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MICROBIOLOGY REVIEW

MICROBIOLOGY REVIEW
DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)
NDA # 20-636 SE7-017; Date Reviewed: 03/25/02

Reviewer: LALJI MISHRA, Ph.D.
Date Submitted: 05/31/01
Date Received: 05/31/01
Date Assigned: 07/09/01
Sponsor: Boehringer Ingelheim Pharmaceuticals, Inc.
900 Ridgebury Rd
P.O. Box 368
Ridgefield, CT 06877-0368

Product Name(s):

Proprietary: Viramune^R

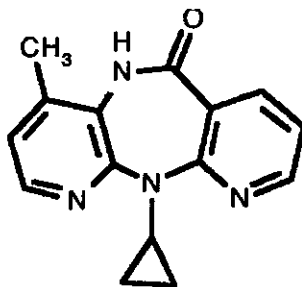
Non-proprietary: Nevirapine

Chemical: 11-cyclopropyl-5,11-dihydro-4-methyl-6H-dipyrido [3,2-b:2',3'-][1,4]diazepin-6-one

Molecular Formula: C₁₃H₁₄N₄O

Molecular Weight: 266.3

Structural Formula:



Route of Administration/Dosage form: Oral/Tablets

Indication: For use in combination with other antiretroviral agents for the treatment of HIV-1 infection.

BACKGROUND

Boehringer Ingelheim Pharmaceuticals, Inc. (BIPI) has submitted a supplemental new drug application in support of a marketing claim for nevirapine (NVP) tablets. NVP received accelerated approval on June 21, 1996 for the treatment of HIV-1 infection in combination with other antiretrovirals. NVP is a non-nucleoside reverse transcriptase

MICROBIOLOGY REVIEW
DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)
NDA # 20-636 SE7-017; Date Reviewed: 03/25/02

inhibitor (NNRTI) of HIV-1. It is a noncompetitive inhibitor of HIV-1 reverse transcriptase (RT) and does not compete with the RNA or DNA templates, or the nucleoside triphosphate substrates. NVP directly binds to the HIV-1 reverse transcriptase (RT) and blocks the RNA-dependent and DNA-dependent polymerase activities by causing a disruption of the catalytic site of the RT. NVP is specific for HIV-1 RT and has no inhibitory activity against HIV-2 RT.

NVP has been demonstrated to exhibit anti-HIV-1 activity in vitro and in vivo. The IC₅₀ (50% inhibitory concentration) of NVP ranged from 10-100 nM against laboratory and clinical isolates of HIV-1. NVP exhibited additive to synergistic anti-HIV-1 activity in combination with zidovudine (ZDV), didanosine (ddI), stavudine (d4T), lamivudine (3TC), saquinavir (SQV) and indinavir (IDV) in vitro.

HIV-1 isolates with reduced susceptibility to NVP have been obtained from patients treated with NVP. Genotypic analysis has shown that NVP resistant isolates contain one or more of the following mutations in the HIV-1 RT gene: A98G, K101E, K103N, V106A, V108I, Y181C, Y188C and G190A. Phenotypic analysis of these isolates has shown a >100-fold decrease in susceptibility to NVP in vitro compared to baseline.

In this supplemental NDA (sNDA), BIPI has presented results of a pivotal study [Trial 1100-1090] and two principal supporting studies Atlantic [1100.1290] and INCAS [1100.1046]. Results of these clinical trials are summarized below. For the detailed agency interpretation of these data, please see the Medical Officer's review.

The sponsor did not conduct resistance testing in the BI 1090 or Atlantic studies submitted in support of traditional approval (letter dated 8/24/01). Limited data on phenotypic resistance for HIV-1 isolates from patients enrolled in the BI-1046 (INCAS) study were provided in the sNDA submission and are reviewed here. In addition, data from a published research paper pertaining to the phenotypic and genotypic analysis of HIV-1 isolates from patients enrolled in the INCAS study are reviewed here. The pertinent microbiology data from the above-mentioned studies are summarized below.

PROTOCOL

I. Trial 1100.1090

Title: Final clinical report with analysis of virological endpoints, clinical progression, safety, and tolerability: an international, double-blind, randomized, phase III study to evaluate the tolerance, safety, and effectiveness of NVP in preventing clinical AIDS progression events or death when used in combination with 3TC and background antiretroviral therapy.

MICROBIOLOGY REVIEW
DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)
NDA # 20-636 SE7-017; Date Reviewed: 03/25/02

Objectives

Primary

1. To evaluate the effect of NVP treatment compared with placebo treatment (PBO), when used in conjunction with 3TC plus additional antiretroviral therapy, on the sustained suppression of plasma HIV-1 RNA, i.e. the proportion of patients who had suppression at 48 weeks.
2. To evaluate the effect of NVP treatment on HIV-1 disease progression.

Secondary

1. To evaluate the effect of NVP compared with PBO on HIV-1 RNA response in patients who were treatment naïve before entering the study (NAÏVE patients), in patients who were only treated with ZDV prior to entering the study (ZDV Only patients), and in all other patients (Other group).
2. To evaluate the effect of NVP treatment on HIV-1 RNA response rate and CD4+ cell count.
3. To evaluate the safety and tolerance of NVP when used in combination with 3TC and other antiretrovirals.

Microbiology Specific Inclusion Criteria

1. HIV-1 infection previously documented by licensed ELISA and confirmed by Western blot or positive HIV-1 culture, or HIV-1 RNA test.
2. CD4+ cell count ≤ 200 cells/mm³. After February 28, 1997, only patients with CD4+ cell counts of ≤ 100 cells/mm³ were eligible to enroll.

Microbiology Specific Exclusion Criteria

1. Prior therapy with NVP or any other NNRTI.
2. Therapy with an antiretroviral agent other than ZDV, d4T, ddI, ddC, 3TC, SQV, IDV, or RTV.
3. Therapy with immunosuppressive or cytotoxic drugs or any other experimental agent within 4 weeks prior to study day 0.

Drug Regimen

Patients were randomly assigned to receive NVP or placebo. In addition, all subjects received 3TC and an additional antiretroviral therapy. Antiretroviral –NAÏVE patients received at least one background antiretroviral agent on study Day 0. Antiretroviral-experienced patients continued their background antiretroviral therapy or had the option to switch their background antiretroviral agent(s) on study day 0.

MICROBIOLOGY REVIEW
DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)
NDA # 20-636 SE7-017; Date Reviewed: 03/25/02

Patients assigned to NVP treatment received a lead-in dose of 200 mg/day for 2 weeks, followed by 200 mg b.i.d. All patients also received 3TC 150 mg b.i.d. and antiretroviral background therapy with any of the following: ZDV, ddI, ddC, SQV, IDV, RTV, and/or d4T (the standard of care doses determined by the physician).

The treatment was given until 18 months after the last patient was enrolled or for 24 months, whichever came first.

Endpoints

The primary endpoint for this trial was the proportion of patients whose plasma HIV RNA levels were below the lower limit of quantification (LLOQ) of 50 copies/mL through 48 weeks, and beyond 48 weeks in the combination treatment regimens. The assay used to quantify HIV-1 RNA was the Amplicor HIV Monitor™ test (ultrasensitive) version 1.5. This assay, more efficient in the detection of the various HIV-1 clades, has yet to be approved by the FDA. The LLOQ of this assay is 50 copies/mL. For a description of the version 1.5 assay, please see the METHODOLOGY section of this review.

SUMMARY

I (a). Pre-therapy and post-therapy Viral Load (Trial 1100.1090):

The median baseline viral load and CD4+ counts for patients enrolled in Trial No. 1100-1090 are shown in Table 1. Results of virologic response for patients treated with NVP, or PBO are shown in Table 2. The proportion of patients whose HIV-1 RNA remained below the LLOQ (responder rate) at 48 weeks was significantly greater in the NVP group than those for the PBO patients. At 48 weeks, the responder rates were 19.5% and 2.6% for NVP and placebo treated patients, respectively. Analyses adjusting for baseline CD4+ cell count, HIV-1 RNA copies and prior antiretroviral therapy showed that NVP suppressed viral load significantly greater than PBO (p<0.001).

Table 1: Baseline CD4+ count and HIV-1 RNA level of patients enrolled in study 1100.1090 (Source: sNDA.20,636; vol 1:page 178; Table 3.4.2.1:1)

	All Patients	First HAART patients* and PBO comparator	Treatment experienced patients
N (NVP/PBO)	2249 (1121/1129)	527 (248/279)	1722 (873/849)
Baseline CD4+ cell count (median)	95.5 cells/mm ³	91.0 cells/mm ³	96.0 cells/mm ³
Baseline HIV RNA (median)	4.583 log ₁₀ copies/mL	5.02 log ₁₀ copies/mL	4.453 log ₁₀ copies/mL

MICROBIOLOGY REVIEW
DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)
NDA # 20-636 SE7-017; Date Reviewed: 03/25/02

* = Those patients who were treatment naïve or who had only ZDV therapy prior to entering the study were categorized as “first HART” patients, while the remaining patients were considered “treatment experienced”, because they had more extensive antiretroviral therapy before entering the trial.

Table 2: HIV-1 RNA suppression below the LLOQ (LLOQ = 50 copies/mL) at 48 week; responder rates by antiretroviral treatment history and baseline characteristics (sNDA.20,636; vol 1: page 179; Table 3.4.2.1:2)

	NVP	PBO	P Value
Patient history/baseline characteristics	% Responders (responders/number in Group)		
Total	19.45 % (218/1121)	2.57 % (29/1128)	<0.001
Treatment History			
First HAART	39.52 (98/248)	3.23 (9/279)	<0.001
NAÏVE	41.88 (49/117)	3.05 (4/131)	<0.001
ZDV only	37.40 (49/131)	3.38 (5/148)	<0.001
Other	13.75 (120/873)	2.36 (20/849)	<0.001
Baseline CD4+ cell count (cells/mm³)			
<25	7.22 (14/194)	1.10 (2/181)	<0.004
25-50	10.71(15/140)	3.60 (5/139)	<0.035
50-100	14.50 (39/269)	4.31 (11/255)	<0.001
>100	28.96 (150/518)	1.99 (11/533)	<0.001
Baseline HIV RNA (copies/mL)			
<5000	29.26 (55/188)	6.76 (15/222)	<0.001
5000-100,000	18.41(104/565)	1.56 (9/578)	<0.001
>100,000	16.91(59/349)	1.61(5/311)	<0.001

Table 2 shows that compared to placebo the 48-week viral suppression rates were significantly higher in the NVP-treated patients.

Comments

1. NVP treated-naïve patients and patients whose only prior experience was with ZDV showed response rates of 41.88 % and 37.40 %, respectively. In contrast, patients with more extensive prior treatment (Other patients) had a response rate of 13.75 %.
2. As expected, patients with higher CD4 + cell counts had higher response rates in the NVP treated group. Similarly, patients with the lowest baseline HIV-1 RNA levels, <5000 copies/mL, had higher response rates in both the NVP and PBO treatment group than those patients with higher baseline HIV-1 RNA levels.

MICROBIOLOGY REVIEW
DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)
NDA # 20-636 SE7-017; Date Reviewed: 03/25/02

II. Trial 1100.1046 (INCAS)

Title: A randomized, placebo-controlled, double-blinded multinational trial comparing the immunologic and virologic effects of NVP, ddI and ZDV combinations for the treatment of antiretroviral naïve HIV-1 infected patients with 200-600 CD4+ T-cells and no AIDS defining disease.

This trial was conducted in Italy, the Netherlands, Canada, and Australia and has been referred to as the INCAS trial.

Objective

Primary

To evaluate the activity, safety and tolerance of NVP in combination with ZDV and ddI or in combination with ZDV alone compared with the active control ddI/ZDV in non advanced HIV-1 infected patients who had had received no prior antiretroviral therapy.

Endpoints:

Primary

The above treatment regimens were compared using the following endpoints.

1. Change in viral load from baseline to the end of the trial (40 to 52 weeks).
2. Proportion of patients with plasma HIV-1 RNA below the limit of detection (LOD = 20 copies/ml) at 40 to 52 weeks.
3. Average daily response (52-week AUCMB).

Secondary

1. Virologic and immunologic activity for NVP/ddI/ZDV vs ddI/ZDV and NVP/ZDV vs ddI/ZDV at early (12 to 16 weeks) and middle (20 to 28 weeks) time points were examined. Virologic and immunologic activity beyond 52 weeks (60 to 76 weeks) for patients who remained on blinded study therapy while the last patient was completing 52 weeks were examined.
2. Proportion of patients with plasma HIV-1 RNA below the LOD = 200 copies/mL and the LOD= 400 copies/mL.
3. Phenotypic resistance to antiretroviral agents was analyzed in HIV-1 isolates from patients from whom virus could be amplified.

MICROBIOLOGY REVIEW
DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)
NDA # 20-636 SE7-017; Date Reviewed: 03/25/02

Comments for BIPIs-specified LLOQ =20, and 200 copies/mL:

The sponsor used the Roche Amplicor HIV-1 Montor™ assay (standard) prior to its FDA approval and specified its LOD as = 200 copies/mL. Six month later, FDA approved this assay with a LOD = 400 copies/mL. Similarly, the sponsor used the Roche Amplicor HIV Monitor™ (ultrasensitive). The sponsor referred to it as the ultra-direct assay with a LOD = 20 copies/mL (NDA 20-636, vol 1.70, page 26; and pages 45-46).

One hundred fifty-three HIV-1 infected patients were enrolled in the study. Treatment regimens were: 1) NVP 200 mg BID in combination with 600 mg ZDV and ddI placebo; 2) NVP placebo with 600 mg ZDV and 250 or 400 mg ddI; 3) NVP 200 mg BID with 600 mg ZDV and 250 or 400 mg ddI. Patients were to continue on the study with blinded medication until the last patient reached 52 weeks.

Comments

The trial 1100.1046 was conducted between July 1994 and July 1996. The final study report was completed in May 1997 (NDA 20,636, vol 1.70, pages 1-221). Subsequent to the completion of the report, the FDA issued its draft Guidance for Industry, which identified preferred analyses for evaluating HIV progression endpoints based on virologic criteria. With these preferred analyses in mind, BIPI updated this study report with the 1100.1046 Efficacy Report contained in this sNDA. The analyses from the full clinical trial report and those of the most recent report are summarized in the Integrated Summary of Efficacy. The primary analyses were based on the Amplicor assay (LLOQ: 400 copies/mL) and the ultra direct assay (LLOQ: 20 copies/mL).

The primary objective of the efficacy report analysis was to compare the virologic response to treatment with NVP/ZDV/PBO vs PBO/ddI/ZDV and NVP/ddI/ZDV vs PBO/ddI/ZDV in antiretroviral naïve patients. The primary endpoints of this analysis were time to virologic failure and virologic failure rate at 48 weeks.

The following criteria were used for analysis of virologic failure:

Failure = Day 0 if a patient never achieves the LLOQ (400 copies/mL) while on the originally assigned therapy. If the viral load was ever below the LLOQ while the patient was on the originally assigned therapy (virologic responders), then failure equaled the earliest of the choices below:

- time of treatment switch,
- time of loss to follow-up if lost while below the LLOQ, or
- time of first confirmed value greater than the LLOQ after achieving the LLOQ, or
- time of first plasma HIV-1 RNA = 400 copies/mL, or
- time of death or CDC category C event.

MICROBIOLOGY REVIEW
 DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)
 NDA # 20-636 SE7-017; Date Reviewed: 03/25/02

Patients were categorized as virologic failure or sustained responders based on whether their failure time was < 48 weeks or ≥ 48 weeks.

II (a). Virologic response in patients receiving NVP in combination with other antiretroviral drugs in the INCAS study:

Results of virologic response in patients treated with NVP in combination with ddI/ZDV, ZDV vs ddI/ZDV are summarized in Table 3. For a detailed report, please see Medical Officer's review. Table 3 shows that at 48 weeks, 45.1% of patients treated with the NVP+ZDV+ddI and 18.9% of the patients treated with PBO+ZDV+ddI were virologic responders (below the LLOQ, p<0.006; sNDA, 20,636, page 182). In the NVP/ZDV group, all patients experienced virologic failure by 12 weeks (NDA 20,636; vol 1.2, page 60, Figure 4.2.3.2:2). All patients in the NVP/ZDV group experienced virologic failure by 20 week. The median time to virologic failure for NVP/ZDV patients was only 2 weeks.

Table 3: Proportion of patients with a sustained virologic response through 48 weeks by treatment (Source: NDA 20-636;vol 1.2, page 60, Table 4.2.3.2:3)

LLOQ	Percent Responders ≥ 48 weeks			
	NVP+ZDV	ddI+ZDV	NVP+ddI + ZDV	Total
400	0.00% (0/47)	18.87% (10/53)	45.10% (23/51)	21.85% (33/151)
20	0.00% (0/47)	9.43% (5/53)	35.29% (18/51)	15.23% (23/151)

LLOQ= lower limit of quantification (copies/mL)

Of the 118 patients who met the criteria for failure during the course of the trial 107 (90.7%) failed because of an increase in plasma HIV-1 RNA to ≥ 400 copies/mL, or never achieved a plasma HIV-1 RNA level ≤ 400 copies/mL. Only 11 (9.3%) had other (non-virologic) criteria for failure.

II (b). Other virologic analyses:

Analyses from a full clinical report (U97-3062) examined virologic response using other measures of outcome such as changes from baseline viral load and AUCMB (area under the curve minus baseline, or daily average viral load) of the plasma HIV-1 RNA over the duration of the study.

The baseline CD4+ cell counts and percentage of CD4+ cells did not differ significantly among the three treatment groups (NDA 20,636, vol 1.70, page 71, Table 8.3.1). CD4+ cell count (cells/mm³) ranged from 346-387 for the three treatment groups and percentage

MICROBIOLOGY REVIEW
DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)
NDA # 20-636 SE7-017; Date Reviewed: 03/25/02

CD4+ cells were 20.9, 21.6 and 21.7 for the NVP/ZDV, ddI/ZDV and NVP/ddI/ZDV groups, respectively. Similarly, baseline HIV-1 RNA for the NVP/ZDV, ddI/ZDV and NVP/ddI/ZDV groups were 4.54, 4.47 and 4.24 log₁₀ copies/mL, respectively (NDA 20,636; vol 1.70, page 71, Table 8.3.1).

At week 8, treatment with NVP/ZDV, ddI/ZDV and triple therapy (NVP/ddI/ZDV) resulted in an approximately 0.90 log₁₀ decline, a 1.55 log₁₀ decline, and a 2.18 log₁₀ decline from baseline, respectively. The mean change in HIV-1 RNA in the NVP/ddI/ZDV group continued to decline to a nadir of 2.33 log₁₀ copies/mL below baseline at week 16. Table 4 shows the mean change in plasma HIV-1 RNA from baseline during therapy.

Table 4: Mean change in plasma HIV-1 RNA log₁₀ copies/mL from baseline at:

Treatment group	12-16 weeks	20-28 weeks	40-52 weeks	60-76 weeks
NVP/ddI/ZDV	-2.45 (n = 41)	-2.43 (n = 40)	-1.91(n = 40)	-1.46 (n = 25)
ddI/ZDV	-1.69 (n = 46)	-1.67 (n = 41)	-1.35 (n = 36)	-0.72 (n = 23)
NVP/ZDV	-0.60 (n = 38)	-0.55 (n = 32)	-0.27 (n = 28)	-0.13 (n = 19)

Table 4 shows that the decrease in plasma HIV-1 RNA (log₁₀ copies/mL) from baseline to weeks 40 to 52 was greater for the triple therapy (NVP/ddI/ZDV) group than the ddI/ZDV group (1.91 versus 1.35 log₁₀ copies/mL, p = 0.21). The difference from baseline to week 60 to 76 for the same groups was 0.74 log₁₀ copies/mL (p= 0.016). These results indicated that treatment with both NVP/ddI/ZDV and ddI/ZDV were superior to the NVP/ZDV therapy.

III. Phenotypic analysis of HIV-1 isolates from patients enrolled in the INCAS study:

Plasma specimens were assayed for susceptibility to drugs (phenotypic resistance) using a recombinant DNA based method (see Methodology) at _____
 Although 57 patients were initially tested, phenotypic resistance data for matched baseline and post-therapy (6 months) HIV-1 isolates were available from only 23 patients from Australia, Italy and the Netherlands.

HIV-1 was less commonly amplifiable from the NVP/ddI/ZDV group (3/22) at 6 months than the NVP/ZDV (11/16) and ddI/ZDV (9/19) treatment groups. Phenotypic resistance (a decrease in susceptibility to a drug) of HIV-1 isolates from patients after 6 months of treatment is shown in Table 5.

Baseline HIV-1 isolates from most patients (n = 11) receiving NVP and ZDV drug regimens were susceptible to NVP except for the isolate from one patient (# 3336; NDA 20-636, vol 1.74, page 91, Appendix 15.12.4, listing 4.6.1). Baseline HIV-1 isolates

MICROBIOLOGY REVIEW
DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)
NDA # 20-636 SE7-017; Date Reviewed: 03/25/02

from patient # 3336 exhibited a 100-fold reduction in susceptibility to NVP compared to the wild type HIV-1 control. All of these baseline isolates (n = 11) were susceptible to ZDV or ddI.

Similarly, baseline HIV-1 isolates from 8 patients in the ZDV/ddI treatment group (n = 9) were susceptible to NVP, ZDV or ddI. However, baseline HIV-1 isolates from one patient (# 3322) were resistant to NVP, but susceptible to ZDV or ddI (NDA 20-636, vol 1.74, page 92, Appendix 15.12.4, listing 4.6.1). Baseline isolates from patients in the ZDV/ddI/NVP treatment group (n=3) were susceptible to NVP, ZDV or ddI

Table 5: Phenotypic resistance of HIV-1 isolates obtained at 6 months of therapy (Source: Appendix 15.12.4, Listing 4.6.1)

Drug Tested for Resistance	Treatment Group		
	NPV/ZDV (n = 11)	ddI/ZDV (n = 9)	NVP/ddI/ZDV (n = 3)
NVP	100%	0%	100%
ZDV	9%	44%	0%
ddI	0%	0%	0%

Table 5 shows the following results.

1. HIV-1 isolates from all evaluable patients (n = 11) receiving NVP/ZDV therapy developed resistance to NVP at or around 6 months of treatment. Post-therapy isolates from 10/11 patients were highly resistant to NVP ($IC_{50} > 100 \mu M$). As mentioned before, baseline HIV-1 isolates from one of these 11 patients in the NVP/ZDV treatment group exhibited a 100-fold decrease in susceptibility to NVP.
2. HIV-1 isolates from all 3 patients in the NVP/ddI/ZDV treatment group developed resistance to NVP.
3. Resistance to ZDV developed more frequently in isolates from patients in the ddI/ZDV treatment group than that from NVP treated patients. HIV-1 isolates from one patient in the NVP/ZDV treatment group developed resistance to ZDV, and none of the HIV-1 isolates from patients treated with NVP/ddI/ZDV showed resistance to ZDV.
4. None of the HIV-1 isolates from any of the above three treatment groups developed resistance to ddI.

MICROBIOLOGY REVIEW
DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)
NDA # 20-636 SE7-017; Date Reviewed: 03/25/02

Comment

This is a very small study. Data on the genotypes of pre-therapy and post-therapy HIV-1 isolates are lacking. Studies should be carried out to identify the genotypes of NVP resistant HIV-1 isolates and correlate it with the phenotypes.

IV. Data from the published paper on resistance to NVP, ddI, ZDV in pre-therapy and post-therapy HIV-1 isolates from patients enrolled in the INCAS Trial (AIDS 2001,15:1269-1274).

Title: Development of drug resistance in patients receiving combination of ZDV, ddI and NVP.

Objective: To evaluate the development of phenotypic and genotypic resistance to ZDV, ddI and NVP as a function of the virologic response to therapy in a group of drug-naïve individuals receiving various combinations of these agents.

No patient had received antiretroviral drug previously. The duration of therapy was 52 weeks. Extensive virologic evaluations were performed at baseline, and repeated at 6 and 12 months.

For selected isolates found to be resistant to NVP, the RT gene was sequenced to establish the genotypic correlate of the phenotypic resistance.

IV (a). Phenotypic analysis of pre-therapy and post-therapy HIV-1 isolates:

Phenotypic resistance was determined using a recombinant virus assay _____
_____ Drug susceptibility was determined for baseline HIV-1 isolates from 24 patients taking NVP/ZDV/ddI, 16 patients taking NVP and ZDV, and 19 patients taking ddI and ZDV (Table 6).

Table 6 shows the following results:

1. Baseline HIV-1 isolates from 5 patients enrolled in the three treatment arms were resistant to NVP. These patients were antiretroviral therapy naïve at the time of enrollment.
2. After 24 weeks, all available on-therapy HIV-1 isolates (32/32) from patients receiving NVP as part of two (NVP/ZDV; n = 19), or three drug combinations (NVP/ddI/ZDV; n= 13) were resistant to NVP.
3. On-therapy HIV-1 isolates obtained at 30-60 weeks from 18/21 (86%) patients receiving NVP/ddI/ZDV therapy were resistant to NVP.
4. None of the baseline HIV-1 isolates showed any evidence of resistance to ddI. On-therapy HIV-1 isolates obtained at weeks 30-60 from 1/21 (5%) patients receiving NVP/ddI/ZDV combination therapy exhibited resistance to ddI.

MICROBIOLOGY REVIEW
DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)
NDA # 20-636 SE7-017; Date Reviewed: 03/25/02

5. None of the baseline HIV-1 isolates from patients receiving ZDV in any of the three treatment arms showed resistance to ZDV. However, on-therapy HIV-1 isolates obtained at week 24 from 1/19 (5%) patients receiving NVP/ZDV and from 3/21 (13%) patients receiving ddI/ZDV therapy exhibited resistance to ZDV. After 30-60 weeks of treatment, HIV-1 isolates from 13/25 (52%) patients receiving ddI/ZDV, and from 4/21(19%) patients receiving NVP/ddI/ZDV triple therapy exhibited resistance to ZDV.

Table 6: Phenotypic analysis of pre-therapy and post therapy HIV-1 isolates from patients enrolled in the INCAS trial

Week of therapy with drug regimen	<u>Resistance * (No. (%))</u>			
	No. patients	NVP	ddI	ZDV
NVP/ZDV				
0	16	1 (6)	0 (0)	0 (0)
24	19	19 (100)	0 (0)	1 (5)
ddI/ZDV				
0	19	1 (5)	0 (0)	0 (0)
24	21	0 (0)	0 (0)	3 (13)
30-60	25	0 (0)	0 (0)	13 (52)
NVP/ddI/ZDV				
0	24	3 (13)	0 (0)	0 (0)
24	13	13 (100)	0 (0)	0 (0)
30-60	21	18 (86)	1 (5)	4 (19)

*defined as >four-fold increase in IC₅₀ compared with a wild type control.

IV (b). Genotypic analysis of post-therapy HIV-1 isolates:

The genotypes of HIV-1 isolates (n = 12) obtained from plasma of 11 patients receiving triple combination therapy were determined and correlated with the phenotypic resistance to NVP. All isolates contained at least one mutation associated with NVP-resistance (K101E, K103N, V106A, Y181C, G190A). The frequency of NVP resistance-associated mutations in these isolates are shown in Table 7. The most frequent mutation was K103N, followed by G190A and Y181C.

Resistant genotype was documented at 12-18 weeks in six samples, and at 40-52 weeks in the other six samples (Table 8). Nine of these 12 on-therapy isolates had multiple mutations.

MICROBIOLOGY REVIEW
 DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)
 NDA # 20-636 SE7-017; Date Reviewed: 03/25/02

Table 7: Mutations associated with NVP resistance in 12 post-therapy HIV-1 isolates taken from 11 patients* receiving NVP/ddI/ZDV.

Mutations	Frequency (n)
K101E	2
K103N	8
V106A	2
Y181C	5
G190A	6

= Eight of the 11 patients had never achieved maximal virologic suppression. HIV-1 isolates from three patients developed phenotypic resistance despite the reduction in plasma viral load to <20 copies/mL.

Table 8: Mutational patterns observed in post-therapy HIV-1 isolates from patients receiving NVP/ddI/ZDV therapy

Mutations	Frequency	Week (s) isolated
K103N	2	47, 52
Y181C	1	48
K103N/Y181C	3	16, 18, 48
K103N/G190A	2	15, 52
V106A/G190A	2	12, 16
K101E/G190A	1	50
K101E/K103N/Y181C/ G190A	1	12

V. Stability of HIV RNA in stored specimens

The objective of this study was to evaluate the effect of long term storage of plasma samples on HIV-1 RNA levels.

Methods

Plasma samples were collected from all patients at screening, on the first day of treatment, and at each subsequent trial visit. The plasma was immediately separated, frozen at -70°C, and shipped on dry ice to one of several central laboratories for storage at -70°C.

Specimens were assayed in 1999 using the Amplicor Monitor version 1.5 ultrasensitive and Standard methods. Specimens were categorized into those with no detectable virus (Not Detectable), detectable virus with below the LLOQ (<50 copies/mL), virus below the LLOQ for the Standard Amplicor HIV-1 assay (50-400 copies/mL), and virus above the LLOQ for the Standard assay (>400 copies/mL). Random samples were selected from

MICROBIOLOGY REVIEW
DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)
NDA # 20-636 SE7-017; Date Reviewed: 03/25/02

within each category: 20 from Not Detectable, 40 from <50 copies/mL, 40 from 50-400 copies/mL, and 50 from 400 copies/mL. Previously unthawed second aliquots were identified and assayed. Each specimen was analyzed, comparing initial assay results with the second assay results.

Results

Specimens were stored for an average of approximately two years (from 10.5 months to 3.5 years) prior to the initial assay. Specimens for repeat testing had an additional 10.5 months to 1.5 years in the freezer prior to their repeat testing.

All specimens that had no detectable viral RNA on initial testing were below the LLOQ (50 copies/mL) upon repeat assay. For those with detectable RNA below the LLOQ, 67.5% were below the LLOQ upon repeat assay (Table 9). Those with RNA levels between the assay limit for the ultrasensitive assay (50 copies/mL) and the assay limit for the Amplicor assay (400 copies/mL) remained above the ultrasensitive assay limit in 87.5% of repeat assays.

Table 9: Results of First and Second assays of stability testing of plasma samples

Second assay	Results of First assay			
	Not Detected	<50	50-400	>400
Not detected	14 (70%)	13 (32.5%)	1 (2.5%)	0 (0%)
<50	6 (30%)	14 (35%)	4 (10%)	0 (0%)
50-400	0 (0%)	13 (32.5%)	23 (57.5%)	0 (0%)
>400	0 (0%)	0 (0%)	12 (30%)	50 (100%)

The degree to which results were replicated was examined by comparing the results of first assay with the second assay for specimens with initial assay results of HIV-1 RNA detectable <50 copies/mL, 50-400 copies/mL, and >400 copies/mL (NDA 20-636, vol 1.2 pages, 91-92, Figures 4.4.3.2:1-3). There was a median increase of 0.15 log₁₀ copies/mL observed upon repeat testing (p = 0.002).

These results suggested that there was no deterioration in the viral RNA copy number due to storage for up to 5.0 years.

CONCLUSIONS

With respect to microbiology, this sNDA is approvable. Results from clinical trials 1100.1090 and INCAS showed that NVP in combination with other antiretroviral agents was effective in suppressing viral load. The suppression in viral load below the LLOQ of 50 copies was the primary endpoint. In both these trails NVP in combination with two other antiretroviral agents demonstrated significant anti-HIV-1 activity. Several factors affected the efficacy of NVP. In study 1100.1090 naïve patients, and patients with prior

MICROBIOLOGY REVIEW
DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)
NDA # 20-636 SE7-017; Date Reviewed: 03/25/02

treatment experience with one antiretroviral drug showed a significantly higher response rate compared to the patients with longer antiretroviral treatment experience. Both cohorts showed statistically significant viral load response compared to placebo. Patients with a higher CD4+ cell count (>100 cells/mm³) and <500 copies/mL HIV-1 RNA at baseline responded better than patients with CD4+ cell counts of <25 cells/mm³ and HIV-1 viral load of $>100,000$ copies/mL at baseline.

Results from the INCAS study showed that NVP in combination with ddI and ZDV exhibited significantly greater anti-HIV-1 activity than the combination of ddI/ZDV. However, the combination of NVP and ZDV showed very little anti-HIV-1 activity after 12 weeks. Limited data available suggested that the failure of the NVP/ZDV combination was due to the emergence of NVP resistance-associated mutations in patients treated with NVP/ZDV.

Phenotypic resistance to NVP was prevalent in HIV-1 isolates from patients receiving the NVP/ZDV and NVP/ddI/ZDV therapies. Genotypic analysis of HIV-1 isolates from patients ($n = 12$) receiving the NVP/ddI/ZDV combination therapy (INCA study) showed mutations K101E, K103N, V106A, Y181C, and G190A in the HIV-1 RT gene. The mutation K103N was more frequent followed by G190A and Y181C. Most of these patients had virologic failure ($n=8$) and NVP resistance developed as early as 12 weeks. These NVP resistance mutations have been amply demonstrated to cause decrease in susceptibility to NVP *in vitro* (Stanford University Resistance Database). In addition, mutations A98G, V108I, Y188C/L/H have been shown to confer varying degree of resistance to NVP.

The mutation K103N alone or in combination with Y181C has been shown to confer cross-resistance to the currently approved NNRTIs efavirenz and delavirdine (Bachelier *et al.* 2001; Casado *et al.*, 2001; Deeks 2001;). Similarly, K103N enhanced phenotypic resistance conferred by K101E and V108I to efavirenz and the other NNRTIs tested (NVP, delavirdine). It should be noted that Y181C alone does not confer cross-resistance to efavirenz (Hanna *et al.*, 2000).

HIV-1 viral RNA log₁₀ copies/mL were quantified using the _____ ultrasensitive and standard methods. The performance characteristics of this assay were described in this sNDA submission. The _____ assay quantified HIV-1 RNA from plasma of patients infected with HIV-1 subtypes A-H. The sponsor has evaluated the effect of plasma storage time on the detectability and quantification of HIV-1 RNA (ultrasensitive and standard methods). There was no deterioration in viral load due to storage of plasma for up to 5 years.

MICROBIOLOGY REVIEW
DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)
NDA # 20-636 SE7-017; Date Reviewed: 03/25/02

METHODOLOGY:

Methodology for Genotypic Analysis:

The HIV-RT gene was sequenced from the plasma of patients using standard sequencing methods (Conway et al., 2001).

Methodology for Phenotypic Analysis (Antivirogram™):

Phenotypic analysis was performed using a recombinant virus assay. ~~This is an experimental assay, and its accuracy, sensitivity, and reproducibility has not been validated.~~ The Antivirogram™ assay is described by Hertogs et al., 1998. The sponsor did not provide any details of the plasmid used for co-transfection with PCR amplified DNA fragments to produce a recombinant virus in MT-4 cells.

Assays for recombinant virus infectivity (TCID₅₀) and susceptibility are performed using the dye uptake method with the vital dye 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) to quantify cell viability. For each assay, 10 concentrations of each compound are used over a 2-fold serial dilution range with each dilution set up in quadruplicate. The concentration of compound which protects 50% of the cells from virus killing (IC₅₀) is determined from regression analysis of the plot of percentage of cell death against drug concentrations. The relative susceptibility compared with the HIV-1 reference strain HXB-2 analyzed in the same assay is calculated. The inherent variability of the phenotypic assay (Antivirogram) is estimated to be 4-fold.

Methodology for HIV RNA copy number determination:

~~_____~~ was used to determine HIV-1 copy number in all study participants plasma samples. The procedure is fully described in the test kit package insert (sNDA 20-636; vol 1.3, appendixes 2 & 3 pages 175-199). The quantitation of HIV-1 viral RNA is performed using a Quantitation Standard (QS). The QS is a non-infectious RNA transcript that contains the identical primer binding sites as the HIV-1 target and a unique probe binding region that allows QS amplicon to be distinguished from HIV-1 amplicon. The QS is incorporated into each individual sample at a known copy number and is carried through the sample preparation, reverse transcription, PCR amplification, hybridization and detection steps along with the HIV-1 target and is amplified together with the HIV-1 target. HIV-1 RNA levels in the test samples are determined by comparing the HIV-1 signal to the QS signal for each sample. The ~~_____~~ (standard) can quantitate plasma associated HIV-1 RNA at concentrations between ≥ 400 to 750,000 copies/mL. On the other hand, the ~~_____~~ (ultrasensitive) can quantitate plasma associated HIV-1 RNA in the linear range of \geq ~~_____~~ copies/mL.

MICROBIOLOGY REVIEW
DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)
NDA # 20-636 SE7-017; Date Reviewed: 03/25/02

Both / _____ gave similar HIV-1 RNA copies/mL for the plasma specimens from patients containing Group M, subtype B, HIV-1 virus. The correlation (Sperman) of the two assay versions was 0.94 for three assay sites indicating equivalent performance by the two assay versions.

Studies of Group M, subtype A-H HIV-1 plasma specimens were carried out using 16 isolates each diluted to 7 concentrations of HIV-1 RNA (80-600,000 copies/mL). The samples were assayed for viral load using both the version 1.0 and 1.5. The results showed that at all concentrations and both sample methods, the version 1.5 assay provided comparable or improved viral load estimates to the nominal concentration values of the panel members.

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 the 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 *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-1 in drug combination regimens with zidovudine (ZDV), didanosine (ddI), stavudine (d4T), lamivudine (3TC), saquinavir, and indinavir. The relationship between *in vitro* susceptibility of HIV-1 to nevirapine and the inhibition of HIV-1 replication in humans has not been established.

Resistance:

HIV-1 isolates with reduced susceptibility (100-250-fold) to nevirapine emerge *in vitro*. Genotypic analysis showed mutations in the HIV-1 RT gene Y181C and/or V106A 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

MICROBIOLOGY REVIEW
DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)
NDA # 20-636 SE7-017; Date Reviewed: 03/25/02

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 K103N, V106A, V108I, Y181C, Y188C and G190A were detected in HIV-1 isolates from 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-1 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 Y181C mutation regardless of the dose. Nevirapine+ZDV combination therapy did not alter the emergence rate of nevirapine-resistant virus or the magnitude of nevirapine resistance *in vitro*. The clinical relevance of phenotypic and genotypic changes associated with nevirapine therapy has not been established.

Cross-resistance:

Rapid emergence of HIV-1 strains which are cross-resistant to NNRTIs has been observed *in vitro*. Nevirapine resistant HIV-1 isolates were cross-resistant to the NNRTIs efavirenz and delavirdine. However, nevirapine-resistant isolates were susceptible to the nucleoside analogues ZDV and ddI. Similarly, ZDV-resistant isolates were susceptible to nevirapine *in vitro*.

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MICROBIOLOGY REVIEW
DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)
NDA # 20-636 SE7-017; Date Reviewed: 03/25/02

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RECOMMENDATIONS

With respect to microbiology, this sNDA is approvable.

Phase IV Commitments

As a part of Phase IV commitments, the sponsor is requested to perform the following studies:

Determine the genotypes and phenotypes of multiple nevirapine resistant HIV-1 isolates from patients receiving nevirapine in combination with other antiretroviral agents. In addition, please provide data on the correlation of nevirapine plasma concentrations and plasma viral load with the emergence of nevirapine resistance mutations.

Microbiologist

CONCURRENCES:

HFD-530 /J. Farrelly/Assoc Dir Date _____

HFD-530/J. O'Rear /ATL Micro Date _____

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