CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

201152Orig1s000

MICROBIOLOGY REVIEW(S)

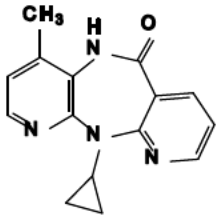
MICROBIOLOGY REVIEW
DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
NDA201152BL SDN 025; Review Completed: 03/21/11

Reviewer: LALJI MISHRA, Ph.D.
Date Submitted: 03/08/11
Date Received: 03/08/11
Date Assigned: 03/09/11
Sponsor: Boehringer Ingelheim Pharmaceuticals, Inc.
900 Ridgebury Rd
P.O. Box 368
Ridgefield, CT 06877-0368

Product Name(s):

Proprietary: Viramune®
Non-proprietary: Nevirapine
Chemical: 5, 11-dihydro- 11-cyclopropyl-4-methyl-6H-dipyrido-[3,2-b:2',3'-e][1,4] diazepin-6-one

Structure:



Nevirapine (NVP)

Molecular formula: $C_{15}H_{14}N_4O$
Molecular mass: 266.30
Drug category: Antiviral
Indication: Treatment of HIV infection in combination with other antiretroviral agents
Dosage Form/Route of administration: 400 mg extended release tablet/oral
Supporting documents: NDA 20-636

BACKGROUND

Boehringer Ingelheim Pharmaceuticals, Inc. (BIPI) submitted a new drug application for an extended release (XR) tablet of nevirapine for once daily dosing in combination with other antiretroviral agents for the treatment of HIV-1 infection. This NDA was reviewed previously and placed in DARRTS (Microbiology Review of NDA 201152 SDN 000 dated 01/04/11). In the current submission, BIPI has submitted final revised version of Viramune XR label. Microbiology label is reviewed here.

MICROBIOLOGY LABEL (03/15/11 version)

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, ß, γ or δ) are not inhibited by nevirapine.

Antiviral Activity

The antiviral activity of nevirapine has been measured in a variety of cell lines including peripheral blood mononuclear cells, monocyte-derived macrophages, and lymphoblastoid cell lines. In an assay using human embryonic kidney 293 cells, the median EC_{50} value (50% inhibitory concentration) of nevirapine was 90 nM against a panel of 2923 wild-type isolates of HIV-1 that were primarily (93%) clade B clinical isolates from the United States. The 99th percentile EC_{50} value was 470 nM in this trial. The median EC_{50} value was 63 nM (range 14-302 nM, n 29) against clinical isolates of HIV-1 clades A, B, C, D, F, G, and H, and circulating recombinant forms CRF01_AE, CRF02_AG and CRF12_BF. Nevirapine had no antiviral activity in cell culture against group O HIV-1 isolates (n 3) or HIV-2 isolates (n 3) replicating in cord blood mononuclear cells. Nevirapine in combination with efavirenz exhibited strong antagonistic anti-HIV-1 activity in cell culture and was additive to antagonistic with the protease inhibitor ritonavir or the fusion inhibitor enfuvirtide. Nevirapine exhibited additive to synergistic anti-HIV-1 activity in combination with the protease inhibitors amprenavir, atazanavir, indinavir, lopinavir, nelfinavir, saquinavir and tipranavir, and the NRTIs abacavir, didanosine, emtricitabine, lamivudine, stavudine, tenofovir and zidovudine. The anti-HIV-1 activity of nevirapine was antagonized by the anti-HBV drug adefovir and by the anti-HCV drug ribavirin in cell culture.

Resistance

HIV-1 isolates with reduced susceptibility (100- to 250-fold) to nevirapine emerge in cell culture. Genotypic analysis showed mutations in the HIV-1 RT gene encoding Y181C and/or V106A substitutions depending upon the virus strain and cell line employed. Time to emergence of nevirapine resistance in cell culture was not altered when selection included nevirapine in combination with several other NNRTIs.

Phenotypic and genotypic changes in HIV-1 isolates from treatment-naïve subjects receiving either nevirapine (n 24) or nevirapine and ZDV (n 14) were monitored in Phase 1 and 2 trials over 1 to ≥12 weeks. After 1 week of nevirapine monotherapy, isolates from 3/3 subjects had decreased susceptibility to nevirapine in cell culture. One or more of the RT mutations resulting in amino acid substitutions K103N, V106A, V108I, Y181C, Y188C, and G190A were detected in HIV-1 isolates from some subjects as early as 2 weeks after therapy initiation. By week eight of nevirapine monotherapy, 100% of the subjects tested (n 24) had HIV-1 isolates with a >100-fold decrease in susceptibility to nevirapine in cell culture compared to baseline, and had one or more of

the nevirapine-associated RT resistance substitutions. Nineteen of these subjects (80%) had isolates with Y181C substitutions regardless of dose.

Genotypic analysis of isolates from antiretroviral-naïve subjects experiencing virologic failure (n 71) receiving nevirapine once daily (n 25) or twice daily (n 46) in combination with lamivudine and stavudine (trial 2NN) for 48 weeks showed that isolates from 8/25 and 23/46 subjects, respectively, contained one or more of the following NNRTI resistance-associated substitutions: Y181C, K101E, G190A/S, K103N, V106A/M, V108I, Y188C/L, A98G, F227L, and M230L.

For trial 1100.1486, genotypic analysis was performed for baseline and on-therapy isolates from 23 and 34 virologic failure subjects in the VIRAMUNE XR and immediate-release VIRAMUNE treatment groups, respectively. Nevirapine resistance-associated substitutions developed in the on-therapy isolates of 78% (18/23) of the virologic failures subjects in the VIRAMUNE XR treatment group and 88% (30/34) of the subjects in the immediate-release VIRAMUNE treatment group, respectively. The Y181C nevirapine resistance-associated substitution was found alone or in combination with other nevirapine resistance-associated substitutions (K101E, K103N, V106A, V108I, Y188 C/F/H/L/N, G190A, P225H, F227L, M230L) in isolates from 14 subjects failing VIRAMUNE XR treatment and 25 subjects failing immediate-release VIRAMUNE treatment. On-therapy isolates from 1 subject in the VIRAMUNE XR treatment group developed a novel amino acid substitution Y181I and isolates from another subject in the immediate-release VIRAMUNE treatment group developed a novel amino acid substitution Y188N. Phenotypic analysis showed that Y188N and Y181I substitutions conferred 103- and 22-fold reductions in susceptibility to nevirapine, respectively.

Cross-resistance

Rapid emergence of HIV-1 strains which are cross-resistant to NNRTIs has been observed in cell culture. Nevirapine-resistant HIV-1 isolates were cross-resistant to the NNRTIs delavirdine and efavirenz. The Y188N conferred 22- and 7-fold reductions in susceptibility to delavirdine and efavirenz, respectively, but no decrease in susceptibility to etravirine. Similarly, the Y181I substitution reduced susceptibility to delavirdine and etravirine 3- and 8-fold, respectively, but did not reduce susceptibility to efavirenz. However, nevirapine-resistant isolates were susceptible to the NRTIs ddI and ZDV. Similarly, ZDV-resistant isolates were susceptible to nevirapine in cell culture.

RECOMMENDATIONS

With respect to virology, the revised version of the Microbiology label is approvable.

_____
Lalji Mishra, Ph.D.
Microbiologist

MICROBIOLOGY REVIEW
DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
NDA201152BL SDN 025; Review Completed: 03/21/11

CONCURRENCES:

_____ Date_____
HFD-530/J. O'Rear /TL Micro

CC:
HFD-530/NDA 201152
HFD-530/ Division File
HFD-530/ Micro/L. Mishra
HFD-530/CSO/Himaya, A.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

LALJI MISHRA
03/22/2011

JULIAN J O'REAR
03/22/2011

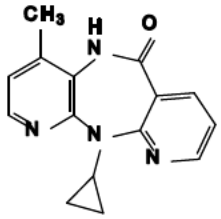
MICROBIOLOGY REVIEW
DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
NDA201152 SDN 000; Review Completed: 01/04/11

Reviewer: LALJI MISHRA, Ph.D.
Date Submitted: 06/03/10
Date Received: 06/03/10
Date Assigned: 06/03/10
Sponsor: Boehringer Ingelheim Pharmaceuticals, Inc.
900 Ridgebury Rd
P.O. Box 368
Ridgefield, CT 06877-0368

Product Name(s):

Proprietary: Viramune®
Non-proprietary: Nevirapine
Chemical: 5, 11-dihydro- 11-cyclopropyl-4-methyl-6H-dipyrido-[3,2-b:2',3'-e][1,4] diazepin-6-one

Structure:



Nevirapine (NVP)

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Drug category: Antiviral
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Dosage Form/Route of administration: 400 mg extended release tablet/oral
Supporting documents: NDA 20-636

Additional submissions reviewed:

(b) (4)

BACKGROUND

Boehringer Ingelheim Pharmaceuticals, Inc. (BIPI) has submitted a new drug application for an extended release (XR) tablet of nevirapine for once daily dosing in combination with other antiretroviral

agents for the treatment of HIV-1 infection. Nevirapine (NVP) is an FDA approved non-nucleoside reverse transcriptase inhibitor (NNRTI) of HIV-1. The prescribed dose of an extended release solid formulation of NVP is one 200 mg tablet daily of immediate release once daily for the first 14 days followed by one 400 mg tablet once daily.

The sponsor has cross-referred to IND 36026 and NDA 20636 for NVP (Viramune®) for Microbiology and Pharmacology/Toxicology information. Please refer to the Microbiology label (Pages 21-23) for information on the antiviral activity, resistance and cross-resistance of NVP. Data on the antiviral activity of NVP is reproduced here from the Microbiology label.

Antiviral Activity
The antiviral activity of nevirapine has been measured in a variety of cell lines including peripheral blood mononuclear cells, monocyte-derived macrophages, and lymphoblastoid cell lines. In an assay using human embryonic 293 kidney cells, the median EC_{50} value (50% inhibitory concentration) of nevirapine was 90 nM against a panel of 2923 wild-type isolates of HIV-1 that were primarily clade B clinical isolates from the United States, but which included 7% non-clade B isolates. The 99th percentile EC_{50} value was 470 nM in this study. The median EC_{50} value was 63 nM (range 14-302 nM, n 29) against clinical isolates of HIV-1 clades A, B, C, D, F, G, and H, and circulating recombinant forms (CRF) CRF01_AE, CRF02_AG and CRF12_BF. Nevirapine had no antiviral activity in cell culture against group O HIV-1 isolates (n 3) or HIV-2 isolates (n 3) replicating in cord blood mononuclear cells. Nevirapine in combination with efavirenz exhibited strong antagonistic anti-HIV-1 activity in cell culture and was additive to antagonistic with the protease inhibitor ritonavir or the fusion inhibitor enfuvirtide. Nevirapine exhibited additive to synergistic anti-HIV-1 activity in combination with the protease inhibitors amprenavir, atazanavir, indinavir, lopinavir, nelfinavir, saquinavir and tipranavir, and the NRTIs abacavir, didanosine, emtricitabine, lamivudine, stavudine, tenofovir and zidovudine. The anti-HIV-1 activity of nevirapine was antagonized by the anti-HBV drug adefovir and by the anti-HCV drug ribavirin in cell culture.

Resistance
One or more of the RT mutations resulting in amino acid substitutions K103N, V106A, V108I, Y181C, Y188C and G190A were detected in HIV-1 isolates from patients (n 24) on nevirapine monotherapy. Nineteen of the 24 patients (80%) had isolates with Y181C substitutions by week 8 regardless of dose.

Genotypic analysis from the 2NN study of isolates from antiretroviral naïve patients experiencing virologic failure (n 71) receiving nevirapine once daily (n 25) or twice daily (n 46) in combination with lamivudine and stavudine for 48 weeks showed that isolates from 8/25 and 23/46 patients, respectively, contained one or more of the following NNRTI resistance-associated substitutions: Y181C, K101E, G190A/S, K103N, V106A/M, V108I, Y188C/L, A98G, F227L and M230L.

Cross-resistance
Rapid emergence of human immunodeficiency virus type 1 (HIV-1) strains which are cross-resistant to NNRTIs has been observed in cell culture. Nevirapine-resistant HIV-1 isolates were cross-resistant to the NNRTIs delavirdine and efavirenz. Efavirenz shares a similar resistance profile with nevirapine with one exception: Y181C is the most common mutation selected by nevirapine and it is not selected by efavirenz.

In support of this NDA, the sponsor has submitted data from two clinical studies 1100.1486 and 1100.1526.

Study 1100.1486 is a randomized, double blind, double dummy, parallel group, active controlled trial to evaluate the antiviral efficacy of 400 mg QD nevirapine extended release formulation in comparison to 200 mg BID nevirapine immediate release in combination with Truvada® in antiretroviral therapy naïve HIV-1 infected subjects.

The primary endpoint of this study was sustained virologic response through Week 48. A virologic response was defined by two consecutive viral load (VL) measurements of <50 copies/mL, at least two weeks apart without virologic rebound or change of antiretroviral (ARV) therapy through Week 48. The time window of Week 48 was defined as 48 ± 4 weeks from Day 0 (baseline was the day a subject initiated dosing with nevirapine IR). HIV-1 RNA in plasma was assayed using the COBAS TaqMan quantitative assay.

Please see reviews of Statistical Reviewer Susan Zhou Ph.D. for efficacy and Medical Officer. Peter Miele, MD for safety analysis.

The sponsor has also submitted a substudy (Study U10-3152-01) which compares the genotypes of viruses isolated from subjects who experienced virological failure or transient increase in viral load in Study 1100.1486 with the viral genotypes of the corresponding baseline samples. Please note that baseline isolates refer to the genotype of plasma samples collected at the screening (visit 1) prior to administration of NVP loading dose. The resistance study is reviewed here.

I. Baseline characteristics of subjects enrolled in study 1100.1486

One thousand sixty-eight (1068) subjects were entered into the study and started to receive the nevirapine 200 mg IR QD lead in dose. Out of these 1068 subjects, 1011 were randomized and treated with either nevirapine 400 mg XR QD or nevirapine 200 mg IR BID.

For randomized subjects, the demographic data and baseline characteristics were similar between nevirapine IR and nevirapine XR treatment groups. The mean baseline viral load was 4.7 $\log_{10}$ copies/mL for subjects in both treatment groups (Table 1, Page 3). About one third of the subjects had baseline viral load >100,000 copies/mL for both treatment groups. The mean baseline $CD4^+$ cell count was 228 cells/mm^3 for nevirapine IR and 230 for nevirapine XR, with 91% of subjects in the >50-350 category for both treatment groups. The percentage of HIV-1 sub-type B was 71% and 75% for nevirapine IR and XR groups, respectively; sub-type A consisted of 7% and 6% of subjects in the IR and XR groups, respectively; 13% and 11% subjects had subtype C in the IR and XR groups, respectively.

Table 1: Baseline characteristics of subjects enrolled in study 1100.1486 (Source: NDA201152, report U10-3212-01, Table 11.2:2, Pages 74-75)

	Not Randomized	NVP IR 200mg BID	NVP XR 400mg QD	Total
Number of patients	55 (100.0)	508 (100.0)	505 (100.0)	1068 (100.0)
Baseline HIV (log10 copies/mL)[1]				
Mean	4.7	4.7	4.7	4.7
SD	0.7	0.6	0.7	0.7
Median	4.8	4.7	4.7	4.7
Baseline HIV RNA (copies/mL)[1] (N [%])				
<1,000	0 (0.0)	2 (0.4)	3 (0.6)	5 (0.5)
1,000 - 10,000	8 (14.5)	82 (16.1)	77 (15.2)	167 (15.6)
>10,000-100,000	29 (52.7)	250 (49.2)	260 (51.5)	539 (50.5)
>100,000-1,000,00	17 (30.9)	167 (32.9)	155 (30.7)	339 (31.7)
>1,000,000	1 (1.8)	7 (1.4)	10 (2.0)	18 (1.7)
Baseline CD4+ count (cells/mm^3)[1]				
N	55	507	503	1065
Mean	225.5	227.6	229.6	228.4
SD	83.0	85.9	81.4	83.6
Median	234.5	224.5	227.5	225.5
Baseline CD4+ count (cells/mm^3)[1] (N [%])				
N	55 (100.0)	508 (100.0)	505 (100.0)	1068 (100.0)
≤50	2 (3.6)	1 (0.2)	0 (0.0)	3 (0.3)
>50-200	20 (36.4)	200 (39.4)	179 (35.4)	399 (37.4)
>200-350	30 (54.5)	263 (51.8)	282 (55.8)	575 (53.8)
>350-<400	2 (3.6)	31 (6.1)	34 (6.7)	67 (6.3)
≥400	1 (1.8)	12 (2.4)	8 (1.6)	21 (2.0)
Missing	0 (0.0)	1 (0.2)	2 (0.4)	3 (0.3)
HIV sub-type (N [%])				
A[2]	9 (16.4)	37 (7.3)	30 (5.9)	76 (7.1)
B	35 (63.6)	360 (70.9)	379 (75.0)	774 (72.5)
C	4 (7.3)	67 (13.2)	55 (10.9)	126 (11.8)
Other[3]	6 (10.9)	43 (8.5)	38 (7.5)	87 (8.1)
Missing	1 (1.8)	1 (0.2)	3 (0.6)	5 (0.5)

[1] Baseline values are calculated as the average of the last two measurements prior to the start of lead-in treatment. HIV-1 RNA viral load is based on TaqMan assay results.
[2] Including A, A/C, A/D, A/G, A1, AE, AG and AG/B.
[3] Including B/C, BF, Complex, D, F, F1, F2 and G.

II. Viral load in subjects treated with NVP IR and NVP XR

Efficacy results are briefly summarized here. For a complete analysis of efficacy data, please see review of Susan Zhou, Ph.D. statistical reviewer.

The primary efficacy endpoint was sustained virologic response at Week 48 using LLOQ 50 copies/mL. Table 2 (Page 4) shows the virologic response for NVP IR and NVP XR treated subjects.

The overall sustained virologic response proportion was 75.9% for nevirapine IR and 81.0% for nevirapine XR treatment group. The virologic failure proportion was 5.9% in IR and 3.2% in XR treatment group. There were 18.2% and 15.8% of subjects who discontinued study drug prior to Week 48 in the nevirapine IR and XR group, respectively.

Table 2: Summary of study outcome at Week 48 with LLOQ 50 copies/mL (Amplicor-corrected, TLOVR) – FAS (Source: NDA 201152, Study Report U10-3201-10, Page 77, Table 11.4.1.1.1: 2)

Outcome (LLOQ = 50 copies/mL)	NVP IR 200mg BID N (%)	NVP XR 400mg QD N (%)
Total	506 (100.0)	505 (100.0)
Responder	384 (75.9)	409 (81.0)
Failure	122 (24.1)	96 (19.0)
Virologic failure	30 (5.9)	16 (3.2)
Never suppressed through Week 48	13 (2.6)	5 (1.0)
Rebound	17 (3.4)	11 (2.2)
Discontinued study drug prior to Week 48	92 (18.2)	80 (15.8)
Death or events leading to death	3 (0.6)	1 (0.2)
Adverse events	42 (8.3)	32 (6.3)
Loss to follow-up	6 (1.2)	7 (1.4)
Consent withdrawn	9 (1.8)	3 (0.6)
Non-compliance	7 (1.4)	5 (1.0)
Lack of efficacy	24 (4.7)	23 (4.6)
Pregnancy	0 (0.0)	6 (1.2)
Other	1 (0.2)	3 (0.6)

Comment:

Virological failure proportion (%) as presented by the sponsor in Table 2 does not match with the DAVP analysis. Please see review of Dr. Susan Zhou, Ph.D. Statistical reviewer. According to Dr. Zhou's analysis, proportion of patients with virological failure in NVP IR and NVP XR treatment groups were 13% and 11%, respectively.

Table 3: Proportion of virologic response at Week 48 using LLOQ 50 copies/mL by baseline characteristics and lead-in duration (Amplicor – corrected, TLOVR) – FAS (Source: NDA201152, report U10-3202-01, Page 81, Table 11.4.1.1.3: 2)

	No. with response/total No. (%)	
Baseline characteristics	NVP IR 200mg BID	NVP XR 400mg QD
Total number of patients	384/506 (75.9)	409/505 (81.0)
Baseline HIV-1 RNA stratum copies/mL [N (%)]		
≤100,000	240/303 (79.2)	267/311 (85.9)
>100,000	144/203 (70.9)	142/194 (73.2)
HIV-1 sub-type [N(%)]		
A[1]	30/ 37 (81.1)	20/ 30 (66.7)
B	272/358 (76.0)	314/379 (82.8)
C	52/ 67 (77.6)	43/ 55 (78.2)
Other[2]	29/ 43 (67.4)	29/ 38 (76.3)
Missing	1/ 1 (100)	3/ 3 (100)

[1] Including A, A/C, A/D, A/G, A1, AE, AG and AG/B.
[2] Including B/C, BF, Complex, D, F, F1, F2 and G.

In summary, subjects with low baseline HIV-1 viral load (≤100,000 copies/mL) responded better.

III. Treatment emergent amino acid substitutions in isolates from virologic failure subjects

Genotypes of baseline isolates were available from 1008 of the 1011 subjects treated with either nevirapine 200 mg IR BID or nevirapine 400 mg XR QD. Eighty six of the 1008 subjects were selected to have nucleotide sequence analysis of on treatment specimens based on a review of the virologic profiles. These 86 subjects represent only the subjects for which genotype results were reported; in some other subjects, amplification failed or was not attempted because of low viral load (<500 copies/ml) and a genotype was not reported. The genotyped subjects were classified into 4 distinct groups of subjects based on the data available at the time of the 48 week database lock:

1. 48-week responders: Met the primary endpoint for the study after experiencing transient increase in viral load during the course of the study, or failed after week 48 (n 4).
2. Discontinued treatment for reasons other than efficacy. These subjects stopped treatment early for one or more of the following reasons: adverse events, being noncompliant with the protocol or withdrawal of consent (n 25). Three additional subjects whose on-therapy isolates developed NVP resistance-associated substitutions are considered virologic failure for resistance analysis.
3. Partial response: Achieved a ≥1 $\log_{10}$ decline from baseline in VL but never achieved a VL nadir of <400 copies/mL (n 25).
4. Rebounders: Achieved a VL nadir of <400 copies/mL with subsequent VL increase to >500 copies/mL (n 32)

The rebounders (Group 4) were further classified based on VL nadir reaching <50 copies/mL and the timing of virologic rebound (at or before Week 16 classified as early):

4a. VL nadir <50 copies/mL with early rebound (n 6)
4b. VL nadir <50 copies/mL with late rebound (n 7)
4c. VL nadir 50 399 copies/mL with early rebound (n 16)
4d. VL nadir 50 399 copies/mL with late rebound (n 3)

Although some subjects had multiple on-treatment specimens sequenced, the genotypic and phenotypic analysis of emergence of resistance was based exclusively on the first available post-failure specimen that was successfully amplified and genotyped.

All genotypic testing was conducted by [redacted] (b) (4) using their commercially available [redacted] (b) (4). All phenotypic testing was conducted by [redacted] (b) (4) using their commercially available [redacted] (b) (4) assay.

Baseline characteristics of virologic failure subjects are shown in Table 4.

The majority of the subjects (56/86, 65.1%) included in the resistance study had high HIV-1 viral load at baseline (>10^6 copies/mL). Clade B was the most predominant subtype (65/86, 75.6%) among the substudy subjects

Table 4: Baseline characteristics of subjects included in resistance substudy (Source; NDA 201152, study report U10-3152-01, Page 17, Table 7.1:1)

	Total [(N(%)]
Number of subjects	86 (100.0)
Viral load (copies/mL)	
<10,000	7 (8.1)
10,000-100,000	23 (26.7)
>100,000	56 (65.1)
HIV-1 subtype	
Subtype B	65 (75.6)
Non-B	21 (24.4)

IV. Genotypic analysis of isolates from virologic failure subjects

On-therapy HIV-1 isolates were genotyped from a total of 86 subjects who were either 48-week responders, discontinued therapy for reasons other than efficacy, responded only partially, or responded to at least a viral load (VL) nadir of <400 copies/mL and subsequently experienced virologic rebound. The breakout of the 86 subjects by these failure groupings is shown below in Table 5. Four subjects were responders to treatment at Week 48, 3 in the nevirapine IR group and 1 in the nevirapine XR treatment group. Twenty-five out of 86 subjects discontinued for reasons other than virologic failure prior to Week 48. The reasons for discontinuation were adverse events consent withdrawn or non-compliance.

A total of 57 subjects out of the 86 subjects experienced virologic failure, with 25 being classified as a partial responder, 32 as virologic rebounders.

Table 5: Summary of failure groupings for the subjects included in the substudy (Source; NDA 201152, study report U10-3152-01, Page 18, Table 7.2:1)

	NVP IR	**NVP XR**
Number genotyped	54	32
48 week responder	3	1
Discontinued due to reasons other efficacy i.e., AE, non-compliance	17	8
Partial Responder	14	11
Early Rebounder (≤Weeks 16)	14	8
Late rebounder (>Week 16)	6	4

V. Genotypes of baseline and on-therapy isolates from virologic failure subjects

Genotypes of isolates from virologic failure subjects receiving NVP IR are shown in Table 6 below. Please note that genotypes of isolates from subjects (n 25) who discontinued due to adverse reaction, non-compliance etc are not included. However, on-therapy isolates from 3 subjects who discontinued due to adverse reactions or non-compliance, but developed NVP resistance-associated amino acid

substitutions are included for resistance analysis. Baseline isolates refer to the genotype of plasma samples collected at the screening (visit 1) prior to administration of NVP loading dose.

Table 6: Emergence of NVP resistance-associated substitutions in RT of isolates from subjects receiving NVP IR (Source: NDA 201152, study report U10-3152-01, Pages 140-161, Listing 1.31 and Resistance data in DAVP Template Format)

Subject ID	Response	Clade	Week	NVP resistance-associated amino acid substitutions
10167	Partial responder	BF	BL	V179D
			WK 6.3	V179D, Y181C
10347	Early rebounder	B	BL	-
			WK 6	V108I/V,Y181C
			WK 15.9	V90I/V,Y181C
11332	Early rebounder	B	BL	-
			WK 16.1	Y181C
11386	Late rebounder	B	BL	-
			WK 25	Y188N
11549	Partial responder	B	BL	V106I
			WK 8.1	V106I, Y181C
			WK 10	V106I, Y181C
11579	Partial responder	B	BL	V90I
			WK 6	V90I, Y181C/Y, Y188L
			WK 12.1	V90I, Y188L
11821	Partial responder	B	BL	-
			WK 6.9	V106A, F227L
12234	Early rebounder	C	BL	K103N, E138G, P225H/P
			WK 16.1	V90I/V, K103N, E138G, P225H/P
12416	Partial responder	B	BL	-
			WK 5.9	K101E/K/Q, Y181C
			Wk 7.9	K101E/K/Q, Y181C
12452	Early rebounder	B	BL	-
			WK 15.9	K103K/N, Y181C
			WK 23.9	K103N, V108I/V, Y181C
13079	Early rebounder	B	BL	-
			WK 17.3	K103N, Y181C/Y
13968	Partial responder	B	BL	-
			WK 8	K101E/K, E138E/G, Y181C/Y, G190A
14105	Partial responder	B	BL	-
			WK 3.9	K101E/K, Y181C
			WK 5.9	K103K/N, Y181C
14187	Early rebounder	B	BL	V106I, V179I, Y188L
			WK 7.9	V106I, V179I, Y188L

14188	Early rebounder	B	BL	-
			WK 8	V108I/V, Y181C
			WK 12	K101E/K, V108I/V, Y181C
14205	48-Week responder	B	BL	-
			WK* 65.3	V106A
14208	Early rebounder	B	BL	-
			WK 15.9	K103N, Y181C
14374	Partial responder	B	BL	-
			WK 8	K103K/N
			Wk 12	K103N
14386	Late rebounder	B	BL	-
			WK 48.3	V106A, Y181C/Y, F227L
14930	Partial responder	B	BL	-
			WK 11.9	V108I, Y181C
15348	Early rebounder	B	BL	-
			WK 16	K101E/K, V108I, Y181C
			WK 23.7	V108I, Y181C
15709	Partial responder	Complex	BL	V179D/V
			WK 8	K103N, Y181C
15996	Partial responder	B	BL	A98S
			WK 23.6	A98S, K101E/K, Y181C
16003	Late rebounder	G	BL	-
			WK 32	Y181C
			WK 40	Y181C
16037	Partial responder	G	BL	-
			WK 12.6	K103N, Y181C/Y
16249	Early rebounder	C	BL	-
			WK 16.9	K103K/N, V106M/V, V179I/V
16250	Early rebounder	C	BL	-
			WK 16.9	K103K/N, V108I/V, Y181C/Y
16251	Early rebounder	C	BL	-
			WK 15.7	K103K/N, Y181C/Y
16812	Early rebounder	C	BL	-
			WK 23.2	K103N, V106M
17200	48-Week responder	B	BL	-
			WK 16	V106I/V
17549	Partial responder	B	BL	V179I
			WK 4	V179I, Y181C
			WK 6	V179I, Y181C
17596	Early rebounder	B	BL	V179E
			WK 8	V179E, Y181C
17615	Partial responder	B	BL	-
			WK 5.7	Y181C

			WK 7	Y181C
18637	Late rebounder	B	BL	-
			WK 19	Y181C
			WK 24.1	Y181C

Comment: Subjects #15087 and #16052 in the NVP IR treatment group were late rebounders. Baseline and WK 24 isolates for subject #15087 or the WK 23 isolate for subject #16052 did not contain any NNRTI resistance-associated substitutions. These subjects were not included in Table 6.

(b) (4)

On-therapy isolates at WK 65.3 from 48 Week responder subject #14205 and at WK 16 from 48 Week responder subject #17200 contained amino acid substitutions V106A and V106I, respectively.

Table 7: Emergence of NVP resistance-associated substitutions in RT of isolates from subjects receiving NVP XR (Source: NDA 201152, study report U10-3152-01, Pages 140-161, Listing 1.31 and Resistance data in DAVP Template Format)

Subject ID	Response	Clade	Week	NVP resistance-associated amino acid substitutions
10238	Late rebounder	BF	BL	-
			WK 39.4	V179D/E/V
10881	Partial responder	D	BL	-
			WK 13.7	V90I/V, Y181C
10990	Partial responder	B	BL	V106I, Y188L, P225H
			WK 6	V106I, Y188L, P225H
11026	Partial responder	B	BL	-
			WK6.1	Y181C
11078	Early rebounder	B	BL	-
			WK 15.6	V106A, Y181C
11276	Late rebounder	B	BL	-
			WK 39.1	V90I, V108I/V, Y181C
11383	Partial responder	A/G	BL	-
			WK 7.6	Y181C, M230L/M
11494	Partial responder	B	BL	-
			WK 4	K101K/R, V108I/V, Y181C
			WK 6	K101K/R, V108I/V, Y181C, G190A/G
11560	Partial responder	B	BL	-
			WK 12	Y181I
			WK 12.9	Y181I/V
12349	Early rebounder	B	BL	-
			WK 8.3	K101E/K, K103K/N, V106A/V, Y181C

12362	Partial responder	B	BL	E138A
			WK 3.9	E138A
13070	Partial responder	B	BL	V106I/V, E138A, V179D
			WK 12	V106I, E138A, V179D, Y188C, M230L
14201	Early rebounder	B	BL	-
			WK 16	K103K/N, Y181C/Y, G190A/G
14575	Early rebounder	B	BL	-
			WK 25.1	K103K/N, Y188F/H/L/Y
16036	Late rebounder	B	BL	-
			WK 31.7	K103K/N, Y181C
16820	WK 48 Responder	C	BL	-
			WK 27	K103K/N
17100	Partial responder	B	BL	-
			WK 16.9	Y181C, M230L
			WK 24.3	Y181C, M230L
17198	Partial responder	B	BL	-
			WK 6	V108I/V, Y181C
			WK 12	V108I/V, Y181C
17556	Early rebounder	B	BL	-
			WK 3.7	Y181C
			WK 16	V90I/V, K103K/N, Y181C, G190A/G
17645	Early rebounder	B	BL	V90I, K103R, V179I/V
			WK 8	V90I, K103R, V108I, Y181C/Y
			WK 12.3	V90I, K103R, V108I, Y181C
18407	Partial responder	B	BL	-
			WK 8.1	K103K/N, Y181C
			WK 25.9	K103K/N, Y181C

Comment: Subjects #11325 and #17981 in NVP XR treatment group were early rebounders, baseline and on-therapy isolates from subject #11325 at WK 11.9 and subject #17981 at WK 21.4 did not develop NNRTI-resistance associated substitutions. Similarly, baseline and WK 37 isolates from subject #14718 (late rebounder) did not develop NNRTI resistance-associated substitutions.

On-therapy isolates from subject #16820 (48 Week responder) developed a K103N substitution in RT at WK 27.

(b) (4)

Table 8: Frequency of nevirapine resistance-associated substitutions emerging in on-therapy isolates from virologic failure subjects (Source: Data from Table 6 and Table 7 above)

	NVP IR	NVP XR
Partial Responder	Y181C (n 1)	V90I/V, Y181C (n 1)
	K101E/K/Q, Y181C (n 1) K101E/Q, Y181C (n 2)	Y181C, M230L/M (n 1) Y181C, M230L (n 1)
	K101E/Q, E138E/G, Y181C/Y, G190A (n 1)	K101K/R, V108I/V, Y181C, (n 1)
	K103N, Y181C (n 1), K103K/N (n 1)	K103K/N, Y181C (n 1) Y181I (n 1), **E138A** (n 1)
	V90I, Y181C/Y, Y188L (n 1) V106A, F227L (n 1) **V106I**, Y181C (n 1) V108I, Y181C (n 1) **V179D**, Y181C (n 1) **V179I**, Y181C (n 1)	**V106I, E138A, V179D**, Y188C, M230L (n 1) **V106I, Y188L, P225H** (n 1) V108I/V, Y181C (n 1)
Early Rebounder	Y181C (n 1)	V106A, Y181C (n 1)
	K101E/K, V108I, Y181C (n 1) K103N, Y181C (1) K103K/N, Y181C (n 1) K103N, Y181C/Y (n 1) K103K/N, Y181C/Y (n 1) K103K/N, V108I/V, Y181C/Y (n 1) K103K/N, V106M/V, V179I/V (n 1) K103N, V106M (n 1) V90I/V, **K103N, E138G, P225H/P** (n 1) **V106I, V179I, Y188L** (n 1) V108I/V, Y181C (n 2) **V179E,** Y181C (n 1)	K101E/K, K103K/N, V106A/V, Y181C (n 1) K103K/N, Y181C/Y, G190A/G (n 1) K103K/N, Y188F/H/L/Y (n 1) V90I/V, K103K/N, Y181C, G190A/G (n 1) **V90I, K103R**, V108I, Y181C/Y (n 1)
Late Rebounder*	V106A,Y181C/Y, F227L (n 1)	V90I, V108I/V, Y181C (n 1)
	Y181C (n 2) Y188N (n 1)	K103K/N, Y181C (n 1)
Discontinued but developed NVP resistance - associated substitutions	K103N, Y181C/Y (n 1)	V179D/E/V (n 1) Y181C (n 1)

Note: Amino acid substitutions present in baseline isolates are shown in bold. Substitutions emerging in the earliest time points are shown in Table 7

*= NNRT resistance-associated substitutions not detected in isolates from 2 late rebounder subjects in NVP IR treatment group and 1 late rebounder subject in NVP XR group**.** Similarly, NNRT resistance-associated substitutions not detected in isolates from 2 early rebounder subjects and 1 late rebounder subjects in the NVP XR treatment group.

Results presented in Table 6, 7 and 8 (above) are summarized here. Only NVP resistance-associated substitutions are counted in the mixed population of mutants for this analysis, i.e., K103K/N is counted as K103N.

1. The nevirapine resistance-associated substitution Y181C developed either alone (n 1) or in combination with other NVP resistance-associated substitutions in on-therapy isolates from partial responder subjects (n 10) in the NVP IR treatment group. Other NVP resistance-associated substitutions detected in on-therapy isolates were: K101E/Q, K103N, V106A, V108I, V179D/I, Y188L, G190A and F227L. Additionally, amino acid substitution E138G in combination with K101E/Q, Y181C and G190A was also detected in on-therapy isolates from 1 partial responder subject in the NVP IR treatment group. Similar to NVP IR treatment group, on-therapy isolates from 6 of the 10 partial responder subjects in the NVP XR treatment group developed Y181C amino acid substitution in combination with other NVP resistance-associated substitutions. An additional amino acid substitution, Y181I, developed in on-therapy isolates from 1 subject in the NVP XR treatment group. This is a new amino acid substitution which confers resistance to nevirapine and also confers reduced susceptibility to etravirine (see phenotypic analysis data, Table 9, Page 14). The amino acid substitution E138A alone was present in both baseline and on-therapy isolates from 1 partial rebounder subject and in combination with other NVP resistance-associated substitution in on-therapy isolates from another partial rebounder subject in the NVP XR treatment group. Other NVP substitutions detected in on-therapy isolates from partial rebounder subjects in the NVP XR treatment group were: V90I, V106I, V108I, Y188C/L, P225H, and M230L.
2. The amino acid substitution Y181C developed either alone (n 1) or in combination with other NVP resistance-associated substitutions in on-therapy isolates from early rebounder subjects (n 9) in the NVP IR treatment group. Other NVP resistance-associated substitutions which developed in on-therapy isolates from early rebounder subjects were: V90I, K101E, K103N, V106M, and V108I. The amnio acid substitution K103N in combination with Y181C was detected in on-therapy isolates from 5 early rebounder subjects in the NVP IR treatment group. Amino acid substitutions K103N, E138G, and P225H (n 1); V106I, V179I, and Y188L (n 1) and V179E (n 1) were present in baseline and on-therapy isolates from early rebounder subjects in the NVP IR treatment group. Similar to NVP IR treatment group, on-therapy isolates from 5 early rebounder subjects in the NVP XR treatment group developed the amino acid substitution Y181C in combination with other NVP resistance-associated substitutions. Other NVP resistance-associated substitutions detected in combination with Y181C substitutions were K101E, K103N, V106A, V108I, and G190A. On-therapy isolates from 1 early rebounder subject in the NVP XR treatment group developed amino acid substitution Y188F/H/L/Y in combination with K103N. The amino acid substitutions V90I and K103R were present in baseline and on-therapy isolates from 1 early rebounder subject. Baseline and on therapy isolates from 2 early rebounder subjects (#11325 and #17981) in the NVP XR treatment did not develop any NNRTI resistance-associated substitutions.

3. The amino acid substitution Y181C developed either alone (n 2) or in combination with amino acid substitutions V106A and F227L in on-therapy isolates from late rebounder subjects (n 1) in the NVP IR treatment group. On-therapy isolates from 1 late rebounder subject developed amino acid substitution Y188N. The amino acid substitution Y188N is a new substitution identified in this study to be associated with NVP resistance. Baseline and on therapy isolates from 2 late rebounder subjects (#15087, #16052) in the NVP IR treatment group did not develop any NNRTI resistance-associated substitutions. The amino acid substitution Y181C in combination with V90I, K103N, V108I substitutions developed in on-therapy isolates from 2 late rebounder subjects in the NVP XR treatment group. Baseline and on-therapy isolates from 1 late rebounder subject (# 14718) in the NVP XR treatment group did not develop any NNRTI-resistance-associated substitutions.
4. [redacted] (b) (4)

Comment

According to Stanford database, V90I and V106I are polymorphisms (http://hivdb.stanford.edu/pages/GRIP/ETR.html) that occur in isolates of 1% of treated and untreated persons and are not associated with NNRTI resistance. V179I, a common polymorphism, occurs in isolates of 2% of untreated person and 12% of persons receiving NNRTI (Stanford database). K103R and E138A are listed as polymorphisms.

VI. Phenotypic analysis of failure isolates with new NVP resistance-associated substitutions or no NNRTI resistance-associated substitutions

Phenotypic analysis of paired baseline and on-therapy isolates with no known NVP-resistance-associated substitution or of isolates with no NNTI resistance-associated substitutions was performed using [redacted] (b) (4) assay.

Table 9: Susceptibility of isolates to NNRTIs (Source; NDA 201152, study report U10-3152-01, Pages 162-168, Listing 2.2)

Subject ID	Treatment	NVP substitution	Fold change in EC_{50} values				
			Isolate	NVP	DLV	EFV	ETR
10238	NVP XR	None	BL	0.3	0.3	0.4	0.5
		V179D/E/V	WK 39.4	0.3	0.4	0.8	0.7
11325	NVP XR	None	BL	1.9	1.4	1.1	1.3
			WK 11.9	1.2	1.	0.9	1.
11386	NVP IR	None	BL	0.75	1.2	1.1	0.9

		Y188N	WK 25	77	26	7.2	0.3
11560	NVP XR	None	BL	8.2	3.4	1.2	1.8
		Y181I	WK 12	184	11	1.1	14
12362	NVP XR	E138A	BL	6.5	8.8	2.7	5.1
		E138A	WK 3.9	3.5	6.1	2.4	4.8
13070	NVP XR	V106I, E138A, V179D	BL	1.3	4.9	1.8	2.3
		V106I, E138A, V179D, Y188C, M230L	WK 12	168	147	104.	7.7
16052	NVP IR	None	BL	0.3	0.5	0.6	0.6
			WK 23	0.1	0.3	0.4	0.5

Table 9 lists the fold change in EC_{50} values of NVP, DLV, EFV and ETR for baseline and on-therapy isolates with new NVP resistance-associated substitution or with no NNRTI resistance-associated substitution but grouped as failure.

Comment: Fold change in EC_{50} values are rounded off except for EC_{50} value of NVP for baseline isolates of subject #11386.

Table 9 shows the following:

The Y188N substitution conferred103-, 22- and 7-fold deceases in susceptibility to NVP, DLV and EFV, but no decrease in susceptibility to ETR. Similarly, the Y181I substitution decreased susceptibility to NVP, DLV and ETR by 22-, 3- and 8-fold. However, the Y181I substitution did not confer reduced susceptibility to EFV. Both Y181I and Y188N substitutions were observed for the first time in this study to confer reduced susceptibility to NVP. However, Y181I has been previously shown to confer reduced susceptibility to ETR.

Amino acid substitution V179D/E/V observed in on-therapy isolates from a subject #10238) in the NVP XR treatment did not confer reduce susceptibility to NVP, DLV, EFV and ETR. BIPI regrouped this subject as non-compliant. However, for resistance analysis, this subject was considered as a virologic failure.

Amino acid substitution E138A was present in baseline isolates and did not decrease susceptibility of on-therapy isolates to NVP, DLV, EFV and ETR. The amino acid substitutions Y188C plus M230L, previously shown to confer resistance to NVP, decreased susceptibility of on-therapy isolates to NVP, DLV, EFV, and ETR by 129-, 30- , 58- and 3-fold, respectively.

Isolates from subjects #11325 and #16052 with no NNRTI resistance-associated substitutions were susceptible to NVP, DLV, EFV, and ETR. These subjects were early and late rebounders, respectively.

Phenotypes of baseline and on-therapy isolates from late rebounder subjects #14718, #15087 and #17981 were not available. Baseline and on-therapy isolates from these subjects did not contain any NNRTI-resistance-associated substitutions.

VII. Emergence of NVP resistance substitutions by HIV-1 baseline characteristics and NVP trough levels

Table 10: Correlation of viral load, NVP plasma trough concentration and emergence of NVP resistance-associated substitutions in subjects receiving NVP IR treatment (Source; NDA 201152, study report U10-3152-01, data presented in DAVP Resistance template Format)

Subject ID	Virologic Response	HIV-1 RNA copies/mL at BL	HIV-1 RNA copies/mL at the time of emergence of substitution	NVP trough conc. µM (ng/mL)	Treatment-emergent NVP resistance-associated substitution
10167	Partial responder	339,053	101,000	19.3 (5130)	Y181C
10347	Early rebounder	90,280	16,500	16.4 (4370)	V108I/V, Y181C
11332	Early rebounder	406,357	1,750	12.5 (3340)	Y181C
11386	Late rebounder	204,814	75,100	2.1 (556)	Y188N
11549	Partial responder	65,194	17,800	14.3 (3810)	Y181C
11579	Partial responder	2,029,241	397,000	17.7 (4720)	Y1811C/Y, Y188L
11821	Partial responder	190,871	35,500	20.1 (5360)	V106A, F227L
12234	Early rebounder	128,312	4,340	15.7 (4190)	V90I, ***K103N, E138G, P225H/P**
12416	Partial responder	382,903	74,300	15.2 (4040)	K101E/K/Q, Y181C
12452	Early rebounder	155,119	9,080	20.5 (5450)	K103K/N, Y181C
13079	Early rebounder	9,664	3,550	0.4 (113)	K103N, Y181C/Y
13968	Partial responder	170,235	151,000	16.6 (4420)	K101E/K, E138E/G, Y181C/Y, G190A
14105	Partial responder	2,185,132	200,000	16.2 (4310)	K101E/K, Y181C

14187	Early rebounder	19,473	44,100	11.2 (2990)	***V106I, V179I, Y188L**
14188	Early rebounder	133,675	78,100	13.0 (3470)	V108I/V, Y181C
14208	Early rebounder	15,174	4,320	9.4 (2490)	K103N, Y181C
14374	Partial responder	93,631	1,120	5.3 (1420)	K103K/N
14386	Late rebounder	109,117	1,290	10.6 (2830)	V106A, Y181C/Y, F227L
14930	Partial responder	212,915	7,350	23. 1 (6140)	V108I, Y181C
15348	Early rebounder	343,103	14,500	18.4 (4910)	K101E/K, V108I, Y181C
15709	Partial rebounder	2,960,000	11,200,000	11.8 (3150)	K103N, Y181C
15996	Partial rebounder	103,719	16,900	9.5 (2520)	K101E/K, Y181C
16003	Late rebounder	35,601	2,590	[#]0.8 (222)	Y181C
16037	Partial responder	451,949	12,500	NA	K103N, Y181C/Y
16249	Early rebounder	414,097	729,000	5.9 (1570)	K103K/N, V106M/V, V179I/V
16250	Early rebounder	131,225	4,020	19.0 (5060)	K103K/N, V108I, Y181C/Y
16251	Early rebounder	81,421	117,000	BLQ	K103K/N, Y181C/Y
16812	Early rebounder	32,243	2,320	33.3 (8850)	K103N, V106M
17549	Partial responder	222,486	6,470	11.9 (3160)	Y181C
17596	Early rebounder	40,786	7,790	21.6 (5750)	Y181C
17615	Partial rebounder	145,000	26,900	20.1 (5360)	Y181C
18637	Late rebounder	41,202	8,320	11.5 (3050)	Y181C

[#] anomalous results, WK 24 and WK 40 NVP concentration for this subject were 6.1 µM (1630 ng/mL) and 18.5 µM (4930 ng/mL), respectively.
* substitutions shown in bold were also present in BL isolates
**No new substitution: substitution present in BL isolates were also present in on therapy isolates
BLQ below the limit of quantification.

Table 10 shows that most subjects in the NVP IR treatment group whose on-therapy isolates developed NVP-resistance amino acid substitutions had baseline viral load higher than >100,000 copies/mL (n 21). Ten subjects (#s 10347, 11549, 14187, 14208, 14374, 16003, 16251, 16812, 17596, 18637) in the NVP IR treatment group with on-therapy isolates containing NVP resistance-associated substitutions had baseline HIV-1 RNA levels of >10,000 to 100,000 copies/mL. Only one subject (#13079) in the NVP IR treatment group with on-therapy isolates containing NVP resistance-associated substitutions had baseline viral load <10,000 copies/mL.

Except for three subjects, NVP plasma trough concentration ranged from 5.3 to 33.3 µM (1420 to 8850 ng/mL). NVP trough concentrations at or near the time of emergence of NVP drug resistance-associated substitutions were 0.4, 0.8, and 2.1 µM (113, 222 and 556 ng/mL) for subjects #13079, #16003 and #11386, respectively. The median EC_{50} value of NVP against clinical isolates of HIV-1 was 90 nM (0.0234 µg/mL). NVP trough plasma concentrations exceeded the EC_{50} values 60- to 378-fold for most subjects with HIV-1 isolates developing NVP-resistance-associated substitutions. NVP plasma trough concentration in viral failure subjects did not correlate with the development of NVP-resistance-associated substitutions in failure isolates

Table 11: Correlation of viral load, NVP plasma trough concentration and emergence of NVP resistance-associated substitutions in subjects receiving NVP XR treatment (Source; NDA 201152, study report U10-3152-01, data presented in DAVP Resistance template Format)

Subject ID	Virologic Response	HIV-1 RNA copies/mL at BL	HIV-1 RNA copies/mL at the time of emergence of substitution	NVP trough conc. µM (ng/mL)	Treatment-emergent NVP resistance associated substitution
10238	Late rebounder	157,493	71,100	NA	V179D/E/V
10881	Partial rebounder	496,741	211,000	10.0 (2660)	V90I/V, Y181C
10990	Partial responder	313,033	27,100	12.1 (3230)	***V106I, Y188L, P225H**
11026	Partial responder	773,873	58,300	16.1 (4280)	Y181C
11078	Early rebounder	160,754	519	8.4 (2250)	V106A, Y181C
11276	Late rebounder	216,166	722	9.4 (2510)	V90I, V108I/V, Y181C
11383	Partial rebounder	1,608,478	102,000	11.5 (3060)	Y181C, M230L/M
11494	Partial responder	709,126	928,000	8.2 (2180)	K101K/R, V108I/V, Y181C
11560	Partial	88,311	6,330	8.4	Y181I

	responder			(2230)	
12349	Early rebounder	126,554	4,640	11.6 (3100)	K101E/K, K103K/N, V106A/V, Y181C
12362	Partial responder	221,702	1,440	1.5 (411)	***E138A**
13070	Partial responder	293,182	34,400	9.2 (2450)	Y188C, M230L
14201	Early rebounder	9,953	13,400	BLQ	K103K/N, Y181C/Y, G190A/G
14575	Early rebounder	13,316	909	11.9 (3160)	K103K/N, Y188F/H/L/Y
16036	Late rebounder	289,889	5,720	24.1 (6410)	K103K/N, Y181C
16820	48-WK Responder	742,320	668,000	NA	K103K/N
17100	Partial responder	120,851	35,900	16.0 (4260)	Y181C, M230L
17198	Partial responder	115,269	118,000	9.9 (2630)	V108I/V, Y181C
17556	Early rebounder	21,373	5,620	13.5 (3580)	Y181C
17645	Early rebounder	148,946	3,500	9.6 (2550)	V108I, Y181C/Y
18407	Partial responder	1,132,078	57,900	12.0 (3200)	K103K/N, Y181C

BLQ below the lower limit of quantification.
*No new substitution: substitution present in BL isolates were also present in on therapy isolates

Table 11 shows that most subjects in the NVP XR treatment group whose on-therapy isolates developed NVP-resistance amino acid substitutions had baseline viral load higher than >100,000 copies/mL (n 17). Three subjects (#11560, #14575, #17556) in the NVP XR treatment group with on-therapy isolates containing NVP resistance-associated substitutions had baseline HIV-1 RNA levels of >10,000 to 100,000 copies/mL. Only one subject (#14201) in the NVP XR treatment group with on-therapy isolates containing NVP resistance-associated substitutions had baseline viral load <10,000 copies/mL.

Except for 2 subjects, NVP trough plasma concentrations for subjects ranged from 8.2-24.1 µM (2180 to 6410 ng/mL). NVP trough concentration for 1 subject was 1.5 µM (411 ng/mL) [# 12362]) and for another subject, NVP plasma trough concentration values were below the limit of quantification. Thus for most subjects NVP trough concentrations exceeded the EC_{50} values of HIV-1 clinical isolates 93- to 273-fold.

METHODOLOGY

Genotyping

Plasma samples for HIV-1 genotyping were collected at Visit 1 and analyzd in real time. A plasma sample for storage was collected at every visit, stored frozen at [redacted] (b) (4). On-study samples selected by BIPI based on subject response to treatment (in cases of virologic failure) were tested. All genotypic testing was conducted by [redacted] (b) (4) using their commercially available [redacted] (b) (4) The report notes all amino acid changes from a "wild-type" reference sequence. The [redacted] (b) (4) algorithm for identifying resistance-associated amino acids and predicting resistance is based on published scientific literature and proprietary information from the [redacted] (b) (4) phenotype-genotype database.

Phenotyping

All phenotypic testing was conducted by [redacted] (b) (4) using their commercially available [redacted] (b) (4) assay. Results are presented as fold changes in the EC_{50} value relative to a reference strain. Changes in EC_{50} value of failure sample relative to baseline (BL) sample are presented as Δ FC values.

CONCLUSIONS

Genotypic analysis was performed for baseline and on-therapy isolates from 34 and 23 virologic failure subjects in the NVP IR and NVP XR treatment groups, respectively in study 1100.1486. Nevirapine resistance-associated substitutions developed in the on-therapy isolates of 88% (30/34) of the subjects in the NVP IR treatment group, and 78% (18/23) of the virologic failures subjects in the NVP XR treatment group, respectively. The Y181C nevirapine resistance-associated substitution was found alone or in combination with other nevirapine resistance-associated substitutions (K101E, K103N, V106A, V108I, Y188 C/F/H/L/N, G190A, P225H, F227L, M230L) in isolates from 25 subjects failing NVP IR treatment and 14 subjects failing NVP XR treatment. Thus, Y181C was the predominant amino acid substitution emerging in on-therapy isolates from virologic failure subjects receiving either NVP IR or NVP XR treatment in this study. The Y181C substitution has also been observed as a predominant substitution in isolates from subjects failing NVP therapy in other studies (Banister et al., 2008; de Bethune, 2010). On-therapy isolates from 1 subject in NVP IR treatment group developed a novel amino acid substitution Y188N and isolates from another subject in the NVP XR treatment group developed a novel amino acid substitution Y181I. Amino acid substitutions Y181I and Y181C have been also shown to be associated with etravirine resistance (Vingerhoets et al., 2010).

V90I substitution was detected alone or in combination with other NVP resistance-associated substitutions in baseline and on-therapy isolates from virologic failure subjects in both the NVP IR and NVP XR treatment groups. The amino acid substitution E138A alone was present in both baseline and on-therapy isolates from 1 partial rebounder subject and in combination with other NVP resistance associated substitution in on-therapy isolates from another partial rebounder subject in the NVP XR treatment group. Both V90I and E138A substitutions have been also shown to be associated with etravirine resistance (Vingerhoets et al., 2010).

Phenotypic analysis showed that Y188N substitution conferred 103-, 22- and 7-fold deceases in susceptibility to nevirapine, delavirdine and efavirenz, but no decrease in susceptibility to etravirine.

Similarly, Y181I substitution decreased susceptibility to nevirapine, delavirdine and etravirine by 22-, 3- and 8-fold. However, the Y181I substitution did not confer reduced susceptibility to efavirenz.

Baseline viral load was important determinant for the emergence of NVP resistance-associated substitutions. Most subjects in the NVP IR (n 21) and NVP XR (n 17) treatment groups whose on-therapy isolates developed NVP resistance-associated amino acid substitutions had baseline viral load higher than >100,000 copies/mL. On-therapy isolates from ten subjects in the NVP IR treatment group and 3 subjects in the NVP XR treatment group with a baseline HIV-1 RNA levels of >10,000 to 100,000 copies/mL also developed NVP resistance-associated substitutions. On the other hand, on-therapy isolates from only one subject in the NVP IR treatment group and 1 subject in the NVP XR treatment group with baseline HIV-1 RNA <10,000 copies/mL developed NVP resistance-associated substitutions.

A direct correlation between NVP plasma concentration and the emergence of NVP resistance-associated substitutions was not observed. NVP plasma trough concentrations at the time of emergence of NVP resistance-associated substitutions in on-therapy isolates ranged from 5.3 to 33.3 µM (1420 to 8850 ng/mL) for most subjects in the NVP IR treatment group and 8 to 24 µM (2180 to 6410 ng/mL for most subjects in the NVP XR treatment. The median EC_{50} value of NVP against clinical isolates of HIV-1 was 90 nM (0.0234 µg/mL). NVP trough plasma concentrations exceeded 60- to 378-fold the EC_{50} values for most subjects with HIV-1 isolates developing NVP resistance-associated substitutions in the NVP IR treatment group and 93- to 273-fold the EC_{50} values for subject in the NVP XR treatment group. In a study by Rey et al., 2009, in subjects receiving once daily NVP in combination with lamivudine and tenofovir DF, mean NVP trough plasma concentrations were not significantly different in subjects who had full viral suppression compared to those with viral failure. Gonzalez de Requena et al., 2005 attempted to correlate the selection of NVP-resistance associated substitutions with NVP plasma concentrations. In virologic failure subjects, HIV-1 strains carrying NVP resistance-associated substitutions were more frequently detected (19/20, 95%) in subjects with NVP trough plasma concentrations of 12 to 16 µM (3,100 to 4,300 ng/mL). Gonzalez de Requena et al., 2005 stated that prolonged maintenance of plasma concentrations at this level could allow the replication of HIV-1 strains with reduced susceptibility, thus promoting the appearance of resistant strains. However, in this study NVP resistance-associated substitutions were found in isolates from 13/23 (56.52%) and 13/16 (81.2%) virologic failure subjects with NVP trough concentrations of <11.65 µM (<3100 ng/mL) and >16.16 µM (>4300 ng/mL), respectively. Thus, a correlation of NVP trough plasma concentration and emergence of NVP-resistance associated substitutions could not be established.

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Microbiology Label (current version)

BIPI has added new information in the Microbiology label under resistance section (shown in bold). This paragraph will be striked out and replaced with the DAVP proposed changes.

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, ß, γ or δ) are not inhibited by nevirapine.

Antiviral Activity

The antiviral activity of nevirapine has been measured in a variety of cell lines including peripheral blood mononuclear cells, monocyte-derived macrophages, and lymphoblastoid cell lines. In an assay

using human embryonic kidney 293 cells, the median EC_{50} value (50% inhibitory concentration) of nevirapine was 90 nM against a panel of 2923 wild-type isolates of HIV-1 that were primarily (93%) clade B clinical isolates from the United States. The 99th percentile EC_{50} value was 470 nM in this trial. The median EC_{50} value was 63 nM (range 14-302 nM, n 29) against clinical isolates of HIV-1 clades A, B, C, D, F, G, and H, and circulating recombinant forms CRF01_AE, CRF02_AG and CRF12_BF. Nevirapine had no antiviral activity in cell culture against group O HIV-1 isolates (n 3) or HIV-2 isolates (n 3) replicating in cord blood mononuclear cells. Nevirapine in combination with efavirenz exhibited strong antagonistic anti- HIV-1 activity in cell culture and was additive to antagonistic with the protease inhibitor ritonavir or the fusion inhibitor enfuvirtide. Nevirapine exhibited additive to synergistic anti-HIV-1 activity in combination with the protease inhibitors amprenavir, atazanavir, indinavir, lopinavir, nelfinavir, saquinavir and tipranavir, and the NRTIs abacavir, didanosine, emtricitabine, lamivudine, stavudine, tenofovir and zidovudine. The anti-HIV-1 activity of nevirapine was antagonized by the anti-HBV drug adefovir and by the anti-HCV drug ribavirin in cell culture.

Resistance

HIV-1 isolates with reduced susceptibility (100- to 250-fold) to nevirapine emerge in cell culture. Genotypic analysis showed mutations in the HIV-1 RT gene encoding Y181C and/or V106A substitutions depending upon the virus strain and cell line employed. Time to emergence of nevirapine resistance in cell culture was not altered when selection included nevirapine in combination with several other NNRTIs.

Phenotypic and genotypic changes in HIV-1 isolates from treatment-naïve patients receiving either nevirapine (n 24) or nevirapine and ZDV (n 14) were monitored in Phase 1 and 2 trials over 1 to $\geq$12 weeks. After 1 week of nevirapine monotherapy, isolates from 3/3 patients had decreased susceptibility to nevirapine in cell culture. One or more of the RT mutations resulting in amino acid substitutions 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 cell culture compared to baseline, and had one or more of the nevirapine-associated RT resistance substitutions. Nineteen of these patients (80%) had isolates with Y181C substitutions regardless of dose.

Genotypic analysis of isolates from antiretroviral-naïve patients experiencing virologic failure (n 71) receiving nevirapine once daily (n 25) or twice daily (n 46) in combination with lamivudine and stavudine (trial 2NN) for 48 weeks showed that isolates from 8/25 and 23/46 patients, respectively, contained one or more of the following NNRTI resistance-associated substitutions: Y181C, K101E, G190A/S, K103N, V106A/M, V108I, Y188C/L, A98G, F227L, and M230L.

(b) (4)

(b) (4)

DAVP proposed changes (02/14/11) and accepted by BIPI (02/18/11)

For trial 1100.1486, genotypic analysis was performed for baseline and on-therapy isolates from 23 and 34 virologic failure subjects in the NVP XR and NVP IR treatment groups, respectively. Nevirapine resistance-associated substitutions developed in the on-therapy isolates of 78% (18/23) of the virologic failures subjects in the NVP XR treatment group and 88% (30/34) of the subjects in the NVP IR treatment group, respectively. The Y181C nevirapine resistance-associated substitution was found alone or in combination with other nevirapine resistance-associated substitutions (K101E, K103N, V106A, V108I, Y188 C/F/H/L/N, G190A, P225H, F227L, M230L) in isolates from 14 subjects failing NVP XR treatment and 25 subjects failing NVP IR treatment. On-therapy isolates from 1 subject in the NVP XR treatment group developed a novel amino acid substitution Y181I and isolates from another subject in the NVP IR treatment group developed a novel amino acid substitution Y188N. Phenotypic analysis showed that Y188N and Y181I substitutions conferred 103- and 22-fold reductions in susceptibility to nevirapine, respectively.

Cross-resistance

Rapid emergence of HIV-1 strains which are cross-resistant to NNRTIs has been observed in cell culture. Nevirapine-resistant HIV-1 isolates were cross-resistant to the NNRTIs delavirdine and efavirenz. The Y188N conferred 22- and 7-fold reductions in susceptibility to delavirdine and efavirenz, respectively, but no decrease in susceptibility to etravirine. Similarly, the Y181I substitution reduced susceptibility to delavirdine and etravirine 3- and 8-fold, respectively, but did not reduce susceptibility to efavirenz. However, nevirapine-resistant isolates were susceptible to the NRTIs ddI and ZDV. Similarly, ZDV-resistant isolates were susceptible to nevirapine in cell culture.

RECOMMENDATIONS

With respect to microbiology, this NDA is approvable.

_____
Lalji Mishra, Ph.D.
Microbiologist

CONCURRENCES:

_____ Date_____

HFD-530/J. O'Rear /TL Micro

CC:
HFD-530/NDA 201152
HFD-530/ Division File
HFD-530/ Micro/L. Mishra
HFD-530/CSO/Himaya, A.

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/s/

LALJI MISHRA
02/24/2011

JULIAN J O'REAR
02/24/2011

VIROLOGY FILING CHECKLIST FOR NDA or Supplement

NDA Number: 201152 **Applicant:** Boehringer Ingelheim Pharamceuticals, Inc. **Stamp Date:** 06/03/10

Drug Name: Viramune ®XR™(extended release tablets) **NDA Type:**000

On **initial** overview of the NDA application for filing:

	Content Parameter	Yes	No	Comments
1	Is the virology information (nonclinical and clinical) provided and described in different sections of the NDA organized in a manner to allow substantive review to begin?	X		Non-clinical studies reviewed in the original NDA20-636
2	Is the virology information (nonclinical and clinical) indexed, paginated and/or linked in a manner to allow substantive review to begin?	X		
3	Is the virology information (nonclinical and clinical) legible so that substantive review can begin?	X		
4	On its face, has the applicant submitted cell culture data in necessary quantity, using necessary clinical and non-clinical strains/isolates, and using necessary numbers of approved current divisional standard of approvability of the submitted draft labeling?	X		Reviewed previously and incorporated in Viramune Label
5	Has the applicant submitted any required animal model studies necessary for approvability of the product based on the submitted draft labeling?		X	Not needed
6	Has the applicant submitted all special/critical studies/data requested by the Division during pre-submission discussions?	X		
7	Has the applicant submitted the clinical virology datasets in the appropriate format as described in the relevant guidance documents and are the datasets complete?		X	Resistance data not submitted in DAVP Template Format
8	Has the applicant used standardized or nonstandardized methods for virologic outcome measures? If nonstandardized methods were used, has the applicant included complete details of the method, the name of the laboratory where actual testing was done and performance characteristics of the assay in the laboratory where the actual testing was done?	X		
9	Has the applicant submitted draft labeling consistent with current regulation, divisional and Center policy, and the design of the development package?	X		
10	Has the applicant submitted annotated microbiology draft labeling consistent with current divisional policy, and the	X		

VIROLOGY FILING CHECKLIST FOR NDA or Supplement

	Content Parameter	Yes	No	Comments
	design of the development package?			
11	Have all the study reports, published articles, and other references been included and cross-referenced in the annotated draft labeling or summary section of the submission?	X		
12	Are any study reports or published articles in a foreign language? If yes, has the translated version been included in the submission for review?		X	

IS THE MICROBIOLOGY SECTION OF THE APPLICATION FILEABLE?

If the NDA is not fileable from the microbiology perspective, state the reasons and provide comments to be sent to the Applicant.

The NDA is fileable.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

The sponsor is requested to provide resistance data (Study U10-3152-01) in the DAVP Template Format.

Lalji Mishra, Ph.D. 07/13/10

Reviewing Microbiologist Date

Microbiology Team Leader Date

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-201152	ORIG-1	BOEHRINGER INGELHEIM PHARMACEUTICALS INC	Nevirapine Extended Release Tablets

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/s/

LALJI MISHRA
07/27/2010

JULIAN J O'REAR
07/27/2010