, or TSAO-m^3T to evaluate resistance development to nevirapine when combined with other non-nucleoside reverse transcriptase inhibitors (NNRTI). HIV-1 variants selected with nevirapine monotherapy were again detected as early as passage 1. By passage 4 the virus isolate had a >300-fold decrease in nevirapine susceptibility compared to parent virus (enclosure 9). When parent $HIV\text{-}1_{IIIB}$ virus was passaged with nevirapine and one additional NNRTI at subinhibitory concentrations the following observations were made (6). Variants arising *in vitro* from double-combination (nevirapine included) pressure basically had the same level of nevirapine-resistance as that seen in nevirapine monotherapy selection. Likewise, the time to development of a nevirapine-resistant phenotype was the same as that seen with monotherapy. Therefore, *in vitro*, nevirapine in combination with other NNRTIs afforded no advantage in maintaining a nevirapine-sensitive virus population.

These data demonstrate that it is biologically possible for both laboratory strains and low passage clinical isolates to develop a measurable degree of nevirapine resistance. and that when nevirapine is combined with other NNRTIs a nevirapine-resistant virus population still emerges with similar temporal development and intensity. The clinical ramifications of these data are not predictable at this time.

Genotypic analysis of nevirapine-resistant isolates

Genotypic analysis of the RT genes from representative virus clones from each of the nevirapine-resistant virus populations described above were performed. A prominent nucleotide substitution mutation which should alter the deduced amino acid sequence at position 181 (Tyr to Cys) was observed in nevirapine-resistant $HIV\text{-}1_{BRU}$ and A018A isolates (4). In ZDV-resistant laboratory derived HIV-1 strains, HXB41L/215Y and RTMC, nevirapine-resistance apparently manifested its phenotype through a substitution mutation at amino acid 106 (Val to Ala). Therefore, in ZDV-sensitive background nevirapine induces a mutation at amino acid position 181, and in ZDV-resistant backgrounds nevirapine induces a mutation at amino acid position 106 *in vitro* (5).

Balzarini et al. (6) reported that when HIV-1_{IIIB} was subjected to nevirapine alone RT mutations were detected at amino acid positions 106 (Val to Ala) and/or 181 (Tyr to Cys) (enclosure 10). When nevirapine was combined with either BHAP, TSAO-m3T or TIBO the same two amino acid substitutions were detected at positions 106 (Val to Ala) and 181 (Tyr to Cys). These data demonstrate that a variety of HIV-1 variant populations grown under nevirapine pressure develop amino acid mutations in the RT gene sequence which may or may not contribute to the phenotypic decrease in nevirapine susceptibility.

To address the question of whether a causative relationship exists between these genetic mutations and the phenotypically expressed nevirapine resistance *in vitro* site-directed mutagenesis was used to construct infectious clone HIV-1 variants (4). These cloned virus variants only contain the mutation 181 (Try to Cys) in the RT gene against a wild type HIV-1 genetic background. Nevirapine susceptibility testing was performed on these virus constructs using the plaque reduction assay in acutely infected HT4-6C cells. The single mutation evaluated at position 181 (Tyr to Cys) produced a shift in nevirapine susceptibility; approximately 100-fold (enclosure 11).

In a similar study conducted by Larder (5), infectious-clone derived HIV-1 variants which contained the 181 RT mutation showed a >200-fold decrease in susceptibility to nevirapine in vitro as measured by plaque reduction (enclosure 12).

To further define the mechanism of nevirapine resistance development the mutation at position 181 (Tyr to Cys) was introduced into a recombinant purified RT. Purified, recombinant wild type and mutant RT were tested for enzyme activity and sensitivity to nevirapine (4). Those data demonstrated that although the enzyme activity between the wild type and mutant RT was the same the nevirapine IC_{50} of the mutant RT was 100-fold higher than the wild type recombinant enzyme (enclosure 7). A study by Larder (5) reported corroborating results.

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

Cross-resistance analysis of laboratory-derived nevirapine-resistant HIV-1 isolates.

The potential for HIV-1 cross-resistance between non-nucleoside RT inhibitors has been evaluated in *in vitro* derived nevirapine-resistant laboratory isolates. Several reports have consistently found that laboratory derived nevirapine-resistant HIV-1 isolates are generally highly cross-resistant to other non-nucleoside RT inhibitors in phenotypic analyses performed in vitro (4, 5, 6, 22). Preparation of nevirapine-resistant HIV-1 isolates in vitro and the phenotypic analysis of those isolates as performed by Balzarini et al. (6), Richman et al. (4), and Larder (5) were briefly described and referenced in the above section.

Richman et al. (4) found that nevirapine-resistant HIV-1 (250-fold decrease in nevirapine susceptibility) displayed no cross-resistance to foscarnet or ZDV, however, the susceptibility to several NNRTI's was significantly reduced (enclosure 7). Those studies, although incomplete, provide additional data which suggest that the RT amino acid position 181 mutation (Thr to Cys)

is at least partially responsible for the NNRTI cross-resistant phenotype. Consistent with these data Larder (5) presented data which demonstrated that nevirapine-resistant isolates (100-350 fold decrease in nevirapine susceptibility) retained their original susceptibility to ZDV *in vitro* (enclosure 8).

Recombinant RT enzymes which contained point mutations known to confer nevirapine resistance *in vitro* were cloned into the HIV-1 infectious clone HXB2. HIV-1 variants produced from the infectious clone were tested for drug susceptibility *in vitro*. All variants which contained the Thr to Cys 181 point mutation displayed a significant decrease in nevirapine and CL-TIBO susceptibility (enclosures 12 and 13), but not ZDV or ddI. Other variants which contained the RT amino acid position 41 (Leu to Val) mutation, a genetic marker for ddI resistance *in vitro*, displayed a consistent loss of ddI susceptibility independent of other co-existing mutations (enclosure 12). Larder (5) further defined the mechanism of point mutation driven cross-resistance by preparing and testing enzymatically recombinant RT enzymes *in vitro* which contain RT mutations of interest. Consistently, whenever the Thr to Cys position 181 mutation was present, regardless of whether known ZDV or ddI inhibitory mutations were present, CL-TIBO inhibition on enzyme activity was significantly reduced (enclosure 14).

HIV-1_{IIIB} mutant strains were selected for nevirapine-resistance in vitro (6). Phenotypic analyses demonstrated that those strains were highly cross-resistant to all other HIV-1 specific NNRTIs tested (enclosure 9 and 15).

Genotypic analysis of the variants and the correlative or causative relationships between those RT point mutations and the degree of phenotypic resistance to a given alternate antiretroviral appear to be somewhat complex. Additional studies are needed in order to unequivocally define the impact of one or more RT mutations on drug susceptibility. The only consistent findings amongst these studies are that single agent selection using nevirapine and a HIV-1 wild type isolate will quickly produce a nevirapine-resistant variant, the variant will possess a significant degree of cross-resistance to most other NNRTI's, it will also contain a RT amino acid position 181 point mutation (Thr to Cys), and the Thr to Cys 181 point mutation will have no immediate impact on variant susceptibility to ZDV or ddI. These conclusions are further supported by data reported by Byrnes et al. (23).

No comprehensive studies have been reported which systematically evaluate the laboratory-derived nevirapine-resistant HIV-1 isolate's degree of phenotypic susceptibility to nucleoside analogue RT inhibitors or HIV Protease Inhibitors.

Phenotypic and genotypic analysis of HIV isolates from patients during therapy with nevirapine.

Phase I and II clinical trials with nevirapine have confirmed *in vivo* the rapid selection for resistant virus that has been seen in cell culture (7, 8, 24, 25). Phenotypic and genotypic changes in HIV-1 isolates from patients treated with either nevirapine (n=24) or nevirapine and zidovudine (ZDV) (n=14) were monitored in phase I/II trials over a period of 1 to ≥12 weeks (7). HIV isolates from 3/3 patients showed a decrease in susceptibility to nevirapine *in vitro* after 1 week of therapy (enclosure 16). RT mutations at amino acid positions 103, 106, 108, 181, 188, and 190 were detected as early as 2 weeks after initiation of nevirapine monotherapy (enclosure 16). By week eight 100% of the patients tested had HIV isolates with a >100-fold

decrease in susceptibility to nevirapine *in vitro* compared to baseline, and had one or more of the nevirapine-associated RT resistance mutations (enclosure 16). Nineteen of 24 (80%) patients receiving nevirapine monotherapy had viral isolates with a position 181 mutation regardless of the nevirapine dose (enclosure 17). These findings are consistent with that reported by Havlir et al. (24) (enclosure 18).

Nevirapine/ZDV combination therapy did not alter the emergence rate of viral isolates with reduced susceptibility to nevirapine nor the magnitude of nevirapine resistance *in vitro* (7). However, a different RT mutation pattern was observed. The predominant mutations were distributed amongst amino acid positions 103, 106, 188, and 190. Conversely, in those patients (6/14) whose baseline isolates possessed a wild type RT gene, nevirapine/ZDV combination therapy did not appear to delay the emergence of ZDV-resistance RT mutations.

A pilot study (ACTG 208), conducted by Havlir et al. (25), was designed to evaluate the development of HIV-1 isolates which possessed phenotypic resistance to nevirapine in asymptomatic HIV-infected patients with >500/mm^3 CD4 cell counts. The results suggested that lower levels of HIV RNA and of immunosuppression did not retard the rate of emergence nor the magnitude of nevirapine resistant virus (enclosure 19).

Finally, de Jong et al. (8) attempted to evaluate whether alternating nevirapine and ZDV therapy in the treatment of HIV-1 infected adults would prolong nevirapine antiviral activity (more accurately, prolong the time to development of nevirapine resistance). Ten antiretroviral naive persons were treated for 9-13 weeks with an alternating regimen of 1 week of nevirapine and 3 weeks of ZDV. HIV-1 isolates from 2 patients after 8 weeks of therapy were 40- and 1000-fold less susceptible to nevirapine in vitro as compared to baseline measurements. The authors suggest that, based on these data, the treatment regimen will not delay nevirapine resistance emergence nor its magnitude. Definitive results were obtained from 2/10 participants. These findings while interesting are not significant.

Nevirapine-resistant HIV-1 appears to develop rapidly and in all subjects tested following nevirapine therapy (both monotherapy and in combination with ZDV). However, preliminary resistance data from trial BI1046 participants suggests that nevirapine in triple therapy combination with ZDV and ddI may effect the evolution of nevirapine-resistant clinical isolates. Sixteen trial participant's HIV isolates, nevirapine/ZDV/ddI (n=4), nevirapine/ZDV (n=4), and ZDV/ddI (n=8), were evaluated at baseline and at 6 months for phenotypic susceptibility to nevirapine *in vitro;* employing a modified ACTG methodology. Those modifications primarily consist of the use of cord blood mononuclear cells instead of PBMCs as the source of donor cells, and using a measurement of RT rather than p24 as an indicator of HIV growth (Boehringer Ingelheim document submitted to DAVDP 21 May 1996) Both the control arm and the double combination arm revealed fully nevirapine resistant clinical isolates at the 6 month measurement. However, only 2/4 patients who had received nevirapine/ZDV/ddI arm had isolates with high level resistance to nevirapine (enclosure 20), and 2/4 patients had isolates which were fully sensitive to nevirapine with no detectable nevirapine-resistant isolates. The mechanism by which nevirapine susceptibility was maintained, in the 2 patients, is not clear. The clinical significance of this observation is not known.

Immunosuppressive Potential of Nevirapine

The effect of nevirapine (BIRG 0587) on immunologic function was assessed *in vitro* by the mixed lymphocyte reaction (MLR) and the development of IgM antibody response in response to antigen stimulus of cells in culture. *In vivo*, immunologic effects were measured by delayed type hypersensitivity assays and antibody assays following immunization of animals. In general, no substantial immunosuppressive activity was observed in any of the test systems, except at the highest concentrations of nevirapine tested which were substantially greater than (100X) antiviral concentrations.

The addition of nevirapine to human peripheral blood mononuclear cells did not suppress the MLR response at concentrations up to 37.5 uM. At 187uM (the next higher concentration tested) MLR responses were suppressed by 70%. IgM plaque forming cell (PFC) responses of murine spleen cells immunized *in vitro* with sheep red blood cells were not suppressed by nevirapine at concentrations of 10 uM or less. In fact lower concentrations enhanced PFC responses. At concentrations of 100 uM, however, PFC responses were depressed by 50%.

In vivo, none of the concentrations of nevirapine (1-100 mg/kg/day p.o.) administered to Lewis rats suppressed the induction of DTH responses to methylated bovine serum albumin. Similarly, treatment of mice with nevirapine (10-50 mg/kg/day orally) had no appreciable effect on the induction of IgM antibody responses following *in vivo* immunization with sheep erythrocytes. As observed in the in vitro immunization study, low concentrations of nevirapine (1 mg/kg) slightly enhanced antibody production (as measured by PFCs/spleen) and higher concentrations (100 mg/kg) suppressed PFC responses

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Microbiology Labeling Proposed by the Sponsor.

CLINICAL PHARMACOLOGY: ***Mechanism of Action***: VIRAMUNE® is a potent, highly selective, non-nucleoside inhibitor of HIV-1 reverse transcriptase (RT). The enzymatic activity of RT is required for replication of HIV-1. Nevirapine binds directly to RT and blocks the RNA-dependent and DNA-dependent DNA polymerase activities by causing a disruption of the enzyme's catalytic site. The inhibitory activity of nevirapine is not competitive with respect to template or nucleoside triphosphates. Reverse transcriptase from HIV-2 and eukaryotic DNA polymerases (such as human DNA polymerases α, ß, γ, or δ) are not inhibited by nevirapine.

Microbiology: Antiviral Activity in vitro: The relationship between *in vitro* susceptibility of HIV-1 to nevirapine and the inhibition of HIV-1 replication in humans has not been established. The antiviral activity of nevirapine was measured in peripheral blood mononuclear cells and in a variety of cell lines where 50% inhibitory concentration (IC_{50}) values ranged from 10-100 nM. Potent antiviral activity was observed when nevirapine was tested against both laboratory strains of HIV-1 and against fresh clinical isolates. Furthermore, nevirapine was equally effective in inhibiting the replication of both zidovudine-sensitive and zidovudine-resistant strains of HIV-1.

Resistance: Resistance to nevirapine has been studied by site-directed mutagenesis experiments, cell culture selection and by *in vivo* clinical studies. Mutations of the RT gene which confer reduced susceptibility of the enzyme to nevirapine are all localized to the non-nucleoside binding pocket. Specifically, changes of RT amino acid residues at positions 103, 106, 108, 181, 188, and 190 have been shown to engender resistance to nevirapine in the recombinant enzymes and viruses which carry these mutations. Treatment of HIV-1 positive individuals with nevirapine resulted in the appearance of HIV-1 variants with decreased susceptibility to nevirapine within 1 to 8 weeks of initiation of therapy. Clinical mutations were observed singly or in combinations and have been demonstrated to confer from 10- to 1000-fold reduction in susceptibility to nevirapine. All these amino acids are located within the nevirapine binding site and are distinct from mutations which confer resistance to nucleoside inhibitors. Consequently, nevirapine-resistant HIV-1 variants remain fully susceptible to nucleoside RT inhibitors. Nevirapine resistant variants are also susceptible to protease inhibitors which target viral protease, and not RT. Conversely, nevirapine is effective against mutant viruses resistant to nucleoside analogues. Currently, monitoring of viral resistance generally is not considered useful in the management of individual patients.

Combination Therapy: A variety of studies using biochemical, crystallographic, and mutational approaches have documented that the mechanism of action of nevirapine is unique

from nucleoside RT inhibitors and protease inhibitors. Results from laboratory experiments using nevirapine in multiple two- or three- drug combination regimens, showed nevirapine acted synergistically, or at least additively to suppress viral replication with a variety of other anti-HIV-1 drugs, including zidovudine (ZDV), didanosine (ddI), stavudine (d4T), lamivudine (3TC), and saquinavir. In clinical trial ACTG 241, a statistically significant improvement of VIRAMUNE®+ZDV+ddI over ZDV+ddI was demonstrated in both virologic and immunologic response throughout the 48 week trial. (See ***Description of Clinical Studies***)

FDA Proposed Microbiology Labeling

MICROBIOLOGY:

Mechanism of Action: VIRAMUNE® is a non-nucleoside inhibitor of HIV-1 reverse transcriptase (RT). Nevirapine binds directly to RT and blocks the RNA-dependent and DNA-dependent DNA polymerase activities by causing a disruption of the enzyme's catalytic site. The inhibitory activity of nevirapine is not competitive with respect to template or nucleoside triphosphates. Reverse transcriptase from HIV-2 and eukaryotic DNA polymerases (such as human DNA polymerases α, ß, γ, or δ) are not inhibited by nevirapine.

In Vitro HIV Susceptibility: The relationship between *in vitro* susceptibility of HIV-1 to nevirapine and the inhibition of HIV-1 replication in humans has not been established. The in vitro antiviral activity of nevirapine was measured in peripheral blood mononuclear cells, monocyte derived macrophages, and lymphoblastoid cell lines. IC50 values (50% inhibitory concentration) ranged from 10-100 nM against laboratory and clinical isolates of HIV-1. In cell culture, nevirapine demonstrated additive to synergistic activity against HIV in drug combination regimens with zidovudine (ZDV), didanosine (ddI), stavudine (d4T), lamivudine (3TC) and saquinavir.

Resistance:

HIV-1 isolates with reduced susceptibility (100-250 fold) to nevirapine emerge in vitro. Genotypic analysis of these isolates showed mutations in the HIV reverse transcriptase (RT) gene at amino acid positions 181 and/or 106 depending upon the virus strain and cell line employed. Time to emergence of nevirapine-resistant isolates in vitro was not altered when selection included nevirapine in combination with several other non-nucleoside RT inhibitors.

Phenotypic and genotypic changes in HIV-1 isolates from patients treated with either nevirapine (n=24) or nevirapine and zidovudine (ZDV) (n=14) were monitored in phase I/II trials over a period of 1 to ≥12 weeks. HIV isolates from 3/3 patients receiving nevirapine monotherapy showed a decrease in susceptibility to nevirapine in vitro after 1 week of therapy. RT mutations at amino acid positions 103, 106, 108, 181, 188 and 190 were detected in some patients as early as 2 weeks after initiation of nevirapine monotherapy. By week eight, 100% of the patients tested (n=24) had HIV isolates with a >100 fold decrease in susceptibility to nevirapine in vitro compared to baseline, and had one or more of the nevirapine-associated RT resistance mutations. Nineteen of 24 (80%) patients receiving nevirapine monotherapy had viral isolates

with a position 181 mutation regardless of the nevirapine dose. Nevirapine/ZDV combination therapy did not alter the emergence rate of viral isolates with reduced susceptibility to nevirapine nor the magnitude of nevirapine resistance in vitro, however, a different RT mutation pattern was observed. The predominant mutations were distributed amongst amino acid positions 103, 106, 188, and 190. Conversely, in those patients (6/14) whose baseline isolates possessed a wild type RT gene, nevirapine/ZDV combination therapy did not appear to delay the emergence of ZDV-resistance RT mutations. The clinical relevance of phenotypic and genotypic changes associated with nevirapine therapy has not been established.

Cross-resistance: Rapid emergence of HIV strains that are cross-resistant to non-nucleoside RT inhibitors has been observed in vitro. Data on cross-resistance between the non-nucleoside RT inhibitor nevirapine and nucleoside analogue RT inhibitors are very limited. Four clinically derived ZDV-resistant HIV isolates tested in vitro retained susceptibility to nevirapine, and six clinically derived nevirapine-resistant HIV isolates were susceptible to ZDV and ddI. Cross-resistance between nevirapine and HIV protease inhibitors is unlikely because the enzyme targets involved are different.

CONCLUSIONS.

1. With respect to microbiology, VIRAMUNE is approvable pending final review of the label.

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

3. Drug interaction studies in vitro have demonstrated additive to synergistic anti-HIV-1 activity.

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

5. Rapid emergence of HIV strains that are cross resistant to non-nucleoside RT inhibitors has been observed in vitro. Data on cross-resistance between the non-nucleoside RT inhibitor nevirapine and nucleoside analogue RT inhibitors are limited. No data are available on cross-resistance profiles between nevirapine and protease inhibitors.

6. Rapid emergence of nevirapine-resistant HIV-1 has been observed following monotherapy and combination therapy with ZDV in phase I/II clinical trials.

7. Preliminary data from four patients who had received concomitant therapy with ZDV, ddI and nevirapine revealed that two individuals possessed HIV-1 isolates that were fully nevirapine resistant while the isolates from the other two individuals remained sensitive to nevirapine after six months on therapy. The clinical signficance of this observation is not known.

RECOMMENDATIONS:

1, With respect to microbiology, NDA 20-636 (VIRAMUNE) is approved pending final review of the label.

2. The sponsor may wish to continue monitoring of patients who are enrolled in ongoing clinical trials for development of resistance to nervirapine. In particular, the development of resistance should be evaluated in those patients who are receiving combination therapy.

Nalla L Dumpsey
Microbiologist

Microbiologist

CONCURRENCES:

HFD-530/Deputy Dir. _____ **Signature** _____ **Date**
HFD-530/SMicro _____ **Signature** 6/17/96 **Date**
cc:
HFD-530/Original IND
HFD-530/Division File
HFD-530/Div Dir Reading file
HFD-530/Pre-Clin Dep
HFD-530/MO
HFD-530/Pharm
HFD-530/Chem
HFD-530/SMicro
HFD-530/Review Micro
HFD-530/CSO Zecolla

Tramontano, E. and Cheng, Y. Biochem. Pharmacol. 1992: 43: 1371-1376.

Table 1. Template-primer dependence of HIV-1 RT inhibitors

Template-primer	Units used	Relative rate	BI-RG-587	R8215/	PFA	HEPT
			IC_{50} (μM)			
RNA-dependent DNA polymerase associated activity						
Poly(rC)-oligo$(dG)_{12-18}$	4×10^{-3}	30	0.082 ± 0.01	0.07[illegible] ± 0.007	3.9 ± 0.8	23 ± 6
Poly(rA)-oligo$(dT)_{10}$	1.6×10^{-3}	100	0.650 ± 0.02	0.5[illegible]0 ± 0.035	0.2 ± 0.01	49 ± 2
Poly(rU)-oligo$(dA)_{12-18}$	0.16	0.4	0.185 ± 0.02	0.[illegible]80 ± 0.01	170 ± 20	56 ± 10
Poly(rG)-oligo$(dC)_{12-18}$	0.32	0.1	0.125 ± 0.03	0.130 ± 0.02	260 ± 50[a]	33 ± 10[a]
DNA-dependent DNA polymerase associated activity						
Poly(dC)-oligo$(dG)_{12-18}$	4×10^{-3}	68	0.240 ± 0.005	0.265 ± 0.01	1.6 ± 0.08	53 ± 10
Poly(dA)-oligo$(dT)_{12-18}$	0.32	0.5	0.090 ± 0.005	0.090 ± 0.007	5.0 ± 0.6	37 ± 5

A unit was defined as the amount of enzyme necessary to incorporate 1 nmol of [^{3}H]dTMP into the poly(rA)-oligo$(dT)_{10}$ template in 1.0 min at 37° [14]. All the reaction rates were normalized to the reaction rate obtained using poly(rA)-oligo$(dT)_{10}$. The calculations of IC_{50} (concentration inhibiting 50% of control) and standard deviations were done from at least three determinations, with the exception of two indicated cases (a) in which only two determinations were performed and the average values ± range are presented. The reaction mixture used is described in Methods with the exception of dTTP (final concentration 30 μM), dATP (final concentration 100 μM) and poly(rU)-oligo$(dA)_{12-18}$ (final concentration 0.085 OD_{260} units/mL).

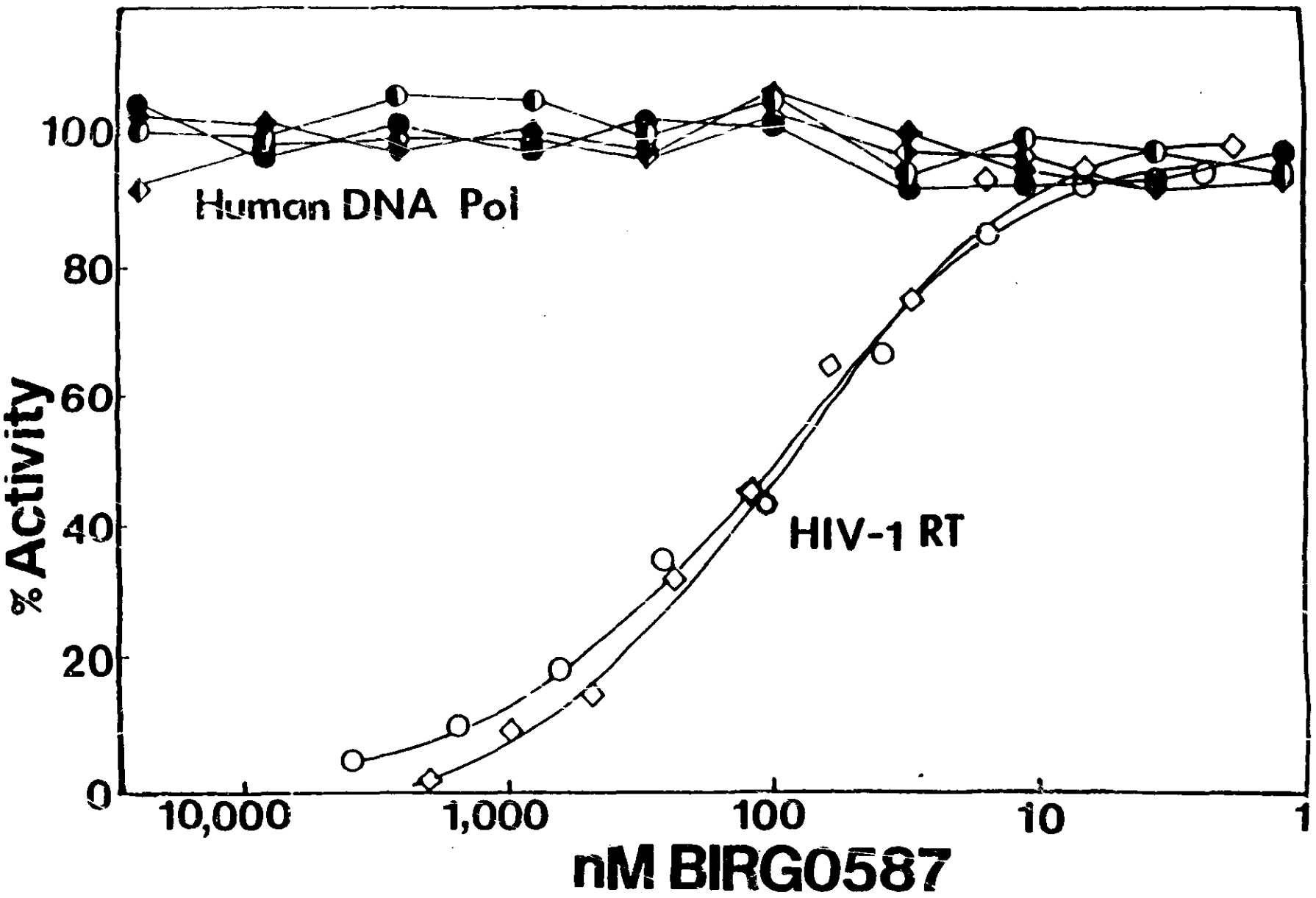
Enclosure 1

Merluzzi, V.J. et al. Science 1990: 250: 1411-1413

Enzyme	Enzyme specificity of BI-RG-587	
	Concentration (μM)	IC_{50} (nM)
HIV-1 RT polymerase	160*	84 ± 4
HIV-1 RT RNase H	160†	50 ± 6
Simian RT	>>185‡	
Feline RT	>>185‡	
Human DNA polymerase α	>> 24‡	
Human DNA polymerase β	>> 24‡	
Human DNA polymerase γ	>> 24‡	
Human DNA polymerase δ	>> 24‡	
Calf thymus DNA polymerase α	>>185‡	

*Maximal achievable inhibition by BI-RG-587 at 160 μM (I_{max}) = 100%. †I_{max} = 54%. ‡Insignificant inhibition at these concentrations.

Enclosure 2


100
80
60
40
20
0
% Activity
Human DNA Pol
HIV-1 RT
10,000
1,000
100
10
1
nM BIRG0587

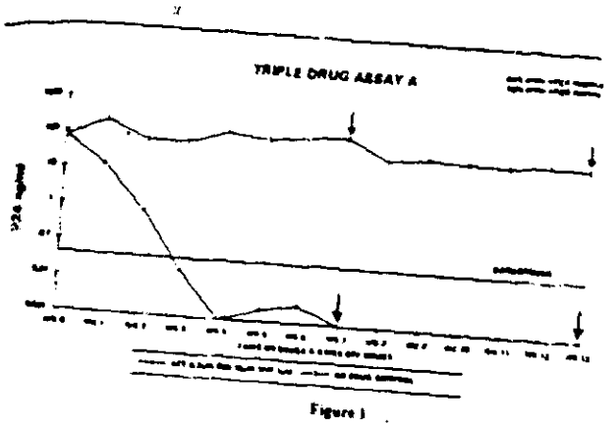


Figure 1

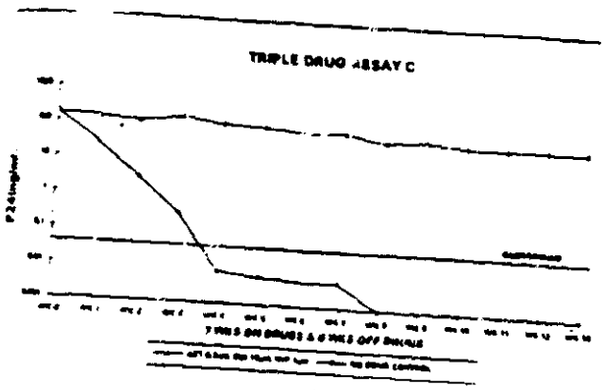


Figure 3

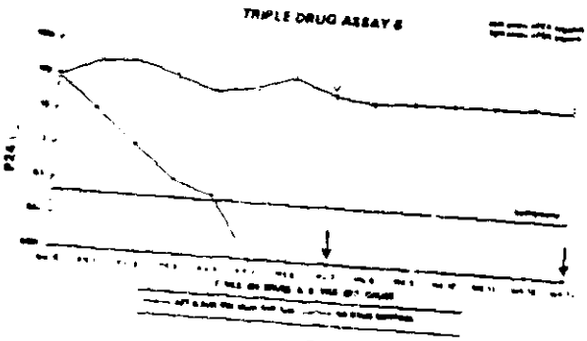


Figure 2

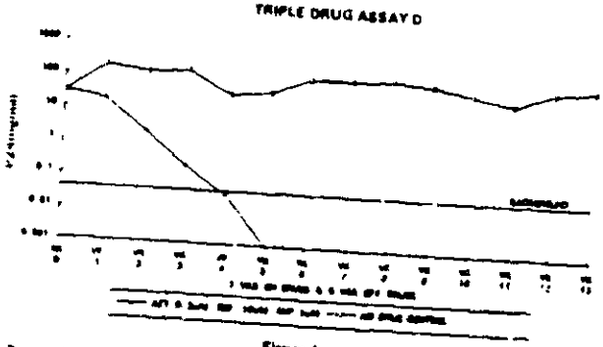


Figure 4

Figures 1-4: P24 values of HIV-1 (IIIB) infected PBMC cultures maintained for 13 weeks with and without drugs are shown. ZDV, DDI, NVP were added at the indicated concentrations to the cultures 7 days post infection while viral replication was at peak levels (week 0). Drugs were present for 7 weeks. At week 7, drugs were withdrawn and cultures were continued for six more weeks. P24 values ≤ 0.01 ng/ml are considered negative (background).

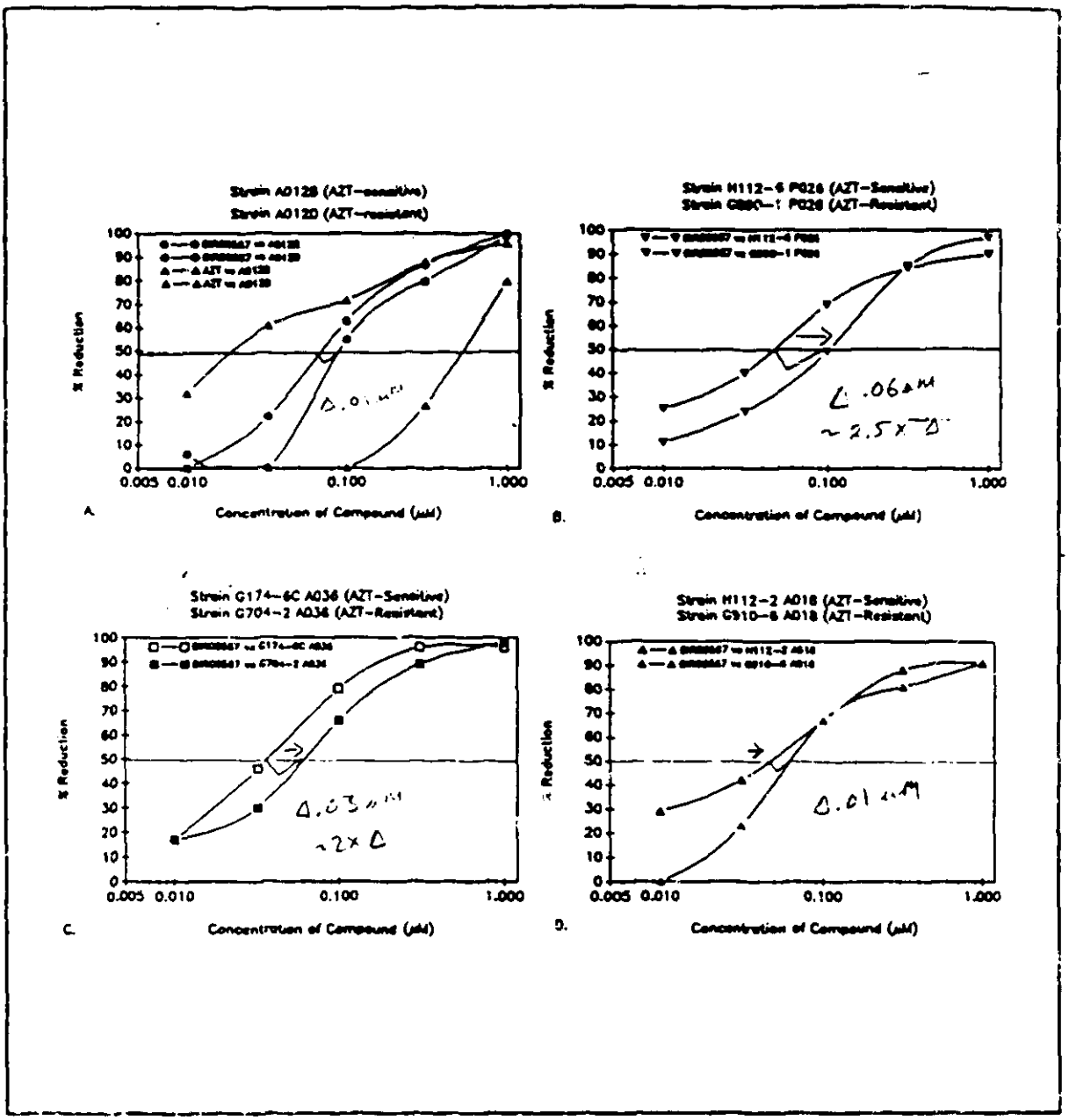


Figure Legend 5. BIRG0587 was tested in a plaque reduction assay versus AZT-sensitive and resistant strains of HIV-1 from four pairs of patient isolates (A012, PO26, A036, A018). In each pair the AZT-sensitive strain was isolated prior to the patient's treatment with AZT, and the resistant strain was isolated subsequent to AZT treatment. Graph A. shows the effect of AZT on a representative pair of isolates. BIRG0587 effectively reduces plaque formation for both AZT-sensitive and AZT-resistant isolates.

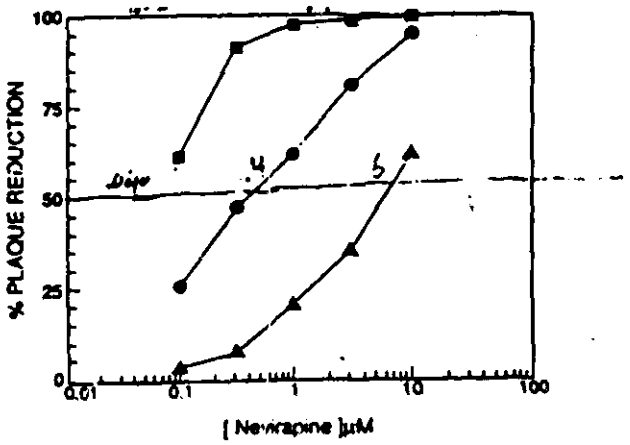


FIG. 1. Susceptibility of HIV-1$_{RTU}$ passaged in the presence of 1 µM nevirapine. ■, Passage 0; ●, passage 12; ▲, passage 20.

4. Richman, D. et al. PNAS 1991: 88: 11241-11245.

Enclosure 6

Table 1. Susceptibility of nevirapine-resistant variant to other RT inhibitors

	IC_{50}, μM					
	Nevirapine	AZT-TP	Foscarnet	TIBO	E-EPU	E-BPU-S
Virus						
Passage 0	0.04	0.01	30	0.05	0.02	0.003
Passage 12	10.0	0.01	30	0.2	1.0	1.0
Recombinant enzyme						
Wild type	0.06 ± 0.01	0.04 ± 0.02	18.6 ± 11.1	0.13 ± 0.03	ND	ND
Y181C mutant*	4.83 ± 0.71	0.02 ± 0.01	17.2 ± 9.3	1.62 ± 0.08	ND	ND

The 1 μM nevirapine passage 12 supernatant of strain A018A and the parental virus were used. Polymerase assays were performed in triplicate at each drug concentration, and averages of triplicate data were used for analysis. Results from two independent experiments were analyzed to derive IC_{50} ± SE. ND, not done; E-EPU, 1-ethoxymethyl-5-ethyl-6-(phenylthio)uracil; E-BPU-S, 1-benzyloxymethyl-5-ethyl-6-phenylthio-2-thiouracil.
*Tyr-181 to Cys-181 mutation.

4. Richman, D. et al. PNAS 1991: 88: 11241-11245.

TABLE 2. Passage of HIV-1 strains in BI-RG-587[a]

Passage no.	IC_{50} (μM)			
	HXB41L/215Y		HIVRTMC	
	BI-RG-587	AZT	BI-RG-587	AZT
0	0.05	0.7	0.06	1.5
3	17.8 (356x)	1.1 Ø	5.6 (93x)	1.4 Ø

[a] Virus was passaged three times in 1 μM BI-RG-587. Virus recovered in MT-2 cell culture supernatants after passage 3 was assessed for susceptibility to BI-RG-587 and AZT by plaque reduction assay in HT4LacZ-1 cell monolayers. Both virus strains were initially AZT resistant and were derived from infectious molecular clones.

5. Larder, B.A. Antimicrob. Agents Chemother. 1992: 36:2664-2669.

TABLE 1. EC_{50}s of the test compounds for HIV-1(III_B) isolated in the presence of the HIV-1-specific inhibitors TSAO-m^3T, nevirapine, and TIBO R82913 in CEM cells[a]

Compound	No drug[b]	EC_{50} (μg/ml) in the presence of increasing concs of: TSAO-m^3T				Nevirapine				TIBO R82913		
Passage #		1[c]	2	3	4	1	2	3	4	1	2	3
TSAO-T	0.03	2	5	2	5	1	2	3	3	0.11	0.12	0.23
TSAO-m^3T	0.03	>50	>50	>50	>50	50	>50	50	>50	0.04	0.03	0.15
TSAO-e^3T	0.03	>50	>50	>50	>50	≥50	>50	≥50	>50	0.06	0.07	0.23
Nevirapine	0.03	0.015	0.003	0.005	0.01	2.6	5	≥5	8.3	0.05	0.08	1.5
Pyridinone	0.007	0.10	0.02	0.05	0.05	2	2	2	3	0.09	0.04	0.5
TIBO R82913	0.02	0.04			0.04	0.4			0.6	0.12	0.20	≥5
BHAP	0.03	0.02			0.03	0.85			8.0	0.02		2

Selection drug

.02 EC50

[a] Virus was isolated after full breakthrough of cytopathicity in CEM cell cultures in the presence of TSAO-m^3T at 0.1 μg/ml (passage 1), 0.5 μg/ml (passage 2), 2.5 μg/ml (passage 3), and 10 μg/ml (passage 4); in the presence of nevirapine at 0.04 μg/ml (passage 1), 0.2 μg/ml (passage 2), 2.5 μg/ml (passage 3), and 10 μg/ml (passage 4); and in the presence of TIBO R82913 at 0.01 μg/ml (passage 1), 0.25 μg/ml (passage 2), and 1.0 μg/ml (passage 3).

[b] Values for wild-type HIV-1(III_B) that had not been exposed to any test compound before evaluation for susceptibility to the different HIV-1-specific inhibitors.

[c] Passage number of HIV-1(III_B) cells.

6. Balzarini, J. et al. J. Virol. 1993: 67: 5353-5359.

TABLE 4. Amino acid changes in HIV-1 mutant strains selected for resistance against the HIV-1-specific inhibitors nevirapine, TIBO R82913, and TSAO-m^{5}T used either as single agents or in combination

Amino acid position	HIV-1(IIIB)	Amino acid in: HIV-1/Nev (expt 1)		HIV-1/TIBO			HIV-1/TSAO-m^{5}T + Nev				HIV-1/TSAO-m^{5}T + TIBO			HIV-1/Nev + TIBO		
		1[a]	4	1	2	3	1	2	3	4	1	2	3	1	2	3
100	Leu (TTA)	Leu	Leu	Leu	Leu	Leu	Leu	Leu	Leu	Leu	Leu	Leu	Leu	Leu	Leu	Leu
103	Lys (AAA)	Lys	Lys	Lys	Asn[b] (AAC)	Asn (AAC)	Lys	Lys	Lys	Lys	Lys	Lys	Lys	Lys	Lys	Lys
106	Val (GTA)	Ala[c] (GCA)	Ala[c] (GCA)	Val	Val	Val	Val	Val	Val	Ala (GCA)	Val	Val	Val	Val	Ala (GCA)	Ala (GCA)
138	Glu (GAG)	Glu	Glu	Glu	Glu	Glu	Glu	Glu	Glu	Glu	Glu	Glu	Glu	Glu	Glu	Glu
181	Tyr (TAT)	Cys[c] (TGT)	Cys[c] (TGT)	Tyr	Tyr	Tyr	Cys (TGT)	Cys (TGT)	Cys (TGT)	Cys (TGT)	Cys (TGT)	Cys (TGT)	Cys (TGT)	Cys[d] (TGT)	Cys (TGT)	Cys (TGT)

[a] Passage number.
[b] Partially wild type (Lys-103) and mutant (Asn-103), with predominance of wild type.
[c] Mixture of Cys-181 (TGT) and Ala-106 (GCA).
[d] Partially wild type (Tyr-181) and mutant (Cys-181).

TABLE 5. Amino acid changes in HIV-1 mutant strains selected for resistance against the HIV-1-specific inhibitors TSAO-m^{5}T, nevirapine, and BHAP used either as single agents or in combination

Amino acid position	HIV-1(IIIB)	Amino acid in: HIV-1/TSAO-m^{5}T		HIV-1/Nev (expt 2)			HIV-1/BHAP			HIV-1/TSAO-m^{5}T + BHAP			HIV-1/Nev + BHAP		
		1[a]	4	1	2	3	1	2	3	1	2	3	1	2	3
100	Leu (TTA)	Leu	Leu	Leu	Leu	Leu	Ile[b] (ATA)	Ile (ATA)	Ile (ATA)	Leu	Leu	Leu	Leu	Leu	Leu
103	Lys (AAA)	Lys	Lys	Lys	Lys	Lys	Lys	Lys	Lys	Lys	Lys	Lys	Lys	Lys	Lys
106	Val (GTA)	Val	Val	Ala[c] (GCA)	Ala (GCA)	Ala (GCA)	Val	Val	Val	Val	Val	Val	Val	Val	Ala[c] (GCA)
138	Glu (GAG)	Lys (AAG)	Lys (AAG)	Glu	Glu	Glu	Glu	Glu	Glu	Glu	Glu	Glu	Glu	Glu	Glu
181	Tyr (TAT)	Tyr	Tyr	Tyr	Tyr	Tyr	Tyr	Tyr	Tyr	Cys (TGT)	Cys (TGT)	Cys (TGT)	Cys (TGT)	Cys (TGT)	Cys (TGT)

[a] Passage number.
[b] Partially Ile-100 (ATA) and Met-100 (ATG).
[c] Partially wild-type Val-106 and mutant Ala-106.

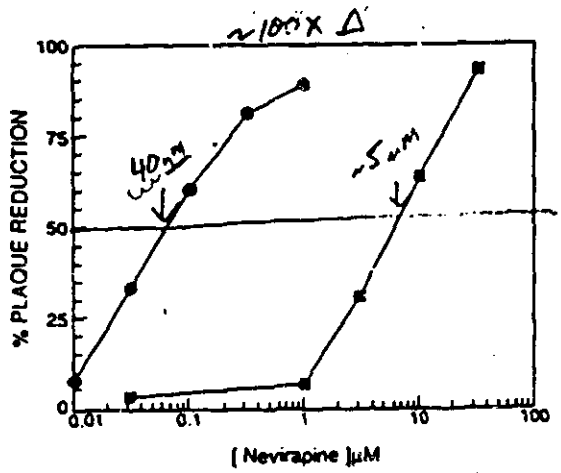


FIG. 3. Susceptibility to nevirapine of HIV reconstructed to contain the Cys-181 mutation in RT. Virus was prepared by transfecting Jurkat cells with mutant or wild-type proviral DNA. Nevirapine susceptibility of the progeny virus was then assayed by plaque reduction in CD4 HeLa (HT4-6C) cells. ●, Wild type; ■, Cys-181 mutant.

Richman, D. et al. PNAS 1991: 88: 11241-11245.

TABLE 4. Susceptibilities of viruses with L74 to V and Y181 to C mutations to nucleoside and nonnucleoside inhibitors

Virus	RT genotype			IC_{50} (μM)[a]			
	Leu-74	Tyr-181	Thr-215	AZT	ddI	Cl-TIBO	BI-RG-587
HXB2				0.01	1.4	0.11	0.06
HXB2(74V)	Val			0.02	11.2	0.16	0.05
HXB2(181C)		Cys		0.01	1.3	4.5	12.6
HXB2(74V/181C)	Val	Cys		0.02	8.9	4.0	4.0
HIVRTMF(74V)	Val		Tyr	0.02	10.0	0.1	0.05
HIVRTMF(74V/181C)	Val	Cys	Tyr	0.02	10.0	2.5	3.2

[a] Inhibitor susceptibility was determined by a plaque reduction assay in HT4LacZ-1 cells.

Larder, B.A. Antimicrob. Agents Chemother. 1992: 36:2664-2669.

TABLE 5. Susceptibility of HIV-1 containing AZT resistance mutations and the Y181 to C mutation to nucleoside and nonnucleoside inhibitors

Virus	RT genotype						IC_{50} (μM)[a]			
	Met-41	Asp-67	Lys-70	Thr-215	Lys-219	Tyr-181	AZT	ddI	Cl-TIBO	BI-RG-587
HXB2							0.01	1.4	0.11	0.06
HXB2(181C)						Cys	0.01	1.3	4.5	12.6
HXB2(41L/215Y)	Leu			Tyr			0.7	1.6	0.11	0.05
HXB2(41L/181C/215Y)	Leu			Tyr		Cys	0.02	0.6	2.8	10.0
RTMC		Asn	Arg	Phe	Gln		1.5	2.0	0.14	0.05
RTMC(181C)		Asn	Arg	Phe	Gln	Cys	0.05	1.4	2.5	5.6

[a] Inhibitor susceptibility was determined by plaque reduction assay in HT4LacZ-1 cells.

Larder, B.A. Antimicrob. Agents Chemother. 1992: 36:2664-2669

TABLE 3. Inhibition of mutant RT activities by Cl-TIBO[a]

HIV-1 RT genotype	RT activity (% of wild type)	Cl-TIBO inhibition (IC_{50} [μM])
Wild type	100	0.4
Y181C	88	10
L74V	79	0.7
L74V Y181C	100	9
L74V T215Y	52	0.4
L74V T215Y Y181C	100	7
M41L T215Y Y181C	54	ND
D67N K70R T215F K219Q	50	0.7
D67N K70R T215F K219Q Y181C	53	7

[a] RT activity in *E. coli* lysates infected with M13 clones was determined by using poly(rA) · oligo(dT) as the primer-template and [^{3}H]TTP as the substrate. Inhibition of RT activity by Cl-TIBO was assessed with poly(rC) · oligo(dG) as the primer-template and [^{3}H]dGTP as the substrate. ND, not done.

Larder, B.A. Antimicrob. Agents Chemother. 1992: 36:2664-2669.

TABLE 2. EC_{50}s of the test compounds for HIV-1(III_B) isolated in the presence of the HIV-1-specific inhibitors nevirapine, BHAP, and TSAO-m^3T as single agents or in combination in CEM cells[a]

Compound	EC_{50} (μg/ml)													
	No drug	Nevirapine			BHAP			TSAO-m^3T + BHAP			Nevirapine + BHAP			
		1[b]	2	3	1	2	3	1	2	3	1	2	3	
TSAO-T	0.03	0.50	0.77	1.93	0.07	0.06	0.07	1.0	1.4	0.8	0.75	0.63	2.5	(83x)
TSAO-m^3T	0.03	0.87	0.90	2.5	0.04	0.04	0.04	0.71	0.95	0.44	0.70	0.75	1.4	(~47x)
TSAO-e^3T	0.03	4.3	14	26	0.13	0.13	0.08	1.5	30	8	25	14	50	(1700x)
Nevirapine	0.03	1.2	1.1	2.3	0.04	0.03	0.04	1.9	3.0	2.2	0.75	1.75	4.60	(153x)
Pyridinone	0.007	0.24	0.08	0.24	0.15	0.12	0.10	5.8	≥10	4.7	5.0	7.0	5.2	(742x)
TIBO R82913	0.02	0.50	0.63	0.50	0.42	0.41	0.27	0.33	0.37	0.28	0.43	1.1	1.1	(55)
BHAP	0.03	0.20	0.20	0.40	0.27	0.63	2.5	1.1	1.3	1.3	1.1	1.1	2.8	(93x)

[a] Virus was isolated after full breakthrough of cytopathicity in CEM cell cultures in the presence of nevirapine, BHAP, TSAO-m^3T plus BHAP, or nevirapine plus BHAP at 0.1 μg/ml (passage 1), 0.5 μg/ml (passage 2), or 2.5 μg/ml (passage 3).

[b] Passage number of HIV-1(III_B) cells.

Balzarini, J. et al. J. Virol. 1993: 67: 5353-5359.

TABLE 1. Emergence of isolates of HIV with reduced susceptibility and resistance mutations with nevirapine therapy[a]

Wk of therapy	Cumulative proportion with:		
	Reduced susceptibility	Known resistance mutation[b]	Either
1	3/3		3/3
2	12/14	6/8	12/15
4	23/26	18/21	24/26
8	32/32	30/30	33/33
≥12	38/38	38/38	38/38

[a] Table combines results from 24 patients receiving nevirapine monotherapy and 14 patients receiving combination therapy with AZT.

[b] Mutations identified: K103N, V106A, V108I, Y181C, Y181S, Y188L, Y188H, Y188D, G190A, G190S, G190L. The effects of some allelic variations (i.e., 188D, 190S, and 190L) on the susceptibility to nevirapine have not been fully characterized by site-directed mutagenesis.

Richman, D.D. J. Virol. 1994: 68: 1660-1666.

Enclosure 16

TABLE 3. Effect of concomitant therapy with AZT on the pattern of nevirapine resistance mutations

Treatment	No. of patients examined	% of patients with isolates developing mutations at the indicated residue of RT:					
		103	106	108	181	188	190
Nevirapine monotherapy	24	33	0	8	79	8	17
Nevirapine plus AZT	14	57	14	0	0	50	50

Richman, D.D. J. Virol. 1994: 68: 1660-1666.

Enclosure 17

Table 3. In vitro susceptibilities to nevirapine and mutations of HIV isolates from patients given 400 mg of nevirapine.

Patient	IC_{50} of isolate (μM) Baseline	Follow-up*		Mutation†	Mean nevirapine trough (μM)
Responders					
1	0.019	4.0 (2 and 14)	210x	Y181C/Y188L (mixed population)	13.7
2	<0.01	4.0 (12)	400x	Y188L	16.8
3	0.065	NA			16.6
4	0.15	7.7 (6)	50x	Y181C	29.0
5	0.019	>5.0 (4)	263x	K103N, Y181C	20.8
6	0.03	20 (8)	670x	Y181C	16.7
7	0.1	9 (13)	90x	K103S, Y181C	17.6
8	NA	NA			26.3
Nonresponders					
9	0.05	6.7 (6)	134x	G190S	11.9
10	0.07	5.8 (8)	83x	Y181C	12.4

NOTE. NA = not available.

* Numbers in parentheses are weeks on nevirapine.

† First letter represents pretreatment, wild type amino acid. Number represents amino acid residue of reverse transcriptase. Second letter indicates mutant amino acid at residue. C = cysteine, G = glycine, K = lysine, L = leucine, N = asparagine, S = serine, Y = tyrosine.

Havlir, D. et al. J. Inf. Dis. 1995: 171: 537-545.

Table 2. Susceptibility of virus isolates to nevirapine.

Patient	Baseline HIV RNA copies/mL	Weeks after therapy initiated	Nevirapine IC_{50} (μM)
1	150	60	1.6
2	200	6	3.6
3	200	12	9.0
5	400	12	>31.6
6	600	24	0.1
7	1000	12	6.8
8	1200	8	7.5
9	4900	12	>31.6
10	7500	12	0.12
11	39,000	8	7.0
12	45,000	8	6.0
13	52,500	12	8.0
14	62,000	24	>10
15	135,000	12	>31.6
16	212,500	4	3.5
17	360,000	12	>10

Baseline IC_{50} $\bar{X} \cong 0.04 \mu M$

Havlir, D. et al. J. Inf. Dis. 1995: 172: 1379-1383.

TABLE 1. SIX MONTH RESISTANCE ACCORDING TO STUDY GROUP AND COMPLIANCE IN 1046 CANADIAN PATIENTS

Treatment=ZDV+NVP

ID	Study Drug Interruptions		6 Month Resistance			
	Any 4 weeks during first 6 months-1*	4 weeks Between 16-28 weeks-2*	VISIT	AZT IC_{50}	NVP IC_{50}	DDI IC_{50}
18	N/I*	N/I	10	0.1	>20.0	3
26	NVP,ZDV	ZDV	11	0.4	>20.0	2.5
31	N/I	N/I	17	0.007	>20.0	1.5
50	N/I	N/I	10	0.25	>20.0	1.8

Treatment=ZDV+ddI

ID	Study Drug Interruptions		6 Month Resistance			
	Any 4 weeks during first 6 months	4 weeks Between 16-28 weeks	VISIT	AZT IC_{50}	NVP IC_{50}	DDI IC_{50}
4	N/I	N/I	10	0.007	0.03	0.8
6	ddI,ZDV	ddI,ZDV	10	0.02	0.035	2
35	N/I	N/I	10	0.15	0.2	15
37	ddI	ddI	10	0.01	0.2	4.5
41	N/I	N/I	10	0.08	0.03	2
45	N/I	N/I	10	0.16	0.22	5.5
46	ZDV	ZDV	10	0.05	0.07	2
49	ddI	ddI	8	0.0009	0.018	0.1

Treatment=ZDV+ddI+NVP

ID	Study Drug Interruptions		6 Month Resistance			
	Any 4 weeks during first 6 months	4 weeks Between 16-28 weeks	VISIT	AZT IC_{50}	NVP IC_{50}	DDI IC_{50}
3	N/I	N/I	10	0.01	0.052	1.5
20	ddI,ZDV	ZDV	10	0.01	>20.0	1.4
24	N/I	N/I	10	0.06	0.12	5
34	ddI	ddI	10	0.05	>20.0	0.45

1* The study drug was not taken for at least 4 consecutive weeks during the first 6 months on study.

2* The study drug was not taken for at least 4 consecutive weeks between 16-28 weeks on study.

*N/I= No Interruption